CHAPTER ONE

1.0 INTRODUCTION

The world's agriculture practices and problems are no doubt facing new challenges. The demand for food, feed and fibre remains unsatisfied, the human population is increasing rapidly, the resources for manipulating the environment to fit the crop requirements are obviously quite limited, and the time pressure to meet the challenges is increasing geometrically (Plaxton, 2004).

For some years, soil salinity has been a major threat facing agriculture. It limits plant growth and causes severe loss of vegetable crops (Allakhverdiev *et al*., 2000). Salinity has a great impact on the quality and quantity of food that people consume as well as the social and environmental effects that it entails. This has led to major changes in the food chain (Cheng *et al*., 2012). Salt-affected areas keep increasing, and, on the other hand, arable land is being abandoned yearly due to saline soils (Mallik *et al.*, 2011). Most of the lands used for agriculture are greatly affected with salt, which often reduces the commercial production of vegetables (Munns, 2005), and other agricultural products worldwide (Akbarimoghaddam *et al*., 2011). Sodium and calcium ions are accumulated in the tissues of of salt stressed plant. Uptake of both sodium and calcium ions in the plant tissues often result to significant physiological disorder(s) due to ionic imbalance. Reports indicate that potassium ion is inhibited due to high concentration of sodium ion, which lower production and consequently leads to plant mortality (James *et al*., 2010). Salinity stress generates free radicals in plant tissues under saline soil (Apel and Hirt, 2004). It often results into damage of macromolecule consistuents (such as proteins, RNA, and DNA) (Jaleel *et al*., 2009). Reactive oxygen species (ROS) cause chlorophyll destruction and reduce the root meristem activity, which consequently leads to poor crop yield (Foreman *et al*., 2003).

The nutritional state of the people can be enhanced by adequate production of foods of good quality (FAO, 2002). Concerted research efforts by scientists have been put in place for the production of foods with high nutrient values to meet the nutritional requirements of the human race (Oludimu and Ayanlaja, 2001). Previous studies have shown that soil and climatic conditions in Nigeria favour the cultivation of various vegetables and fruits like okra, tomato, pepper, onion, oranges and amaranthus (Atayese *et al*., 2004). Majority of Nigerians eat

vegetables and fruits on regular basis with staple food products. Okra (*Abelmoschus esculentus*) is a fruit vegetable crop that is common in various markets throughout Nigeria and it is consumed worldwide (Oyelade *et al*., 2003). It is originated commercially in Asia, Africa and the Southern United States. In term of okra production, India ranked first worldwide (FAOSTAT, 2008). Okra is usually cultivated for its fleshy edible green pods (Franklin, 1982). It was originally referred to as *Hibiscus esculentus*, and in Nigeria, various names have been given to okra based on ethnicity: 'ikhiavbo' in Edo, 'etigi' in Efik, 'atike' in Ibibio, 'okwulu or okwuru' in Igbo, 'bera or dandawan betso' in Hausa and 'ila' in Yoruba (Chauchan, 1972). The matured seeds of okra fruits can be used for coffee, the crude fibre is used in the paper industries. Okra fruit is also rich in vitamins and minerals which are not present in sufficient amount in the diet consumed by the people in low-income countries (IBPGR, 1990). Studies have shown the ameliorating effects of okra extract on various metabolic disorders. It helps in the treatment of ulcers and haemorrhoids (Adams, 1975). In the treated rats, polysaccharide constituents of okra have hypoglycemic and hypolipidemic activities (Tomoda *et al*., 1989; Ngoc *et al*., 2008).

Various techniques of treatments like fertilizer application with the aim of increasing yield of fruits and crops in the time past has been observed to have negative effects both on the environment and the quality of fruit (Alleyne and Clark, 1997). Nitrate leaching, eutrophication and greenhouse-gas emissions are the prevalent environmental threats linked with the applications of fertilizer (Su *et al*., 2003). Also, plant breeding techniques are cost intensive and time consuming (Lindner, 2004). These, coupled with the increasing trend of saline soils calls for research into alternative methods of treatments to avert food insecurity (Pacini *et al*., 2003).

Synthetic plant bioregulators have been developed in recent times with a view to manipulating plant developmental stages in order to aid quantity and quality of food crops (Das *et al*., 2015). The fact that their use tends to ameliorate the menace of saline soils makes this research worthwhile. In particular, their application to the okra crop under saline soil conditions has not been given much attention within the Black African sub-continent.

1.1 RATIONALE, AIM AND OBJECTIVES OF THE STUDY

Taking into consideration the increasing interest in the use of plant bioregulators worldwide for food crop production, improvement as well as the nutritional aspect and ethno medicinal importance of okra, this research aims at evaluating the influence of bioregulators and salinity stress on some biochemical, nutritional and antidiabetic properties of okra. The bioregulators used are salicylic acid (SA) and indole-acetic acid (IAA).

This research is specifically designed to:

(i) determine the ameliorative effect of bioregulators on salt-stressed okra,

(ii) determine the level of some enzymes responsible for various biochemical and physiological changes in the leaves and fruits tissue of treated plants,

(iii) examine the interactive effects of salicylic acid or indole-acetic acid and salt stress on proline contents, total soluble protein accumulation and antioxidants activity in okra plant genotypes,

(iv) screen phytochemical constituents of ethanol and aqueous extracts of bioregulator-treated okra fruits,

(v) evaluate the aqueous and ethanol extracts effects of bioregulator-treated okra fruits on intestinal glucose transporter using *Xenopus lavis* (Frog) oocytes,

(vi) evaluate the effects of bioregulator-treated okra fruits extracts in hyperglycemic and hyperlipidemia animals respectively.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Okra

Okra is an essential vegetable crop across the globe that is cultivated throughout the tropics. It is highly affected by environmental factors, and its cultivation varies from country to country (Kochlar, 1999). Okra is a multifunctional crop, the matured seeds are good sources of essential oils, the crude fibre in okra serves as a raw material in the paper industry, and can also be consumed as a vegetable (Kochlar, 1999).

2.1.1 Chemical composition

Okra is a medicinal vegetable crop on the basis of its chemical composition. Various researches have shown that the fibre component of okra contains α-cellulose, hemicellulose, lignin and pectic matter, while other constituents are in low proportion. Table 1 below shows the nutritional value per 100g of raw okra.

Table 1: Nutritional composition of raw okra (100 g)

Magnesium	57 mg
Protein	2g
Sugars	1.48 g
Energy	33kcal
Calcium	82mg
Iron	0.62 mg
Water	90.19g
Carbohydrates	7.45 g
Ascorbic acid	36μg
Thiamine (Vitamin B1)	0.2 mg
Niacin (Vitamin B3)	1mg
Tocopherol	0.27 mg
Zinc	0.58 mg
Riboflavin(B2)	0.06mg
Vitamin K	31.3 μg
dietary fibres	3.2 g
Potassium	299mg
Fat	0.19g

(Source: USDA database, 1986).

2.1.2 Climate conditions, soil and water management

Okra has the ability to grow on a variety of soils, but the most preferred soil is the one with a high level of organic matter. Fertilizers are required in its cultivation. Saline soil affects the growth and development of okra plant, but a wide range of rainfall system of about 400 mm support its growth and development (Dhankhar *et al*., 2012).

2.1.3 Propagation and planting

2.1.3.1 Land preparation

Okra cultivation requires soil preparation for about two to three months, which allows the decomposition of organic matters on the topsoil. Early cultivation of okra seeds after land preparation has a negative impact on tender weeds before planting. Planting of okra can be done in two major ways namely, direct planting of seeds in the field and nursery method. For proper sprouting, okra seeds need to be soaked in water prior to planting. Seeds planting depth is about 1.5 cm with space of about 45 by 45 cm (Ijoyah *et al*., 2010).

2.1.3.2 Fertilization

Soil analysis is required before planting of okra seeds to determine the soil fertility. Conventional production of fertilizer requires a well composite manure, which must be applied at the rate of 15-20 ton per hectare (17-20 gm/plant). This needs to be mixed properly with the soil before planting. Also, application of NPK fertilizer is recommended at the rate of 120 kilograms per hectare (2 gm/plant). The application of the fertilizer is done around the heaps. Calcium ammonium nitrate (140 kg) can be used to top-dress the plants. Akande *et al*. (2005) reported that urea can be used in place of calcium ammonium nitrate but it should be in the moist soil. The use of urea should be avoided in the alkaline soil as high volatilization of ammonia would occur. Chloride-containing fertilizers should be avoided due to the sensitivity of okra to salinity.

2.1.3.3 Sources of plant nutrients

Okra cultivation flourishes in fertile soil with natural organic matters like compost, manure teas, plant teas such as tithonia for foliar feed are required for the cultivation of okra. Application of a well-composite manure should be between 15-20 t/ha (17-20 gm/plant). During vegetation and flowering periods, additional manure is required. Manure application

can be done on the plant through a drip irrigation system to avoid excessive labour (Akande *et al*., 2005).

2.1.3.4 Irrigation

A high rate of irrigation system is required for the cultivation of okra plant. Postel *et al*. (2001) reported that adequate supply of water is needed for its proper germination. Irrigation can be done during seedling emergence through flowering to pod production.

2.1.3.5 Rotation

Planting of okra can be done through a rotational system of cropping, and this can be rotated with maize, onions, potatoes and peas be in the same family of cotton that has the same pest and diseases (Odeleye *et al*., 2005). Weed control is a necessity during cultivation of okra from the seedling stage through flowering to pod production. Subsistence farmers control the weeds with the use of hoes. The harvesting period of okra occurs when the pod is matured at around 2 to 3 months. Harvesting of the pod is done 4 to 6 days after flowering. Depending on the market requirement as well as varieties, pods are harvested at the tender stage with a length of 7 to 15 cm. Under normal conditions, the pods are produced repeatedly for several months. In every 1 to 2 days, picking of the pod is essential to meet market special specifications. It is not advisable to harvest okra during excessive rainfall, as the excess moisture can develop mould around the pods. Because of the short lifespan, okra fruit should be harvested prior to the market day. Handling of okra fruits should be mild, otherwise, there may be bruise or discolouration of the pods.

2.1.4. Botanical features of okra

Okra is a semi-perennial crop of about 3 to 6 feet tall. It has an erect semi-woody stem with various short branches, it has a green or reddish colour. The stem can reach the heights of 3, 7 or 8 feet depending on the types or varieties. Okra leaves reach up to 12 inches in length, they are lobed with the hairy surface. The leaves are similar to heart shape. The colour is darkish green. Its flower is spherical in shape with about 1.8 to 2.0 inches in diameter, the petal has yellow colour with a red patch. After the flower is fully matured, a small green pod is developed. The flower is bisexual containing both the androecium and the gynoecium. The androecium has stamens with divergent filaments consisting anthers. The gynoecium has

carpels, styles and an ovary with locules, each containing many ovules. The calyx is embedded to protect the flowering parts (Purseglove, 1968). The flowering feature of okra is depicted in Figure 1.

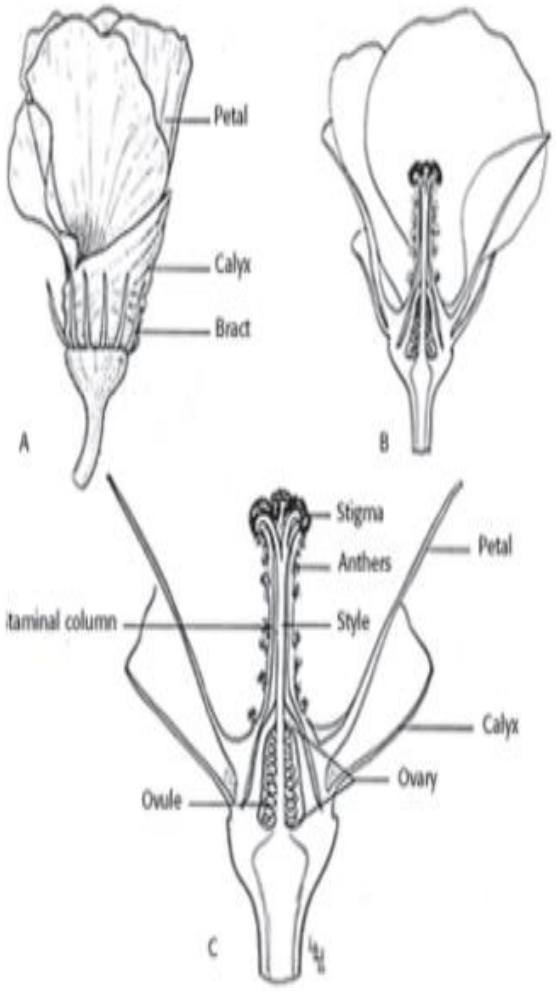


Fig. 1: Okra flower showing a side view (A) and longitudinal sections B and C.

(Source: Purseglove, 1968)

Fruits

The fruit is a conical capsule with five cavities consisting ovules. The fruit is ribbed, long and yellowish green in colour but at times purple or white. The matured pods are plucked at the early stage to prevent the toughness of the pods. The okra seeds are striated with dark green to dark brown colour formation. Figures 2, 3 and 4 depict the fruits, seeds and okra plant respectively.

Fig. 2: Okra fruits.

Fig. 3: Okra seeds.

Fig. 4: Okra plant

2.1.5 Biotic and abiotic factors

2.1.5.1 Insect/pests of okra

Okra plants are prone to attack by shoot and fruit borers. Flea beetle attack seedling and causes significant damage to the leaves (Odebiyi, 1980). Chemical control is required, if they occur in large number on a seedling (Osekre *et al*., 2013). The prominent okra pests include whiteflies, jassids and aphid. These pests cause a reduction in the economic importance of okra by feeding on leaves, stems, branches and pods during the raining season. Grasshoppers and cutworms attack the whole plant of okra, while cottonseed bugs, African bollworms, spiny bollworm and flower beetles damage the flower, pods, and the leaves of germinating okra plants (Fasunwon *et al*., 2010). Control measures against these pests include proper and careful inspection of soil during land preparation, when the numbers of insects are minimal in small fields, handpicking can help to destroy both the eggs and the caterpillars (Obeng-Ofori and Sackey, 2003).

2.1.5.2 Abiotic factors

The common environmental factors that greatly affect okra development are salinity, drought and water stresses (Munns, 2002). Othman *et al*., (2006) reported an increase in the soil soluble salt in response to climate change and thus increases the proportion of salinized land area. Soil fertility is greatly affected by the soil soluble salt, which hampered plant production (Munns and Tester, 2008). The term "salinity" means the accumulation of salts in the soil which above the threshold (IRRI, 2011). Hypertonic conditions and ionic toxicity experienced in the plants' tissues is as a result of the salinity stress. Many metabolic and physiology processes under salt stress are greatly affected (Munns, 2002). Saline soil is primarily caused by the weathering of rock and the subsequent minerals release soluble salts in the soil (Pitman and Lauchli, 2002). Whereas, land clearing with poor water quality for drainage are responsible for the secondary causes of soil salinity (Doering and Sandoval, 1981). Osmotic stress results with the increase in the salt levels outside the roots, which inhibit cell expansion, lateral bud development and water uptake (Munns and Tester, 2008). While excessive accumulation of sodium ion results to ionic stress in the plant tissues, which in turn causes leaf senescence, chlorosis and necrosis of the leaves with a low activity of cellular metabolisms (Glenn *et al*.,1999).

2.1.5.3 Effect of salinity stress in plants

Crop vegetables are sensitive to saline soil because of their nature as glycophytes. The salt compartmentalization mechanism is not well developed in vegetable crops and thus result to increase in the internal concentrations of salt in these vegetables. The ion toxicity occurs in the cell membrane that causes the impairment of ion transporters, which in turn lead to ion imbalances and poor mineral nutrition of the plant. Endogenous enzymes and organelles are affected in the cytoplasm. Salinity alters various biochemical processes and consequently reduces crops viability (Rozema and Flowers, 2008). Induction of osmotic stress due to ion toxicity affects plant vigour (Rahnama *et al*., 2010). Salt concentration in the soil, with an increased rate of transpiration through the leaves reduced the root systems water uptake capacity (Munns, 2005). Increase sodium ion toxicity in the plant tissues inhibits the uptake of K^+ ions and thus responsible for the stunted growth in the plant (James *et al*., 2011; Orthman *et al*., 2006). Salinity causes oxidation reaction in the plant cell, which generates the reactive oxygen species (Ahmad, 2010).

2.1.5.4 Mechanisms of salt tolerance in plants

The salt compartmentalization mechanism is not well developed in vegetable crops and this results to increase in the internal concentrations of salt in vegetables. Therefore, plants employ various mechanisms to overcome salinity stress, such as synthesis of endogenous metabolites, enzymes and hormonal modulation to improve their salt tolerance (Flower and Colmer, 2008).

2.1.5.5 Hormone regulation of salinity tolerance

Bioregulators have gained tremendous attention in alleviating oxidative stress in plants. Salinity reduces plant tissue water content by osmotic pressure with high synthesis of abscisic acid (ABA) (Cabot *et al*., 2009). Bioregulator reduces the salinity effects on plants through improves membrane trafficking and photosynthesis (Popova *et al*., 1995). Potasium ion with organic molecules caused ions homeostasis in the plant tissue (Chen *et al*., 2001). Indole acetic acid (IAA) and brassinosteroids (BR), also participate in salt tolerance in the plant (Clause and Sasse, 1998). Seeds pre-treated with salicylic acid reduced the Na^+ concentration accumulation in the shoot. El-Mashad and Mohamed, (2012) reported that plants enzymes and nonenzymatic compounds activities were increased under the influence of

brassinosteroids (BRs). Brassinosteroids is common in plants, and thus stimulating plants tolerance and productivity in diverse ways (Ashraf *et al*., 2010).

2.1.5.6 Bioregulators

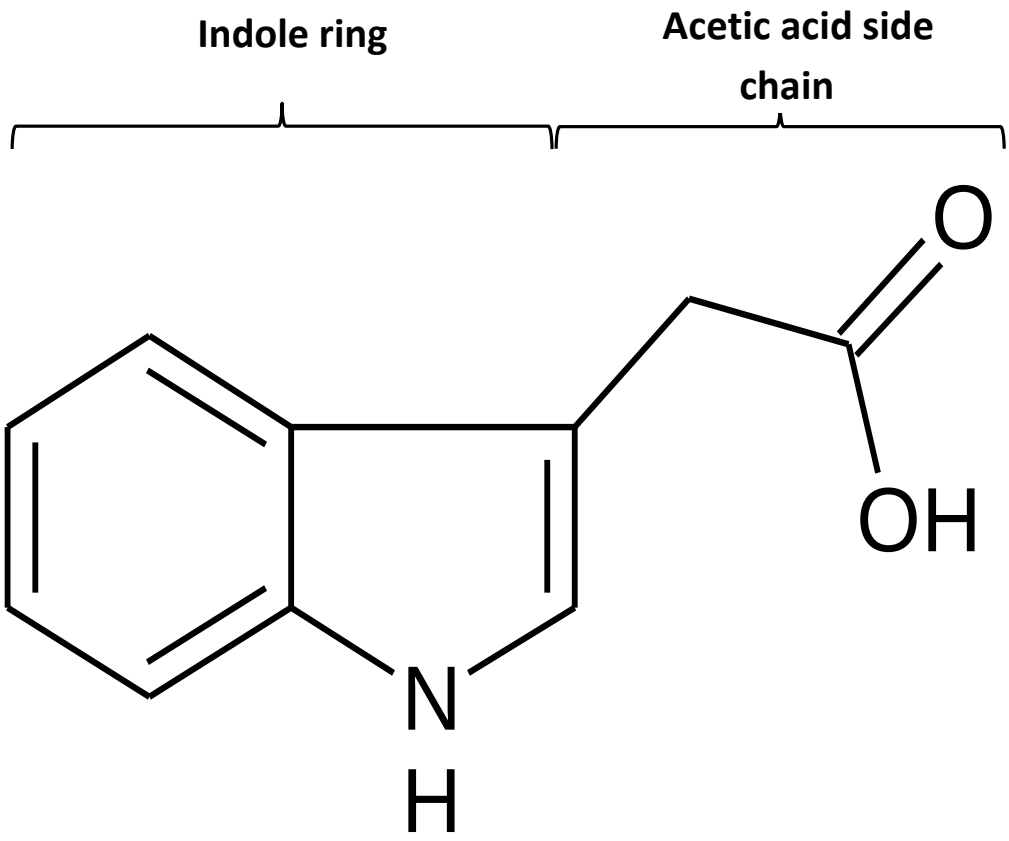
Bioregulators are natural or synthetic biological substances that alter the chemistry of the plant. They improve plant development when the concentrations are low, and inhibit growth at very high concentrations (El-al and Faten, 2009), and with no biocidal or nutritive effect (Rademacher, 2000). Bioregulators are easily assimilated in the plant tissue with the potential of penetrating the surfaces of the cells, and majority of plant parts. Bioregulators are required throughout the entire life of plant. They also help the plant to survive extreme conditions of environmental factors. The biochemical and physiological roles of bioregulators in plants include: germination of seeds and dormancy, plant growth, embryogenesis, flowering and fruiting (El-Rokiek *et al*., 2012), plant defence mechanisms (Kamboh *et al*., 2000; Khan *et al.,* 2002; Deslauriers and Larsen, 2010). During the developmental stages of plants, they are exposed to a various number of stress. The response of plants to these changes is important for their survival (Wang *et al*., 2007). Gibberellic acid (GA_3) is involved majorly in stem and petiole extension, while salicylic acid (SA), sitosterol and putrescine enhance yield in grains and biochemical constituents of plants (Nishi and Anil, 2007). In the same vein, pre-sowing seed treatment of tomato (*Solanum lycopersicon*) with auxins resulted in enhanced seedling emergence (Olaiya and Osonubi, 2009), yield (Olaiya, 2010) and fruit nutritional quality (Olaiya *et al*., 2010).

2.1.5.7 Benefits of chemical manipulation of plant growth with bioregulators

Modification of plants by bioregulator's application at low concentration has more economic importance on food production than plant breeding techniques (Tam *et al*., 1995). The application of commercial chemicals and the time-scale involved to achieve the desired result is always shorter (Cavins *et al*., 2003). Economic importance of bioregulators for food production include; resistance to climate change (Shahid *et al*., 2011); lodging control in vegetables (Szepesi *et al*., 2008); fruit tree shaping (Hayat *et al*., 2007); maturation and inhibition of degradation in sugar contents of sugarcane (Kaydan *et al.,* 2007); inhibition of transpiration and increased water utilization efficiency (Al-Hakim, 2006).

2.1.5.8 Indole acetic acid

Indole acetic acid (IAA) is a heterocyclic compound that belongs to the class of bioregulator called auxin. It is solid, it has no colour and it has the molecular formula $C_{10}H_9NO_2$ and a molar mass of 175.184. Chemical structure of indole acetic acid is shown in Figure 5.



Indole acetic acid

Fig. 5: Structure of indole acetic acid (Source: Bartel, 1997).

Indole acetic acid is produced on the apical meristem in the cells and in the leaves of a plant. Indole acetic acid can be synthesized through the tryptophan pathway and the tryptophan independent pathway. Chemically, indole acetic acid can be synthesized using a chemical reaction between glycolic acid and indole in the presence of a base at a very high temperature (Johnson and Crosby, 1973). Various methods have been adapted for the synthesis of indole acetic acid but originally it can be synthesized by using indole-3- acetonitrile (Majima and Hoshino, 1925).

2.1.5.8.1 Biosynthesis of indole acetic acid

Auxin synthesis occurs in the cytoplasm of germinating plant cells majorly in the apex of the shoot. There are two major pathways for the production of IAA.

1. Tryptophan-dependent Pathway. Many studies have shown that the precursor for the auxin biosynthesis is through an amino acid tryptophan.
2. Tryptophan-independent Pathway. This pathway does not involve tryptophan directly as an auxin biosynthesis precursor.

Chorismate is a precursor for the synthesis of tryptophan through an intermediate indole-3-glycerol phosphate in the chloroplast (Radwanski and Last, 1995). Anthranilate synthase (ASA1 and ASA2) catalysed the initial reaction of the pathway. Indole-3-glycerol phosphate synthase (IGS) catalysed the conversion of 1-(O-carboxyphenylamino)-1-deoxyribulose- 5-phosphate to indole-3-glycerol phosphate (Ouyang *et al*., 2000). In Trp-independent pathway, auxin biosynthesis is directly from indole-3-glycerol phosphate, but there is dearth of information about the pathway (Ouyang *et al*., 2000). Complex enzyme tryptophan synthase is an enzyme with the two subunits namely; tryptophan synthase a gene (TSA1) and tryptophan synthase b genes (TSB1 and TSB2). In plants for the production of various indole containing substances like tryptamine (TAM) derivatives, indole acetic acid, indole glucosinolates and camalexin tryptophan plays a central role (Gaudin *et al*., 1993). Then, the enzyme (indole-3-acetamide hydrolase) with genes; aux2, iaaH, and tms2 catalyses the conversion of indole-3-acetamide (IAM) to indole acdetic acid (Gaudin *et al*., 1993). The pathway is depicted in Figure 6 below.

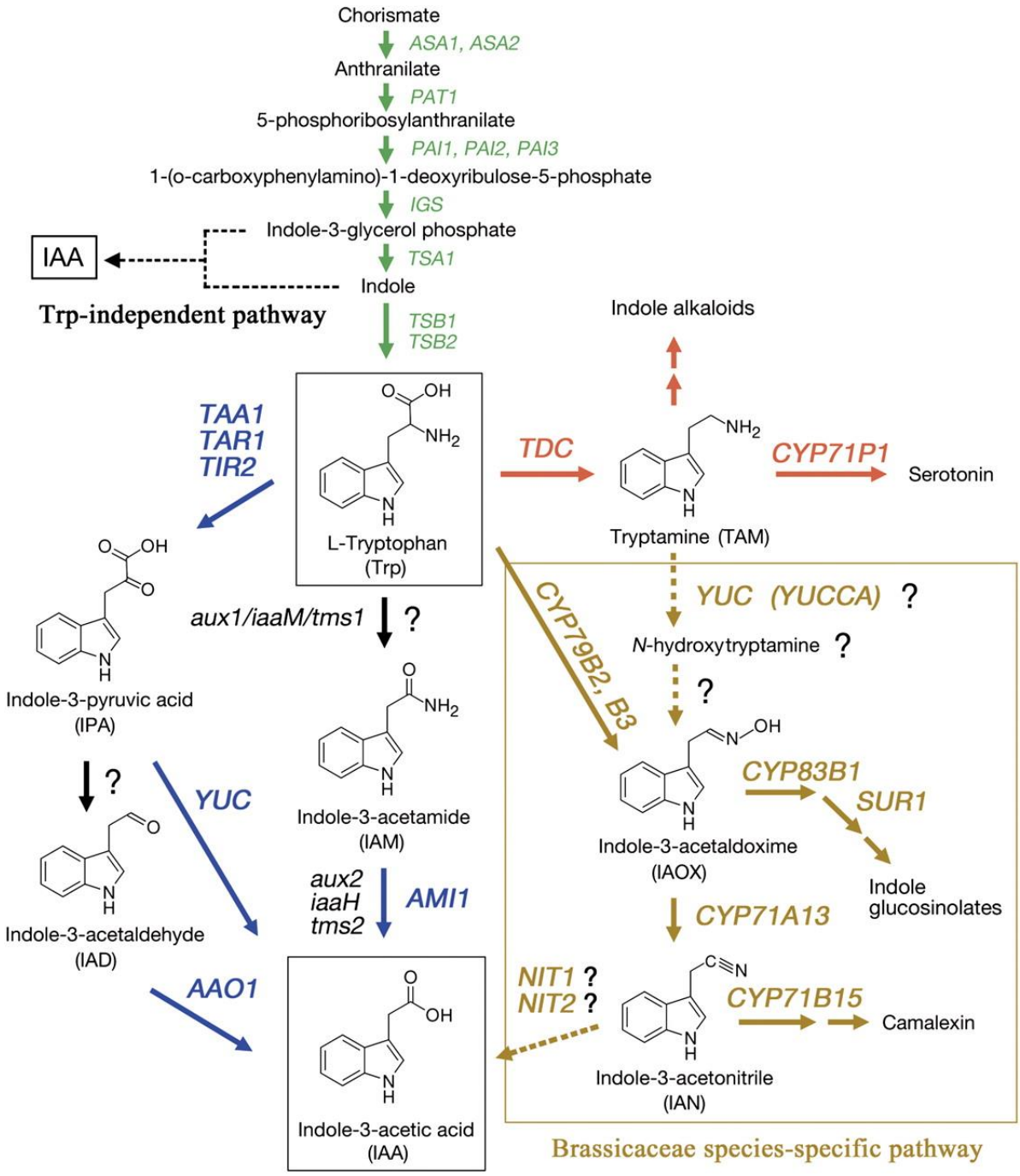


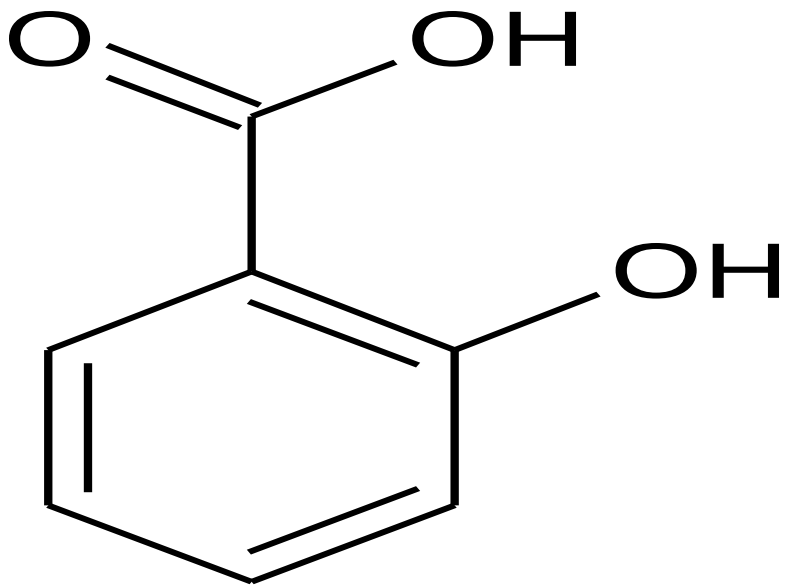
Fig. 6: Biosynthesis of indole acetic acid (IAA). (Source: Normanly, 2010)

2.1.5.9 Salicylic acid

Salicylic acid is a bioregulator that has phenolic ring. It plays crucial roles in the plant tolerance to abiotic factors. Salicylic acid has a molecular mass of 138.2 with chemical formular of $C_6H_6OHCOOH$ (Goldberg, 2009)

.2.1.5.9.1 Synthesis of salicylic acid

Salicylic acid is an aromatic compound containing a hydroxyl group. The most important pathway for the biosynthesis of salicylic acid is through shikimic acid and configuration of trans-cinnamic acid side chain via ortho-coumaric acid or benzoic acid (Schmid and Amrhcin, 1999). Figures 7 and 8 below depict the structure and pathway for the synthesis of salicylic acid respectively.



Salicylic acid

Fig. 7: Structure of salicylic acid (SA) (Source: Schmid and Amrhcin, 1999)

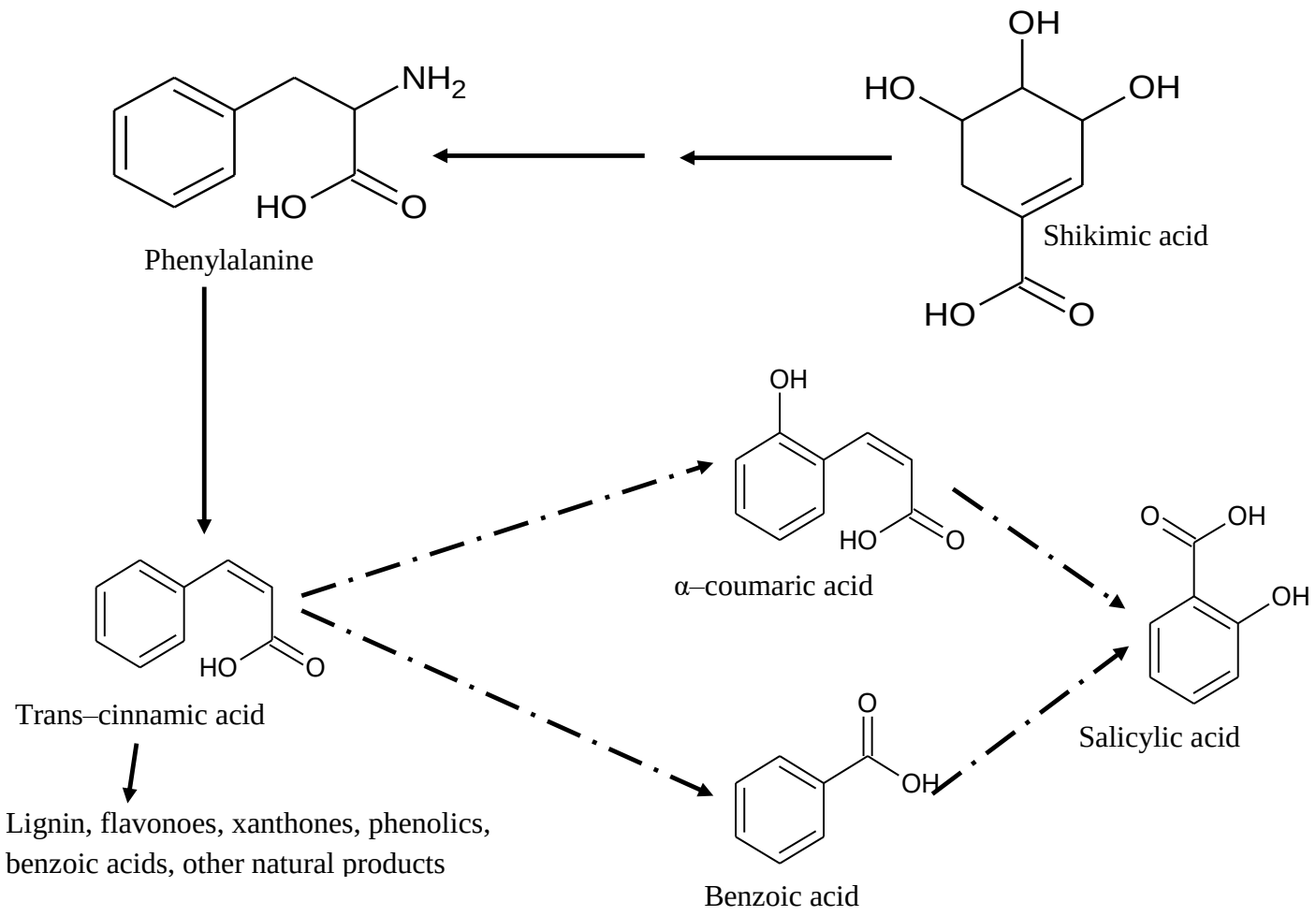


Fig. 8: Pathways of production of the salicylic acid. (Source: Schmid and Amrhcin, 1999)

2.1.5.9.2 SA-regulated physiological functions

2.1.5.9.3 Germination of seeds

Inhibition of *Arabdopsis thaliana* germination at a concentration of salicylic acid (SA) greater than 1 mM was reported by Rajjou *et al.* (2006). Application of salicylic acid at a dose greater than 0.250 mM inhibited the seed germination in barley (Xie *et al.*, 2007). Salicylic acid induces oxidative stress at higher concentration, which is responsible for its negative effect on seed germination. Rao *et al.*, (1997) observed higher hydrogen peroxide (H_2O_2) levels in Arabidopsis plant treated with 1 to 5 mM SA. It is noteworthy that when very low concentration of salicylic acid is applied exogenously, it improves *Arabidopsis* seeds germination by three folds and establish the seedling under varying abiotic stress factors (Rajjou *et al.*, 2006).

2.1.5.9.4 Photosynthesis

Uzunova and Popova, (2000) reported that aerial spray of salicylic acid (as an essential photosynthesis regulator) improves chloroplast and leaf structures of plants, stomatal closure (Melotto *et al.*, 2006), photosynthetic pigment in a dose-dependent on various plant species (Rao *et al.*, 1997). The concentration of about 1–5 mM of salicylic acid showed a decrease chlorophyll contents of *Arabidopsis* (Rao *et al.*, 1997). Low activity of Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) was correlated to low levels of protein relative to the control plants, such changes in the protein levels were associated with precipitation of stroma (Miteva *et al.*, 1992; Uzunova and Popova, 2000). According to Fariduddin *et al.* (2003), the concentration of about 10 μM steadily increase the CO_2 assimilation, photosynthetic rate, chlorophyll content and upregulate the activities of some photosynthetic enzymes. Therefore, the beneficial effects of salicylic acid at a low concentration inhibits photosynthesis oxidation of auxin, increases nitrate reductase activity and photosynthetic rate (Ahmad *et al.*, 2001).

2.2 Photosynthetic Pigments

2.2.1 Chlorophyll

Chlorophyll is a photosynthetic pigment in the chloroplast of the thylakoid membrane of a plant that absorbs sunlight in the electromagnetic spectrum, and uses its energy to produce sugars from H_2O and CO_2. This process is known as photosynthesis and is the basis for sustaining the life processes of all plants. Since plants are the primary producers of food to animals and human beings (Speer, 1997).

2.2.2 Chemical structure of Chlorophyll

Chlorophyll is a photoreceptor molecule that is found in the chloroplast of the green plants, which is responsible for the greenish colouration of plants. Porphyrin ring is the main structure of chlorophyll which is sandwich by a central atom magnesium (Mg) (Scheer, 1988). This is similar to heme found in the haemoglobin, except that the central atom is iron (Fe). The chemical and molecular structures of chlorophyll are shown in Table 2, Figures 9 and 10.

Table 2: Similarities of structures of Chlorophyll a and b

	Chlorophyll a	**Chlorophyll b**
Chemical formula	$C_{55}H_{72}O_5N_4Mg$	$C_{55}H_{70}O_6N_4Mg$
Carbon 3 group	$-CH=CH_2$	$-CH=CH_2$
Carbon 7 group	$-CH_3$	-CHO
Carbon 8 group	$-CH_2CH_3$	$-CH_2CH_3$
Carbon 17 group	$-CH_2CH_2COO$-Phytyl	$-CH_2CH_2COO$-Phytyl
Carbons 17 and 18 bonds	Single	Single
Occurrence	Universal	Mostly plants

(Source: Scheer, 1988)

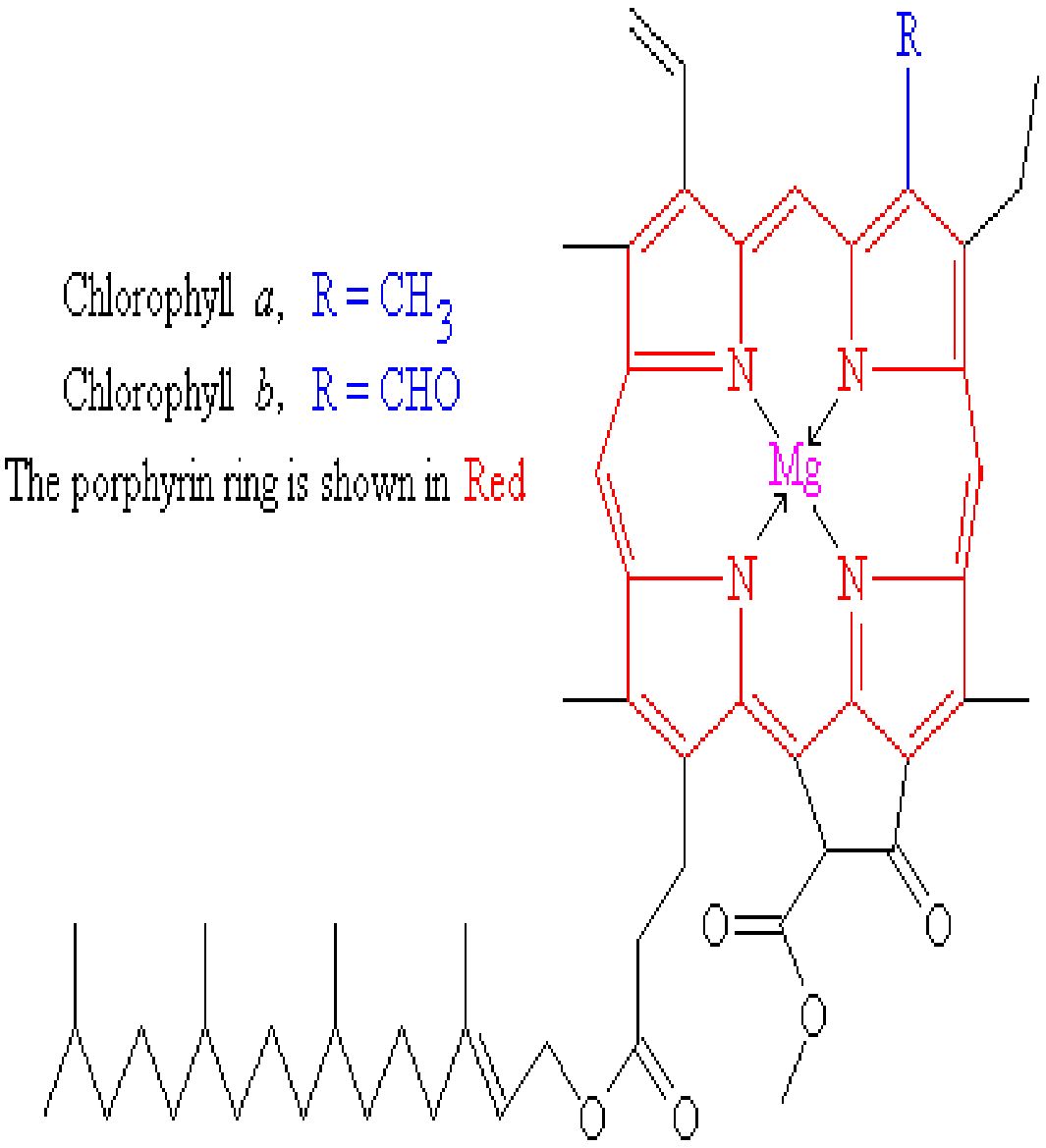


Fig. 9: Structure of chlorophyll (Source: Scheer, 1988)

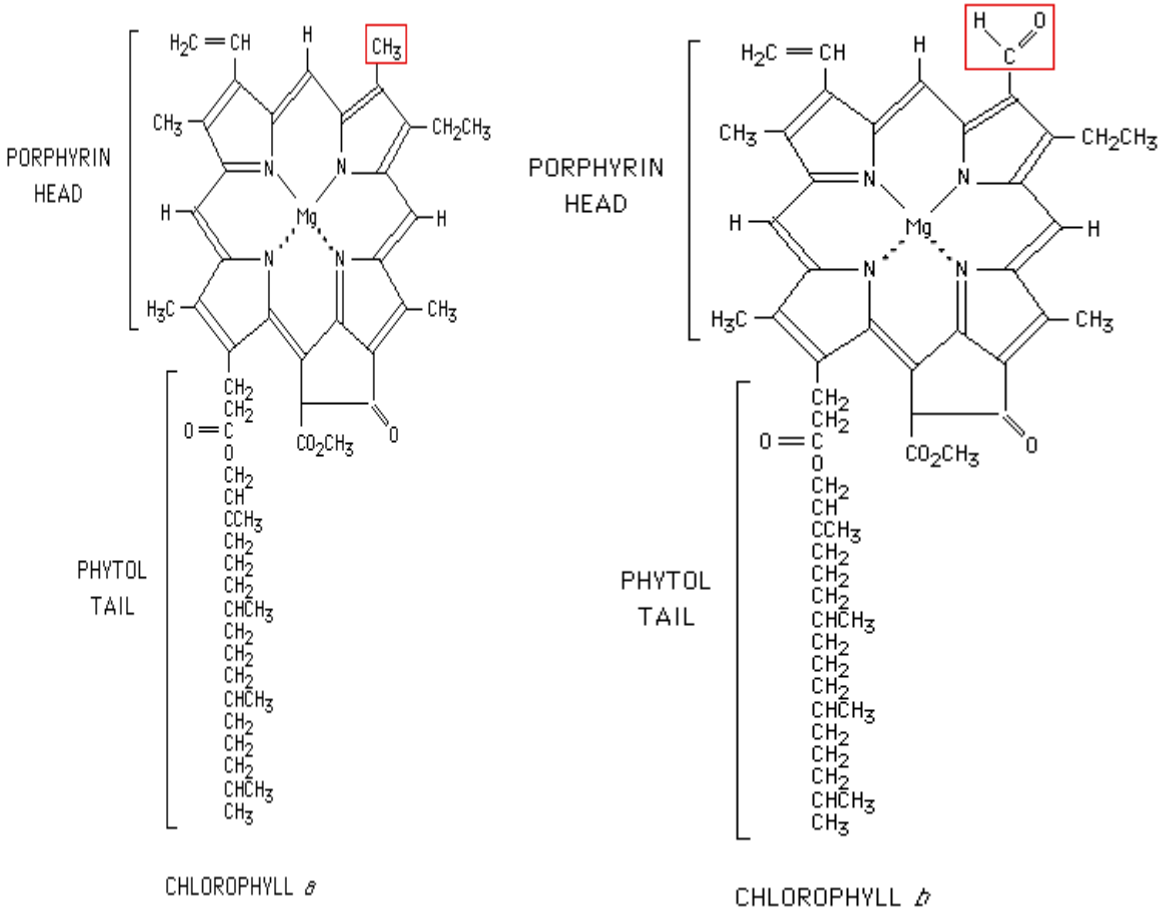


Fig. 10: Structure of chlorophyll a and b (Source: Scheer, 1988)

2.2.3 Biosynthesis of Chlorophyll

Succinyl-CoA and glycine are the major precursors for the synthesis of chlorophyll, but protochlorophyllide is the intermediate precursor to both chlorophylls a and b. Green algae and all other plants that have light-dependent enzyme grow to green in the darkness. Protochlorophyllide acts as a photosensitizer, forming free radicals under light conditions, and can also occur mostly in the free form. In plants, there is need to regulate the amount of chlorophyll precursor, as high toxic level of protochlorophyllide occur through the accumulation of aminolevulinic acid (Meskauskiene, *et al*., 2001).

2.2.4 Carotenoids

Carotenoids are tetraterpenoid organic pigment molecules that are found in the chloroplast of plants and organisms such as algae, species of aphid, some bacteria and fungi. Animal kingdom cannot synthesize carotenoids, but one species of aphid has the genes that can synthesize carotenoid from fungi through horizontal gene transfer (Nancy and Moran, 2010). There are two classes of carotenoids namely; zeaxanthin and xanthophylls. They protect photodamage of the chlorophyll during photosynthesis (Armstrong and Hearst, 1996). Diplock *et al*., (1998) observed that diets rich in carotenoids is essential for the healthy living. Studies have shown the detrimental effects of consuming additional beta-carotene from supplements, although this conclusion is attributed to the smokers (Goran and Christian, 2007).

2.2.5 Physiological effects of carotenoids

Carotenoids are good antioxidants that scavenge free radicals and thus enhance the vertebrate immune system. Physiology and chemical nature of carotenoids improve individual health and wellbeing (Alija *et al*., 2004). Vitamin A and lycopene are the most common carotenoids, which are also the major precursors of beta-carotene. The presence of the chlorophyll, lutein and other carotenoids in the matured leaves are not visible, but the yellowish, reddish and oranges colours are predominant in young foliage when chlorophyll is absent. In a similar way, carotenoids colours are highly predominant in ripe fruits like tomatoes, banana etc. due to the absence of photosynthetic pigment chlorophyll. Vitamin A and β-carotene structure are shown in Figure 11.

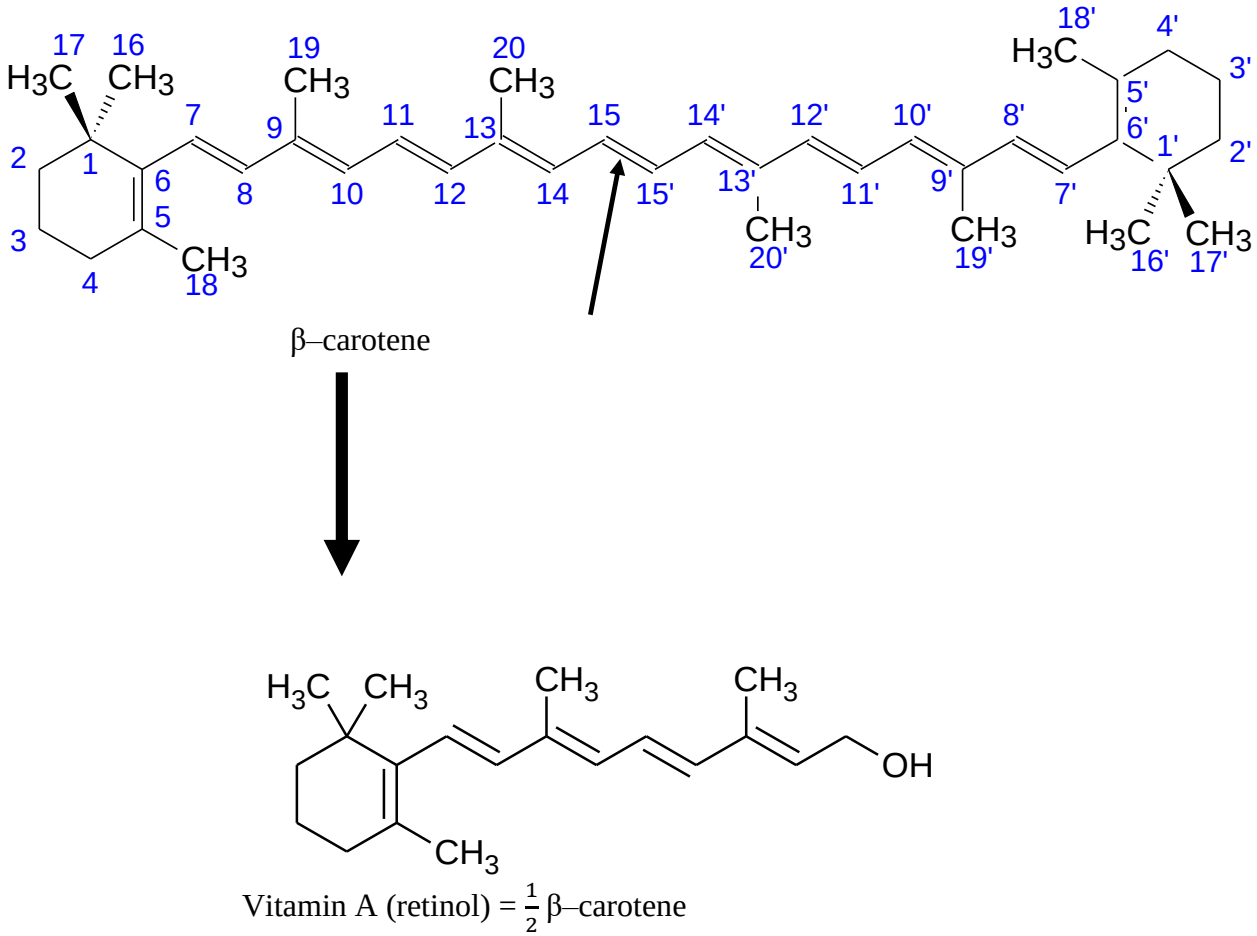


Fig. 11: Structure of vitamin A and β-carotene (Source: DeMan, 1999)

2.3 Antioxidant enzymes in plants

Various environmental factors have negative effects on living organisms, including plants. When this occurs, molecular oxygen acts as an electron acceptor that generates free radicals which compromise the cell membrane integrity (Groß *et al*., 2013). The free radicals generated by salinity stress can be ameliorated with both antioxidant enzymes and non-enzymatic compounds in plants. Kim *et al*. (2013) observed the hormonal and antioxidant enzymes stimulation in the root of salt-stressed rice with the use of silicon (Si). The result showed a reduction in the sodium accumulation which in turn reduced the electrolytic leakage and peroxidation of lipid relative to the control plant. Anthocyanin-impaired-response-1 (air1) mutation has no effect on the biosynthesis of anthocyanin but its response regulates salinity stress (Van Oosten *et al*., 2013). Ascorbate and glutathione peroxidase enzyme ameliorate salt stress effects in various plant species (Munir and Aftab, 2011; Foyer *et al*., 1997). Roy *et al.* (2014) reported three main traits of anthocyanin-impaired-response-1 (air1) in plants, that ensure their adaptability in saline condition through ion exclusion and tissue tolerance, which is coordinated by the antioxidants.

2.4. Uses of Okra

2.4.1 Medicinal Uses of okra

Okra root has a myriad of medicinal uses because of its rich mucilage with antidiabetic and demulcent properties. The juice obtained from the roots is essential for wounds, cuts and boils treatment. The leaves and the fruits can be used for catarrh infections, dysuria, and gonorrhoea. The seeds have antispasmodic and sudorific properties (Franklin and Martin, 1982).

2.4.2 Other Uses of okra

The okra stem is very rich in crude fibres which serve as an alternative to jute. It is a good raw material for paper and textiles industries. The fibre has a length of about 2.4 mm, the stems are harvested at a tender stage immediately after harvesting the edible fruit for the paper industries, during this process, the leaves are harvested from the stems, and subjected to steam for about 2 hours until the fibre is obtained. Nutritionist reviewed that okra has essential properties which are beneficial to health. Okra helps to maintain normal physiological and mental alertness such as depression and general weakness, the fibre can be used for a sore

throat, bowel inflammation and proper functioning of the intestine after steaming or on low heat (Grieve, 1984). Also, the fibre helps to maintain blood glucose level by regulating sugar absorption through the intestinal tract (Ramachandran *et al*., 2011).

2.5 DIABETES

Diabetes is one of the metabolic-related syndromes occurs through improper insulin secretion and action that affects vast majority of population (Lindberg *et al*., 2004). Disturbances in macromolecules metabolism in chronic hyperglycemia is a hallmark for insulin deficiency (Beverley and Eschwège, 2003). Diabetes mellitus effect is prominent in tissue or vascular, which in turn result to various complications (Saely *et al*., 2004; Seki *et al*., 2004). By 2025, there will be a high risk of diabetes due to a growing population worldwide (Zimmet, 2000). WHO, (2002) reported an increase in the percentage of the world population affected by diabetes. Therefore, diabetes mellitus is a global phenomenon that develops as a result of lifestyle changes and western-style diet.

2.5.1 Type 1 diabetes

Insulin-dependent diabetes (Type I diabetes) develops through autoimmune alteration of beta-cells in the pancreas, which in turn decrease insulin secretion. This diabetes is mediated by immune system, in which a T-cell attacks leading to an impaire β-cells and insulin secretion (Rother, 2007). At the inception of type I diabetes, the affected people look healthy, and the response of the body cells to insulin are normal. This type of diabetes is referred to as "juvenile diabetes" because is very rampant among the children. In type I diabetes, various chronic complications of hyperglycemia are set in such as metabolic disorder of glucose, infection, gastroparesis (which bring about malabsorption of carbohydrates), and endocrinopathies (Dorner *et al*., 1977).

Stage 1: Genetic Susceptibility

One major autoimmune syndromes have been reported in type I diabetes namely: autoimmune polyendocrine syndrome type 1 (Hagopian *et al*., 2006). Alterations in the *FoxP3* gene regulates T cells, which in turn brings about IPEX syndrome (Eisenbarth and Gottlieb, 2004). As a result of a deficient of *FoxP3* gene, autoimmunity occurs in children, and 80% of children develop type 1 diabetes. When children are diagnosed with IPEX syndrome bone marrow,

transplantation is the alternative therapy for the provision of T cells (Bacchetta *et al*., 2006). Mukherjee *et al*. (2003) reported potential therapy for IPEX syndrome through the generation of T-cell in FoxP3 that are specific to the antigen gene. These diseases are recessive, but the report by Rao *et al*. (2007) showed a single family and animal model with a dominant mutation. The autoimmune regulator (AIRE) gene is essential in producing insulin within the medullary epithelial cells (Su *et al*., 2008). The study has shown a patient with APS-1 syndrome with various disorders and more autoantibodies than specific diseases (Hagopian *et al*., 2006).

Stage II: Triggering-environment

Environmental factors that trigger the occurrence of type I diabetes in the developed countries over the past years is not yet clear (Barker *et al*., 2004). The lifestyles in the western countries may be attributed to the "hygiene" which increase multiple immune-mediated disorders such as asthma and diabetes (Bach, 2002). Gale, (2002) reported alteration of diabetes in experimental animals raised in a germ-free environment, this study is now suggesting a decrease in the rate of diabetes due to the intestinal microbiota in the animal model. Some medications have contributed to the occurrence of diabetes in the experimental rats (Fourlanos *et al*., 2004), and in human these medications can induce islet autoantibodies leading to ketoacidosis through increase diabetes progression (Schreuder *et al*., 2008).

2.5.1.1 Insulin dependent diabetes and metabolic syndrome

Metabolic syndrome (Isomaa *et al*., 2001) and impaired glycemic control (Gerich, 2006) increased the risk of cardiovascular diseases. Alberti and Zimmet, (1998) described metabolic syndrome as a condition associated with various environmental factors, which in turn result to atherosclerotic disease and failure of glycemic control. Despres *et al*. (2008) observed an increase in the abdominal adipose tissues like visceral fat in patients with metabolic syndrome. Visceral adipocytes can be considered as an endocrine organ as a result of its secretory activity (Tilg and Moschen, 2006). Proinflammatory cytokines and free fatty acids through insulin resistance increase incidence of obesity (Hotamisligil, 2006). Low level of adiponectin occurs in obesity with an inflammatory action (Yamauchi *et al*., 2004). Increase in cardiovascular disease is traced to insulin impairment and result to the cytokines released by adipose tissue with proinflammatory activity (Guzik *et al*., 2006). Havel, (2002) reported a decrease in the

triglycerides synthesis with increased fatty acids metabolism and enhance insulin action as a result of leptin and adiponectin production in the experimental animals. The metabolic disorder mechanism in Diabetes mellitus is described by Schmidt *et al*. (1999) in Figure 12.

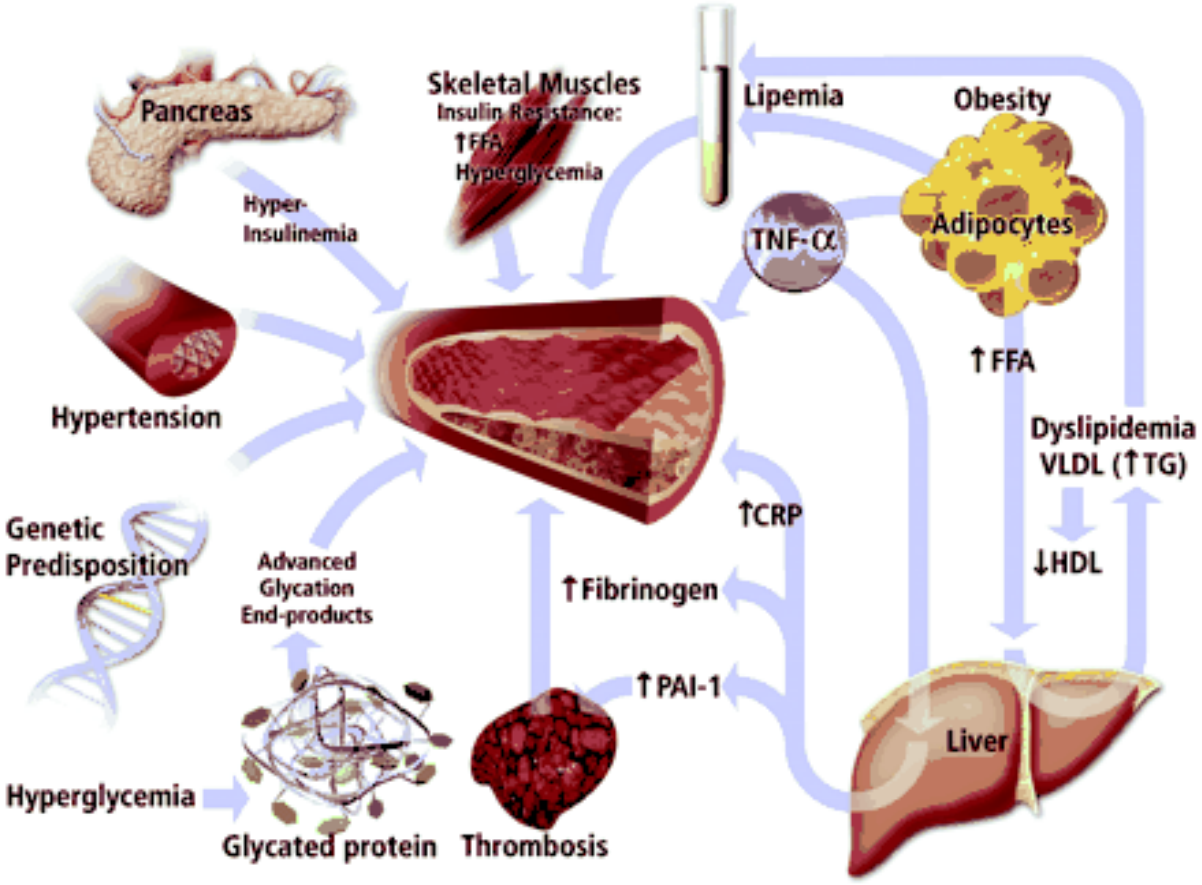


Fig. 12: Mechanism of metabolic derangement in Diabetes mellitus (Source: Schmidt *et al*., 1999).

2.5.2 Type II Diabetes

Type II Diabetes is a condition when the cell fails to insulin action. This also refer to as noninsulin-dependent diabetes mellitus (WHO, 2013). Deficiency of insulin in the body results to the on set of type II diabetes (*RSSDI* textbook of diabetes mellitus, 2012). Insulin resistance may combine with an impaired pancreatic beta cells which make type II and type I diabetes differ from each other (David and Gardner, 2011). Type II diabetes is predominant worldwide. At the inception of this diabetes, the pronounced abnormality is the insulin insensitivity. Therefore, hyperglycemia at this stage can be corrected with insulin sensitive medications or through regulation of glucose production in the liver. The genesis of type II diabetes is correlated with hereditary and genetic factors, as well as lifestyle modifications (Risérus *et al*., 2009). Lack of exercise, poor diet, stress, urbanization and obesity can cause noninsulin-dependent Diabetes mellitus (Williams's textbook of endocrinology, 2011). The percentage of excess body fat varies from one continent to the other, and those who that are not obese are still having a high ration of waist-hip. (David and Gardner, 2011). Malik et *al*., (2010) observed that the consumption of excess sugar in a drink or high-calorie food intake contribute to the risk factors for type II diabetes. The fats content in diets also increases the risk of noninsulin-dependent diabetes mellitus. Trans saturated fatty acids promote the risk of type II diabetes, while unsaturated fatty acids like monounsaturated and polyunsaturated mitigate the risk of the disease (Risérus *et al*., 2009).

2.5.2.1 Diagnostic definition of Type II diabetes

Fasting blood glucose concentrations (7 or 11.1 mmol/L) with oral glucose load of about 75 g after 2 hours is use to diagnosis noninsulin-dependent diabetes mellitus (Alberti and zimmet, 1998). However, the consensus has not been made for the upper threshold for a normal glucose level during insulin insensitivity. The maintenance of glucose homeostasis is described by Alberti and Zimmet (1998) in Figure 13.

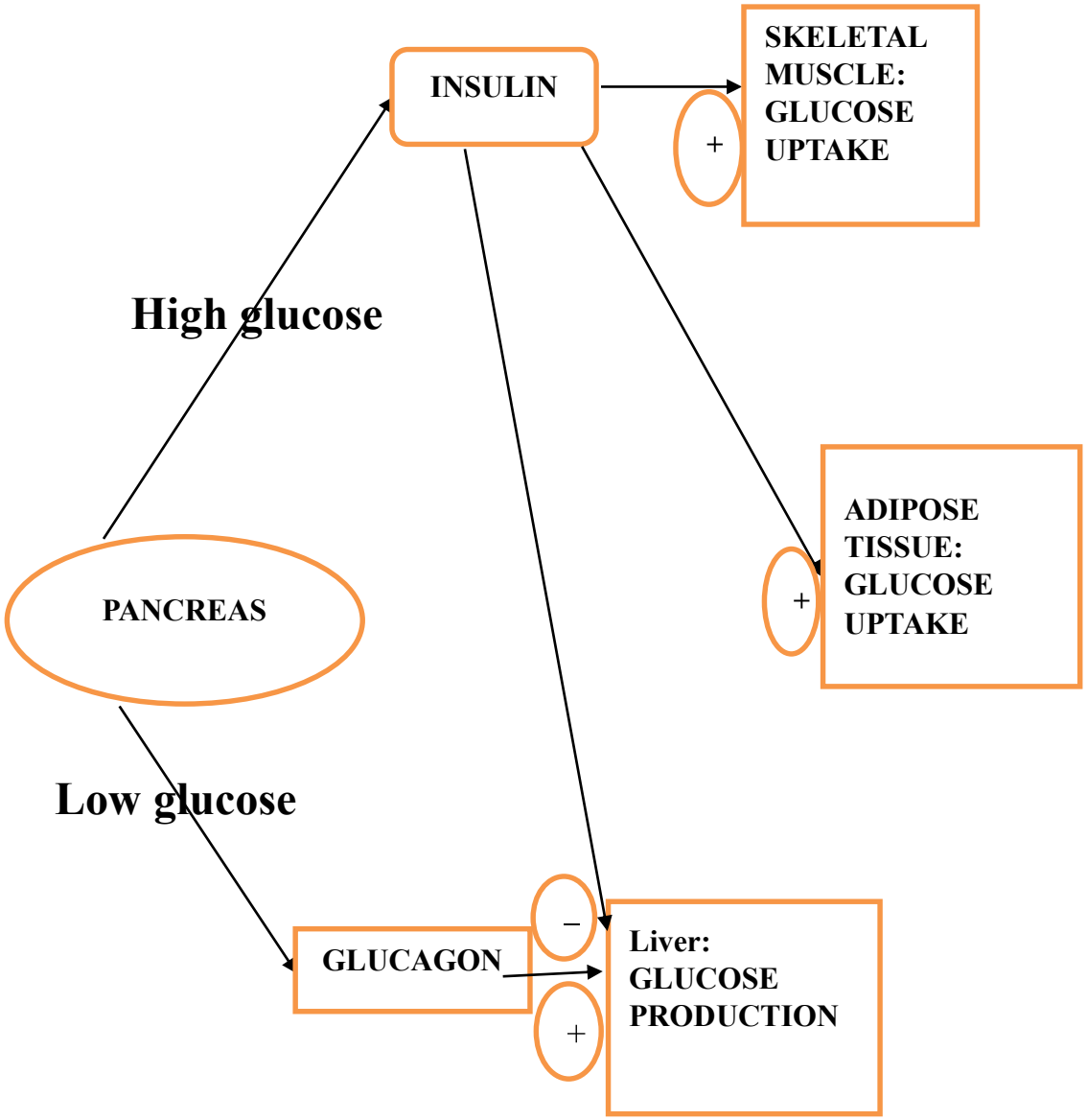


Fig. 13: Maintenance of glucose homeostasis (Source: Alberti and Zimmet, 1998).

2.5.2.2 Developmental stages of type II diabetes

Pathogenesis of type II diabetes starts with the skeletal muscle and adipocytes cells insulin resistance. In this situation, β-cells secrete insulin but fail to metabolize the glucose in the circulation after a meal. This causes glucose tolerance impairment. Fasting glucose impairment is considered in plasma glucose level ranging from 6.1 to 6.9 mmol/L (Alberti and Zimmet, 1998). The mathematical minimal modelling technique proposed in 1981 ensured the quantitative measurement of pancreatic beta cells and sensitivity of insulin to glucose tolerance test (Bergman *et al*., 1981). With this technique, a hyperbolic relationship was established between the pancreatic responsiveness and insulin sensitivity (Kahn *et al*., 1993), as depicted in Figure 14 below.

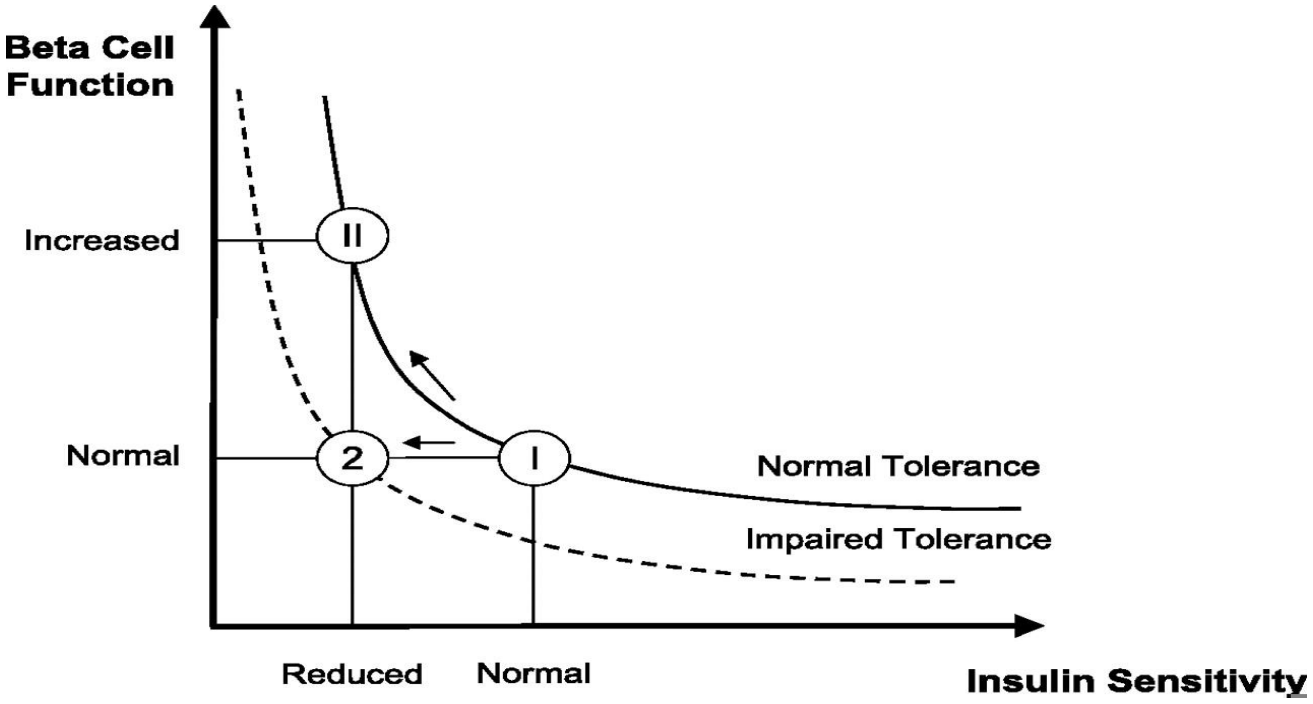


Fig. 14: Minimal model the hyperbolic relationship pancreatic responsiveness and insulin sensitivity (Source: Stumvoll *et al*., 2005).

2.5.2.3 Noninsulin-dependent diabetes mellitus and metabolic disorder

Dyslipidemia and hypertension are also metabolic syndromes associated with insulin resistance (Moller and Kaufman, 2005). High triglyceride and LDL-cholesterol levels with decreased plasma concentration of HDL-cholesterol are the hallmarks of dyslipidemia (Alberti and Zimmet, 1998). The type II diabetes metabolic disorders are numerous. Hanson *et al*. (2002) reported hyperinsulinemia and obesity as the major cause of noninsulin-dependent diabetes mellitus.

2.5.2.4 Type II diabetes and inflammatory disease

Duncan *et al*. (2000) reported that insufficient systemic inflammation begins and predicts type II diabetes development in the adults. Adipose tissue releases various metabolic mediators. Some of these mediators are cytokines that mediate resistance of insulin and regulates metabolism (Hotmisligil *et al*., 1993; Kershaw and Flier, 2004). Pickup *et al*. (1997) observed elevated serum concentrations in type II diabetes, and in another study of Weyer *et al*. (2001) who reported a reduce level in the concentration of adiponectin, which is related to both hyperinsulinemia and insulin resistance.

2.5.3 Gestational diabetes

WHO (2013) observed the development of gestational diabetes in a pregnant woman with no high blood glucose level and diabetes history. However, after delivery, about 5 to 10 % women with gestational diabetes still develop the most common type II Diabetes mellitus (NDIC, 2011). Gestational diabetes can be managed medically by mean of dietary changes, blood glucose monitoring, and insulin replacement. A lot of risks is posed with untreated gestational diabetes, which includes central nervous system abnormality, congenital cardiac, high birth weight, skeletal muscle malformations and destruction of red blood cell. In many cases, maternal death may occur, as a result of placenta function impairment, labour induction may occur, which in turn can lead to a caesarean section due to an increased risk of injury associated with high birth weight (WHO, 2013).

2.5.3.1 Pathophysiology of gestational diabetes

During pregnancy, various factors that bring about insulin resistance include alteration in cortisol secretion and growth hormones, human placental lactogen secretion, and insulinase

secretion. Also, estrogen and progesterone which are female's hormones cause a disruption of the glucose-insulin balance. Glucose intolerance can be resulted from increase caloric intake, increase adipose deposition and a decrease in exercise.

2.5.3.2 Risk Factors for gestational diabetes mellitus

The prevalent factors for gestational diabetes mellitus include high birth weight greater than 400g, history of type II diabetes and spontaneous abortions with unexplained stillbirths, obesity and previous gestational diabetes in the last pregnancy with persistent glycosuria (Marion, 2008). About 50% of patients with gestational diabetes are diagnosed with unidentified risk factors (Marion, 2008).

2.6 Diabetes-related symptoms

Gestational diabetes symptoms are somewhat related to noninsulin-dependent diabetes and type I diabetes. But in noninsulin-dependent diabetes greater risk of atherosclerosis associated with hyperlipidaemia, hypertension, and obesity are commonly reported (Eriksson *et al*., 1991). Cardiovascular complications as well as end stage renal disease are the hallmark of type II diabetes (Ramachandran *et al*., 1997). Cross section of related symptoms in diabetes mellitus as described by Cooke and Plotnick, (2008) is shown in Figure 15.

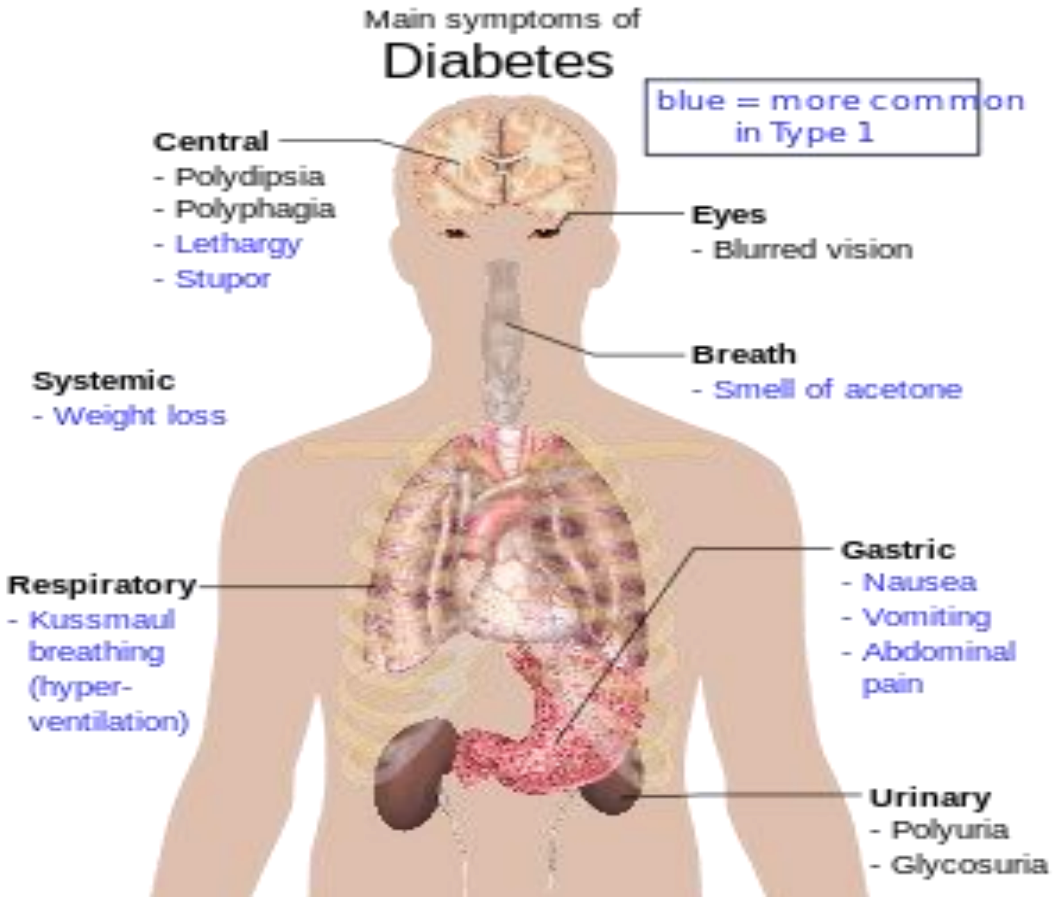


Fig. 15: Cross section of diabetes-related symptoms. (Source: Cooke and Plotnick, 2008).

2.7 Control of diabetes

There are various control measures for Diabetes mellitus, but the major one is insulin therapy, in insulin-dependent diabetes patients while noninsulin-dependent diabetes disease is preventable. O'Dea, (1984) reported improved metabolism of carbohydrate and lipid in noninsulin-dependent diabetes patients living with traditional lifestyle. In another study of Knowler *et al*. (2002) who observed the prevention of diabetes with lifestyle changes. Pharmacotherapy and non-pharmacotherapy are the two major means of controlling Diabetes mellitus.

2.7.1 Pharmacotherapy

The main reasons for the management of diabetes are to save lives as well as to prevent chronic diabetic complications. Type I Diabetes mellitus patients can be managed through insulin replacement while lifestyle modifications and diet are considered for noninsulin-dependent diabetes patient's management (DCCTRG, 1993). However, insulin replacement is essential for type II Diabetes patients when diet, weight loss, exercise, and oral medications cannot control high levels of blood glucose (Pan *et al*., 1997). Chemical drugs can also be used as a therapy for noninsulin-dependent diabetes, which is important in correcting the underlying metabolic disorders, like insulin resistance and insulin secretion (UKPDS, 1998).

2.7.2 Lifestyle modification in the management of noninsulin-dependent diabetes

Stoffers *et al*. (1997) reported reduce insulin resistance, reduce body weight and high glucose tolerance as a result of an increase in daily energy expenditure. Lifestyle modification is essential for the management of noninsulin-dependent diabetes. Also, caloric intake should be avoided in an obese patient (Stoffers *et al*., 1997).

2.7.2.1 Diet and exercise

Routine exercise is an important method for the prevention and management of type II Diabetes mellitus. Over the years, various studies have shown increased physical activity as a means of controlling type II Diabetes mellitus (Ross *et al*., 2000). Also, Ross *et al*. (2000) reported various levels of routine exercise with a decrease in disease episode at a long time of monitoring diabetes in both genders at varying age groups. Studies revealed little or no effect of regular physical activity for type I diabetes management because of the likelihood of

consuming additional carbohydrates in an attempt to prevent hypoglycemia. Felig *et al*. (1982) reported the occurrence of hypoglycemia in type I diabetic patients during exercise unless the extra carbohydrate is consumed or insulin dose is reduced. Physical activity has negative and positive impacts on diabetic patients. Physiological effects are prominent and the metabolic benefits are lower in the insulin-dependent diabetes patients than their noninsulin-dependent diabetes patients' counterparts. Therefore, dietary and lifestyle modifications are essential for diabetic therapy.

2.7.3 Insulin therapy for diabetes mellitus

Insulin replacement is a major therapy for insulin-dependent diabetes patients. Owens *et al*. (2001) reported a significant number of type I diabetic patients whom their lives have been saved with the use of insulin therapy in the year 2000. UKPDS, (1998) reported the motive of preventing chronic diabetes complications with insulin replacement in diabetes. Insulin therapy is essential in preventing hyperglycemia and hypoglycemia between meals (Ciofeta *et al*., 1999). But on the other hand, pharmacological problems complicate insulin therapy (Bethel and Feinglos, 2002).

2.7.4 Oral hypoglycemic agents

2.7.4.1 Sulfonylureas

This is one of the various drugs, it was discovered in 1942 in experimental animals. In the 1950s twenty different classes of sulfonylureas have been in use worldwide. Garrino *et al*. (1986) reported insulin secretion with the use of non-sulfonylurea analogues moiety. Sulfonylureas are classified as the first generation oral hypoglycemic agents and in term of potency, the second generation is superior to the first generation.

2.7.4.1.1 Sulfonylureas mechanism of action

Sulfonylureas bind to the cell membrane of pancreatic β-cell on its receptor, which leads to the closure of potassium ion (K^+) channel that is sensitive to ATP, which in turn depolarize the membrane cell. Thus opens the Ca^+ ion channel, and allows an influx of Ca^+ which lead to the secretion of an insulin. According to Levine, (1984) who observed an increase in plasma glucose with decline insulin release from the pancreas after the sulfonylureas administrations respectively to the noninsulin-dependent diabetes patients.

2.7.4.1.2 Pharmacokinetics

The absorption rates of all the classes of sulfonylureas are different, but the absorption at the gastrointestinal tract is common for all. However, food-drug interaction affects the rate of digestion. Sulfonylureas with high clearance rate are more active when taken prior the meal due to their poor pharmacokinetics. The short-acting sulfonylureas include glipizide with five hours' clearance rate and inactive metabolites, others have about 12 hours' clearance rate (Ferner and Chaplin, 1987). In all the classes of sulfonylureas in use, the one with the longest clearance rate is chlorpropamide, which is active within 24 to 48 hours (Ferner and Chaplin, 1987). The next sulfonylureas are gliquidone with an elimination half-life of twenty-four hours, but there is little information on its clearance rate and risk factor because its use is very limited. Lebovitz (1983) reported the potency of second-generation agents over the first generation agents in spite of their shorter half-lives of about 3 to 5 hours. Initially, several studies have shown a short elimination half-life of about 2-10 hours for glibenclamide, not until when Jönsson *et al*. (1994) reported glibenclamide with a half-life between 15-20 hours. Based on the longer elimination half-life, this drug has been largely used clinically and implicated in various cases of hypoglycemic conditions relative to other sulfonylureas (Ferner and Chaplin, 1987).

2.7.4.2 Biguanides

In 1958, metformin and phenformin were introduced. They are major drugs for the management of noninsulin-dependent diabetes across the Europe countries for almost 20 years. Because of the side effects associated with fatal lactic acidosis (Schafer, 1983), they were withdrawn from the circulation in the 1970s. Metformin with a low rate of lactic acidosis is commonly used in European countries than the phenformin. DeFronzo and Goodman, (1995) revealed high macromolecules levels in patients with no appetite to diet under the influence of metformin alone or in combination with a sulfonylurea. Bailey, (1992) observed that metformin increases insulin cells resistance in patients.

2.7.4.2.1 Biguanides mechanism of action

Clarke and Duncan, (1979) observed that metformin has no effect on insulin secretion or causes hypoglycemia, even at high concentrations. Metformin causes hormonal secretion, and thus increases the peripheral uptake of glucose (Hundal *et al*., 1992), and it also reduces the hepatic glucose output when given orally (Perriello *et al*., 1994) but not intravenously (Sum

et al., 1992). Metformin also decreases fatty acid concentrations and triglycerides in the serum (Perriello *et al*., 1994).

2.7.4.2.2 Pharmacokinetics of metformin

Metformin is a stable drug, it does not bind to plasma protein, its absorption is through the small intestine, and the excretion in urine is unchanged with 2 hours' clearance rate. In the United States, the daily recommended dose is 2.5g.

2.7.4.2.3 Side effects of metformin

Metallic taste, abdominal discomfort, anorexia or diarrhoea, nausea, headache, agitation, dizziness, and tiredness are always noticed in the patients under the influence of metformin drug. However, these negative situations can be reduced with a high daily dose. Bauman *et al*. (2000) observed drug interaction between metformin and vitamin B_{12} absorption, which can be reversed with calcium supplements. Metformin-associated lactic acidosis is reported in a patient with renal impairment and hyperfussion (Lalau *et al*., 1995). Therefore, accumulation of metformin can be removed with the use of dialysis (Lalau *et al*., 1987). The risk of mortality is similar to that of hypoglycemia in patients treated with a sulfonylurea (Campbell, 1984).

2.7.4.3 Thiazolidinediones

Chemical structure and function of Thiazolidinediones (TZDs) are not related to other classes of hypoglycemic agents. All hypoglycemic agents are analogue of thiazolidinediones. Rosiglitazone and pioglitazone are the two thiazolidinediones which are currently in use (Papoushek, 2003). Troglitazone was removed from circulations because of its toxicity on the liver (Bae *et al*., 2003).

2.7.4.3.1 Mechanism of action of thiazolidinediones

The Thiazolidinediones bind to PPAR-gamma (Peroxisome proliferator-activated receptor gamma) that result to the activation of insulin-responsive genes which regulate both lipid and carbohydrate metabolism. There are three sub-types of PPAR: PPAR- alpha, gamma, and delta or beta (Desvergne and Wahli, 1999). Thiazolidinediones lower peripheral tissue insulin resistance, but glucose lowering action in the liver has also been reported (Matsuda *et al*., 1998). Thiazolidinediones increase the transportation of glucose into the body cells through

biosynthesis of glucose transporters (Lemberger *et al*., 1996). The mechanism of action of thiazolidinediones as described by Desvergne and Wahli, (1999) is shown in Figure 16.

2.7.4.3.2 Pharmacokinetics of thiazolidinediones

The thiazolidinediones are rapidly absorbed and reach peak concentrations within a few hours (Mudaliar and Henry, 2001). Steady-state is usually reached within one week, but perhaps because of the importance of fatredistribution, the full benefit may take 4-12 weeks to become evident. Rosiglitazone and pioglitazone are strongly protein bound in the circulation, predominantly to albumen (Mudaliar and Henry, 2001). No significant drug interactions have been reported with the thiazolidinediones, but it should be noted that in combination with the sulfonylureas, hypoglycaemia may occur due to the combination of enhanced insulin sensitivity (thiazolidinediones) and enhanced insulin sensitivity (sulfonylureas).

2.7.4.3.3 Side effects of thiazolidinediones

The effect of the thiazolidinediones on lipid concentrations is complex. HDL cholesterol concentrations tend to increase while triglyceride concentrations decrease (Schoonjans and Auwerx, 2000). Although LDL cholesterol concentrations may increase initially, this effect lessens over time and particles are now larger and more buoyant (Gurnell *et al*., 2003). The outcomes of ongoing large clinical trials may clarify the effect of thiazolidinediones on cardiovascular risk. Pioglitazone has some PPAR- alpha activity, which may account for the data suggesting a more favourable effect on triglyceride and LDL cholesterol levels (Parulkar *et al*., 2001).

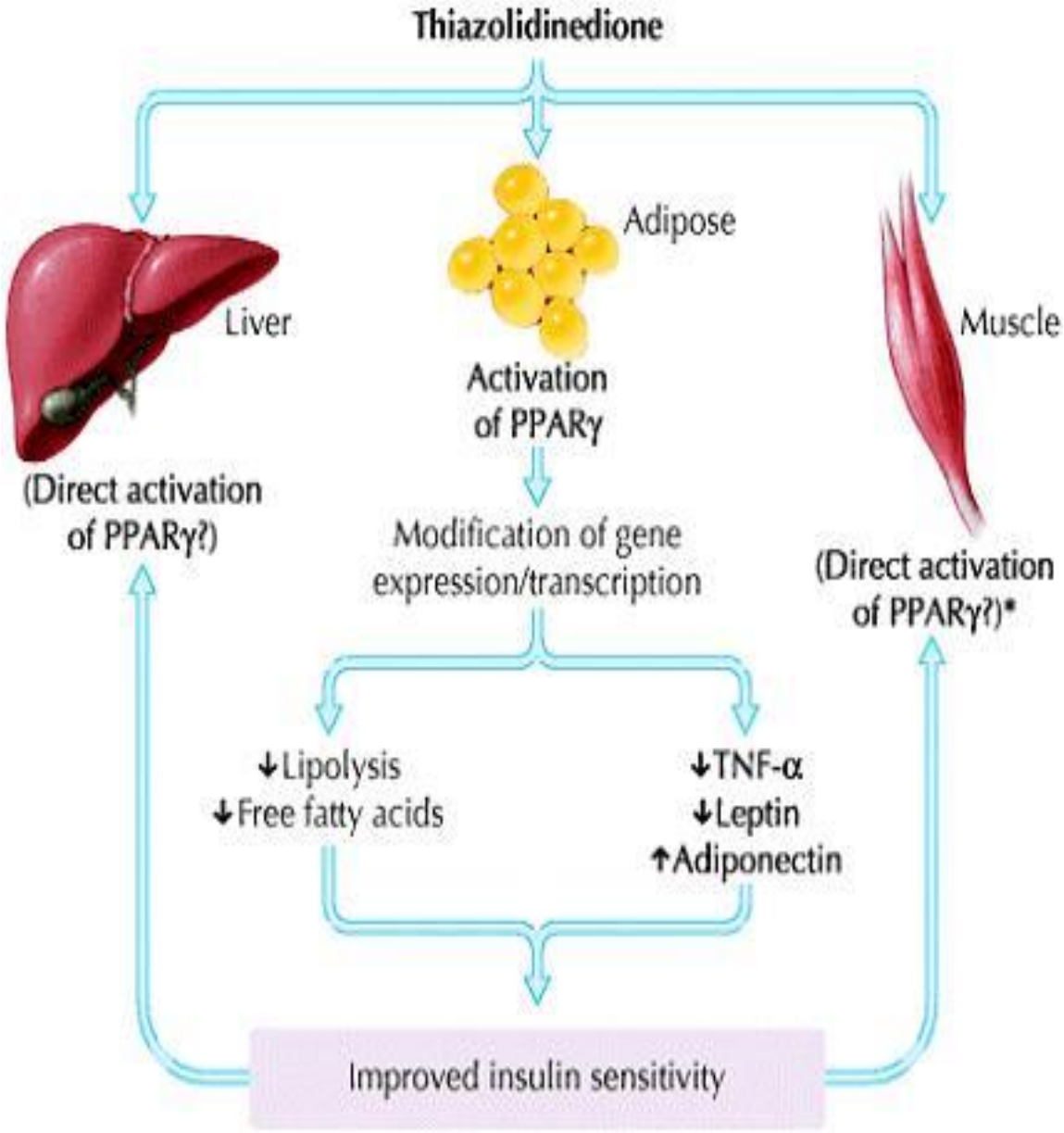


Fig. 16: Mechanism of action of thiazolidinediones. (Source: Desvergne and Wahli, 1999).

2.7.4.4 Alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors major function is to prevent the digestion of complex carbohydrate and thus reduce blood glucose level (Puls, 1996). These actions significantly reduce postprandial glycemic and insulinemic increase whether they are used as monotherapy or combined in the management of noninsulin-dependent and insulin-dependent diabetes.

2.7.4.4.1 Mechanism of action of alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors are competitor of small intestine brush border enzymes and thus block the hydrolysis of oligo and polysaccharides to monosaccharides (Bischoff, 1995). Carbohydrates absorption is slow through the inhibition of this enzyme, and therefore, the postprandial high level of plasma glucose is not found in patients (Reabasa-Lhoret and Chiasson, 1998). Three types of α-glucosidase inhibitors have been known with similar pharmacological profiles; acarbose, miglitol, and voglibose. Kageyama *et al*. (1997) reported limited information on voglibose clinical trial. Acarbose inhibits glucoamylase and sucrase with no effect on pancreatic α-amylase. Hypoglycemia does not occur in α- glucosidase inhibitors due to its inability to stimulate insulin release.

2.7.4.4.2 Pharmacokinetics of alpha-glucosidase inhibitors

Acarbose is poorly absorbed (less than 2%) on oral administration and 35% of an oral dose appears as metabolites in the urine.215 Miglitol is not metabolized and is excreted quantitatively by the kidney.215 However, because of its close resemblance to the glucose molecule, miglitol is significantly absorbed through a jejunal transport mechanism identical to that of glucose (Lebovitz, 1998). It then circulates and concentrates in enterocytes of the small intestine. Acarbose is given initially at a dose of 25 mg at the start of a meal for 4-8 weeks followed by an increase at 4- to 8-weeks interval up to 75 mg before each meal. This will reduce gastrointestinal side effects. Smaller doses are given with snacks. Acarbose is most effective when given with a starchy, high-fibre diet with restricted amounts of glucose and sucrose (Bressler and Johnson, 1992).

2.7.4.4.3 Uses and adverse effects of alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors is a major therapy in patients with postprandial hyperglycemia. Alpha-glucosidase inhibitors administration with insulin or other antidiabetic drugs is possible, they could be used before or during the meal (Bischoff, 1995). Kageyama *et al*.

(1997) reported that the potency of voglibose was less than acarbose. Relatively, alpha-glucosidase inhibitors seem to have low potent to sulfonylureas with 0.85% reduction of HbA1c versus 1.02% with sulfonylureas (Coniff *et al*., 1995). Acarbose was comparable to metformin for reducing HbA1c in patients previously subjected to sulfonylureas treatment (Willms and Ruge, 1999). Indigestion and low assimilation of carbohydrate diets occur using α-glucosidase inhibitors (Reabasa-Lhoret and Chiasson, 1998).

2.7.4.5 Glibenclamide

Glibenclamide (glyburide) is an antidiabetic drug that was discovered in 1966 in the study of Boehringer and Marble, (1971) Majorly glyburide involves in the management of noninsulin-dependent diabetes. Glyburide is an important medicine for hyperglycemia (WHO, 2011). Glibenclamide was the most popular sulfonylurea in the United States as of 2003 (Riddle, 2003).

2.7.4.5.1 **Mechanism of action** of **glibenclamide**

Glibenclamide mechanism involves its binding to the membrane cell of pancreatic β-cell on the receptor, and lead to the closure of potassium ion (K^+) channel that is sensitive to ATP, which in turn depolarizes the membrane cell. Thus opens calcium ion channel, and allows an influx of Ca^+ which prompts the secretion of insulin (Serrano-Martín *et al*., 2006). Ortega *et al*. (2012) observed efficient binding of glibenclamide to the ischemic hemisphere. The mechanism of action of glibenclamide by Serrano-Martín et *al*., (2006) is shown in Figure 17.

2.7.4.5.2 Pharmacokinetics of glibenclamide

Following oral administration of glibenclamide, the extent of absorption is 75% in rats and > 90% in rabbits and dogs (Heptner *et al*., 1969). In humans after oral administration of 5 mg glibenclamide, a C_{max} value of 90 nM was obtained (Rupp *et al*., 1969). Glibenclamide is a substrate for human organic anion-transporting polypeptide OATP-B, which is responsible for its uptake on the luminal membrane of intestinal epithelial cells (Satoh *et al*., 2005).

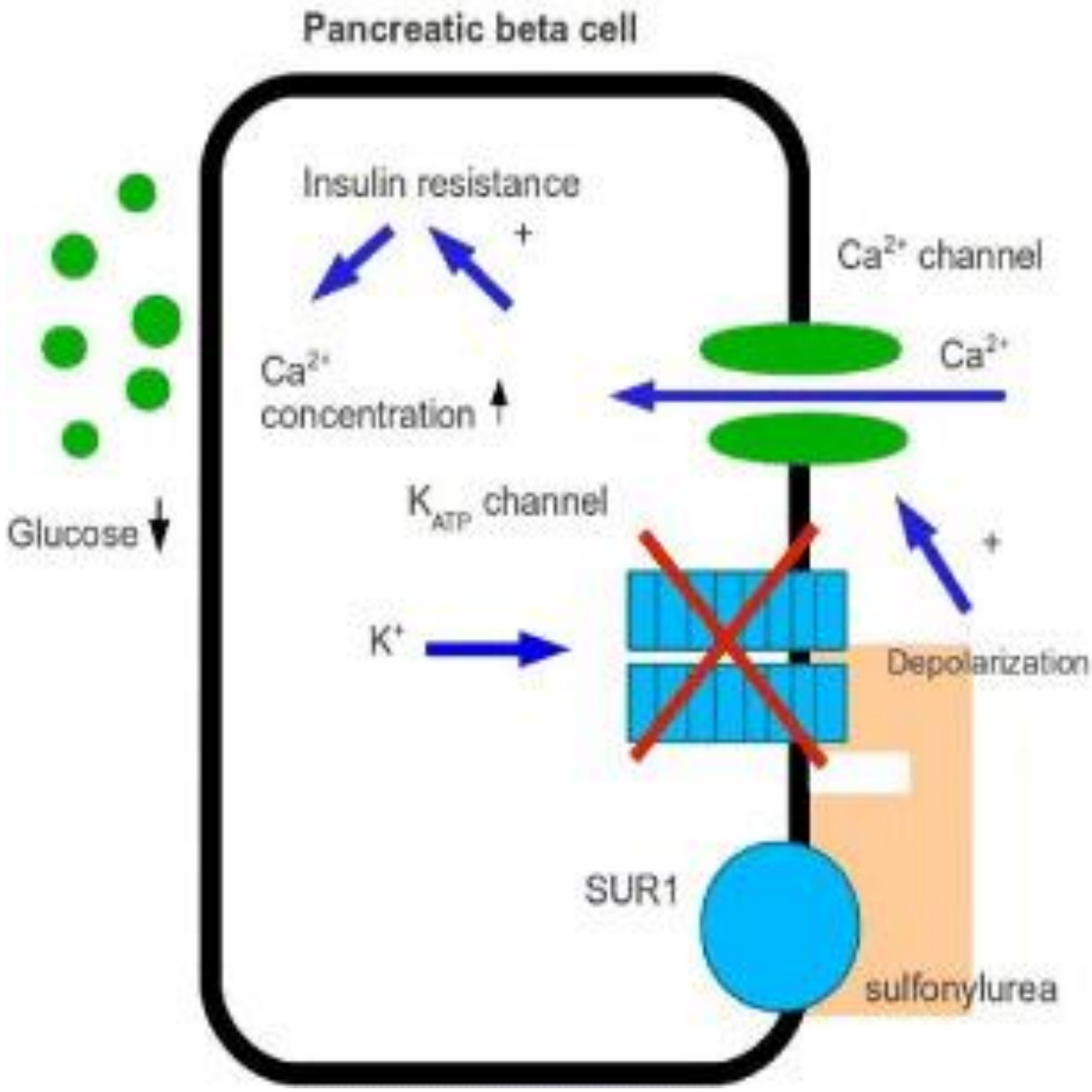
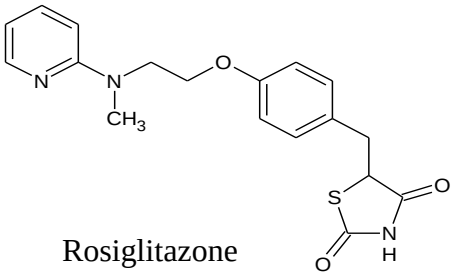


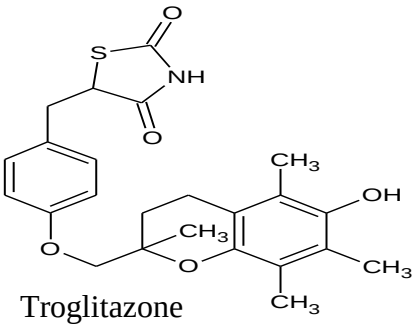
Fig. 17: Mechanism of action of glibenclamide. (Source: Serrano-Martín et *al*., 2006).

2.7.4.5.3 **Side effects and contraindications of glibenclamide**

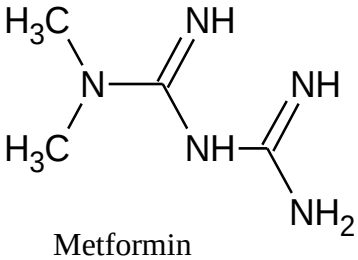
Glibenclamide has a lot of contraindication which includes drug-induced hypoglycemia. The contraindication of glibenclamide is discovered in a patient with the deficiency of an enzyme glucose-6-phosphate dehydrogenase and thus leads to acute hemolytic anaemia (Meloni and Meloni, 1996). The data obtained in the study of Monami *et al.* (2006) suggested high mortality rate when glibenclamide combined with metformin than other oral hypoglycemic agents. The management of diabetes with various oral hypoglycemic agents elicit a number of side effect. Therefore, the interest is now diverting toward the use of ethnomedicine. A plant containing biologically active compounds has become a target for producing new drugs (Yeh *et al*., 2003). The chemical structures of some antidiabetic drugs as described by Yeh *et al*., (2003) is shown in Figure 18.



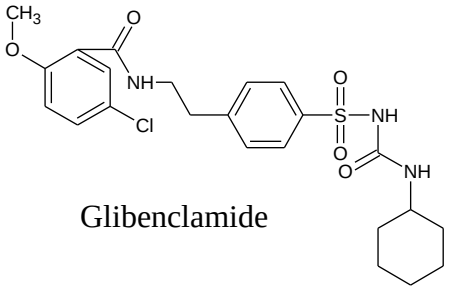
Rosiglitazone



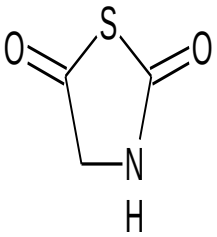
Troglitazone



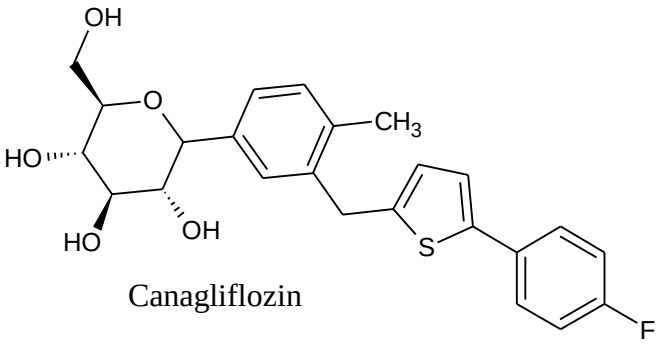
Metformin



Glibenclamide



Thiazolidinediones



Canagliflozin

Fig. 18: Chemical structures of some antidiabetic drugs. (Source: Yeh *et al*., 2003)

2.7.5 Herbal management of diabetes

Several types of studies by various researchers have shown the use of phytochemicals for ameliorating hyperglycemia. About three hundred species of plant have been reported in the literature with hypoglycemic properties (Atta-ar-Rahman and Zaman, 1989). One of these plants is okra plant that is cultivated majorly in Nigeria as a vegetable crop. Khomsug *et al*. (2010) reported its wide distribution through various continents. Okra is very rich in vitamins and mineral salts (IBPGR, 1990). Medicinally, okra has been involved in the management of various diseases and disorders. Tomoda *et al*. (1989) revealed okra hypoglycemic activity in diabetic mice. Recent research of Uraku *et al*., (2011) reported okra as an antidiabetic agent.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials and Equipment

Glassware

Cotton wool

Whatman filter paper

Syringe and needles

Surgical gloves

Lithium heparin bottles

Ethylenediaminetetraacetic acid (EDTA) bottles

Paper tape

Screw-cap tubes

Permanent markers

Micropipettes

Mortar and pestle

Blender

Freezers (-20°C, -80°C)

Hot air oven

Weighing balance

Vortex mixer

Incubator

Water bath

Rotary evaporator

UV spectrophotometer

Scintillating machine

Freeze dryer

3.2 Reagents

Tween-20

Indole acetic acid (IAA)

Salicylic acid (SA)

Sodium hypochlorite (NaOCl)

Analytical sodium chloride

KOPF Needle and Pipette Puller

2-deoxy-D-[1-^{3}H]-2-DG

Sodium dodecyl sulphate (SDS)

75% Ethanol

Copper sulphate ($CuSO_4$)

Paraffin

Rabbit Anti-Mouse Insulin antibody

Goat Anti-Rabbit IgG HRP conjugated Millipore

Normal Horse Serum Blocking Solution

Impact peroxidase DAB substrate

Potassium tartrate ($KNaC_4O_{64}H_2O$)

Potassium iodide (KI)

Sodium hydroxide (NaOH)

Metaphosphoric acid

2, 6-dichlorophenolindophenol (DPPH)

Mayer’s Hematoxylin Solution0

Periodic Acid Solution

Schiff reagent

Vitamin C

10% methanol

Trichloroacetic acid (TCA)

Bradford reagent

Concentrated tetraoxosulphate (vi) (Conc. H_2SO_4)

Streptozotoxin (STZ)

2,6-dichlorophenol

Chloroform (CCl_3)

Triton -X-100

Carboxymethyl cellulose (CMC)

Sodium lauryl sulphate
Acetic acid

Thiobarbituric acid

Perchloric acid

Ferric chloride

Folin's phenol reagent

Glucose

Magnesium chloride

Dipotassium hydrogen phosphate

Potassium chloride

Calcium chloride

Sodium fluoride

Collagenase

Tris-HCl buffer

Ammonium molybdate

Aminonaphthol sulphonic acid (ANSA)

Molybdic acid

Glutathione peroxidase kit

Superoxide dismutase kit

Catalase kit

Cholesterol kit

Triglycerides kit

A kit for Hi scribe T_7

A kit for PCR purification

3.3 Materials

3.3.1 Plant materials

Two hundred okra seeds of 47-4 and LD88 were collected from NIHORT, Eleyele area in Ibadan. The bioregulators were purchased from BDH Chemical Limited. The seeds were divided into two equal parts each and 1.0% sodium hypochloride was used to sterilized the seeds by immersion for about 120 secs, after which the seeds were rinsed with distilled water and then air dried. Twenty-five okra seeds part each of 47-4 and LD88 were soaked in each of 0 (control), 0.4, 0.5, and 0.6 mM IAA and another twenty-five seeds were soaked in each of 0, 10^{-2}, 10^{-4}, and 10^{-6} mM SA for 24 hours in the dark at 25^{0C}. Thereafter, the bioregulator solutions were removed from the seeds and washed 2-3 times in distilled H_2O. The okra seeds were air dried for 1 hour. After which the seeds (n=5) were sown in a complete randomized design in each 10 kg of soil contained each of 0, 50, 100, 150, and 200 mM NaCl. All 104 pots for each genotype (3 replicates by 5 levels by 4 treatments) were irrigated with tap water three times a week till maturity. Experimental design is as:

I. Control

a. (No salinity and no bioregulator)

II. Salt treated group

(a) 50, 100, 150 and 200 mM NaCl

III. IAA and salt levels group (IAA treatment)

0.4 mM IAA+ 0 mM NaCl	0.5 mM IAA+ 0 mM NaCl	0.6 mM IAA+0 mM NaCl
0.4 mM IAA+50 mM NaCl	0.5 mM IAA + 50 mM NaCl	0.6 mM IAA + 50 mM NaCl
0.4 mM IAA+100 mM NaCl	0.5 mM IAA +100 mM NaCl	0.6 mM IAA + 100mM NaCl
0.4 mM IAA+150 mM NaCl	0.5 mM IAA +150 mM NaCl.	0.6 mM IAA +150 mM NaCl
0.4 mM IAA+200 mM NaCl	0.5 mM IAA + 200 mM NaCl	0.6 mM IAA + 200 mM NaCl

IV. Salicyclic acid and salt treatments group (SA treatment):

10^{-2} mM S.A + 0 mM NaCl	10^{-4} mM S.A +0 mM NaCl	10^{-6} mM S.A + 0 mM NaCl
10^{-2} mM S.A + 50 mM NaCl	10^{-4} mM S.A +50 mM NaCl	10^{-6} mM S.A + 50 mM NaCl
10^{-2} mM S.A + 100 mM NaCl	10^{-4} mM S.A +100 mM NaCl	10^{-6} mM S.A + 100 mM NaCl
10^{-2} mM S.A + 150 mM NaCl	10^{-4}mM S.A + 150 mM NaCl	10^{-6} mM S.A + 150 mM NaCl
10^{-2} mM S.A +200 mM NaCl	10^{-4}mM S.A + 200 mM NaCl	10^{-6} mM S.A + 200 mM NaCl.

3.3.2 Animals

Sprague-Dawley male rats weighing 130 ±15 g of four to five weeks of age were obtained at an animal central house in the Human Nutritional Sciences Department, University of Manitoba, Winnipeg, Canada. They were acclimatized for one week in cages with *ad libitum* feeding.

3.3.3 Frog

Full matured female frog used was obtained at the Human Nutritional Sciences Department, University of Manitoba, Canada. The feeding was done twice a week. Salman pellets, minced beef and live blowfly larvae were used.

3.4 Methods

3.4.1 Preparation of plant extracts

The leaves and fruits of okra were made into powder for the extraction after drying at the room temperature respectively. For aqueous extraction, fruit powder was dissolved into 2500 ml of distilled water in ratio 1:10 respectively. Stirred until when it viscous, it was then heated and boiled for about 10 mins, and then cooled for 10 mins before it was centrifuged. Then the supernatant was decanted and stored at -80°C before transferring into the freeze dryer machine in order to get soluble powder, which is stored for the experiment. For the ethanol extraction, the leaves and fruits powder were dissolved in round bottom flask containing 75% ethanol in ratio 1:10 respectively. The mixture was stired with the use of magnetic stirrer at temperature of 40°C for 3hours. After which they were decanted, sieved and transferred into the rotary evaporator equipment for total removal of ethanol and to concentrate the solution, before transferring it into the freeze dryer machine in order to get soluble powder, which was stored for the experiment.

3.4.2 Phytochemical screening

Screening of the extracts were done by following the described methods of Harborne (1973); Trease and Evans (1989) and Sofowora (1993).

3.4.2.1 Tannins qualitative test (Ferric chloride test)

Harborne (1973) method was applied. Briefly each plant sample powder of about 0.5 g was subjected to boiling and then filtered, 0.1% of ferric chloride was added. Observation of blue-black showed tannins

3.4.2.2 Test for Phlobatannins (Hydrochloric acid test)

Each plant sample powder of about 0.5 g was subjected to boiling in 1% hydrochloric acid. Observation of red precipitate showed Phlobatannins (Trease and Evans,1989).

3.4.2.3 Saponins qualitative test (Foam test)

Each plant sample powder of about 0.5 g was subjected to boiling and then filtered. Then the filtrate was shaking to ensure persistent frothing, which confirmed saponins (Sofowora, 1993).

3.4.2.4 Flavonoids qualitative test (Ammonia test)

A 1% of NH_3 was mixed with 2 ml of sample extract. The observation of yellow colour showed flavonoids (Harborne, 1973).

3.4.2.5 Terpenoids qualitative test (Salkowski test)

According to the method of Trease and Evans (1989). A 2 ml chloroform was mixed with 5 ml of plant aqueous extract and then followed with 3 ml of conc. sulphuric acid. An observation of reddish brown colour showed the presence of terpenoids.

3.4.2.6 Cardiac glycosides qualitative test

Aqueous extract of the plant (5 ml) was reacted with conc. sulphuric acid (1 ml), and then with glacial acetic acid (2 ml) and followed by 1 drop of ferric chloride ($FeCl_3$). A brown ring forming at the test tube edge confirmed cardiac glycoside constituent (Sofowora, 1993).

3.4.2.7 Test for alkaloids (Mayer's test)

A 0.5 g extract of the plant was acidified in hot acidic alcohol and then filtered. Ammonia and chloroform of about 5 ml were added to the filtrate of about 3 ml, and then vortex. Acetic acid (10 ml) was used to extract the chloroform part. The chloroform extracted was divided into two parts. Each portion dissolved with Mayer's reagent and Draggendorf's reagent respectively. Reddish brown precipitate with the two reagents respectively confirmed alkaloids (Trease and Evans, 1989).

3.4.2.8 Test for carbohydrate (Benedict test)

About 5 ml aqueous extract of the plant reacted with copper sulphate (2 ml), and then sodium hydroxide was added and boiled 2-3 minutes. Carbohydrate with free end group reduces the blue cupric ions to a cuprous oxide (red-brown colouration) that confirmed carbohydrate (Lowery *et al*., 1951).

3.4.2.9 Test for starch (Iodine test)

About 5 ml aqueous extract of the plant was mixed to about 0.5 ml of dilute iodine, and then boil for 1-2 minutes. A blue-black colouration confirmed starch (Sofowora, 1993).

3.4.2.10 Test for protein amino acids (Xanthoproteic test)

About 1 ml aqueous extract of the plant was reacted with conc. nitric acid (1 ml). Then the solution was made alkaline with the addition of 40% concentrated sodium hydroxide. A yellow colour in acidic solution turning bright orange in an alkaline medium indicates the presence of amino acids (Harborne, 1973).

3.4.3 Estimation of photosynthetic pigments

Metzner *et al*. (1965) method was applied for the photosynthetic pigments assay of the okra leaves. A 250 mg of okra leaves were homogenized with 85% acetone for about 5 minutes. Then centrifuged (for 10 minutes at 6000 rpm). The reading was taken using UV/VIS spectrophotometer.

3.4.4 Determination of growth characteristics

After 30 days of planting, growth parameters (shoot height and leaf area) were assessed in three plants per group.

3.4.4.1 Shoot height

The okra plants shoot were measured by mean of thread and ruler and the unit was expressed in centimetres.

3.4.4.2 Flag leaf area

Quarrie and Jones (1979) method was used to measure the area of the leaf in cm^2.

3.4.4.3 Determination of shoot dry weight

Fresh shoots of okra plants weight were measured directly after harvesting the plant and then subjected to an oven dried at high temperature until the weight is constant, which determine the dry weight of the okra plant.

3.4.5 Determination of compatible solutes

3.4.5.1 Determination of protein accumulation

Principle

The method of Lowery *et al*. (1951) was followed, in which Copper interacts with a peptide bond of proteins to form purple colouration, which was measured with the use of spectrophotometer at 530 nm.

Reagents

1. 0.75g of $CuSO_4$
2. 3g of $KNaC_4O_{64}H_2O$ (potassium tartrate)
3. 0.5g of KI
4. 15g of NaOH
5. 10% of TCA
6. 0.1g of milk (Standard)

Procedure

About 5 ml of 10% TCA was mixed with 0.5 g leaf and fruit powdered samples respectively. They were centrifuged (10 minutes at 2000 rpm), then 5 ml was decanted from the supernatant and added to 0.1M NaOH (5 ml), followed by adding 8 ml of Biuret reagent. Milk was used as a standard. Incubation of the standards and the sample dilutions were done for 30 minutes at 25°C, before taking the reading at 530nm.

3.4.5.2 Determination of soluble sugar (Reducing sugar)

Principle

The method of Fales (1951) was adopted based on the reaction between the phenol group and the carbohydrates which gives a yellow-orange colouration.

Reagents

1.80% of ethanol

2. 3ml of Conc. H_2SO_4

3. 0.1g of Glucose (Standard)

Procedure

A 0.5 g of leaves and fruits samples were weighed respectively into the test tube into which 10 ml of 80% ethanol was added at 50^{o}C, the reaction occurred at about 15-30 minutes. Then 1 ml of the extract was estimated using spectrophotometer at 530 nm. The glucose was applied as standard.

3.4.5.3 Determination of free proline

Principle

During selective extraction with aqueous sulphosalicylic acid, proteins are precipitated as a complex. Other interfering materials are also presumably removed by absorption to the protein-sulphosalicylic acid complex. The extracted proline is made to react with ninhydrin in acidic conditions (pH 1.0) to form the chlorophore (red color) and read at 520nm.

Reagents

1.10 ml of 30% sulfosalicylic acid
2. 1ml of glacial acetic acid
3. 1ml of ninhydrin
4. 5ml of toluene
5. Proline (Standard)

Procedure

Proline concentrations were assayed by following the method described by Bates *et al*. (1973). A 3% by volume of sulfosalicylic acid (10 ml) was mixed with 0.2 g of samples (leaf and fruit powdered) respectively. Glacial acetic acid and ninhydrin (1 ml) was used to turn the solution acidic under water bath. Toluene was used to extract the mixture. Proline was applied as the standard (0–50 μg/ml) and the reading was taken at 520 nm.

3.4.6 Determination of minerals concentrations

3.4.6.1 Preparation of extract for nutrient content analysis

Perchloric acid, sulphuric acid and nitric acid (4:1:10) respectively were used for the digestion of dried fruit powder sample according to the Chapman and Pratt (1978) method. Plant material powder of about 0.5 g was oven dried at a very high temperature in digestion flask containing digestion reagents. The samples were cooled and poured into the volumetric flask (100 ml) already contained distilled water. The filter paper was used to filter the mixture. Mineral contents were determined with 25ml of the filtrate.

3.4.6.2 Phosphorus

The total phosphorus content of the okra leaves was assayed by using Murphy and Riley (1952) method.

3.4.6.3 Determination of K, Na and Ca in plant tissue digests by flame photometry

Apparatus

Flame photometer, EL

Reagents

1. K standard 100mg/l in aqueous solution: KCl 0.1907g/l
2. Na standard 100mg/l in aqueous solution: NaCl 0.2542g/l
3. Ca standard 100mg/l: $CaCO_3$ 0.2497g/l (final HCl conc. Approx. 0.5%)

Ranges of standard solutions

a. K: 0, 10, 20, 30,40 mg/l K
b. Na: 0, 2, 4, 6, 8,10 mg/l Na
c.Ca: 0, 10, 20, 30,40 mg/l Ca

Procedure

The sample solution was aspirated and read the percentage of optical density (E). The blank and 100% optical density reading was checked with the blank and standard was added after every 10 to 20 sample determinations). The mineral levels were determined in the sample and

K, Na, or Ca contents in plant tissue were calculated. Note: Standard solutions contained the same amount of reagents as in the plant tissue digests.

3.4.6.4 Determination of Mg and Zn by Atomic Absorption Spectrophotometer

Apparatus

Flame photometer and Atomic absorption spectrometer.

Reagents

Standard solution and optimum range for each element are as follows

1. Mg standards 0.1, 0.2, 0.3, 0.4, 0.5 mg/l
2. Zn standards 0.2, 0.4, 0.6, 0.8, 1.0 mg/l

Volumetric flasks containing 10 ml of 10% H_2SO_4 and 20 ml of lanthanum stock solution.

3.4.7 Sample preparation for enzyme assay in plant

Okra leaf and fruit (1g) each was ground in an appropriate buffer solution with EDTA (0.5 mM). Plant extract was centrifuged for 20 minutes at 15000rpm, and the analysis of enzyme was done in the supernatant by using Mukherjee and Choudhuri (1983) method.

3.4.7.1 Determination of catalase (CAT) activity in plant

Catalase activity was evaluated using the method of Chen *et al.* (2000).

Principle

The principle of catalase activity depends on the decomposition of hydrogen peroxide with a concomitance decrease in the wavelength at 240 nm.

3.4.7.2 Determination of superoxide dismutase activity in plant

Dhindsa *et al*. (1981) method was applied to assay for the superoxide dismutase activity.

Principle

Superoxide dismutase principle depends on the enzymatic superoxide radical conversion to H_2O_2 and molecular oxygen. Resulted H_2O_2, in turn, reacts with the 2,4-iodophenyl-3,4-

nitrophenol-5-phenyltetrazolium chloride to give a red species which can be measured at 560 nm.

3.4.7.3 Determination of glutathione peroxidase (GPx) activity in plant

Prabhakar *et al.* (2005) method was used to assay for the glutathione peroxidase activity.

Principle

Glutathione peroxidase is found in plants majorly in the cytosol as well as in the fraction of mitochondrial cells. Glutathione peroxidase involves in the reduction of hydrogen peroxide and hydroperoxides result from oxidation of fatty acids and oxidation of reduced glutathione (GSH). Absorbance was taken at 340 nm.

3.4.8 Determination of total phenolic content

Principle

Singleton and Rossi (1965) method was applied to estimate the total phenolic content of the sample with slight modifications using gallic acid as a standard. Total phenol gave light green colouration with the freshly prepared folin-ciocalteu reagent. The addition of sodium carbonate and incubation neutralized the reaction to give a blue colour.

Reagents

1. 0.5ml of 3, 4, 5-trihydroxybenzoic acid (Folin-Ciocalteu reagent)
2. 2ml of 20%Na_2CO_3
3. 8ml of deionised water
4. 1mg of Garlic acid (Standard)

Procedure

The crude extract of 0.5 g was diluted in 10ml of distilled water separately and the filtration was done with the use of filter paper. An equal volume of 0.5 ml each of the filtrate was reacted with folin-ciocalteu (0.5 ml), 20% Na_2CO_3 (2ml) and then completed with distilled water (10 ml). Incubation was done for about half an hour at 25°C. Reading was taken at 765 nm. The blank reagent was prepared by mixing together 1ml of 20% Sodium carbonate (Na_2CO_3), 1ml of Folin-Ciocalteus reagent (FC) and 8ml of deionised water. A 1mg of garlic

acid was mixed with deionised water (10 ml) as the standard stock concentration to give a stock solution of 100µg/ml concentration. Serial dilution of (1, 2, 4 6, 8 and 10µg/ml) was used.

3.4.9 Total flavonoid content estimation

Principle

Total flavonoid was assayed by using Zhishen *et al*. (1999) method. The principle behind the method involved the formation of intense yellow colouration on the addition of sodium hydroxide.

Reagents

1.0.3ml of 10% $AlCl_3$

2. 0.3ml of 5% $NaNO_2$

3. 2ml of 1MNaOH

4. Catechin (Standard)

Procedure

The crude extract of 0.5 g was diluted in distilled water (10ml) separately, then filtration was done by using filter paper. Equal volume (0.1 ml) of each filtrate was mixed with 0.3 ml of 5% $NaNO_2$, 0.3 ml of 0.3% $AlCl_3$, 2 ml of 1 M NaOH and then completed with distilled water (10 ml). Incubation occurred at about half an hour at 25°C. Then, the reading was taken at 510 nm. A 1mg of catechin was mixed with 10 ml of deionised water as the standard stock concentration of 100µg/ml. All the samples were analyzed in triplicates. The blank reagent was prepared by mixing together 0.3ml of 5% $NaNO_2$, 0.3 ml of 0.3% $Alcl_3$, 2 ml of 1M NaOH was made up to 10 ml with deionized water. A 1 mg of catechin was applied as a standard solution and dissolved into distilled water (10 ml) as a standard stock concentration of 100µg/ml. Serial dilution of (1, 2, 4, 6, 8 and 10 µg/ml) was used.

3.4.10 Crude fibre determination

Principle

This represents the insoluble fraction of the food after boiling in dil. acid and base. Crude fibre is mainly structural carbohydrate such as cellulose, lignin, and hemicellulose. To

determine the crude fibre in the samples, trichloroacetic acid (TCA) digestion method was used. This consists of acetic acid, nitric acid and trichloroacetic acid without any alkali treatment.

Reagents

1. 75% ethanol
2. TCA digestion reagent (containing Conc. HNO_3, acetic acid, trichloroacetic acid) and water.

Procedure

The defatted sample of a known weight was digested with the digestion reagent (TCA). This was then refluxed for about 40 mins. Then the digestest sample was subjected to cooling and then filter paper was used to filter the residues. The residue was rinsed with six-part hot water and one part of ethanol, the weight of the residue was estimated after oven drying at 150^{o} C. The dried residue was then ashed in the muffle furnace until the sample turns greyish. The ash was weighed to determine the difference in weight. Crude fibre percentage was then estimated by method of AOAC (1981) as:

$$Crude\ fibre\ percentage = \frac{Crucible+residue-Crusible+ashed\ residue}{\text{Sample weight}} \times 100 \ldots\ldots\ldots\text{i}$$

3.4.11 Determination of vitamin C (Ascorbic acid) content

Principle

Klein and Perry (1982) method was used to estimate the vitamin C content. This depends on the decrease of 2,6-dichlorophenolindophenol by ascorbic acid.

Reagents

1. 10 ml of 1% metaphosphoric acid
2. 3.9 ml of 2, 6-dichlorophenolindophenol (DPPH)
3. Vitamin C (Standard)
4. 10 ml of 10% methanol

Procedure

A 20 mg of methanol extract of the sample was extracted at room temperature for 45 minutes using metaphosphoric acid and filtered. A 2,6-dichlorophenol (9 ml) was reacted with the filtrate and within 15 seconds, the reading was taken at 515 nm. The vitamin C content was estimated by the calibration curve.

3.4.12 Antioxidant activity determination in plant

Antioxidant activity in plants was assayed by using Zhang and Hamauzu (2004) method.

Procedure

Methanol extracts of fresh leafy and fruit of okra plants used were 10 mg·ml−1 (dry weight).

Extract of the plant was mixed with the reaction mixture. The incubation was set for 1 hour at 25°C, the reading was taken at 517 nm with the use of a spectrophotometer. 2,2-diphenyl-2-picrylhydrazyl radical was estimated as:

$$DPPH\ inhibition\ percentage = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\% \ldots\ldots\ldots \text{ii}$$

3.4.13 Treatment of *Xenopus lavis* oocytes with plant bioactive to block glucose transporter activity

To evaluate the hypoglycemic activity of okra (*Abelmoschus esculentus*) in vitro, the full matured female frog was used; frog was housed in the tank containing water. The water temperature was approximately 19 to 21$^{\circ C}$. Tap water could be used but it is important to remove any high concentration of chlorine or heavy metals, which may be present. These could be avoided by allowing chlorine to evaporate in an open tank or using de-ionized water. The feeding was done twice a week. Salman pellets, minced beef and live blowfly larvae were used. Frog must eat within 3 hrs, afterwards, water must be changed. The frog was euthanized. The scalpel was used to remove frog and pith when the spine was severed from the head, an incision was made on the ventral side of the abdominal wall. Oocytes were removed from the ovary lobe into OR-2 buffer solution without Ca^{2+} in a petri dish. Individual oocytes were release from ovary sac; they were washed 2-3 times with OR-2 (without Ca^{2+}). Oocytes were

drained and split into two batches (one for collagenase treatment and the other for manual removal of collagenation.

3.4.13.1 Collagenase treatment

Oocytes were incubated in OR-2 buffer medium without Ca^{2+} but contained 25 ml of collagenase (100 mg/50 ml) for 30 mins, in petri dish wrapped with aluminium foil to prevent light, and slightly shaking to facilitate the process. The medium was removed and another 25 ml collagenase solution was added the second time for 30 mins. Oocytes were washed extensively (4-6 times) after collagenase treatment in calcium-free OR-2 before transferring them to a calcium-containing the solution. Afterwards, oocytes rinsed 2-3times with freshly prepared OR-2 with Ca^{2+} in order to stop the activity of collagenase. Then oocytes were stored in an incubator and left overnight at 18-20°C.

3.4.13.2 Cell culture

Bacterial *E. coli* strain (JM109XL-Blue) cells were grown on LB Agar, with 100 milliunits of penicillin and 100 µg of streptomycin/ml, so as to purify the medium from unwanted bacteria. Cells were split on the medium in a petri dish and kept under an atmosphere condition. The broth was then taken to the centrifuge tube for centrifugation. After centrifuged, the cells were then taken to miniprep kit to harvest the plasmid. Afterwards, the plasmid (cDNA) was subjected to purification using PCR purification kit. GLUT-14 cDNA was excised from the plasmid on a promoter region known as T_7 by digestion with a restriction enzyme (lyase). In vitro transcription of cDNA to cRNA was done with the use of Ambion mMessage, mMachine kit or Hi scribe T_7 kit. Oocytes were incubated for 30 mins prior to injection of cRNA.

3.4.13.3 Injection of cRNA into oocytes

Microinjection of cRNA into oocytes was done using KOPF Needle and Pipette Puller. The modified syringe was used to fill pipettes with sterile mineral oil. Pipettes were screwed to the automatic micro-injector and then 300 ng/µL of cRNA was filled or with the distilled water. Oocytes were placed in the OR-2 solution in the Petri dish for the purpose of injection. The injection of matured oocytes was done under a light microscope with 10x magnification. After the injection, the oocytes were returned to the buffer solution for incubation at $18^{\circ C}$ for

72 hours. During the incubation for every twelve hours, the media used was changed and non-viable oocytes were removed from the experiment.

3.4.13.4 2-deoxy-D-glucose uptake by oocytes

About 5 to 25 injected oocytes were washed in a medium with no antibiotics and they were selected randomly for the treatment groups and then placed in a 24-well cell culture dish. Radiolabeled glucose used is 2-deoxy-D-[1-^{3}H]-2-DG (sigma). Hexose mixture was kept at a temperature of 4°C but was equilibrated to room temperature 12 hours prior to use. 300 µL of each glucose analogue solutions (2-deoxy-D-[1-^{3}H]-2-DG) was used for the oocytes. The $_{C}$RNA and water injected oocytes were washed three times within one minute with phosphate buffer solution (PBS) before suspending in a 2 ml of 2-deoxy-D-[1-^{3}H]-2-DG containing tritium radiolabeled with plant extracts (bioregulator and non-bioregulator treated aqueous and ethanol extracts respectively) in 7 ml borosilicate glass scintillation vials with 1% sodium dodecyl sulphate (SDS) (0.5 ml) and scintillation fluid (5 ml). Incubation was done for at least 1 hour, which was then vortex to form a homogenous solution. Quantification of the radioactive sample was done using the liquid scintillation analyzer machine (Wallace 1400 DSA scintillation counter). Counting was done at every 180 seconds where indicated.

3.4.14 Treatment of rats with streptozotocin-induced hyperglycemia

Sprague-Dawley rats which weighing 110 ± 15g at age of 5 weeks were involved in the study. They were caged in a standard environment and subjected to *ad libitum* type of feeding. The rats were subjected to streptozotocin (STZ) 65 mg/kg induction through intravenous injection into the tail vein. Rats with the diabetic condition were determined using one stripe glucometer, and those with 250 mg/dl plasma level of glucose or an above were used in the study. To evaluate the hypoglycemic action of the okra, thirty-five rats were used for the experiment and each group contains five rats.

Group A: Control animals received saline (0.9%).

Group B: Experimental animals received saline (0.9%).

Group C: Experimental animals received okra aqueous extract without bioregulator (H_2OEC 100 mg/kg bw).

Group D: Experimental animals received okra aqueous extract with bioregulator ($H_2$0EE 100 mg/kg bw).

Group E: Experimental animals received okra phenolic extract without bioregulator (ETOHEC 100 mg/kg bw).

Group F: Experimental animals received okra phenolic extract with bioregulator (ETOHEE 100 mg/kg bw).

Group G: Experimental animals received 5 mg/kg bw of glibenclamide.

Okra fruit extracts and the drug were administered to all the animals orally for the period of six weeks. The respective weight and level of glucose of experimental animals were measured on weekly basis. Therefore, on the last day of the experiment (sixth week), respective treatments were given to the fasted animals and after 1 hour, euthanization was done to all animals. Cardiac puncture was used to obtain a blood sample, and the sample was stored in containers for the analysis, and thereafter the animals were sacrificed and some tissues were used for the analysis.

3.4.14.1 Determination of glucose level

Barham and Trinder (1972) method was used for glucose determination by using Randox reagent. The Randox reagent is composed of Buffer (100 mmol/L, phosphate buffer, phenol 11 mmol/L and pH of 7.0): Enzyme reagent (1500 U/L glucose oxidase; 0.77 mmol/L 4-aminophenazone; 1500U/L peroxidase and glucose standard (100 mg/L).

Principle

Determination of glucose was done through the glucose oxidation to form the gluconic acid under glucose oxidase enzyme. 4-amino phenazone and phenol react with H_2O_2 in the presence of an enzyme to form a quinoneimine (red-violet) which can be colourimetrically determined.

Procedure

A 0.01 mL of the plasma and glucose standard was reacted with the working reagents and in another tube 1mL of working reagent was served as a blank. The reaction mixture in the tubes were incubated for 25 minutes at 25°C. The absorbance reading was taken at 500 nm.

Plasma glucose was calculated thus:

$$\frac{A\ sample}{A\ standard} \times \frac{Concentration\ of\ standard}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ iii}$$

Where,

$A\ sample = absorbance\ of\ the\ sample$

$A\ standard = absorbance\ of\ the\ standard$

3.4.14.2 Estimation of feeding habit

The feeding habit in all experimental animals was observed on a daily basis throughout the experiment.

3.4.14.3 Preparation of serum

Cardiac puncture technique was used to obtain the blood, then centrifuged (10 minutes at 3000 rpm). Lipid profiles estimation was done from the supernatant.

3.4.14.3.1 Determination of triglycerides

Colourimetric method of Tietz (1990) was used to determined triglycerides.

Procedure

The RANDOX kit procedure was used. The reagent used contained 40 mM PIPES buffer, pH of 7.6, 5.5 mM 4-chlorophenol, 17.5 mM magnesium ions, 0.5 mM 4-aminophenazone, 1 mM ATP, lipases (150 U), glycerol kinase (GK) (0.4 U), glycerol-3-phosphate oxidase (GPO) (1.5 U) and peroxidase (POD) (0.5 U). Sample cuvette contained 0.01 ml of the sample, to which 1 ml of reagent was added. Another cuvette contained standard triglycerides of (0.01 ml) of both solution and the reagent. The blank had only 1 mL of the reagent. Thorough mixing was done for 10 minutes at 25°C. The reading was taken at 500 nm.

Calculation:

$$Triglycerides = \frac{\text{A sample} \times \text{concentration of standard}}{\text{A standard}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots iv$$

Where,

$A\ sample = absorbance\ of\ the\ sample$

$A\ standard = absorbance\ of\ the\ standard$

3.4.14.3.2 Determination of total cholesterol

An enzymatic method of Trinder (1969) was used to determine total cholesterol.

Principle

Cholesterol was determined through hydrolysis and oxidation of cholesterol ester. The resulting H_2O_2 reacts with 4-aminoantipyrine to form the quinoneimine in the presence of peroxidase enzyme.

Procedure

The RANDOX kit procedure was used. The reagent used contained 80 mM pipes buffer, pH 6.8, 4-aminoantipyrine (0.3 mM), phenol (6 mM), peroxides (0.5 U), cholesterol esterase (0.15 U) and cholesterol oxidase (0.1 U). Sample cuvette contained 0.01 ml of sample to which 1 mL of reagent was added while the blank had the only reagent. Similarly, 1 mL of reagent was mixed with 0.01 mL of standard cholesterol (200 mg/dl) in another cuvette. The cuvettes content was incubated (10 minutes at 25°C). The reading was taken at 500 nm.

Calculation

$$Sample\ concentration of\ cholesterol = \frac{\text{A sample} \times \text{concentration of Standard}}{\text{A standard}} \dots\dots\dots\dots\dots v$$

Where,

$A\ sample = absorbance\ of\ the\ sample$

$A\ standard = absorbance\ of\ the\ standard$

3.4.15 Determination of insulin content in the pancreatic β-cell

Materials

1. Rat pancreatic tissue 5 µM paraffin section slide.
2. Rabbit Anti-Mouse Insulin antibody from Abcam catalogue # ab63820
3. Goat Anti-Rabbit IgG HRP conjugated Millipore, catalogue # AP 156P
4. Normal Horse Serum Blocking Solution, Vector lab catalogue # S2000
5. Impact peroxidase DAB substrate, Vector lab catalogue # SK-4105.

Procedure

1. Melt the paraffin section at 65°C for 20 minutes using a heating block.
2. Remove residue paraffin by incubating slide for 10 minutes in Xylene.
3. Remove the Xylene by incubating slide for 10 minutes first in 95% ETOH (Fisher reagent alcohol) and then another 10 minutes in the 80% ETOH.
4. Wash the slide in several changes of tap water.
5. Remove slide from water quickly shake the water off and incubate slide in 0.3% H_2O_2. 200µL is the minimum volume required to completely cover the section for 30 minutes. H_2O_2 will inhibit the endogenous peroxidase.
6. Quick wash the slide in tap water, and incubate section in the Horse Serum Blocking Solution for 30 minutes. After this step, extreme care should be taken to avoid tissue drying.
7. Quick water washes and again dries the slide as above; incubate the tissue in 1:50 dilution anti-mouse insulin for 1 and ½ hours.
8. Quick water washes and incubates the tissue in 1:50 dilution of Goat anti-Rabbit IgG HRP conjugate for 1hour.
9. Quick water washes and keeps all slides in the water. Prepare the DAB substrate use one drop of DAB in the microcentrifuge tube and add 1 mL of peroxide buffer. Remove one slide from water, wipe dry the slide as in step 6 and then add 200µL of the substrate and monitor the reddish brown development in the islet but not the surrounding tissue under microscope around 1-2 minutes. Remove the DAB substrate by place the slide under gentle running tap water and place the slide back into the water container. Continue the step with one slide at the time for the remaining slides.
10. Counter with H&E, dehydration and mounting the slide per mount.

3.4.16 Liver and skeletal muscle glycogen content determination

Reagents

1. Mayer's Hematoxylin Solution0
2. 0.5% Periodic Acid Solution
3. Schiff reagent

Procedure

1. Remove residue paraffin by incubating slide for 10 minutes in Xylene
2. Oxidize the tissue for 5 minutes in 0.5% peroxide acid solution.
3. Quick wash the slide in tap water, and incubate section for 15 minutes in Schiff reagent.
4. Quick wash the slide for 5 minutes in warm tap water for the formation of pink colour
5. Then incubate the tissue for 1 minute with Mayer's hematoxylin.
6. Then Wash the slide with the tap running water for about 5 minutes.
7. Counter with H&E, dehydration and mounting the slide per mount.

3.4.17 Tissue homogenate preparation

About 2.5 g of tissue was homogenized with appropriate buffer in cold condition at pH of 7.0. After the homogenization, the mixture was centrifuged for 10minutes at 1000 rpm. Various analysis was done with the resultant solution.

3.4.17.1 Determination of hexokinase activity

Brandstrup *et al.* (1957) method was used for the estimation of hexokinase activity

Procedure

Hexokinase activity estimation was done in a reaction mixture containing 0.005 M glucose solution, 0.05 M $MgCl_2$, 0.0125 M dipotassium hydrogen phosphate, 0.1 M KCl_2, Tris-HCl and NaF (0.5 M). Incubation of the mixture was done at 37 °C for 5 minutes. The addition of the 1.3 ml of the mixture caused the initiation of the reaction. A 1 mL of 10% trichloroacetic acid was added with 1 mL of the reaction mixture. The second aliquot was removed after half an hour into another tube of 1 mL of 10% TCA. Centrifugation of both mixtures resulted protein precipitation. The glucose residue was determined by using Sasaki *et al*., (1972) method. Blank was run with each test. The amount of glucose phosphorylated was estimated by subtracting the two values. Enzyme activity was expressed in μ / mol of phosphorylated glucose /h1mg protein.

3.4.17.2 Estimation of the activity of glucose 6-phosphatase

The activity of of glucose 6-phosphatase was estimated by using the method of Koide and Oda (1959).

Procedure

The activity of glucose 6-phosphatase was assayed in a reaction medium containing 0.1 M maleic acid, glucose 6-phosphate (0.01 M), 2.5 g ammonium molybdate, 500 g of aminonaphthol sulphonic acid (ANSA) and 10% TCA. Incubation was done at 37°C for 1 hour. The addition of 0.2 mL of the homogenate initiated the reaction, while the addition of 10% TCA stopped the activity of the enzyme. The estimation of phosphate in the solution after centrifugation was done by using Fiske and Subbarow (1925) method. 0.4 mL of ANSA and1 mL of ammonium molybdate was mixed with the 1 mL of the supernatant. The reading was taken at 620 nm after 20 minutes. The standard used was phosphorus.

3.4.17.3 Estimation of the activity of fructose 1, 6-bisphosphatase

The activity of of fructose 1,6-bisphosphatase was done by using the method of Gancedo and Gancedo (1971).

Procedure

Estimation of fructose 1, 6-bisphosphatase activity was done in a reaction medium containing $MgCl_2$ (0.1 M), KCl_2 (0.1 M), EDTA (0.001 M), F- 1,6- bisphosphate (0.05 M), 0.1 M Tris-HCl, 10% of TCA, 2.5% ammonium molybdate, 35.1 mg of potassium dihydrogen phosphate and ANSA reagent. The mixture was incubated for 15 minutes at 37 °C. The addition of 0.2 mL of the homogenate caused the initiation of the reaction, while the addition of 10% TCA stopped the activity of the enzyme. The estimation of phosphate in the solution after centrifugation was done by using Fiske and Subbarow (1925) method. 0.4 mL of ANSA and1 ml of ammonium molybdate was mixed with the 1 mL of the supernatant after 10 minutes. The reading was taken at 620 nm. µ/mol of inorganic phosphorus liberated/h1mg protein was used to express the value of the enzyme.

3.4.17.4 Estimation of the activity of glucose 6-phosphate dehydrogenase

Ellis and Kirkman (1961) method was used for the assay of glucose 6-phosphate dehydrogenase activity.

Procedure

Estimation of the activity of glucose 6-phosphate dehydrogenase was done in a reaction medium containing 0.1 M $MgCl_2$, 0.1 M $NADP^+$, 0.05 M Tris HCl, 0.01% 2, 6-Dichlorophenol indophenol (freshly prepared), 0.005% phenazine methosulphate (freshly prepared) and glucose 6-phosphate (0.02 M). The addition of 0.5 ml of glucose 6-phosphate initiated the reaction. The absorbance reading was taken at 640 nm for 3.5 min interval. The activity of enzyme was estimated by multiply the change in optical density per minute by the factor of 6/17.6 g. mIU/mg of protein was used to express the activity of the enzyme in a tissue.

3.4.17.5 Determination of glycogen synthase and glycogen phosphorylase activities

Leloir and Goldemberg, (1979) method was used to determine liver glycogen synthase and glycogen phosphorylase.

Procedure

Estimation of pyruvate kinase and glycogen synthase activities were determined by the formation of uridyl diphosphate-glucose from glucose-6-phosphate and glycogen. Phosphate transfer from phosphoenolpyruvate to UDP was coordinated by the pyruvate kinase and the pyruvate consequently formed was determined colourimetrically by using Leloir and Goldemberg, (1979) method. μ /moles of uridyl diphosphate formed/ hr/mg protein was used to express the glycogen synthase enzyme activity, while μ /moles of Pi liberated/hr./mg protein was used to express the activity of glycogen phosphorylase enzyme.

3.4.18 Antioxidants enzyme assay in animal tissue

3.4.18.1 Determination of superoxide dismutase activity

Kakkar *et al.,* (1984) method was applied for the assay of the superoxide dismutase enzyme activity.

Principle

The principle involves the inhibition of NADH phenazine methosulphate, nitroblue tetrazolium formazon. The addition of NADH prompts the initiation of the reaction. Addition of glacial acetic acid halts the reaction after 90 secs. incubation. The n-butanol layer was used to extract the developed colour and the reading was measured at 520 nm.

Procedure

Tissue homogenization was done in 0.025 M at pH of 8.3. 1.0 mL of distilled water was added to 0.5 mL of tissue sample, and then followed by the addition of 2.5 ml of ethanol and 1.5 mL of chloroform. This reaction was vortex for more than an hour at a temperature of 4°C and then centrifuged. The activity of superoxide dismutase was assayed in a reaction mixture of 0.025 M sodium pyrophosphate, 10% Chloroform, 4 ml n-butanol, 0.2 mL of 10% Phenazine methosulphate, 0.2 mL Nitroblue tetrazolium and 780 μmol NADH. The addition of 0.2 mL of NADH and 3 mL of homogenate prompt the initiation of the reaction. Addition of 1 mL of glacial acetic acid stopped the activity of the enzyme after incubation for 90 second at temperature of 30°C. Then followed by the mixture of 4 mL n-butanol and vortexed before centrifugation. The reading was taken at 520 nm. Unit/min/mg of protein for tissues was used to express enzyme specific activity.

3.4.18.2 Determination of catalase activity

The method described by Sinha (1972) was used to determine the catalase activity in the tissue.

Principle

The principle involves the conversion of dichromate in acetic acid to per chromic acid which in turn result to the formation of chromic acetate at high temperature in the presence of H_2O_2. The reading was taken at 620 nm. The reaction stopped by adding dichromate - acetic acid mixture at different intervals, the remnant hydrogen peroxide as chromic acetate was colourimetrically estimated. The catalase activity was measured by the difference in absorbance per unit time.

$$H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

Procedure

Catalase activity was done in a reaction medium containing tissue sample (0.1 mL), 0.01 M phosphate buffer, 0.2 M H_2O_2, potassium dichromate (5%), potassium dichromate and glacial

acetic acid (1:3) and hydrogen peroxide (0.2 mM) (standard). After the interval of 30 sec., the addition of 2.0 mL of dichromate-acetic acid to the mixture caused the reaction to be stopped. The reaction medium was set to boiling for about 10 min. and then cooled. The reading was taken at 620 nm. Standard hydrogen peroxide concentration range was between 20-100 μ /mol.

3.4.18.3 Estimation of the activity of glutathione peroxidase

Rotruck *et al.* (1984) method was used to assay for the glutathione peroxidase activity.

Principle

Reduced glutathione peroxidase **(**GSHPx) catalyzes the oxidation of reduced glutathione to its oxidized form (GSSG) under NADPH and glutathione reductase. The oxidized form is in turn converted to the reduced form (GSH) and reduced form of NADPH oxidized to $NADP^+$. The reading was taken at 340 nm.

Procedure

Tissue homogenization was done in tris buffer. The mixture of the reaction contained 0.5 mL tissue homogenate, 0.4 M Tris buffer at pH of 7.0, sodium azide (10 mM), 10% TCA, ethylenediaminetetraacetic acid (0.4 mM), 0.2 mM of hydrogen peroxide and 2 mM of glutathione. The mixture was vortex and incubation was done at the temperature of 37°C for 10 min, in addition with the blank. After incubation for 10 minutes, 10% TCA (0.5 mL) was mixed to stop the reaction. The mixture was centrifuged and reduce glutathione assay was measured in the supernatant according to the method described by Ell man (1959).

3.4.19 Estimation of vitamin C

The content of Vitamin C in the sample was assayed by using the method described by Roe and Kuether (1943). Conversion of vitamin C to dehydroascorbic acid occurred in the presence of thiourea mixed with 2, 4 dinitrophenylhydrazines (DNPH). The addition of sulphuric acid caused the dinitrophenylhydrazine to be converted to a red colour specie compound. The absorbance is taken and read at 540 nm.

Procedure

Tissue homogenization was done in tris buffer. The mixture contained 0.5 mL of sample, 6% TCA, DNPH (2.0 g) in 9N sulphuric acids, 4.0 g of thiourea, 0.3 g acid washed norrit, 85% sulphuric acid and L-ascorbic acid (100 mg) in 100 mL of 6% TCA. The reaction mixture was centrifuged, 0.3 g of washed norrit was added to the supernatant, vortex and filtered, for the conversion of vitamin C to dehydroascorbic acid. 0.5 mL of DNPH was mixed with the 2 mL of the filtrate and stored under heat for 3 hours at 37°C. The mixture cooled, and then 85% sulphuric acid (2.5 ml) was mixed dropwise and vortex. Ascorbic acid was applied as standard at the concentration ranging between 20-100 µg. Blank followed a similar procedure. The reading was taken at 540 nm. µg/mg of protein for a tissue was used to express the value of ascorbic acid.

3.4.20 Estimation of vitamin E

Vitamin E content of the sample was assayed by the method described by Baker *and Davies* (1996).

Principle

Principle involves the conversion of Fe^{+3} to Fe^{+2} by vitamin E to the formation of red species compound by 2, 2' dipyridyl. The reading was taken at 520 nm.

Procedure

Tissue homogenization was done in tris buffer. The reaction mixture contained 0.5 mL of the sample, 60- 80 °C of petroleum ether and 1.5 mL of ethanol. The reaction was vortex, 2, 2' dipyridyl solution ferric chloride solution (0.2 mL) and petroleum ether (3.0 mL) were mixed with the precipitate. The reacting mixture was vortex for about 5 minutes in the dark before adding n-butanol (4.0 mL). The standard used was Vitamin E and the concentration range between 10- 100 µg. A similar procedure was applied to the blank reagent. The reading was taken at 520 nm. µg/mg protein for a tissue was used to express the value of vitamin E in the tissue.

3.4.21 Treatment of rats with Triton-X-100 induced hyperlipidemia

Sprague-Dawley rats which weighing 110 ± 15g at age of 5 weeks were involved. They were allocated in the cage under standard conditions with the *ad libitum* type of feeding. The rats were subjected to Triton-X (100 mg/kg bw) induction through intraperitoneal injection. Rats with 250 mg/dl cholesterol level or higher were used for the study. To evaluate the hypolipidemic action of okra, thirty-five rats (n=5) were used for the experiment as follows:

Group I: animals received 0.2% CMC (p.o) orally 2 hours before feeding the animals with a normal diet.

Group II: served as hyperlipidemia rats treated with 0.2% CMC (p.o) orally 2 hours before feeding the animals with a normal diet.

Group I11: served as *Abelmoschus esculentus* without bioregulator (AWOB) were administered with 200 mg/kg a daily dose of okra extract dissolved in 0.2% CMC (p.o) for 14 days after induction of hyperlipidemia.

Group 1V: served as *Abelmoschus esculentus* without bioregulator (AWOB) were received extract of okra (400 mg/kg) daily for two weeks after induction of hyperlipidemia.

Group V: served as *Abelmoschus esculentus* with bioregulator (AWB) were received extract of okra (200 mg/kg) daily for two weeks after induction of hyperlipidemia.

Group VI: served as *Abelmoschus esculentus* with bioregulator (AWB) were received extract of okra (400 mg/kg) daily for two weeks after induction of hyperlipidemia.

Group V11: The rats were received 30 mg/kg daily dose of atorvastatin dissolved in 0.2% CMC (p.o) for 14 days after induction of hyperlipidemia.

3.4.21.1 Blood glucose level determination

Blood level glucose from the tail was estimated using a one-touch glucometer.

3.4.21.2 Collection of blood

After 24 hours of the last dose, blood samples were obtained at retro-orbital plexus and coagulated at 25°C, centrifuged for 10 minutes at 3000 rpm. Lipid profiles estimation in the serum was done by using Allain *et al*., (1974) method. Friedewald *et al*. (1972) equation was used to determine low and very low lipoprotein fractions as follows:

$$LDL = Total\ cholesterol - (HDL + VLDL) \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{vi}$$

$$VLDL = \frac{Triglyceride}{5} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots vii$$

3.4.21.3 High-density lipoprotein cholesterol estimation

Lopes-Virella *et al*., (1977) method was used to estimate high-density lipoprotein fraction in the sample.

Principle

The addition of phosphotungstic acid with magnesium ions as a cofactor caused the precipitation of the lipoproteins and chylomicron fractions for the estimation of cholesterol concentration.

Procedure

The reagent used is made up of phosphotungstic acid (0.55 mM) and magnesium chloride (25 mM). 0.2 mL of the sample was mixed with 0.5 mL of diluted precipitate. After thorough mixing, the tube was settled and then centrifuged for 2 minutes at 12,000 rpm. Determination of the cholesterol content in the upper layer is outlined in section 3.4.14.2.2.

Calculation

$$Concentration\ of\ HDL = \frac{A\ sample}{A\ standard} \times \frac{Concentration\ of\ standard}{1} \ldots\ldots\ldots\ldots\ldots viii$$

Where,

$A\ sample = absorbance\ of\ the\ sample$

$A\ standard = absorbance\ of\ the\ standard$

3.4.21.4 Low-density lipoprotein cholesterol determination

Estimation of low-density cholesterol was done by the equation described by Friedewald *et al*. (1972).

$$\text{LDL} - \text{CHOL} = Total\ Cholesterol - \frac{Triglycerides}{5} + \text{HDL} - \text{CHOL} \ldots\ldots\ldots\ldots\ldots \text{ix}$$

3.4.21.5 Very low-density lipoprotein cholesterol determination

Determination of very low-density lipoprotein cholesterol concentration was done according to Friedewald *et al*. (1972) by using the formula:

$$VLDL = \frac{\text{TG (Triglyceride)}}{5} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots x$$

3.4.21.6 Determination of atherogenic index

Estimation of atherogenic index was done according to equation described by Friedewald *et al*. (1972). This was determined by the ratio of LDL: HDL.

$$Atherogenic\ index\ (AI) = \frac{LDL}{HDL} \ldots\ldots\ldots\ldots\ldots\ldots\ldots xi$$

3.4.21.7 Measurement of Body Weight, Relative Liver, and Heart

The body weights of the experimental animals were estimated on weekly basis for consecutive five weeks. But relative liver and heart weight of the animals were measured on the 42nd day after which they were sacrificed. The liver and heart weight were estimated by applying Taleb-Dida *et al*. (2011) formula

$$Relative\ weight = \frac{Organ\ weight}{\text{Body weight}} \times 100\ \% \ldots\ldots\ldots\ldots\ldots\ldots\ldots xii$$

3.4.21.8 Preparation of tissue homogenate

Yuan and Kitts (2003) method was used for the homogenization of liver tissue. Briefly, ice-cold homogenizing buffer (10 mL) (Na_2HPO_4 (8 mM), NaH_2PO_4 (12 mM), KCl (1.15%), pH 7.4) was used to homogenize 1 g of liver tissue. The homogenate was centrifuged for 20 minutes at 12,000 rpm. A portion of the homogenate was employed for measuring the oxidation product level (malondialdehyde). Another portion was used for (HMG-CoA reductase activity and protein estimation)

3.4.21.9 Determination of malondialdehyde

Malondialdehyde level was estimated by using Ohkawa *et al*., (1979) method. Oxidation of polyunsaturated fatty acids results to the product of malondialdehyde that shows the degree of peroxidation of lipid reaction.

Principle

The malondialdehyde gives a red species product when reacted with a thiobarbituric acid, and this is readily absorbed at 535 nm wavelength.

Procedure

Homogenization of tissue was prepared with 9.0 mL of 1.15 % KCL, the reaction containing liver sample (0.2 mL), 8.1% sodium dodecyl sulphate (0.2 mL), 20% acetic acid (1.5 mL) at pH of 3.5, 0.8% aqueous solution of thiobarbituric (1.5 mL), n-butanol and pyridine (5.0 mL) (15:1 by volume) and distilled water (1.0 mL). The mixture was heated at a high temperature for I hour. n-butanol and pyridine (5.0 mL) in ratio 15: 1 by volume were mixed with the reaction, and then centrifuged for 10 minutes at 4000 rpm. The reading was taken at 532 nm.

3.4.21.10 Estimation of the activity of HMG-CoA reductase

In the liver homogenate, the activity of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase was measured by using Venugopala Rao and Ramakrishnan (1975) method. Indicator of the enzyme (HMG-CoA reductase) activity is determined by the ratio of HMG-CoA to mevalonate, which convert the HMG-CoA to mevalonate.

Procedure

Homogenization of 100 mg liver sample was carried out in arsenate (1.0 mL). The mixture of the reaction contained 0.2 mL liver sample, diluted perchloric acid (50 mL/L), 0.5 mL of hydroxylamine at pH of 5.5 for HMG-CoA, 0.5 ml hydroxylamine at pH of 2.1 for mevalonate and ferric chloride (1.5 mL) in 0.65 mol/L HCl (50 mL). Centrifugation was done for 10 min. at 2000 rpm. 0.5 mL of hydroxylamine (pH 5.5) was mixed with part of the 1 ml of the upper layer for the HMG-CoA and 0.5 mL of hydroxylamine (pH 2.1) was added to the other part for the mevalonate. 1.5 mL of ferric chloride was reacted with the mixtures and vortex. The

reading was taken at 540 nm after 10 minutes. A similar procedure was carried out on the blank.

3.4.21.11 Determination of total protein

Estimation of the total protein content in the sample was done by adopting the method described by Bradford, (1976).

Principle

The principle involved the interaction between protein peptide bond and Coomassie Brilliant Blue G-250. Force of attraction between the ionic and hydrophobic interactions causes the stabilization of the anionic form of the dye, which brings about a visible colour change.

Procedure

A 5 ml of Bradford solution was mixed with 100 µL of tissue homogenate and then subjected to incubation for about 5 minutes at 25°C. The reading was taken at 595 nm. A protein standard solution was similarly treated.

3.4.22 Statistical analysis

Experimental data were subjected to three-way analysis of variance (ANOVA) with statistical analysis system (SPSS, 2003). Sample means were compared using Tukey-Kramer multiple comparison procedures and data were considered significantly different at $\alpha_{0.05}$. All results are presented as mean ± standard deviation.

CHAPTER FOUR

4.0 EXPERIMENTS AND RESULTS

4.1 Experiment 1: Phytochemical screening of aqueous and ethanol extracts of indole acetic acid -treated okra fruits

Introduction

Phytochemicals are secondary products of the plants (Richter, 1978). They have medicinal value useful for human beings and animals, while the primary metabolites are essential to the plants (Trease and Evans, 1989). Akinmoladun, *et al*. (2007) defined a medicinal plant as any plant that contain substances that are therapeutic in nature with a precursor for making useful drugs. Many vegetable crops have secondary metabolites with biological activities, which interact with various metabolic processes. Traditionally, every part of okra plant is used for medicinal purposes such as diuretic, cooling, aphrodisiac, antiseptic, and in gonorrhoea. This experiment examined the phytochemical and nutritional constituents of okra fruits.

Procedure

The harvested okra fruits were chopped into pieces and dried at room temperature. After which is grounded into powder with used of the mortar and pestle. The powder form of okra fruit was subjected to both aqueous and ethanol extracts. The raw powder of okra fruits along with its ethanol and aqueous extracts were assayed for the qualitative tests as outlined in section 3.4.2.

Results

Table 5 revealed that the okra fruits are rich in flavonoids, terpenoids, tannins, starch, carbohydrate, and protein amino acids. These nutritients and phytochemicals contain medicinal and physiological activities (Sofowara, 1993; Ajayi, *et al.,* 2011). Flavonoids play an important role as an antioxidant that prevents oxidative stress (Sharma, *et al.,*2007). Tannins were reported by Shi *et al*., (2010) to be responsible for antidiarrhoea activities

Conclusion

The medicinal and physiological value of okra fruits are associated with the phytochemicals and nutritional constituents in the okra fruits. Moreover, this result shows vital information on biochemical basis for ethnomedicinal use of okra plant.

TABLE 3: Qualitative tests for nutritional and phytochemical constituents of okra

Tests	**Raw powder**	**Ethanol extract**	**Aqueous extract**
Tannins	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Glycosides	-	-	-
Alkaloids	-	-	-
Terpenoids	+	+	+
Phlobatannins	-	-	-
Carbohydrates	+	+	+
Starch	+	+	+
Protein amino acid	+	+	+

+ = presence, - = absence.

4.2 Experiment 2: Influence of indole acetic acid, salicylic acid and salinity stress on photosynthetic pigments and growth parameters in two genotypes of okra plants

Introduction

Environmental factors such as salinity, drought and water stresses contribute to the stunted growth in the okra plant. Salt stress majorly reduce okra plant productivity, and alters the physiology of many plant species (Ashraf and Harris, 2004). Large portions of land are under the influence of salt stress globally (Ghassemi *et al*., 1995); this then calls for the method of sustaining vegetable crops in harsh environmental conditions (Szabolcs, 1994). The effects of the environmental stress conditions in the plant cause the formation of reactive metabolites (Farooq and Azam, 2006). ROS alter the metabolic composition of macromolecules, hence in the absence of any ameliorative mechanism, metabolic systems of the plants can be seriously damaged through oxidative stress (Noreen and Ashraf, 2009). Antioxidants could be used to mitigate salinity stress effect and thus confer tolerance in the plant (Abdul Hameed *et al*., 2015). Catalase, superoxide dismutase, and ascorbate have been involved in reducing the free radicals in the plants (Sheteawi, 2007). Bioregulators help in the physiology and nutrients uptake in the plant (Nickell, 1978). Recently, indoleacetic acid, salicylic acid has gained tremendous attention in the production of plants (Popova *et al*., 1995). In this experiment, bioregulators (IAA and SA) were used to ameliorate the salinity effects on okra plant photosynthetic pigments and growth parameters.

Procedure

Okra seeds of genotypes LD-88 and 47-4 were pre-treated with bioregulators as described in section 3.3.1.

The procedures for the photosynthetic pigments determination were outlined in section 3.4.3. The growth characteristics method was determined as described in sections 3.4.4 to 3.4.4.3. Minerals content were determined as described in sections 3.4.6.1 - 3.4.6.4

Results

Figure 19 showed a decrease levels of chlorophyll b in the two genotypes with increase or decrease in the levels of chlorophyll a in genotype LD 88 at 100 and 200 mM concentrations also in genotype 47-4 at 100 mM concentration as salinity level increased. However, the increase in concentration of NaCl increased carotenoids levels with exception in genotype LD

88 at 200 mM concentration of salinity stress relative to the control groups. A similar result was observed in genotype 47-4 relative to control (Figure 20).

Result in Table 4 showed an increase carotenoids level in the group treated with 50 mM NaCl at 0.4 mM of IAA. Also, the interaction of 0.5 and 0.6 mM IAA at 200 and 150 mM NaCl respectively increased photosynthetic pigments. Similarly, in Table 5, 50 mM NaCl at 10^{-2} mM of SA increased the level of carotenoids. Interaction of 10^{-4} mM at 150 and 200 mM NaCl increased chlorophyll a and b contents respectively in genotype LD 88. In genotype 47-4, the interaction of all the levels of indole acetic acid with severe salinity stress increased the level of carotenoids. However, increased in chlorophyll a and b were observed at mild and moderate levels of salinity (Table 4). A similar effect was observed on salicylic acid (Table 5).

Figure 21 showed no effect on growth parameters. But the leaf areas were reduced as salinity levels increased, which is more prominent on 200 mM NaCl treated group compared to control group (genotype 47-4). Similar trends were observed in the genotype LD 88 (Figure 22).

In Figures 23 and 24, minerals content tends to increase with increasing salinity stress, but the actual increase was observed on sodium (Na) when compared with other minerals in the control and experimental groups of both genotypes 47-4 and LD 88.

Results in Table 8 revealed greater increase in potassium, zinc and phosphorus under mild and moderate levels of salt stress in all levels of indoleacetic acid treated groups in the genotype 47-4, but reduced sodium concentration at all salinity levels was observed relative to NaCl-treated control. The result in Table 9 revealed that the treatments of salt-stressed okra plant at concentration of 10^{-4} mM SA showed an increase concentrations of potassium (K) at 150 mM and phosphorous (P) at 50 mM and zinc at 10^{-6} mM in 50 mM NaCl relative to NaCl-treated group in 47-4 genotype. However, there was an up-regulation of calcium (Ca) potassium (K) and zinc (Zn) contents in group treated with 10^{-4} mM SA concentration and down-regulation of sodium content in 10^{-6} mM SA treated group at severe salinity level relative to NaCl-treated group in genotype LD 88 (Table 10).

In Table 11, interactive effects of 0.4 mM indole acetic acid and salinity (150, 200 and 100 mM) significantly ($p < 0.05$) increase potassium (K), zinc (Zn) and phosphorous (P) contents respectively in the treated groups relative to NaCl-treated group in LD 88 genotype.

Conclusion

Pretreatment of okra seeds (Genotypes 47-4 and LD 88) with either SA or IAA, under salinity stress increased okra plant tolerance through increased photosynthetic pigments and mineral levels. Therefore, treatments with bioregulators (SA or IAA) reversed the negative effects of salinity on okra plant. Genotype 47-4 responds more positively to IAA treatment than SA treatment, while genotype LD 88 responds better to SA treatment than IAA treatment.

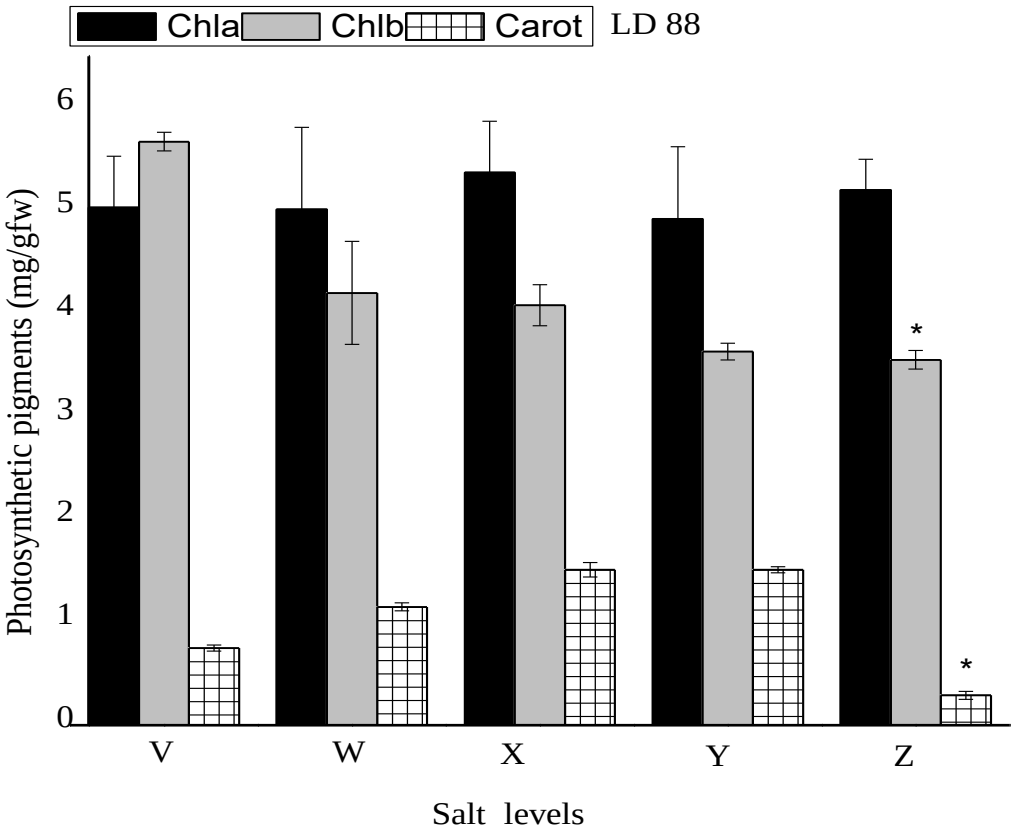


Fig. 19: Influence of salinity on photosynthetic pigments of okra genotypes LD 88

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control

Where V, W, X, Y and Z equal to (0, 50, 100, 150 and 200) mM respectively

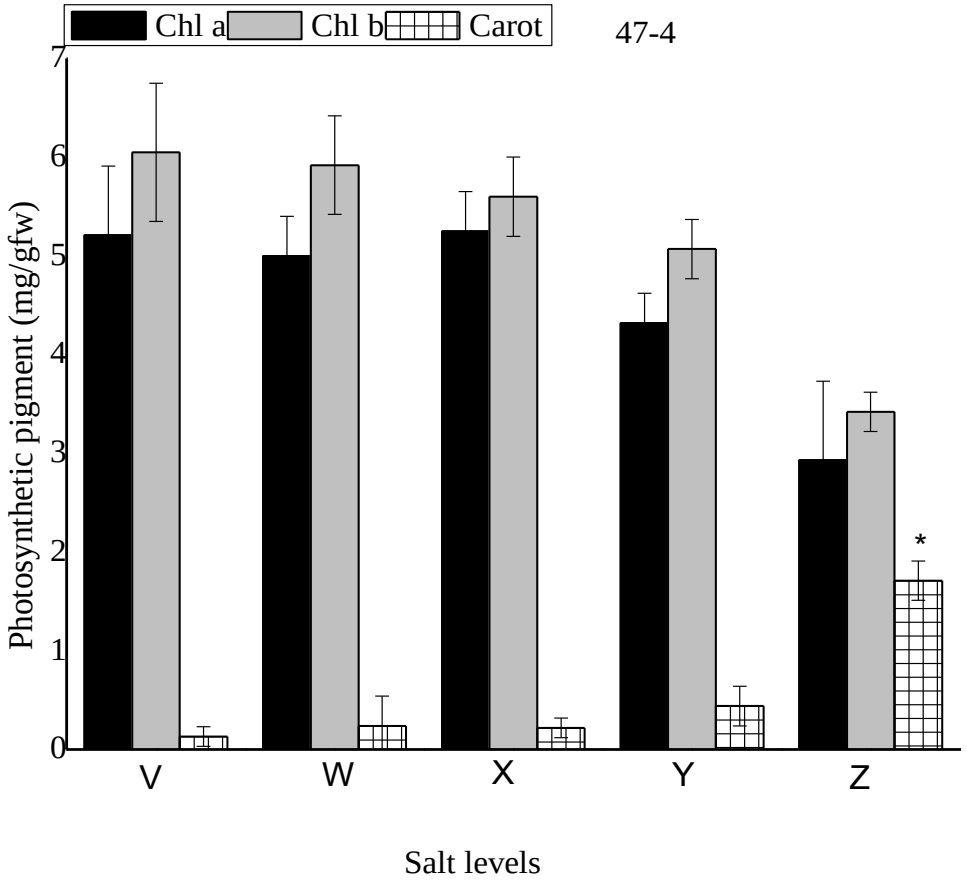


Fig. 20: Influence of salinity on photosynthetic pigments of okra genotypes 47-4

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control

Where V, W, X, Y and Z equal to (0, 50, 100, 150 and 200) mM respectively

TABLE 4: Photosynthetic pigments of okra (Genotype LD 88) under the influence of salinity and indoleacetic acid

Bioregulator IAA (mM)	NaCl (mM)	Chlorophyll A (mg/gfw)	Chlorophyll B (mg/gfw)	Carotenoids (mg/gfw)
0.4	0	5.43 ± 0.03	5.80 ± 0.02	0.54 ± 0.02
	50	5.04 ± 0.02	3.15 ± 0.02	3.26 ± 0.02*
	100	5.44 ± 0.02	4.05 ± 0.02	0.95 ± 0.01
	150	4.84 ± 0.02	4.91 ± 0.01	0.91 ± 0.01
	200	4.80 ± 0.02	4.02 ± 0.01	1.14 ± 0.01
0.5	0	5.02 ± 0.02	4.07 ± 0.02	0.71 ± 0.02
	50	5.20 ± 0.02	4.02 ± 0.02*	1.26 ± 0.02
	100	5.04 ± 0.02	5.04 ± 0.02	1.41 ± 0.02
	150	5.38 ± 0.01	6.04 ± 0.01	0.56 ± 0.01
	200	5.51 ± 0.01	6.52 ± 0.01	0.29 ± 0.02
0.6	0	5.52 ± 0.02	5.63 ± 0.02	0.71 ± 0.02
	50	2.02 ± 0.01*	1.78 ± 0.02*	1.55 ± 0.01
	100	3.03 ± 0.02	2.61 ± 0.01	1.74 ± 0.01
	150	5.53 ± 0.01*	2.70 ± 0.01	1.74 ± 0.02
	200	5.01 ± 0.01	5.72 ± 0.02	0.52 ± 0.01

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the bioregulator treated control

TABLE 5: Photosynthetic pigments of okra (Genotype LD 88) under the influence of salinity and salicylic acid

Bioregulator SA (mM)	NaCl (mM)	Chlorophyll A (mg/gfw)	Chlorophyll B (mg/gfw)	Carotenoids (mg/gfw)
10^{-2}	0	5.43 ± 0.02	5.86 ± 0.08	0.60 ± 0.09
	50	3.47 ± 0.04	2.93 ± 0.04*	2.09 ± 0.07*
	100	5.48 ± 0.05	4.35 ± 0.03	1.74 ± 0.05
	150	4.86 ± 0.01	4.85 ± 0.01	0.90 ± 0.04
	200	4.79 ± 0.04	4.23 ± 0.03	1.13 ± 0.05
10^{-4}	0	5.02 ± 0.01	6.16 ± 0.06	0.31 ± 0.09
	50	5.47 ± 0.06	4.09 ± 0.06	1.16 ± 0.06*
	100	5.36 ± 0.06	3.73 ± 0.04*	1.33 ± 0.05
	150	5.90 ± 0.04*	6.23 ± 0.02	0.56 ± 0.04
	200	5.38 ± 0.03	7.05 ± 0.07*	0.18 ± 0.02
10^{-6}	0	5.19 ± 0.02	5.04 ± 0.02	0.81 ± 0.06
	50	1.31 ± 0.01*	6.69 ± 0.01	1.78 ± 0.06
	100	2.67 ± 0.01	1.35 ± 0.01*	2.23 ± 0.08*
	150	5.34 ± 0.06	2.62 ± 0.03	1.83 ± 0.03
	200	5.44 ± 0.08	5.65 ± 0.07	0.52 ± 0.01

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the bioregulator treated control

TABLE 6: Photosynthetic pigments of okra (Genotype 47-4) under the influence of salinity and indoleacetic acid

Bioregulator IAA (mM)	**NaCl (mM)**	**Chlorophyll A (mg/gfw)**	**Chlorophyll B (mg/gfw)**	**Carotenoids (mg/gfw)**
0.4	0	5.43 ± 0.02	6.09 ± 0.05	0.38 ± 0.03
	50	5.42 ± 0.02	7.26 ± 0.02*	0.24 ± 0.02
	100	5.42 ± 0.02	6.21 ± 0.02	0.22 ± 0.02
	150	5.74 ± 0.02*	5.76 ± 0.02	0.44 ± 0.02
	200	5.44 ± 0.03	5.30 ± 0.02	0.60 ± 0.04*
0.5	0	5.28 ± 0.03	6.85 ± 0.03	0.12 ± 0.03
	50	5.01 ± 0.03	6.36 ± 0.02	0.24 ± 0.04
	100	5.41 ± 0.02*	5.96 ± 0.02	0.31 ± 0.02
	150	4.96 ± 0.04	6.83 ± 0.03	0.57 ± 0.05
	200	5.07 ± 0.02	5.03 ± 0.03	1.13 ± 0.02*
0.6	0	5.34 ± 0.02	6.24 ± 0.02	0.21 ± 0.02
	50	5.40 ± 0.02	6.04 ± 0.04	0.24 ± 0.02
	100	5.31 ± 0.02	5.82 ± 0.02	0.51 ± 0.03
	150	5.59 ± 0.03*	5.99 ± 0.03	0.40 ± 0.04
	200	3.01 ± 0.02*	3.23 ± 0.04*	1.57 ± 0.02*

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the bioregulator treated control

TABLE 7: Photosynthetic pigments of okra (Genotype 47-4) under the influence of salinity and salicylic acid

Bioregulator SA (mM)	**NaCl (mM)**	**Chlorophyll A (mg/gfw)**	**Chlorophyll B (mg/gfw)**	**Carotenoids (mg/gfw)**
10^{-2}	0	5.35 ± 0.03	6.09 ± 0.01	0.39 ± 0.04
	50	5.60 ± 0.01	6.95 ± 0.01	0.41 ± 0.04
	100	5.42 ± 0.02	6.21 ± 0.01	0.60 ± 0.04
	150	5.44 ± 0.01	5.76 ± 0.06	0.49 ± 0.09
	200	5.43 ± 0.06	5.34 ± 0.04	0.67 ± 0.03
10^{-4}	0	5.29 ± 0.08	6.85 ± 0.03	0.06 ± 0.04
	50	5.42 ± 0.02	6.36 ± 0.02	0.24 ± 0.02
	100	5.43 ± 0.04	6.10 ± 0.06	0.32 ± 0.03
	150	5.06 ± 0.06	6.83 ± 0.06	0.57 ± 0.05
	200	5.08 ± 0.05	2.86 ± 0.06*	1.62 ± 0.09*
10^{-6}	0	5.28 ± 0.03	6.35 ± 0.07	0.21 ± 0.02
	50	5.40 ± 0.04	6.95 ± 0.05	0.14 ± 0.07
	100	5.40 ± 0.04	5.76 ± 0.03	0.49 ± 0.06
	150	5.59 ±0.01	5.99 ± 0.06	0.50 ± 0.01
	200	2.57 ± 0.09	3.12 ± 0.01	1.60 ± 0.02*

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the bioregulator treated control

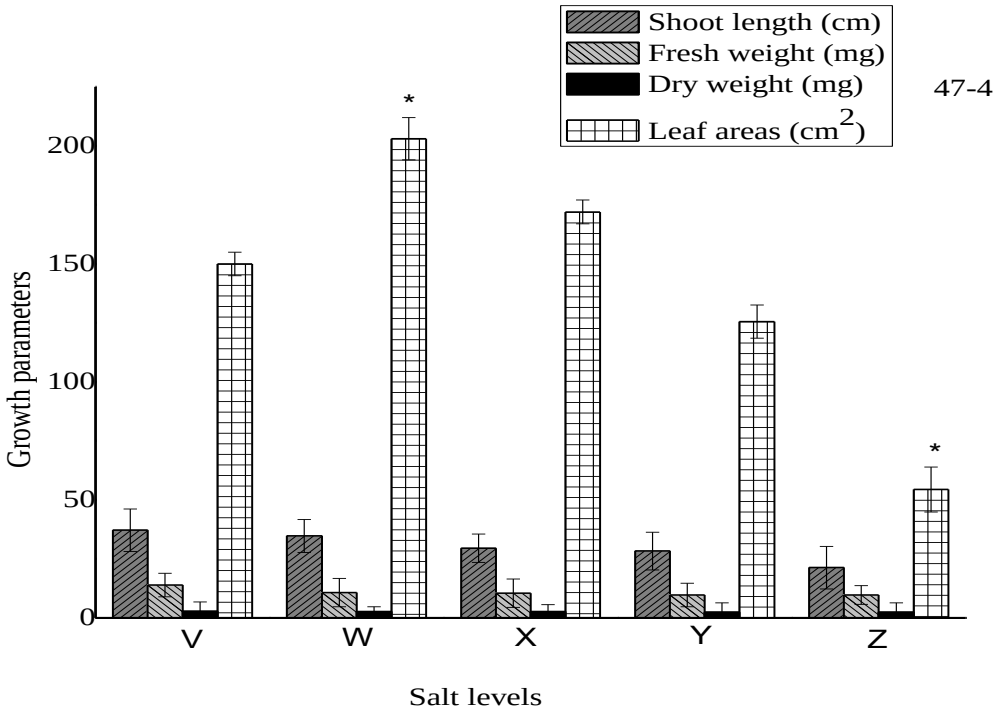


Fig. 21: Growth parameters of okra genotype 47-4 under the effect of salinity stress

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control group

Where V, W, X, Y and Z equal to (0, 50, 100, 150 and 200) mM respectively

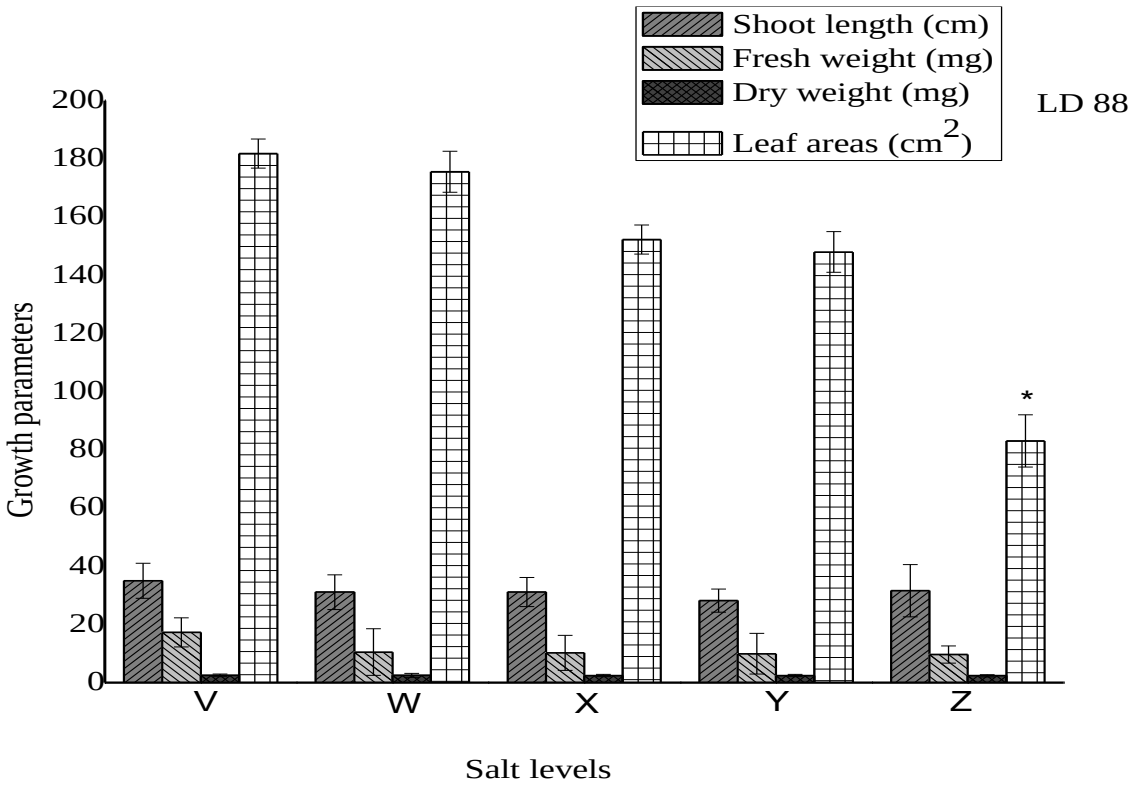


Fig. 22: Growth parameters of okra genotype LD 88 under the effect of salinity stress

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control

Where V, W, X, Y and Z equal to (0, 50, 100, 150 and 200) mM respectively

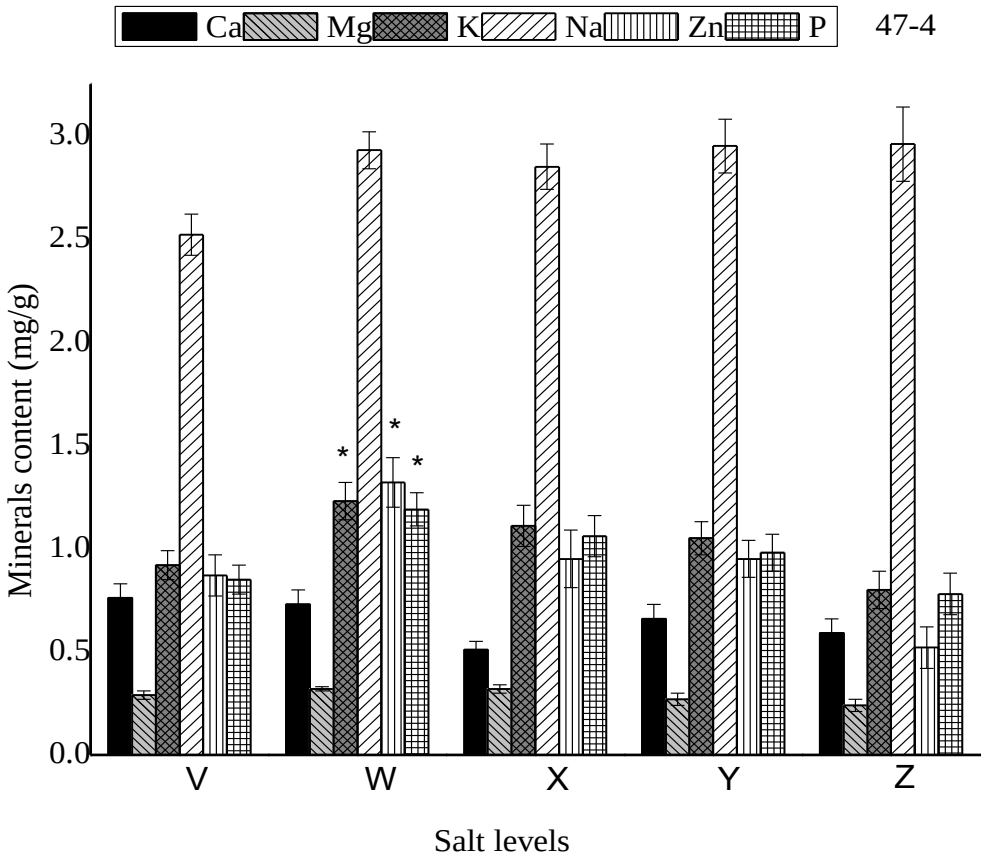


Fig. 23: Influence of salinity on okra genotype 47-4 mineral concentrations

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control

Where V, W, X, Y and Z equal to (0, 50, 100, 150 and 200) mM respectively

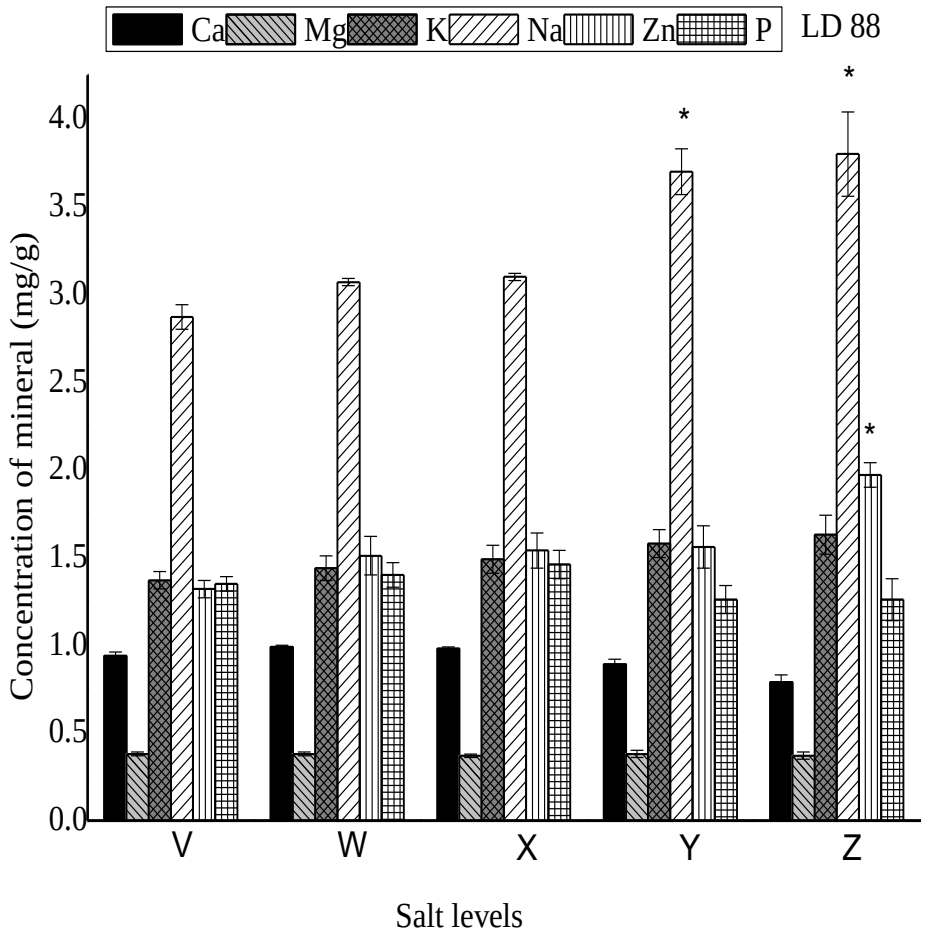


Fig. 24: Influence of salinity on okra genotype LD 88 mineral concentrations

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control

Where V, W, X, Y and Z equal to (0, 50, 100, 150 and 200) mM respectively

TABLE 8: Mineral concentration of okra (Genotype 47-4) under the influence of salinity and salicylic acid

Bioregulator SA (mM)	NaCl (mM)	Calcium (milligram/g)	Magnesium (milligram/g)	Potassium (milligram/g)	Sodium (milligram/g)	Zinc (milligram/g)	Phosphorous (milligram/g)
10^{-2}	0	0.90±0.07	0.12±0.18	1.80±0.03*	2.70±0.11*	2.26±0.12*	1.78±0.06*
	50	0.84 ±0.04	0.71 ±0.16	1.41 ±0.14	2.76 ±0.33	1.96 ±0.06	1.40 ±0.13
	100	0.76 ±0.05	0.66 ±0.14	1.38 ±0.17	2.98 ±0.48	1.94 ±0.02	1.35 ±0.17
	150	0.73 ±0.02	0.39 ±0.07	1.29 ±0.19	2.98 ±0.44	1.49 ±0.16	1.23 ±0.21
	200	0.61 ±0.02	0.44 ±0.11	0.88 ±0.13	2.56 ±0.37	1.41 ±0.12	0.90 ±0.14
10^{-4}	0	1.03±0.04*	0.43±0.01	1.73±0.01*	2.78±0.05*	1.84±0.18	1.74±0.02
	50	1.01±0.05**	0.53 ±0.06	1.42 ±0.14	2.96 ±0.42	1.78 ±0.15	2.40 ±0.11
	100	0.97±0.06**	0.46 ±0.04	1.33 ±0.17	2.97 ±0.04	1.70 ±0.13	1.28 ±0.18
	150	0.87±0.09**	0.43±0.05	2.27 ±0.18	2.75±0.36	1.53 ±0.16	1.26±0.17
	200	0.79±0.10**	0.46 ±0.09	0.99 ±0.16	2.42 ±0.24	1.22 ±0.10	1.01 ±0.07
10^{-6}	0	0.45±0.10	0.41±0.01	1.67±0.06	2.45±0.04	3.93±0.84	1.66±0.06
	50	0.61 ±0.09	0.47 ±0.04	1.46± 0.14	1.88±0.40**	2.96±0.82**	1.47 ±0.13
	100	0.66 ±0.06	0.44±0.05	1.23 ±0.23	1.83±0.40**	2.43±0.61**	1.23 ±0.21
	150	0.43±0.10	0.42±0.06	1.29 ±0.13	1.11±0.12**	2.38±0.24**	1.20 ±0.12
	200	0.64 ±0.07	0.48 ±0.08	0.89 ±0.15	1.09±0.22**	1.72±0.14**	0.90 ±0.14

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control

**Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control

TABLE 9: Effects of salinity and indole acetic acid on the mineral concentration of okra (Genotype 47-4)

Bioregulator IAA (mM)	NaCl (mM)	Calcium (milligram/g)	Magnesium (milligram/g)	Potassium (milligram/g)	Sodium (milligram/g)	Zinc (milligram/g)	Phosphorous (milligram/g)
0.4	0	0.93 ± 0.09*	0.40 ± 0.01	0.59 ± 0.09	3.11 ± 0.08	1.79 ± 0.16*	1.52 ± 0.09*
	50	0.60 ± 0.17	0.34 ± 0.03	1.46 ± 0.10	3.04 ± 0.02	1.60 ± 0.13**	1.40 ± 0.11
	100	0.66 ± 0.15	0.33 ± 0.03	2.08 ± 0.20	2.90 ± 0.09	1.00 ± 0.32**	1.08 ± 0.19
	150	0.56 ± 0.16	0.28 ± 0.05	1.24 ± 0.11	2.78 ± 0.17	1.20 ± 0.17**	1.17 ± 0.14
	200	0.48± 0.17	0.27 ± 0.06	0.76 ± 0.19	3.64 ± 0.13	0.61 ± 0.27**	0.74 ±0.21
0.5	0	0.87 ± 0.05	0.36 ± 0.02	1.35 ±0.07*	3.08 ± 0.02	1.72 ± 0.28	1.39 ± 0.08
	50	0.84 ± 0.06**	0.30 ± 0.02	1.31 ±0.05**	3.12 ± 0.03	2.65 ± 0.21	1.27 ± 0.05
	100	0.81 ± 0.08**	0.30 ± 0.02	1.43 ± 0.18**	3.08 ± 0.02	0.91 ± 0.31	1.34 ± 0.21
	150	0.72 ± 0.09**	0.22 ± 0.03	1.50 ± 0.09**	2.76 ± 0.14	0.74 ± 0.11	0.89 ± 0.16
	200	0.68 ± 0.08**	0.24 ± 0.08	1.82 ± 0.17**	2.25 ± 0.16	0.46 ± 0.16	0.80 ± 0.20
0.6	0	1.03 ± 0.01*	0.42 ± 0.01	1.83 ± 0.02*	3.15 ± 0.01	1.65 ± 0.23	1.75 ± 0.03*
	50	1.00 ± 0.01	0.33 ± 0.02	1.26 ± 0.07	3.12 ± 0.01	0.84 ± 0.16	1.20 ± 0.11
	100	0.51 ± 0.20	0.32 ± 0.03	0.91 ± 0.27	2.35 ± 0.35	1.06 ± 0.36	2.81 ± 0.28
	150	0.47 ± 0.19	0.27 ± 0.06	0.78 ± 0.28	2.22 ± 0.42	0.99 ± 0.22	0.75 ± 0.30
	200	0.43 ± 0.18	0.21 ±0.06	0.61 ± 0.27	1.84 ± 0.59	0.54 ± 0.21	0.59 ± 0.28

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control

**Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control

TABLE 10: Mineral concentration of okra (Genotype LD 88) under the influence of salinity and salicylic acid

Bioregulator SA (mM)	NaCl (mM)	Calcium (milligram/g)	Magnesium (milligram/g)	Potassium (milligram/g)	Sodium (milligram/g)	Zinc (milligram/g)	Phosphorous (milligram/g)
10^{-2}	0	0.90 ± 0.02	0.80 ± 0.02	1.72 ± 0.07	2.61 ± 0.13	1.86 ± 0.03	1.59 ± 0.07
	50	0.82 ± 0.01	0.28 ± 0.01	1.67 ± 0.02**	2.53 ± 0.14	2.33 ± 0.07**	1.46 ± 0.14
	100	0.81 ± 0.02	0.28 ± 0.07	1.35 ± 0.04**	2.31 ± 0.02	2.17 ± 0.07**	1.37 ± 0.07
	150	0.73 ± 0.07	0.26 ± 0.02	1.31 ± 0.05**	2.30 ± 0.01	2.10 ± 0.02**	1.31 ± 0.02
	200	0.54 ± 0.14	0.20 ± 0.07	1.10 ± 0.07**	2.17 ± 0.14	2.00 ± 0.05**	1.12 ± 0.04
10^{-4}	0	1.07 ± 0.03*	0.37 ± 0.02	1.63 ± 0.05	2.64 ± 0.10	1.33 ± 0.04	1.62 ± 0.04
	50	2.00± 0.02	0.59 ± 0.01	2.43 ± 0.07	2.54 ± 0.11	3.60 ± 0.07	1.41 ± 0.07
	100	0.98 ± 0.02	0.55 ± 0.02	1.37 ± 0.02	2.93 ± 0.07	2.35 ± 0.01	1.33 ± 0.07
	150	0.70 ± 0.01	0.47 ± 0.01	1.30 ± 0.07	2.97 ± 0.04	2.60 ± 0.13	1.25 ± 0.07
	200	0.68 ± 0.07	0.47 ± 0.02	1.00 ± 0.02	2.05 ± 0.04	2.60 ± 0.11	1.20 ± 0.03
10^{-6}	0	0.50 ± 0.07	0.35 ± 0.01	1.61 ± 0.07	2.23 ± 0.19	2.39 ± 0.51	1.59 ± 0.07
	50	0.30 ± 0.07	0.30 ± 0.07	1.40 ± 0.03	2.20 ± 0.17	2.32 ± 0.07	1.40 ± 0.02
	100	0.28 ± 0.02	0.26 ± 0.02	1.31 ± 0.07	1.99 ± 0.02**	1.31 ± 0.02	1.38 ± 0.07
	150	0.25 ± 0.02	0.23 ± 0.07	1.27 ± 0.02	1.75 ± 0.07**	1.30 ± 0.02	0.27 ± 0.03
	200	0.17 ± 0.03	0.21 ± 0.02	0.24 ± 0.07	1.63 ± 0.02**	1.00 ± 0.07	0.19 ± 0.04

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control

**Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control

TABLE 11: Mineral concentration of okra (Genotype LD 88) under the influence of salinity and indoleacetic acid

Bioregulator IAA (mM)	NaCl (mM)	Calcium (milligram/g)	Magnesium (milligram/g)	Potassium (milligram/g)	Sodium (milligram/g)	Zinc (milligram /g)	Phosphorous (milligram/g)
0.4	0	0.98 ± 0.03	0.40 ± 0.01	1.38 ± 0.08	2.89 ± 0.17	1.52 ± 0.09	1.36 ± 0.08
	50	0.92 ± 0.01	0.39 ± 0.01	1.45 ± 0.01	3.24 ± 0.12	1.55 ± 0.01	1.34 ± 0.09
	100	0.87 ± 0.14	0.38 ± 0.02	1.47 ± 0.02	3.26 ± 0.01	1.55 ± 0.01	2.32 ± 0.07
	150	0.83 ± 0.11	0.30 ± 0.02	2.50 ± 0.07	3.71 ± 0.03	1.58 ± 0.02	1.21 ± 0.02
	200	0.75 ± 0.02	0.29 ± 0.07	1.65 ± 0.02	3.83 ± 0.01	2.00 ± 0.01	1.17 ± 0.01
0.5	0	0.93 ± 0.02	0.34 ± 0.01	1.38 ± 0.09	2.88 ± 0.12	1.09 ± 0.09	1.31 ± 0.09
	50	0.94 ± 0.01	0.36 ± 0.02	1.27 ± 0.01	2.87 ± 0.02**	1.07 ± 0.02	1.31 ± 0.07
	100	0.89 ± 0.02	0.30 ± 0.03	1.25 ± 0.01	2.88 ± 0.02**	1.03 ± 0.02	1.30 ± 0.07
	150	0.87 ± 0.07	0.31 ± 0.04	1.30 ± 0.07	2.86 ± 0.07**	1.08 ± 0.02	1.29 ± 0.06
	200	0.70 ± 0.02	0.30 ± 0.02	1.20 ± 0.02	2.41 ± 0.09**	1.04 ± 0.02	1.30 ± 0.03
0.6	0	0.91 ± 0.04	0.38 ± 0.01	1.30 ± 0.09	2.85 ± 0.15	1.06 ± 0.03	1.27 ± 0.09
	50	0.70 ± 0.07	0.32 ± 0.01	1.27 ± 0.01	2.84 ± 0.01**	1.01 ± 0.03	1.26 ± 0.02
	100	0.59 ± 0.07	0.33 ± 0.01	1.27 ± 0.02	2.65 ± 0.02**	1.00 ± 0.04	1.19 ± 0.01
	150	0.46 ± 0.07	0.28 ± 0.07	1.25 ± 0.02	2.43 ± 0.01**	0.97 ± 0.01	1.10 ± 0.01
	200	0.31 ± 0.07	0.23 ± 0.01	0.19 ± 0.02	2.41 ± 0.02**	0.72 ± 0.02	0.99 ± 0.02

Data: Means ± SEM for the 3 replicate

**Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control

4.3 Experiment 3: Accumulation of the compatible soluble in the leaves and fruits of two okra plants genotypes under the influence of salicylic acid of indoleacetic acid and salinity

Introduction

Free amino acid (proline) is generated in the salt-stressed plant. Proline is an active metabolite that increases osmotic pressure in plant tissues, which helps to reduce the adverse effects of salt stress in plants (Parviz and Satyawat, 2008). There is dearth of information on how salt tolerance in plant can be improved. However, some known methods such as transgenic techniques and application of bioregulators have gained tremendous attention in ameliorating the adverse effects of salinity on plants (Senaratna *et al*., 2000; Baninasab and Ghobadi, 2011). Studies have shown the positive effects of bioregulator application on seed germination, ion uptake and transport, flowering and fruit yield, transpiration and photosynthesis (Klessig and Malamy, 1994; Khan *et al*., 2003).

Salicylic acid regulates plant growth, stimulate the development of adventitious root and resistance to the environmental factors (Hussein *et al*., 2007; Noreen *et al*., 2009). Plants change their metabolic pathways to confer resistance to both biotic and abiotic factors (Rathinasabapathi, 2000). There are various organic molecules (such as soluble sugar, soluble protein and proline) in plant tissues that act as osmoprotectants which increase plant tissues osmotic pressure (Hasegawa *et al*., 2000). Salt stress tolerance in plants is indicated by an increase level of proline concentration (Kaviani, 2008). The aim of this experiment was to examine the effects of bioregulators and salinity stress in the salt stressed okra plant.

Procedures

The procedures for determining compatible solutes were outlined in the sections 3.4.5.1 – 3.4.5.3.

Results

In Table 12 increase in salt stress levels resulted to high level of leaves and fruits proline concentrations in okra plant genotypes as compared with the normal groups. Reduction in the concentration of salicylic acid affected the accumulation of soluble protein in the leaves, but this has no effect on soluble protein accumulation in the fruits (Figure 25). The group treated with 10^{-4} mM SA concentration revealed the highest level of soluble protein accumulation

relatives to the control group in 47-4 genotype. In Figure 26 accumulation of soluble protein in the two organs of okra plant 47-4 genotype was improved under the influence of salicylic acid and salinity. In Figure 27, the groups subjected to 10^{-2} and 10^{-6} mM SA concentrations showed high levels of sugar accumulation in the fruits, and greater accumulation of sugar was observed in the fruits of 47-4 genotype group treated with 10^{-6} mM SA concentration relatives to the control. In the genotype 47-4 the fruits of the group subjected to severe level of salt stress with 10^{-2} and 10^{-4} mM SA concentrations showed an increase sugar accumulation (Figure 28). In the genotype LD88 decrease in the concentration of salicylic acid decreased protein accumulation in the leaves, but the group subjected to 10^{-4} mM SA concentration revealed an improve in leaves protein accumulation relatives to the control (Figure 29).

The leaves and fruits of groups subjected to combine effects of salicylic acid and salinity showed pronounce alteration in the accumulation of protein. However, the leaves and fruits of groups subjected to 10^{-4} mM of SA at 50 mM NaCl and 10^{-2} mM of SA at 150 mM NaCl respectively showed better protein accumulation in the genotype LD 88 relatives to the control (Figure 30). In Figure 31, reduction in the sugar accumulation was observed in the leaves of genotype LD 88 with decreased salicylic acid concentration. The pronounced reduction in sugar accumulation was observed in the fruits of group subjected to 10^{-4} mM SA relatives to the control. Figure 32 salinity stress at severe level reduced sugar accumulation in the leaves of the okra genotype LD 88. But the fruits of the group subjected to 10^{-6} mM of SA at 50 mM NaCl showed an improve accumulation of sugar.

Figures 33 and 34 showed the effects of bioregulators on protein content. The results revealed that 0.4 mM IAA treated leaves showed better accumulation of protein in the genotype 47-4 relatives to the control (Figure 33). But in the genotype LD 88 an increase level of protein accumulation in fruits was observed in the group treated with 0.5 mM IAA relative to the control group (Figure 34). Figure 35 revealed an increase in the fruits sugar accumulation in the group treated with 0.4 mM IAA at 100 mM NaCl. However, increased level of IAA in genotype 47-4 resulted to a reduction in soluble sugar accumulation in the fruits, but no effect was observed in the leaves relative to the control group. Similarly, in genotype LD 88, increased indole acetic acid concentration resulted to the reduction in the sugar accumulation in both fruits and leaves of the genotype LD 88, but slight sugar accumulation was observed

in the group treated with 0.4 mM IAA at all levels of salinity relatives to the control (Figure 36).

Figures 37 and 38 showed the interactive effects of the bioregulator (IAA) and salinity on the protein content. The interaction in genotype 47-4 showed an increase protein content in the fruits and leaves especially in the groups subjected to indole acetic acid at high and mild salinity levels relative to the control groups (Figure 37). While in genotype LD 88, increased protein accumulation was observed in the fruits of the groups subjected to 0.4 mM IAA at 100 and 150 mM NaCl respectively, but little or no effect was recorded in the leaves of the genotype LD 88 (Figure 38). Among the two studied organs of salinity stressed okra plant in the two genotypes, genotype 47-4 showed more protein accumulation than genotype LD88. Figures 39 and 40 showed reduction of sugar accumulation under the combined influence of bioregulator and salinity on okra plant. In genotype 47-4, an increase sugar accumulation was observed in the fruits under the influence of 0.4 mM and 0.5 mM IAA at severe salt stress relative to the control group. In the genotype LD 88, groups treated with 0.4 mM IAA showed an increase accumulation of sugar in the fruits at all levels of salinity stress (Figure 40).

Conclusion

Fruits and leaves of both genotypes (47-4 and LD 88) showed high accumulation of organic solutes in salinity stress, but higher accumulation of these organic solutes were more in the fruits than in the leaves. The accumulation of sugar and protein in the fruits of both genotypes could be attributed to the fact that fruit serves as storage organ where the metabolic activities occur. Thus, 0.4 and 0.5 mM of indole acetic acid and 10^{-2} and 10^{-4} mM of salicylic acid serve as promising concentrations for alleviating salt tolerance in okra plant.

TABLE 12: Level of proline (mg/g) in the leaves and fruits of okra genotypes under the influence of salinity

NaCl (mM)	47-4		LD88	
	Leaf	**Fruit**	**Leaf**	**Fruit**
0	1.12±0.02	1.08±0.01	2.00±0.02	1.15±0.01
50	2.40±1.00	1.97±0.03	2.35±0.02	2.00±0.02
100	3.50±0.03	2.85±0.04	3.65±0.01	2.86±0.02
150	4.13±1.02*	4.10±0.01*	4.14±0.05*	4.37±0.01*
200	5.21±0.01*	4.79±0.03*	4.99±0.02*	5.11±0.02*

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control

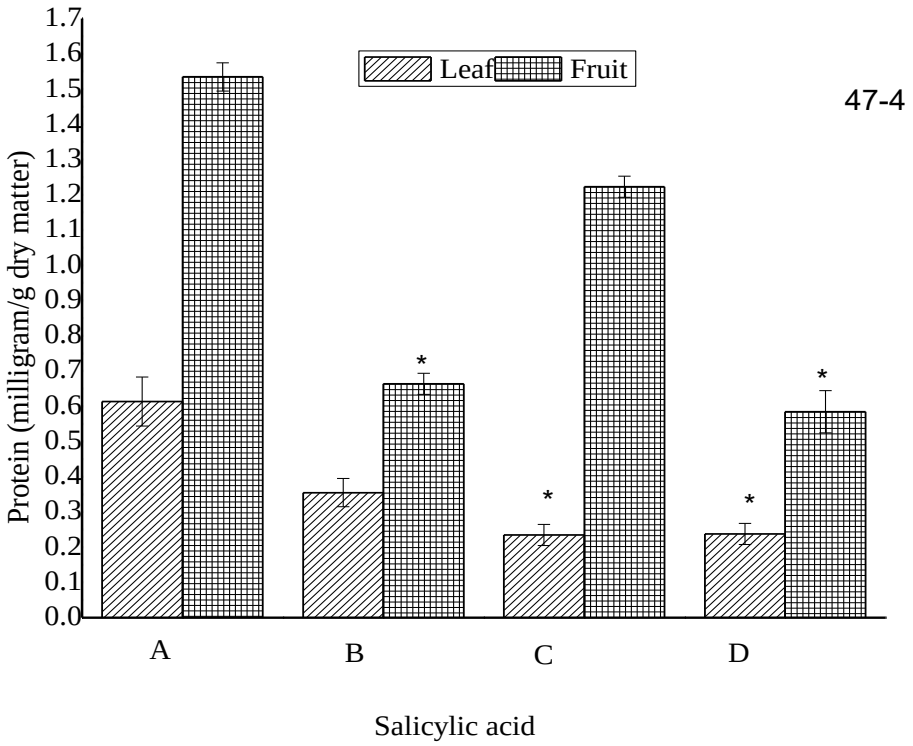


Fig. 25: Proteins accumulation in the leaves and fruits of okra genotype 47-4 under the influence of salicylic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control.

Where A, B, C and D equal to (0, 10^{-2}, 10^{-4} and 10^{-6}) mM respectively

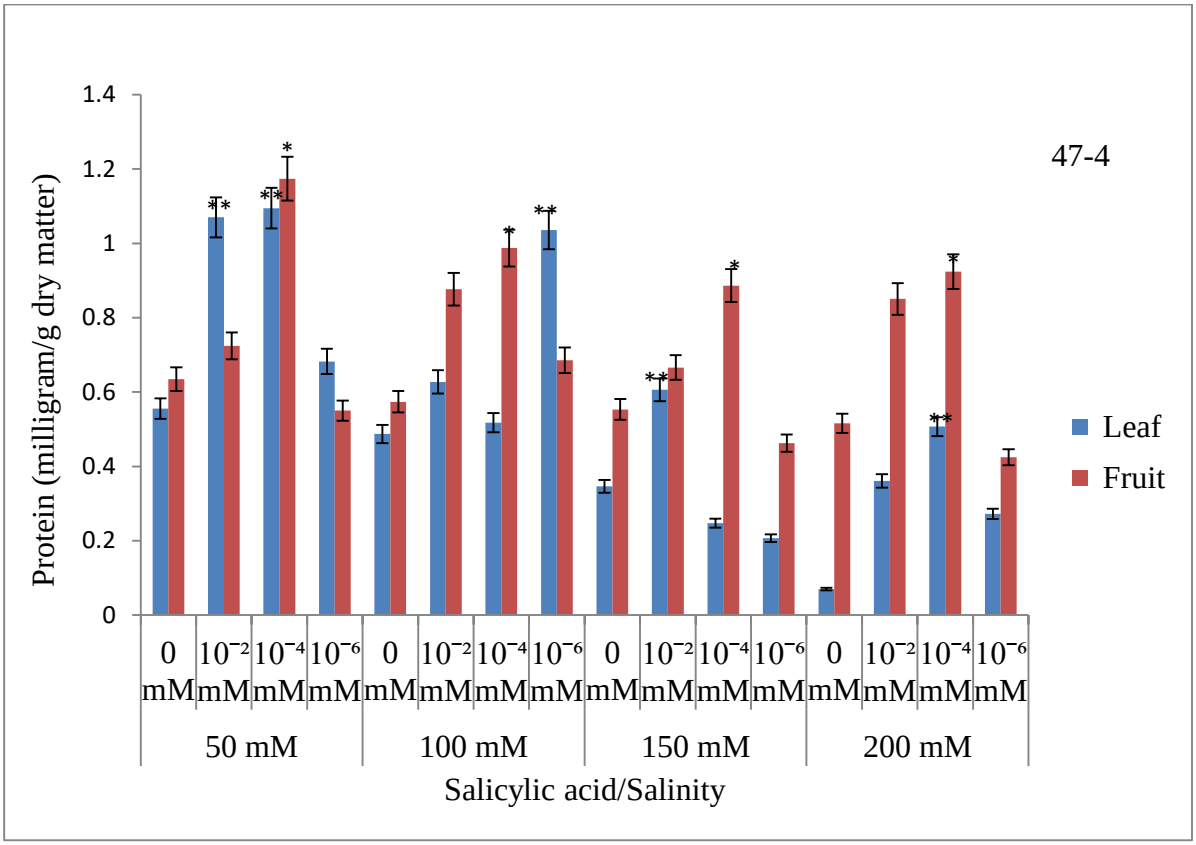


Fig. 26: Proteins accumulation in the leaves and fruits of okra genotype 47-4 under the salinity and salicylic acid influence.

Data: Means ± SEM for the 3 replicate

**Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control leaf

*Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control fruit.

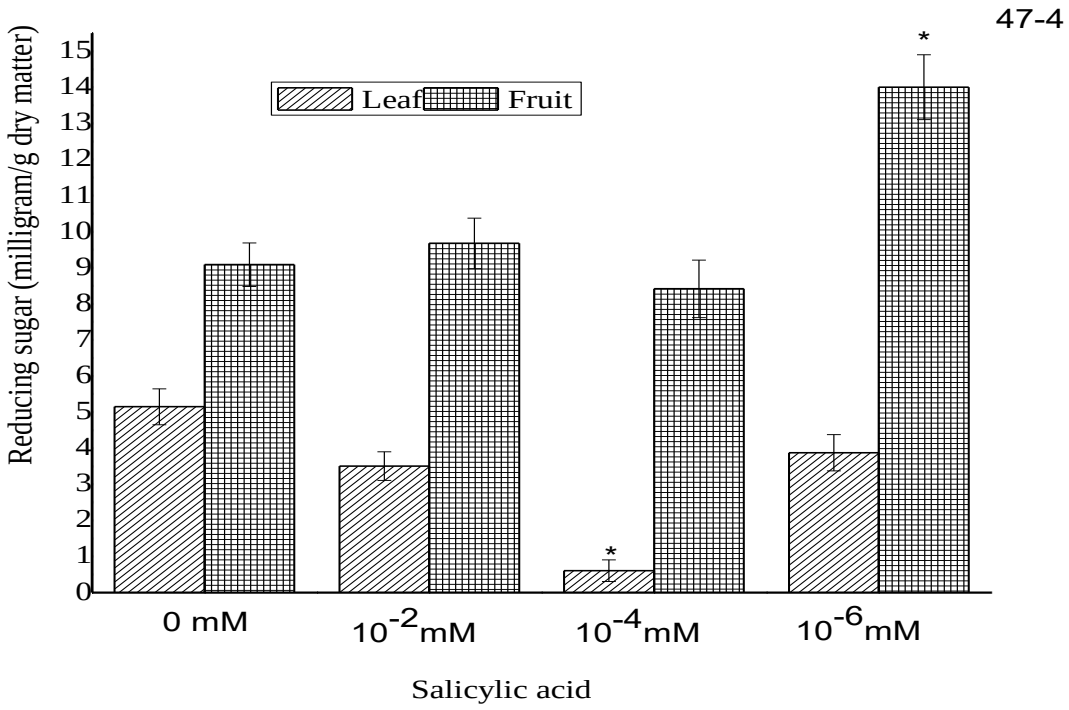


Fig. 27: Accumulation of sugar in the leaves and fruits of okra (genotype 47-4) under the salicylic acid influence

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control

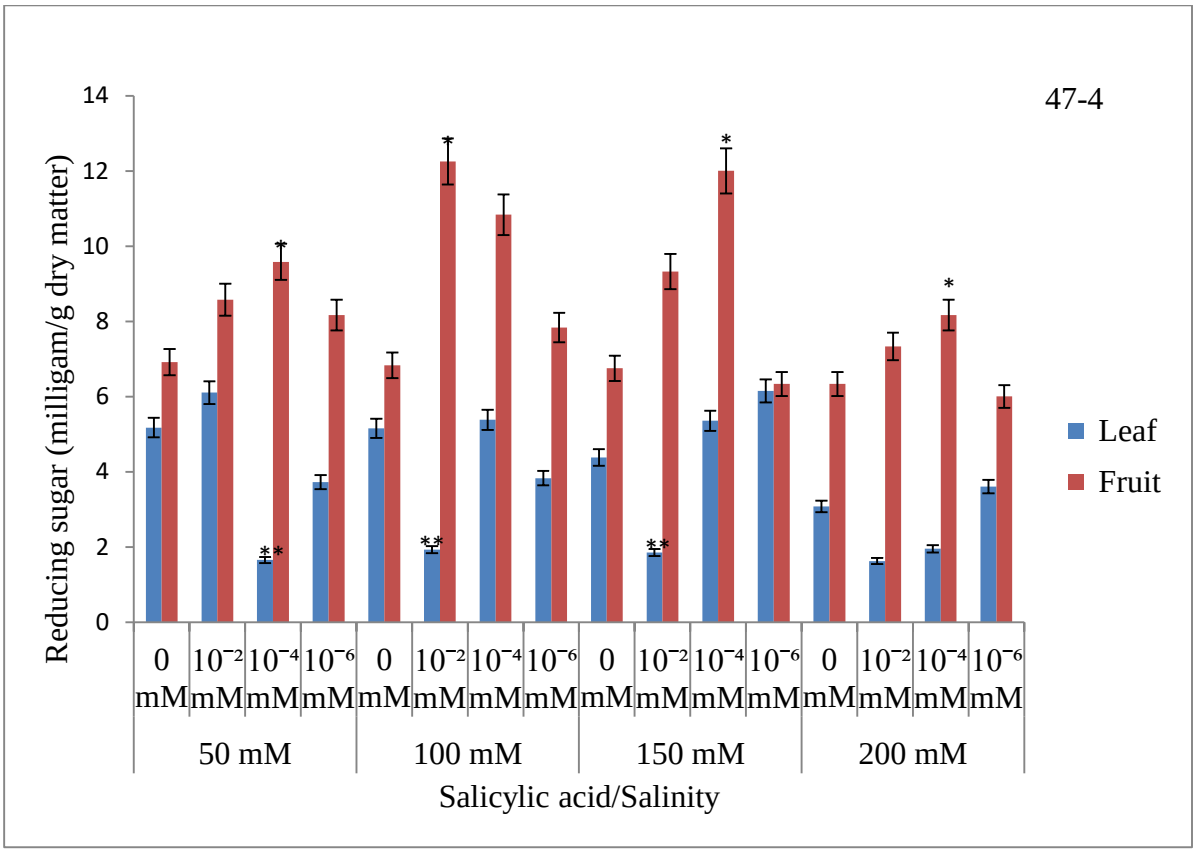


Fig. 28: Accumulation of sugar in the leaves and fruits of okra (genotype 47-4) under the salinity and salicylic acid influence

Data: Means ± SEM for the 3 replicate

**Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control leaf

*Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control fruit.

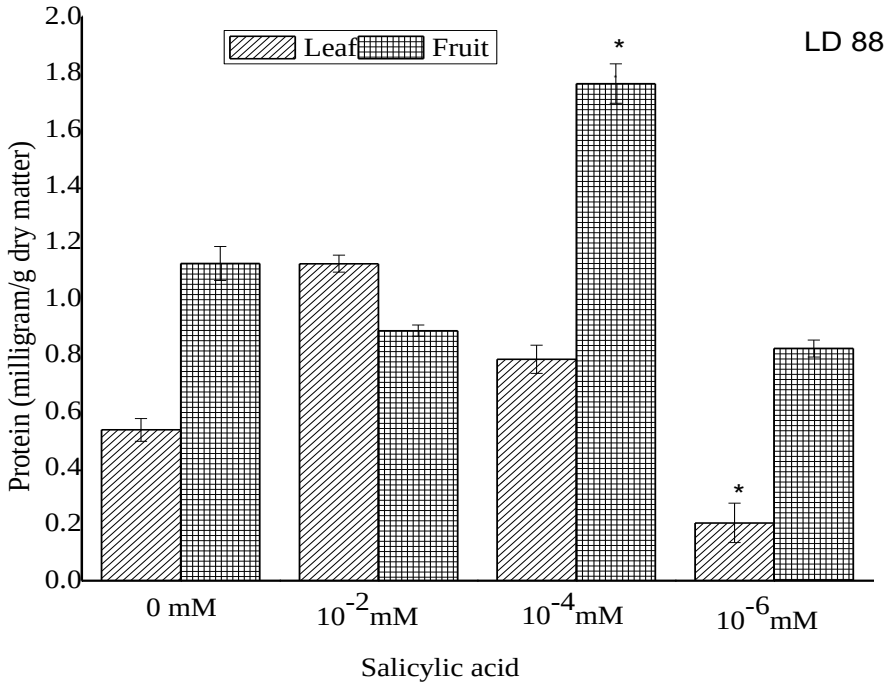


Fig. 29: Proteins accumulation in the leaves and fruits of okra genotype LD 88 under the influence of salicylic acid.

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control group.

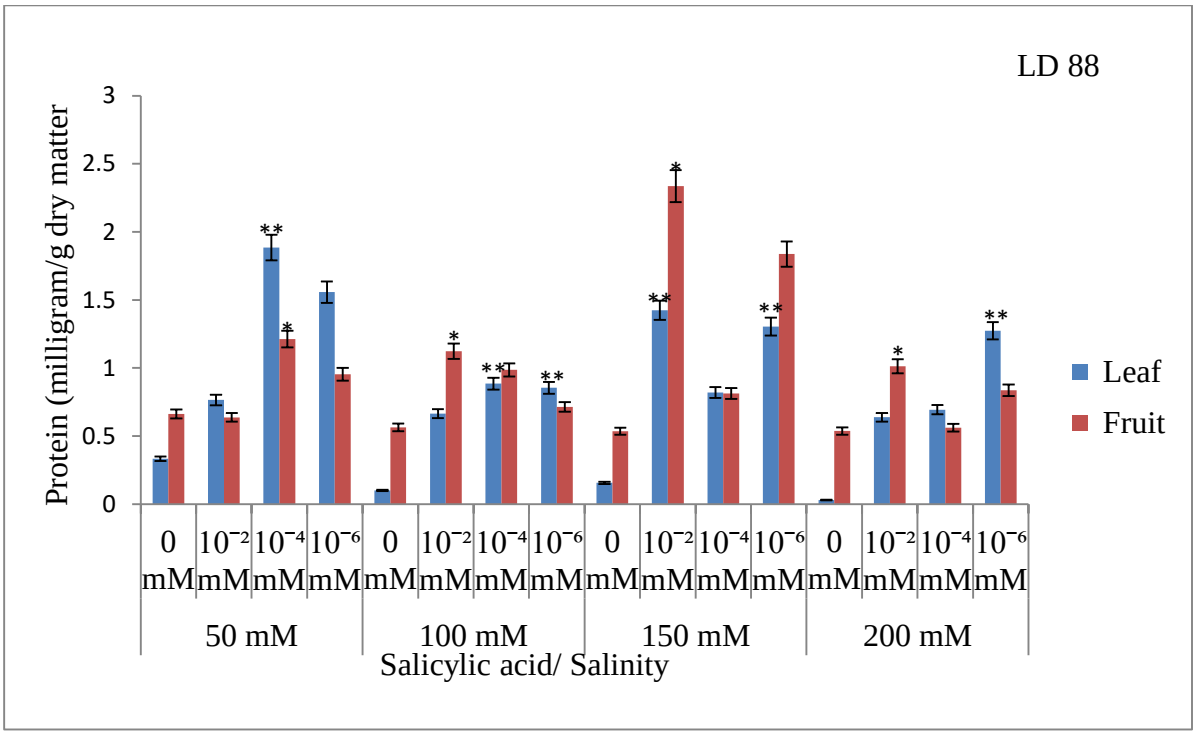


Fig. 30: Proteins accumulation in the leaves and fruits of okra genotype LD 88 under the salinity and salicylic acid influence.

Data: Means ± SEM for the 3 replicate

**Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control leaf

*Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control fruit.

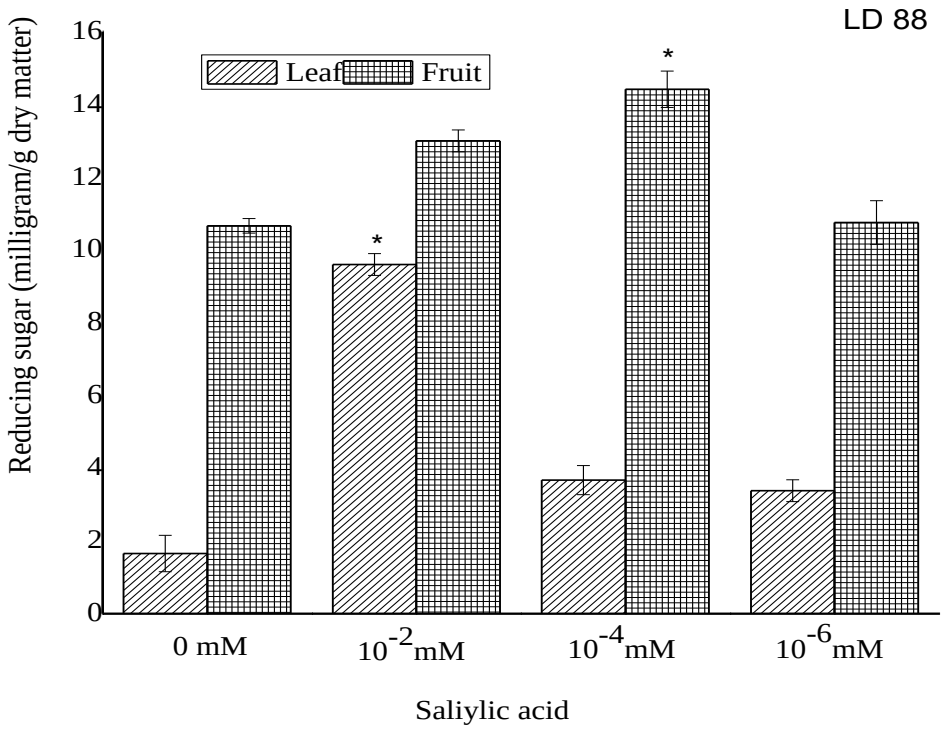


Fig. 31: Accumulation of sugar in the leaves and fruits of okra (genotype LD 88) under the influence of salicylic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control group.

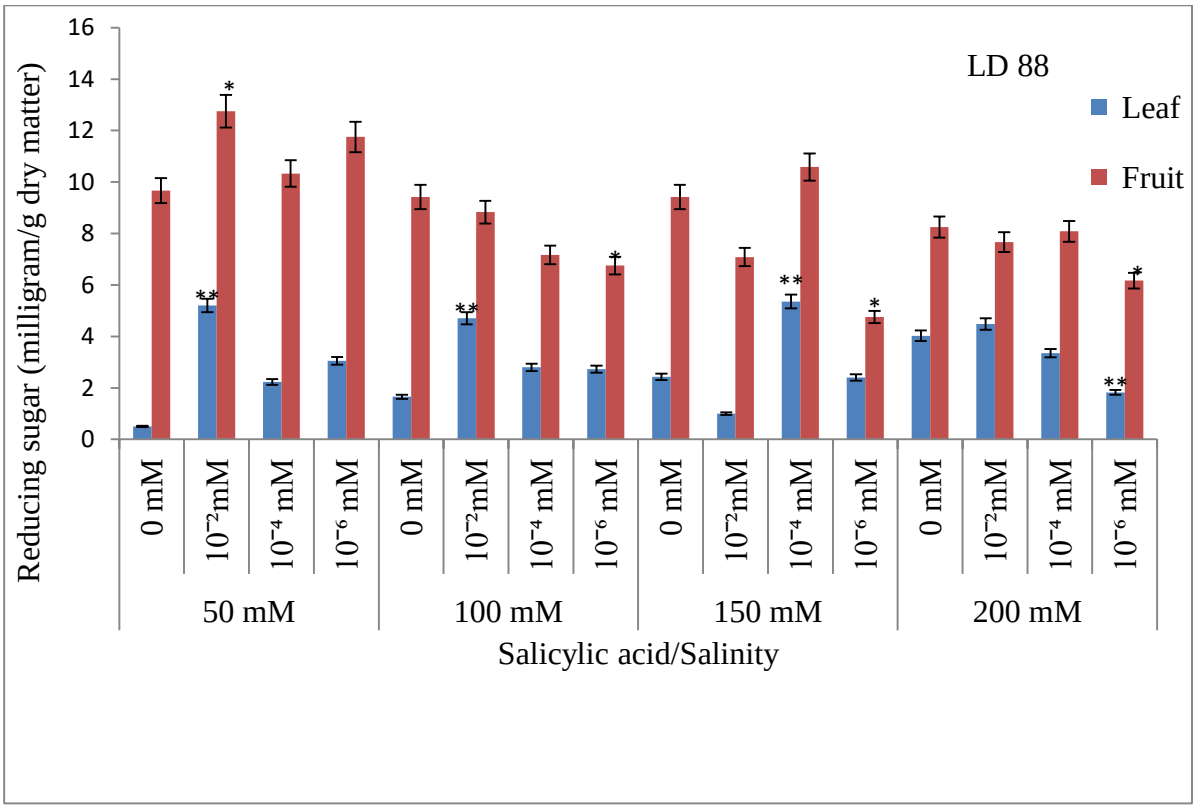


Fig. 32: Accumulation of sugar in the leaves and fruits of okra (genotype LD 88) under the influence of salinity and salicylic acid

Data: Means ± SEM for the 3 replicate

**Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control leaf

*Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control fruit.

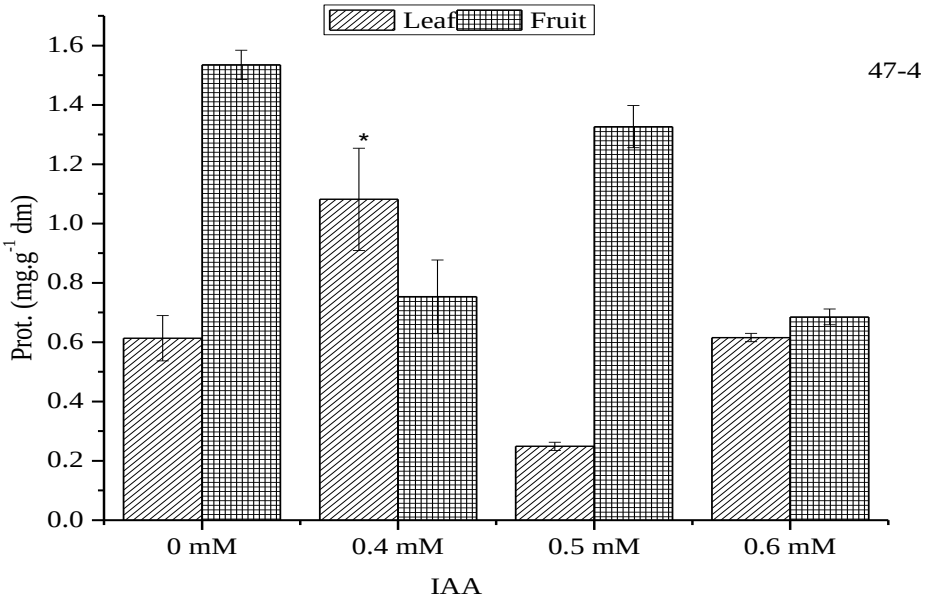


Fig. 33: Proteins accumulation in the leaves and fruits of okra genotype 47-4 under the influence of indole acetic acid influence.

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control group.

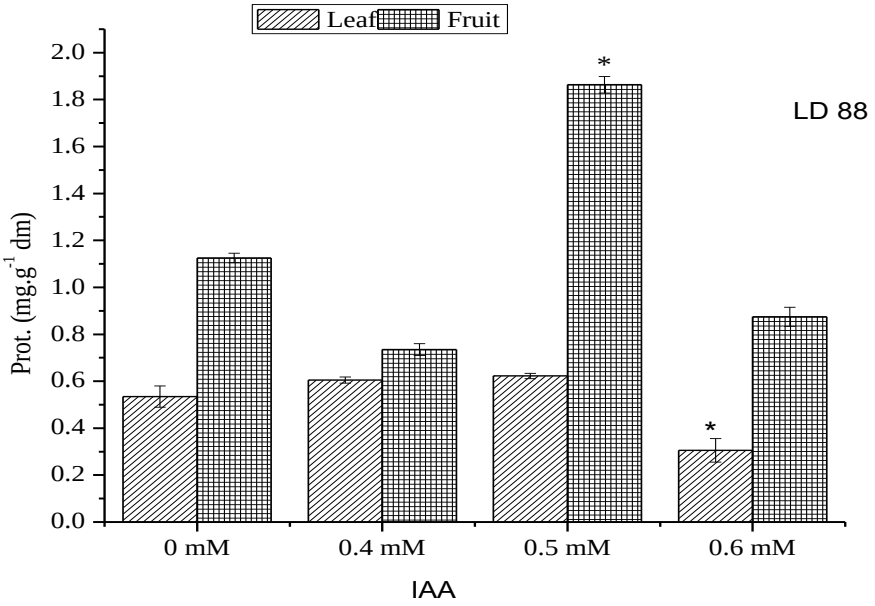


Fig. 34: Proteins accumulation in the leaves and fruits of okra genotype LD 88 under the influence of indoleacetic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control group.

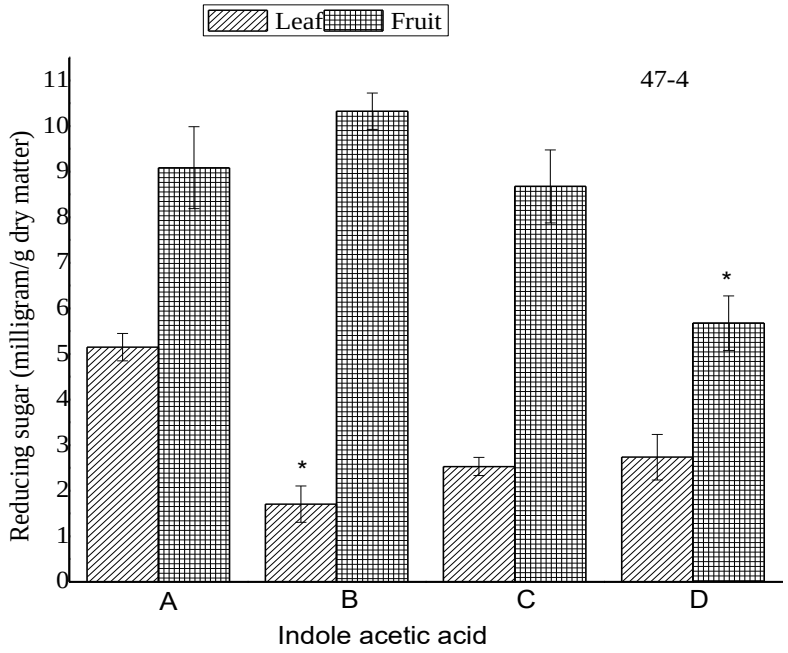


Fig. 35: Accumulation of sugar in the leaves and fruits of okra (genotype 47-4) under the influence of indole acetic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control group.

Where A, B, C and D = (0, 0.4, 0.5 and 0.6) mM respectively

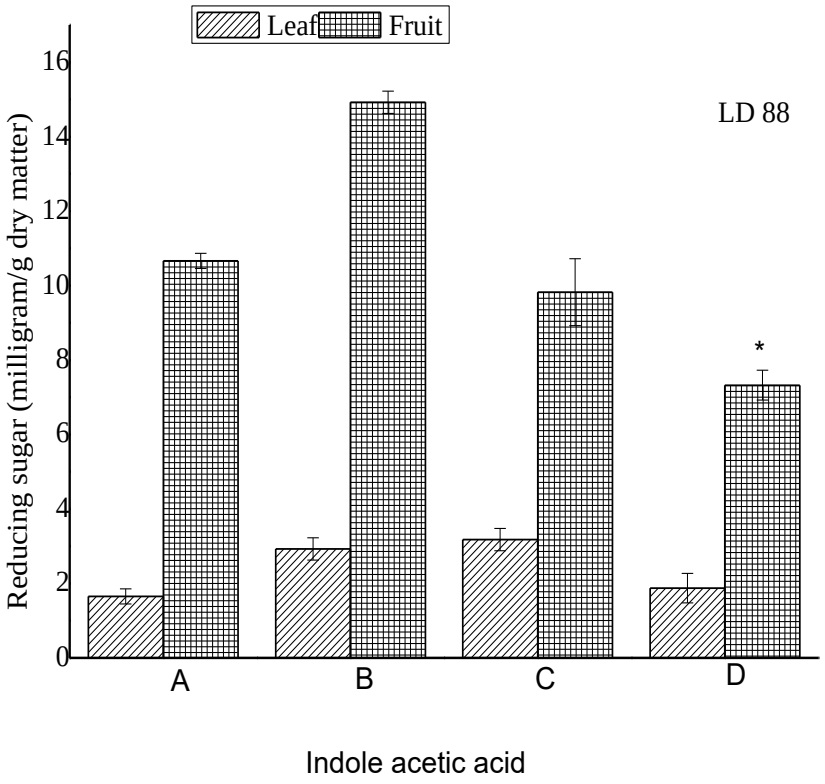


Fig. 36: Accumulation of sugar in the leaves and fruits of okra (genotype LD 88) under the influence of indole acetic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control group

Where A, B, C and D = (0, 0.4, 0.5 and 0.6) mM respectively

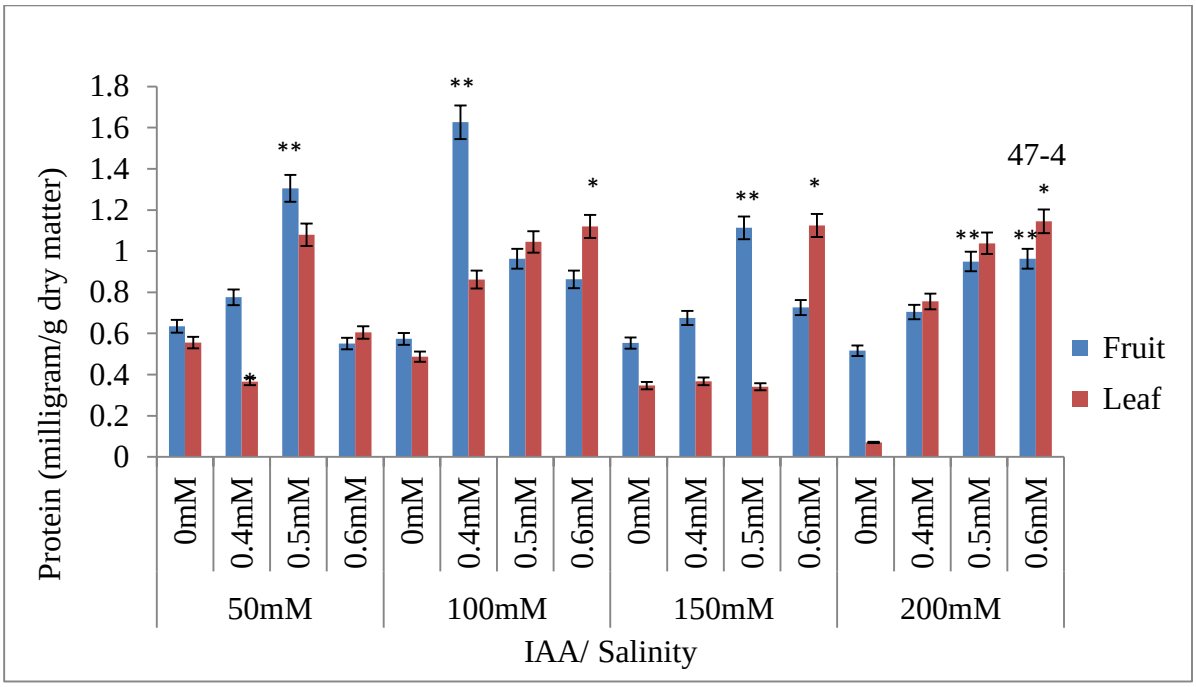


Fig. 37: Proteins accumulation in the leaves and fruits of okra genotype 47-4 under the salinity and indole acetic acid influence

Data: Means ± SEM for the 3 replicate

**Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control fruit

* Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control leaf

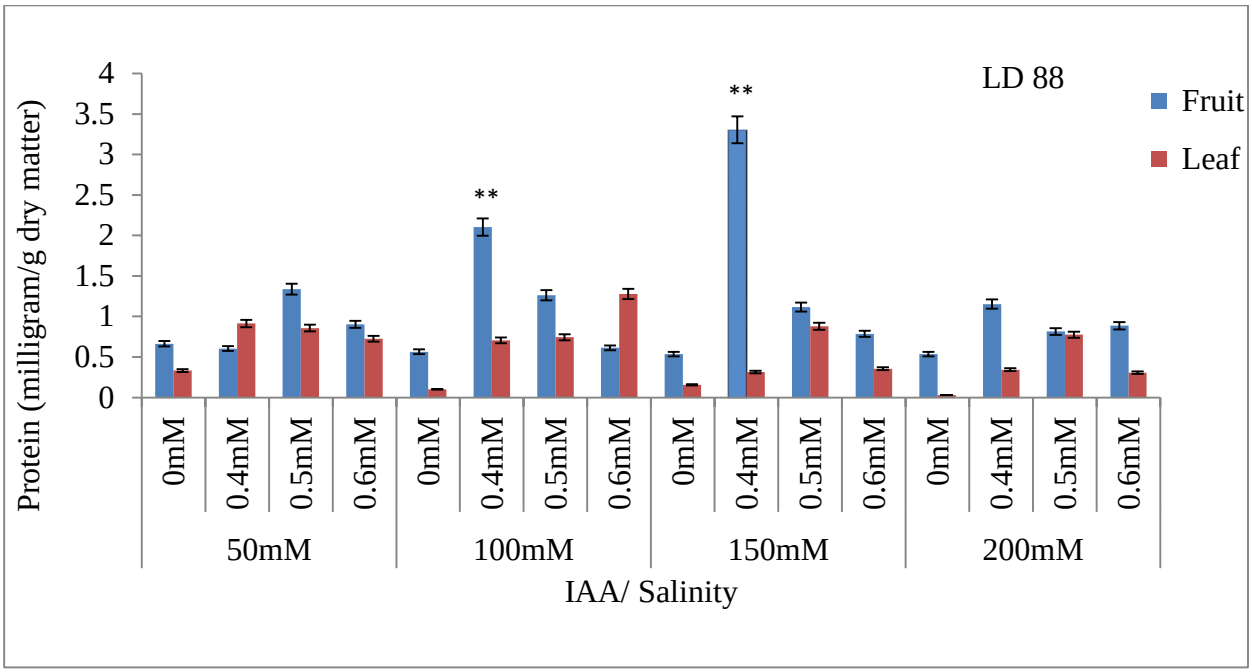


Fig. 38: Proteins accumulation in the leaves and fruits of okra genotype LD 88 under the salinity and indole acetic acid influence

Data: Means ± SEM for the 3 replicate

**Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control fruit

* Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control leaf

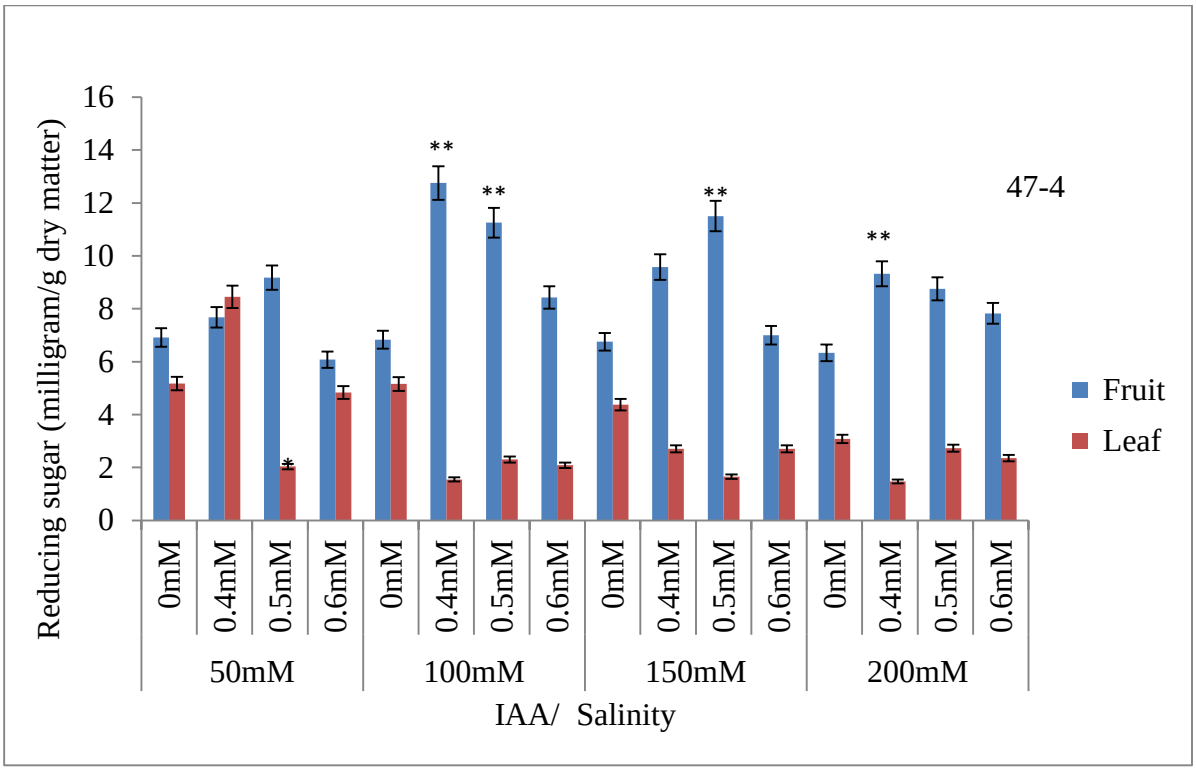


Fig. 39: Accumulation of sugar in the leaves and fruits of okra (genotype 47-4) under the influence of salinity and indole acetic acid

Data: Means ± SEM for the 3 replicate

**Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control fruit

* Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control leaf

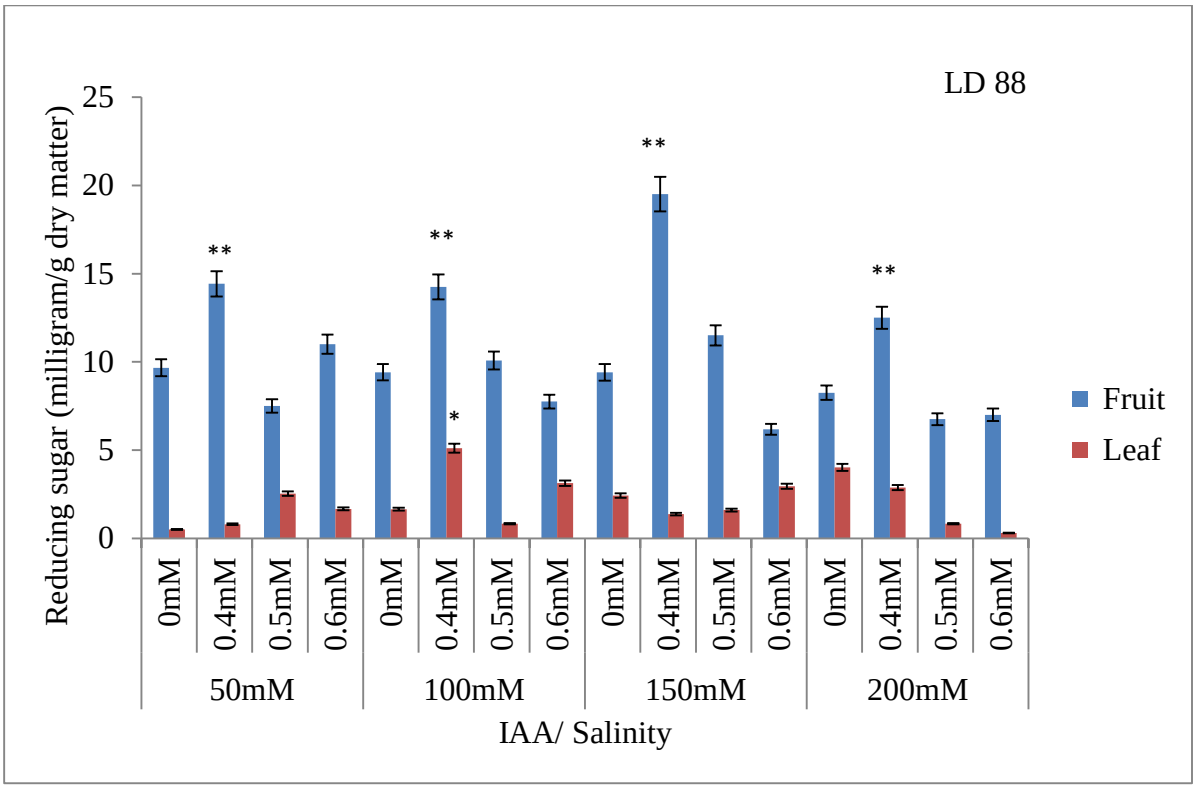


Fig. 40: Accumulation of sugar in the leaves and fruits of okra (genotype LD 88) under the influence of salinity and indole acetic acid

Data: Means ± SEM for the 3 replicate

**Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control fruit

* Significant at $\alpha_{0.05}$ relatives to the NaCl-treated control leaf

4.4 Experiment 4: Influence of indole acetic acid, salicylic acid and salinity on the antioxidant enzymes and phytochemicals constituents on okra fruits genotypes

Introduction

Okra is a vegetable crop that is widely distributed across Africa (Khomsug *et al*., 2010; Shivhare *et al*., 2010). Studies have shown the antioxidant properties of okra (Adelakun *et al*., 2009; Reddy *et al*., 2010). Maritim *et al*. (2003) defined antioxidant as natural chemical substances that scavenge free radical. Many plant phytochemicals are consumed as food, which brings interconnection between nutrition and health (Pieroni and Price, 2006). The constituents of most vegetables serve as basis for ethnomedicine research (El and Karakaya, 2004). In recent time, attention is shifting to phytotherapy as it has no side effect, and it is used to control blood glucose level with its associated complications (Gallagher *et al*., 2003). Antioxidant properties of medicinal plants are responsible for their therapeutic effect (Zhang *et al*., 2001).

Generally, okra plant growth and yield are highly inhibited under salinity stress (Ashraf and Harris 2004). Moreover, as a result of increase in population as well as high demand for food, the need to improve crop production under salt stress is essential (Szabolcs 1994). Bioregulators act as a mediator to suppress plants stress response (Szalai *et al*., 2010). Salicylic acid reduces the negative effects of stress in plant tissues (Bideshki and Arvin, 2010). Salicylic acid aids plants development (Khodary, 2004), also regulates the uptake and the transport of ion and enzyme activities (Khan *et al*., 2010). Under stress conditions indole acetic acid performs a similar function with the abscisic acid (ABA) (Ribaut and Pilet, 1991). Ibrahim *et al*., (2014) reported declined in wheat seed germination under high salinity level, however, these negative effects were reversed under indole acetic acid influence. Kaya *et al*. (2009) reported an increase mineral levels with aerial application of indole acetic acid. Therefore, this study examined bioregulators' effects on the potential of antioxidant enzymes in okra genotypes (47-4 and LD 88) under salinity stress.

Procedure

The catalase, superoxide, and glutathione peroxidase activities were determined as outlined in the sections 3.4.7.1 – 3.4.7.3. DPPH scavenging activity was estimated as described in section 3.4.12.

Total phenol, total flavonoids, crude fibre, and ascorbic acid were determined as outlined in sections 3.4.8 – 3.4.11.

Results

In Figures 41 and 42 results of both genotypes revealed an increase superoxide dismutase activity under the influence of salt stress. At 200 mM concentration of salinity stress superoxide dismutase enzyme showed peak activities in both genotypes. However, at mild salinity, there were steady increased in glutathione peroxidase and catalase activites, but declined enzyme activities were observed at severe salinity stress relative to the control groups.

Figures 43 and 44 showed the influence of IAA and SA on the antioxidant enzymes activities of okra fruit respectively. In the two genotypes, groups treated with bioregulators showed increase catalase activities relative to the control. In the group treated with 0.4 mM IAA and 10^{-4} mM SA, there were high expression of catalase activities. However, in the two treatments poor expression of glutathione and superoxide dismutase activities were observed relative to the control group.

In the genotype 47-4 interactive effect of salicylic acid and NaCl at 50 mM improved GPx activity when compared with the NaCl treated group alone (Figure 45). But at higher NaCl concentrations, the combined effect of SA and NaCl showed no effect on GPx activity. However, in the genotype LD 88 no effect of salinity stress was observed on the GPx activity, but in 10^{-4} mM SA treated groups, the GPx activities were increased (Figure 45).

In the genotype 47-4, 0.4 mM of indole acetic acid in all salt treatments increased the activities of GPx (Figure 46). However, no effect was observed on GPx activity at moderate and severe levels of salinity. But in the genotype LD 88, group treated with 0.4 mM IAA showed higher GPx activity relative to the control.

In Figure 47 at all levels of salinity stress a reduction in the activity of catalase was observed in the okra fruit genotype 47-4. A similar result was obtained in LD 88 genotype. But interaction with salicylic acid improved catalase activity relative to the salinity control group. In Figure 48, in both genotypes (LD 88 and 47-4) activities of catalase decreased under salinity stress. However, interaction of indole acetic acid increased the catalase activity, especially at 0.4 mM IAA relative to the control.

Figure 49 showed the combined influence of salicylic acid and salinity on SOD activity. As salinity levels increased, SOD activities were also increased in the genotype 47-4 and genotype LD 88. But in the genotype 47-4, interactive effect of salicylic acid improved SOD activity in the group treated with 10^{-2} mM of SA at 200 and 100 mM NaCl respectively. Whereas in the genotype LD 88, reduction of superoxide dismutase activity was observed under the combined effects of salicylic acid and salinity. In Figure 50 under the influence of both salinity and IAA, no significant superoxide dismutase activity was observed in 47-4 genotype when compared with NaCl treated group. In LD 88 genotype, the same result was obtained.

In Figure 51 the group treated with 10^{-6} mM of SA in 47-4 genotype revealed the greatest percentage inhibition of DPPH relative to the control group. However, in the LD 88 genotype, a decrease in salicylic acid concentration resulted to a decrease in the percentage inhibition of DPPH relative to the control group. whereas higher percentage inhibition was observed in groups treated with 10^{-2} mM and 10^{-6} mM of SA at all levels of salinity relative to control groups.

In Figure 52 group treated with 0.5 mM IAA at 50 mM NaCl in genotype 47-4 exhibited higher percentage inhibition relative to the control group. But in genotype LD 88, 0.4 mM IAA treated group only exhibited significant ($p < 0.05$) percentage inhibition. Also, a similar effect was observed under the combined effects of IAA and salt stress as compared to the sodium chloride control groups.

Figure 53 showed the phytonutrients composition of the okra genotypes pretreated with bioregulators. In genotype 47-4, treatment with IAA showed an increase phenolic content relative to the control group, However, only group treated with 10^{-4} mM of SA exhibited greatest phenolic content when compared with the control group. Whereas, in genotype LD 88 only the group treated with 0.4 mM of IAA showed higher levels of all the phytonutrients when compared to control group.

Conclusion

Exogenous application of bioregulators (IAA and or SA in combination with salinity stress on okra seeds enhanced the okra plant tolerance. Hence, these bioregulators can be used as promising candidates for salt tolerance in the okra plants especially at concentrations of 10^{-2} mM salicylic acid and 0.4 mM indoleacetic acid.

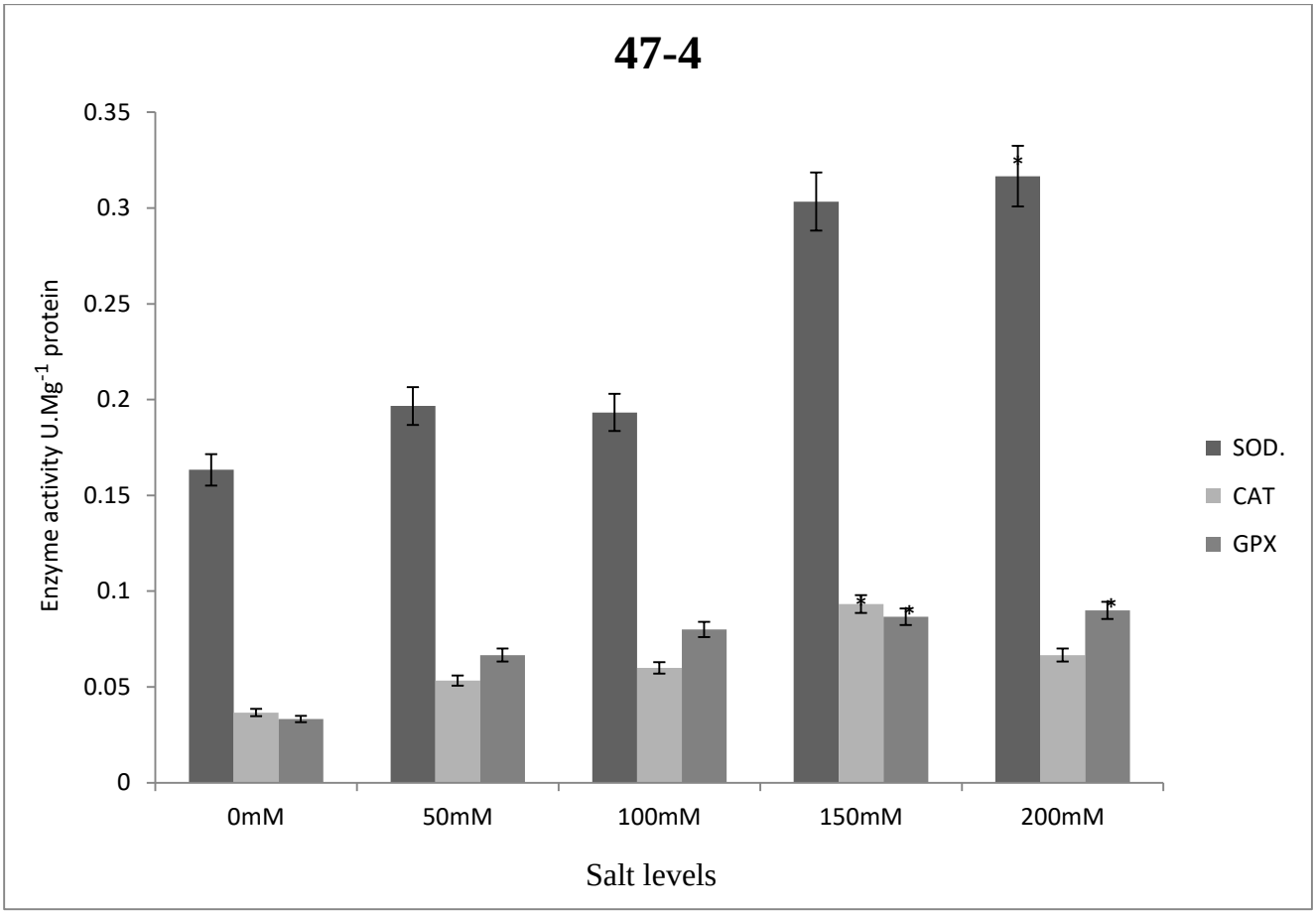


Fig. 41: Influence of salinity on antioxidant activities of okra fruit 47-4 genotype.

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control group

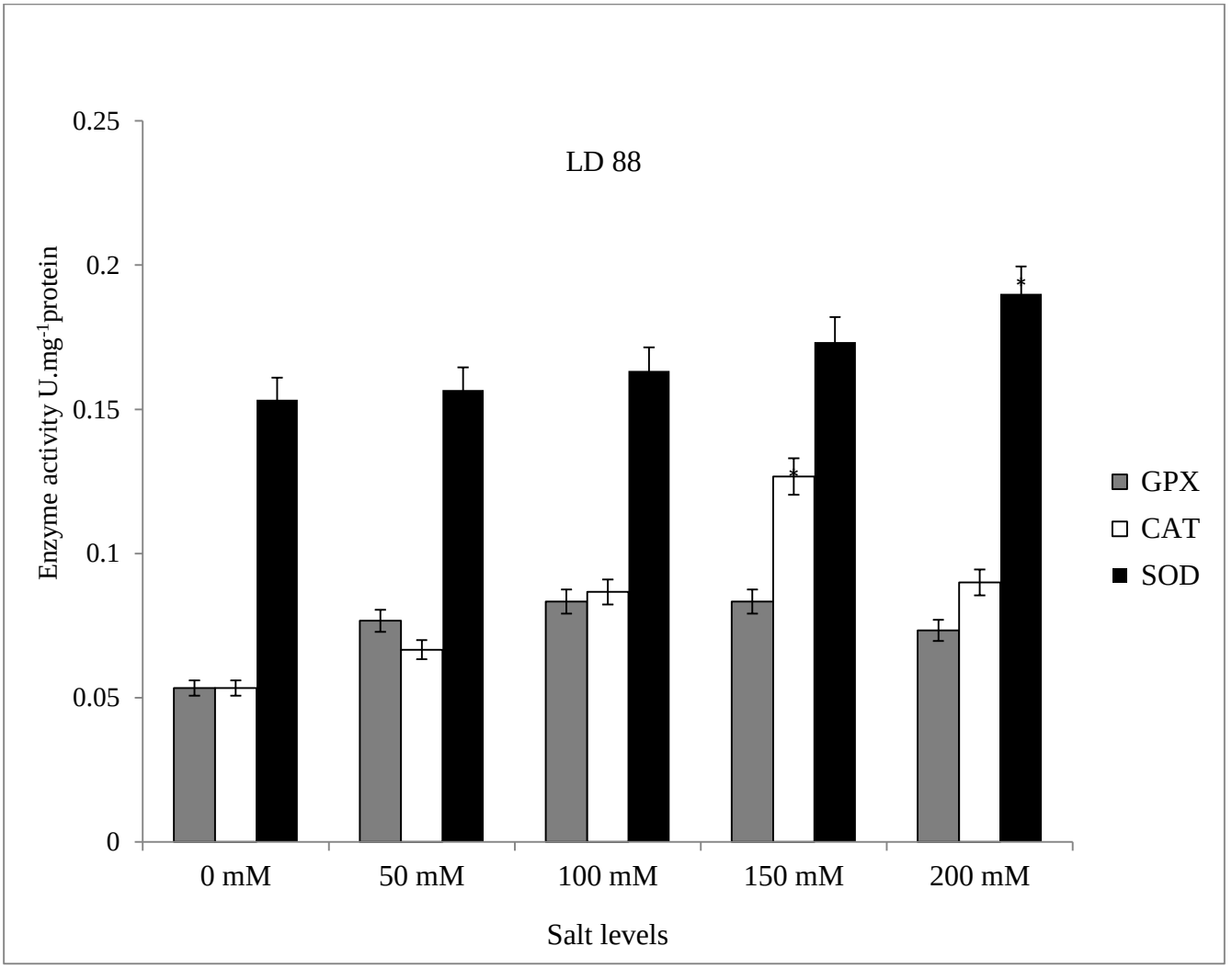


Fig. 42: Antioxidant activities of okra fruits LD 88 genotype under the influence of salinity

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control group

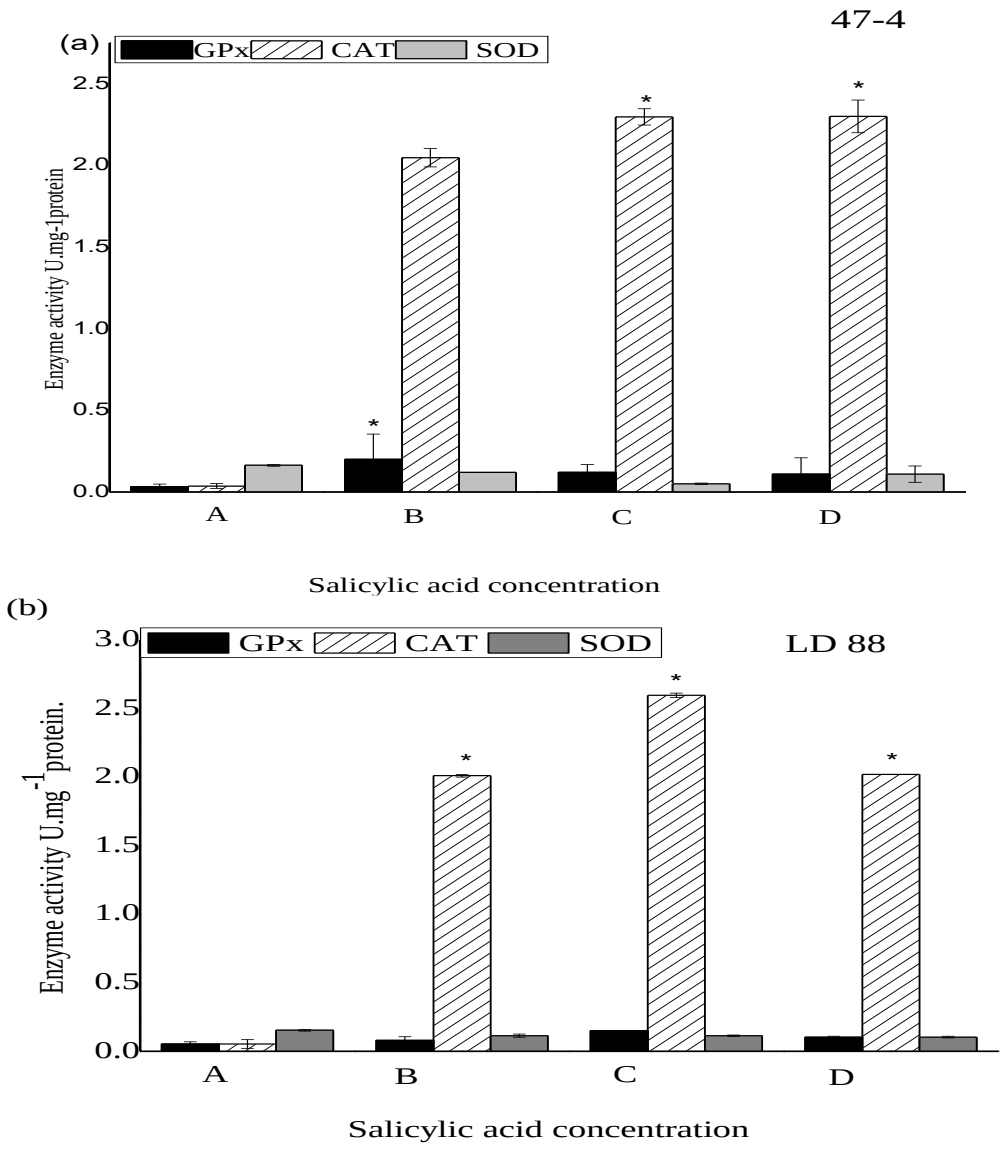


Fig. 43 (a, b): The activities of enzymes in the okra genotypes under the influence of salicylic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control group

Where A, B, C and D equal to (0,10^{-2}, 10^{-4} and 10^{-6}) mM respectively

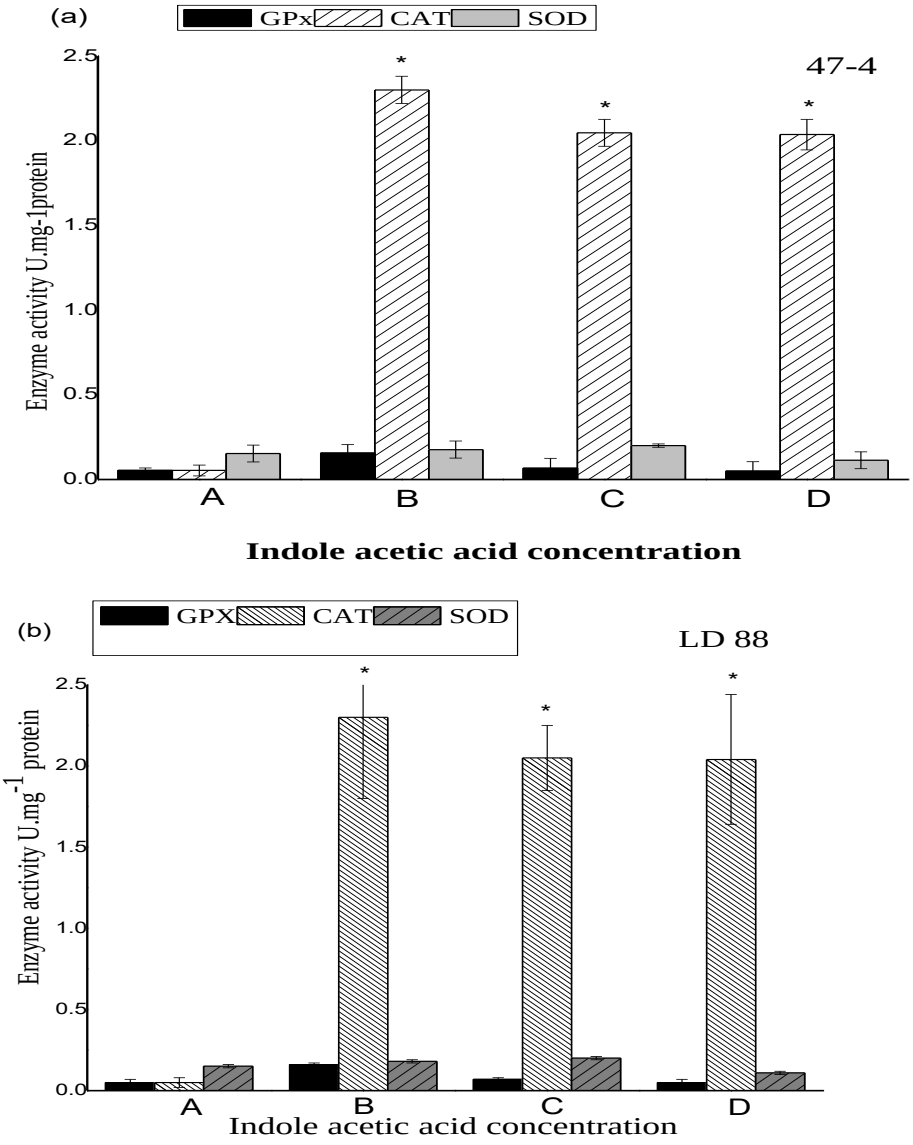


Fig. 44 (a, b): The activities of enzymes in the okra genotypes under the influence of indole acetic acid.

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control group

Where A, B, C and D = (0, 0.4, 0.5 and 0.6) mM respectively

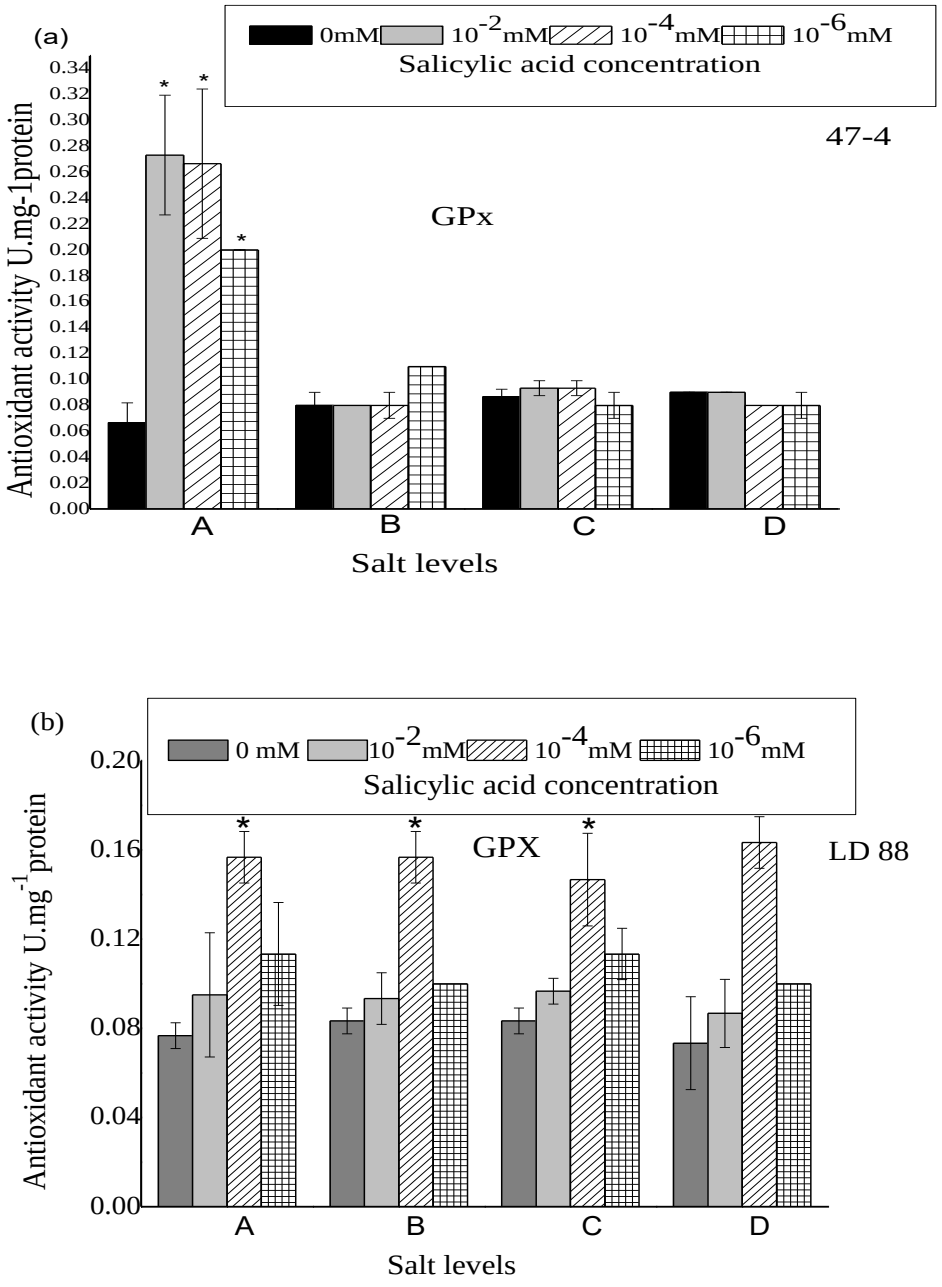


Fig. 45 (a, b): Activity of GPx in okra genotypes 47-4 & LD 88 under the influence of both salinity and salicylic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control (salinity only).

Where A, B, C and D = (50, 100, 150 and 200) mM respectively

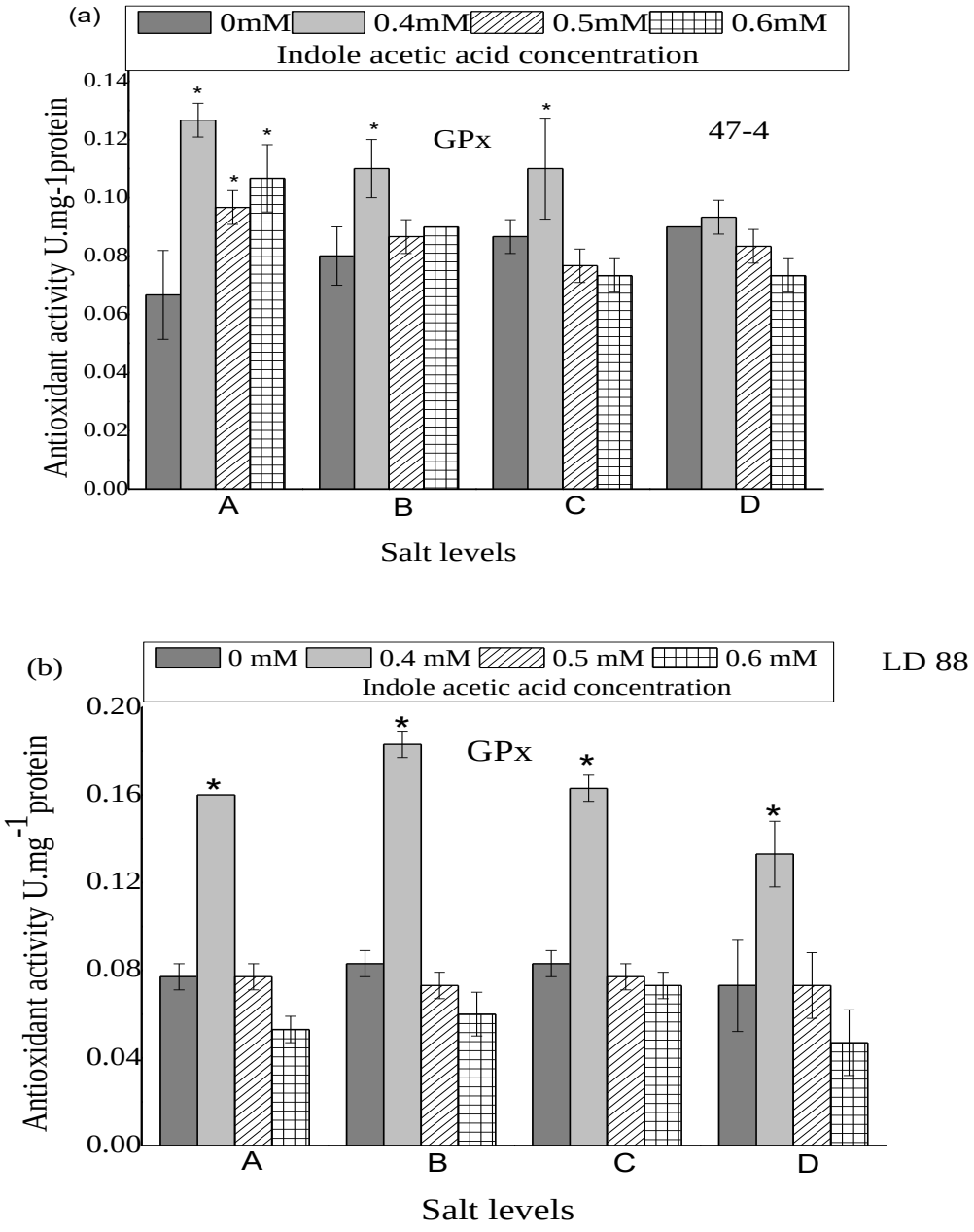


Fig. 46 (a, b): Activity of GPx in okra genotypes 47-4 & LD 88 under the influence of both salinity and indole acetic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control (salinity only)

Where A, B, C and D = (50, 100, 150 and 200) mM respectively

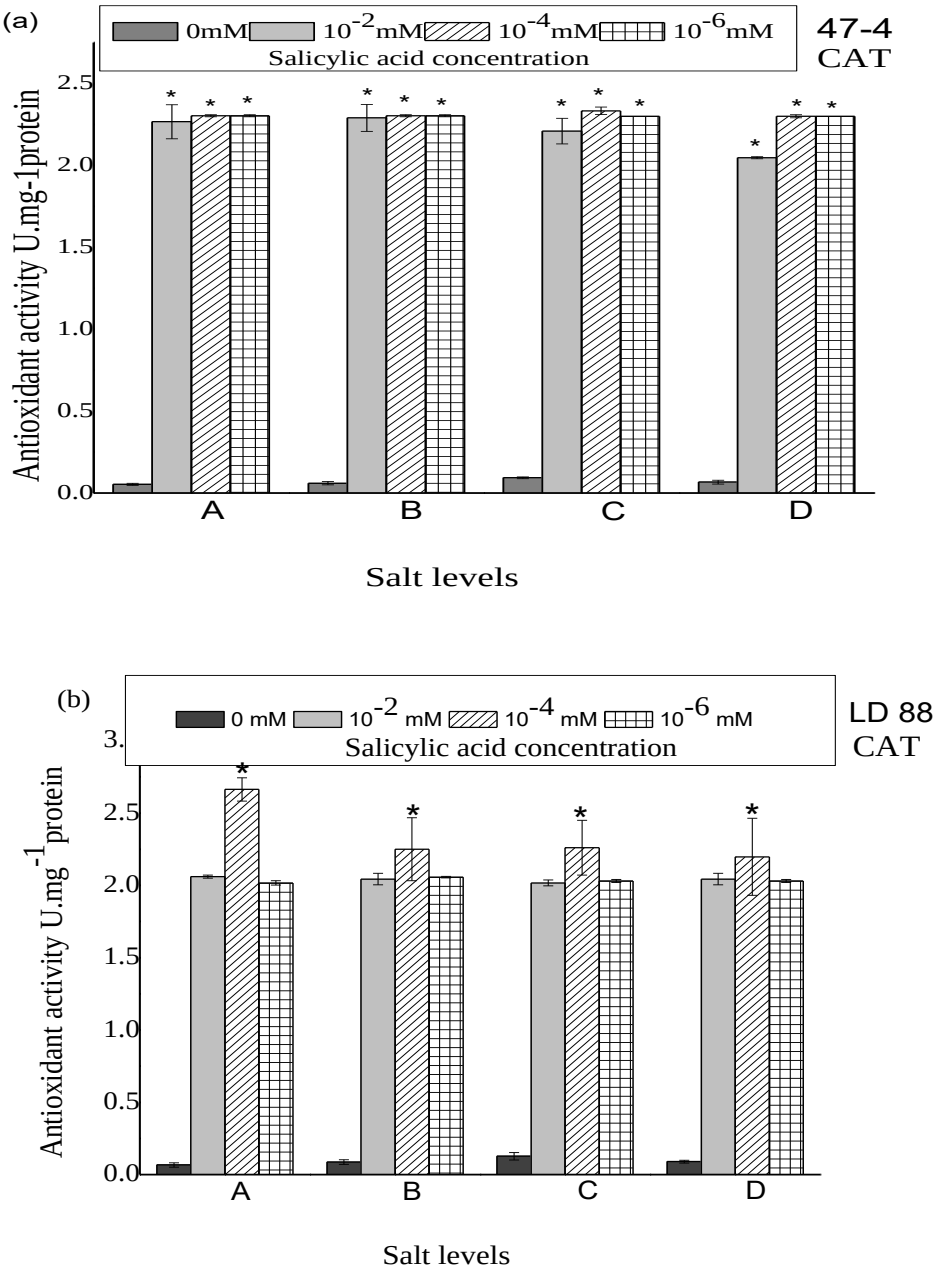


Fig. 47 (a, b): Activity of CAT in okra genotypes 47-4 & LD 88 under the influence of both salinity and salicylic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control (salinity only)

Where A, B, C and D = (50, 100, 150 and 200) mM respectively

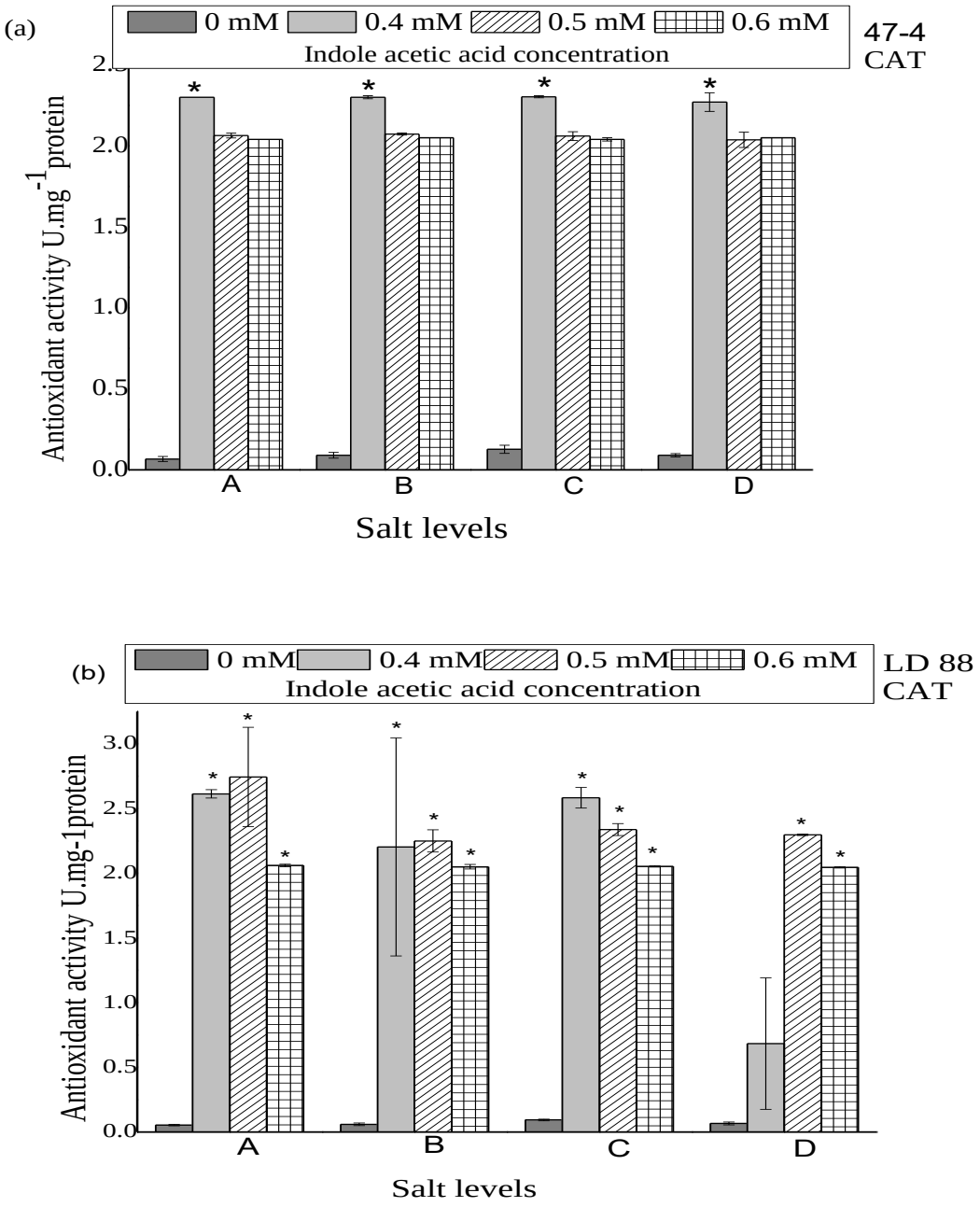
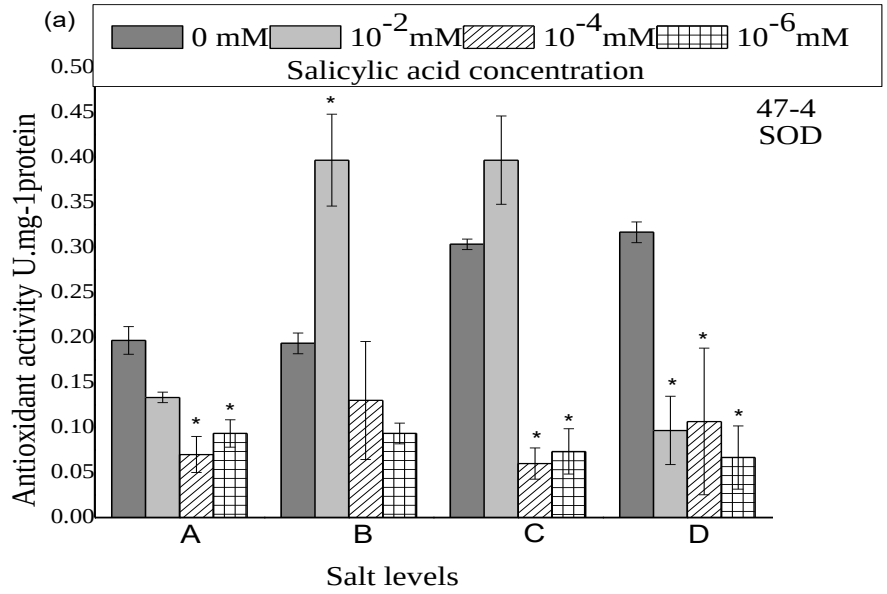


Fig. 48 (a, b): Activity of CAT in okra genotypes 47-4 & LD 88 under the influence of both salinity and indole acetic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control (salinity only).

Where A, B, C and D = (50, 100, 150 and 200) mM respectively



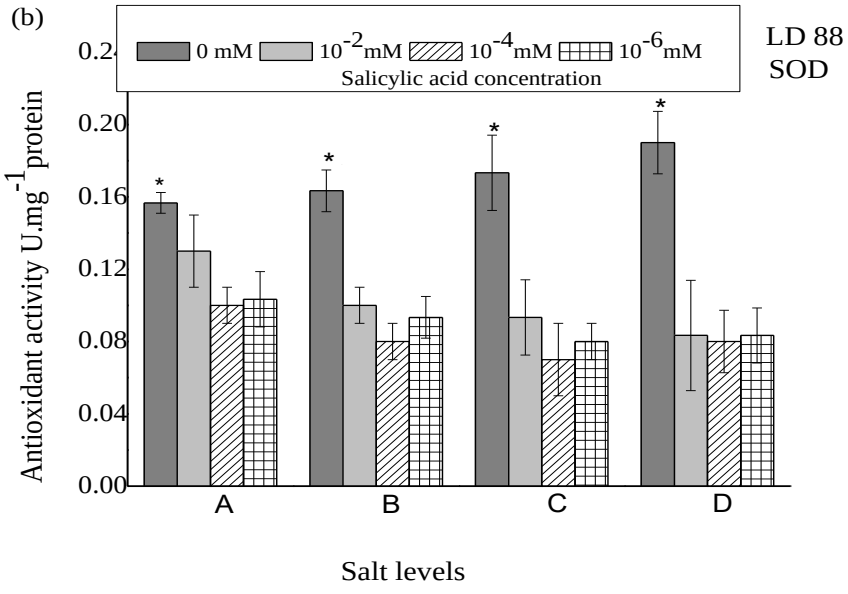


Fig. 49 (a, b): Activity of SOD in okra genotypes 47-4 & LD 88 under the influence of both salinity and salicylic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control (salinity only).

Where A, B, C and D = (50, 100, 150 and 200) mM respectively

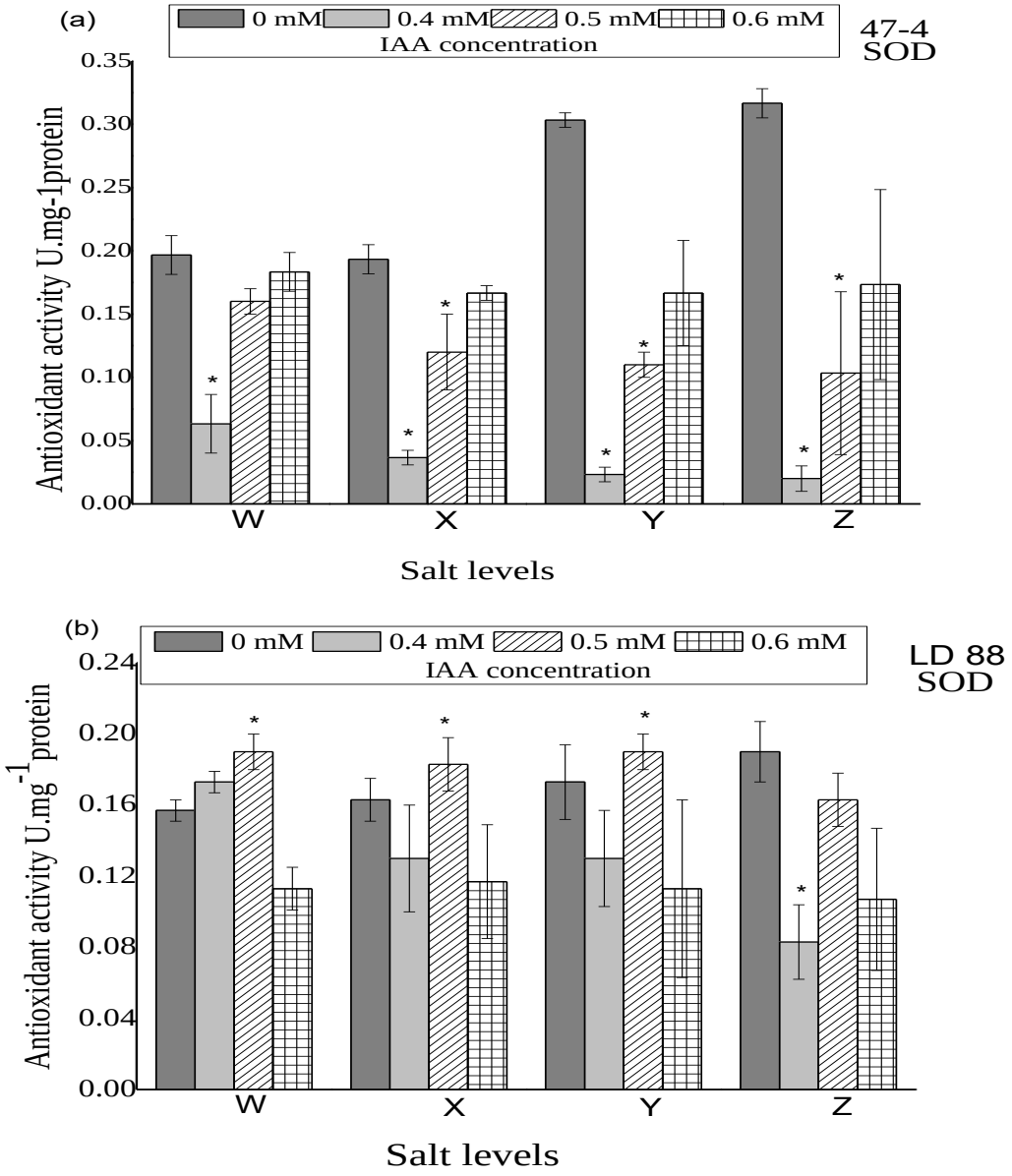


Fig. 50 (a, b): Activity of SOD in okra genotypes 47-4 & LD 88 under the influence of both salinity and indole acetic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the control (salinity only).

Where W, X, Y and Z = (50, 100, 150 and 200) mM respectively

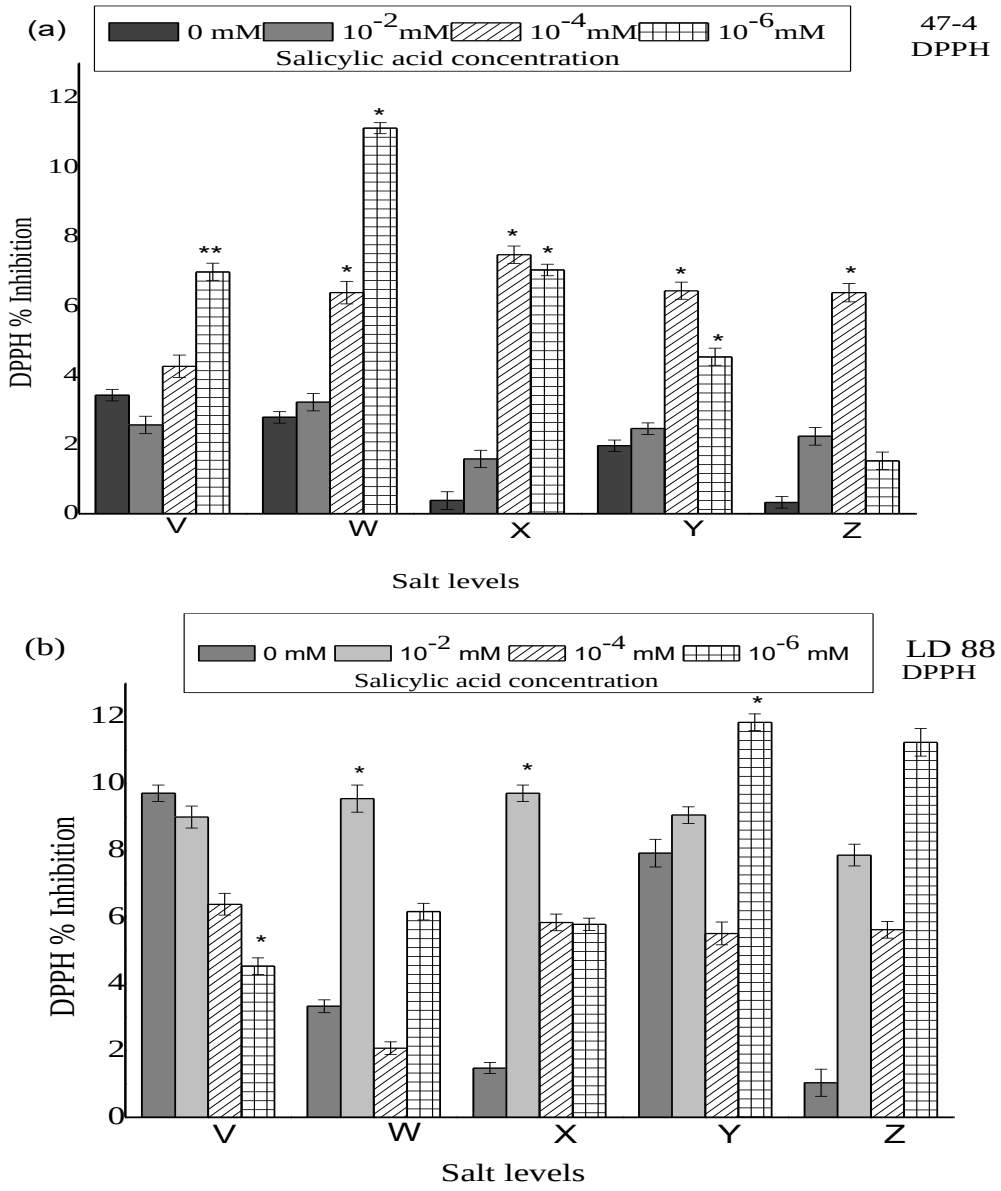


Fig. 51 (a, b): Radical scavenging activity of DPPH in okra genotypes 47-4 & LD 88 under the influence of salinity and salicylic acid

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the normal control

Where V, W, X, Y and Z equal to (0, 50, 100, 150 and 200) mM respectively

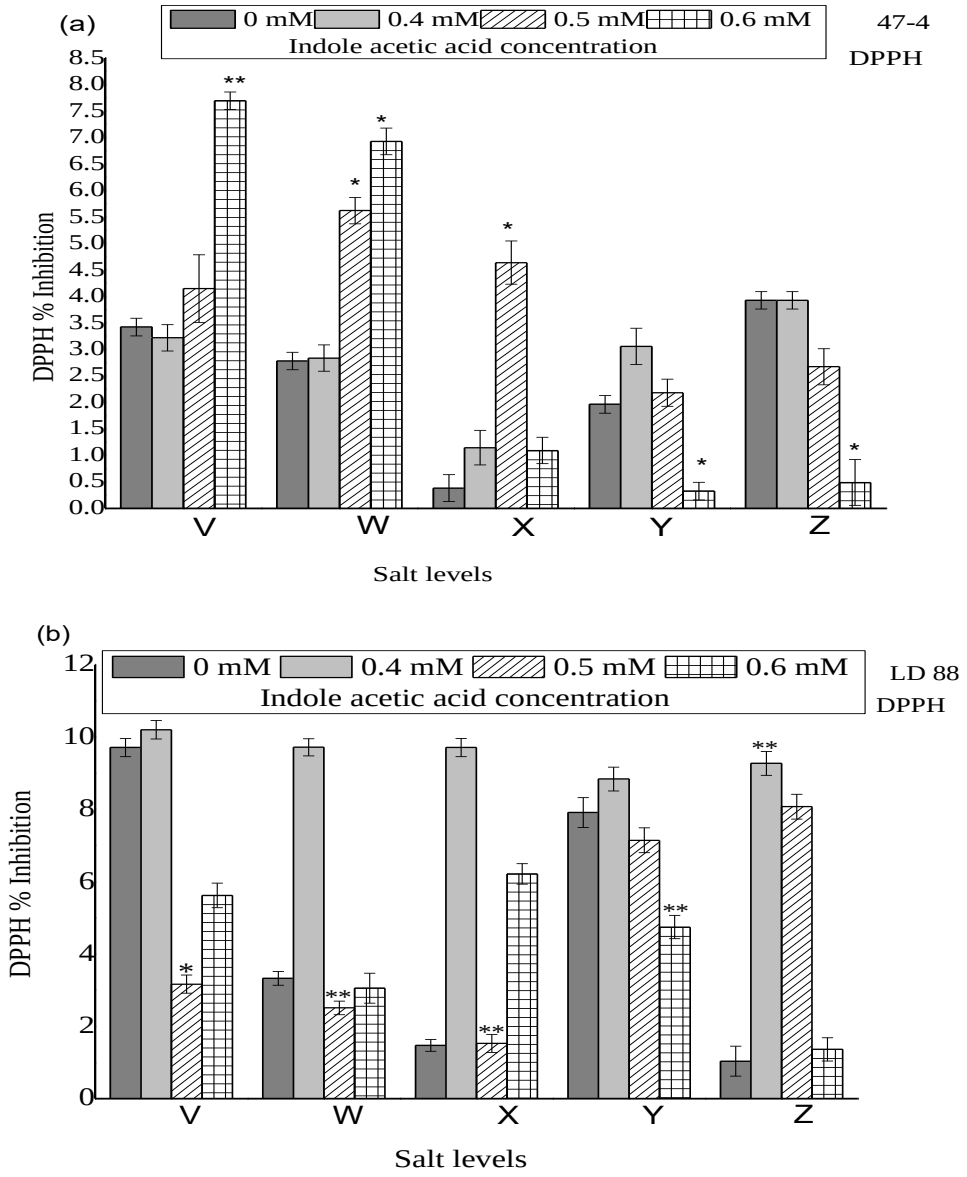


Fig. 52 (a, b): Radical scavenging activity of DPPH in okra genotypes 47-4 & LD 88 under the influence of indoleacetic acid and or both salinity and indole acetic acid.

Data: Means ± SEM for the 3 replicate

*Significant at $\alpha_{0.05}$ relatives to the treated control

** Significant at $\alpha_{0.05}$ relatives to the normal control

Where V, W, X, Y and Z equal to (0, 50, 100, 150 and 200) mM respectively

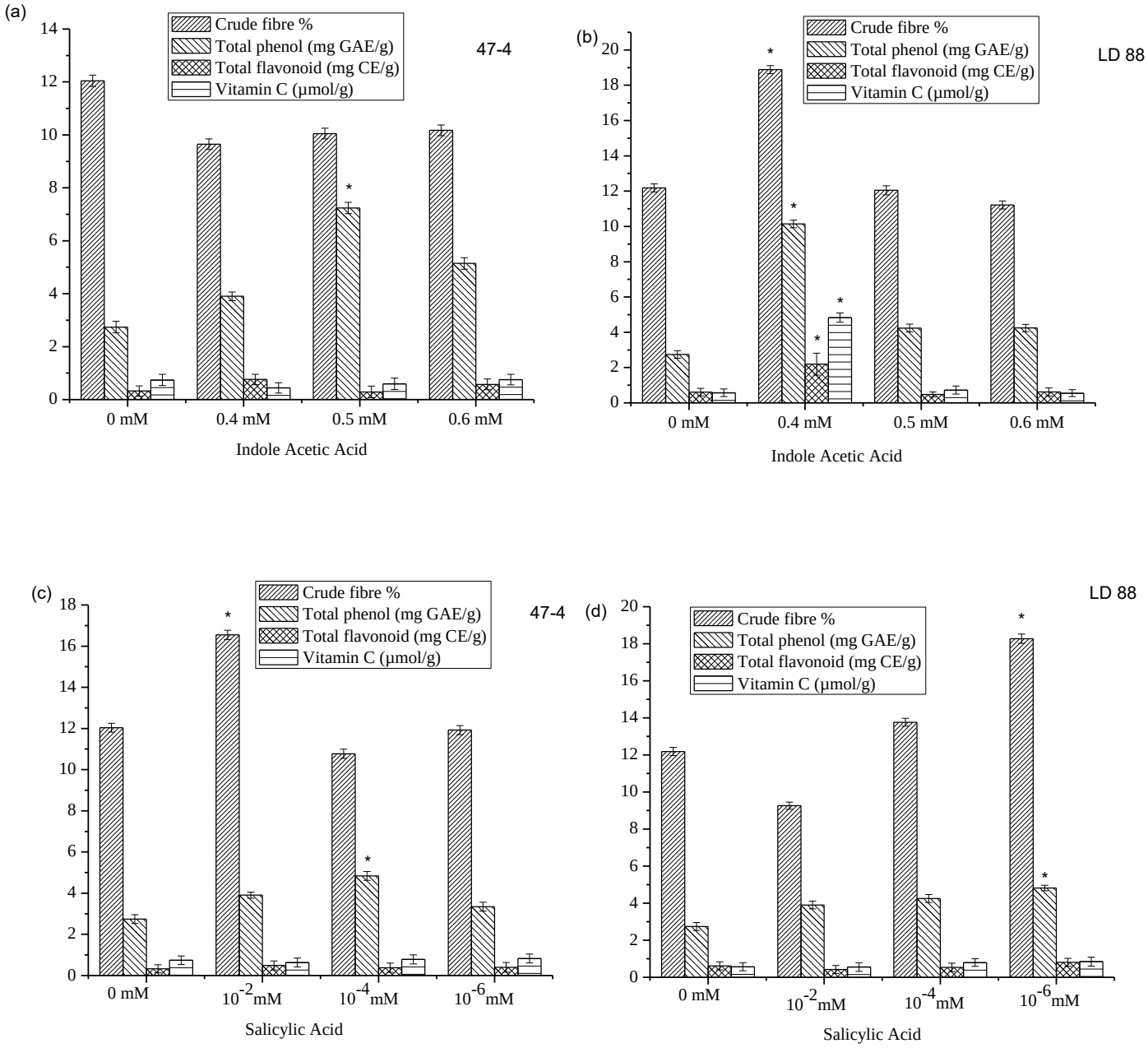


Fig. 53 (a-d): Phytonutrient concentrations in okra genotypes 47-4 and LD 88 under the influence of both indoleacetic acid and salicylic acid.

Data: Means ± SEM for the 3 replicate.

*Significant at $\alpha_{0.05}$ relatives to the control

4.5 Experiment 5: Activity of intestinal glucose transporter under the influence of ethanol and aqueous extracts of okra fruits using frog (*xenopus lavis)* oocytes

Introduction

Glucose transporter (GLUT) mechanism of action in the system greatly depends on facilitated glucose carriers such as glucose transporter 1 and 4 in the internal milieu of plasma cell membrane (James *et al*., 1988). Expression of GLUT 4 is majorly found in the body tissues where glucose transporter activity is facilitated with insulin production, whereas GLUT 1 is widely expressed in the tissues (Holman *et al*., 1990). It involves glucose uptake during lactation (Holman *et al*., 1990). Researchers have shown the expression of the GLUT 1 in *Xenopus lavis* oocytes by microinjection. The activity of GLUT 1 is inhibited by cytochalasin B and phloretin. Therefore, this experiment was designed to examine intestinal glucose transporter activity under the influence of okra fruit extract using *Xenopus lavis* oocytes model and E. coli strain (JMI09XL-Blue) for the GLUT-14 variant of GLUT-2 and GLUT-4.

Procedure

A matured female frog was used, housed in a tank containing water, with the optimum water temperature range between 19 to 21$^{\circ C}$. Tap water could be used but it is important to remove any high concentration of chlorine or heavy metals, which may be present. These could be avoided by allowing chlorine to evaporate in an open tank or using deionized water. The feeding was done twice a week. Salman pellets, minced beef and live blowfly larvae were used. Frog must eat within 3hrs., afterwards, water must be changed. Table 13 shows the stock concentrations of buffer solution.

TABLE 13: Buffer solution(s) for oocytes commonly called OR-2

Reagents	Final conc.	Stock conc.	1litre
NaCl	82.5mM	5M (4.86g)	16.5ml
KCl	2.5mM	1M (7.45g/100ml)	2.5ml
$MgCl_2$	1.0mM	2M	0.5ml
Na_2HPO_4	1.0mM	0.5M	2ml
HEPES	5.0mM	1M	5ml
Gentamycin	100µg/ml	50mg/ml	2ml
Water	-	-	971ml
$CaCl_2$	1.0mM	2M	0.5ml

$CaCl_2$ must be added after water, so as to prevent precipitation

NaOH was used to adjust pH to 7.8. The solution was filtered and sterilized at the end before use. OR-2 was prepared fresh for the experiment with or without calcium ion. OR-2 without calcium ion was used for collagenase treatment during oocytes isolation. Collagenase is inhibited by calcium ion. For maintenance of oocytes, before and after injection, additional 1mM sodium pyruvate (55mg/L) in OR-2 to provide them with energy.

Removal of Oocytes

A matured female frog contains about 30,000 large Oocytes. Through incision, one frog may be opened 2-3 times.

i. The frog was anaesthetized by immersion for about 30mins in cold water containing methanesulfonate salt of 3-aminobenzoic acid ethyl ester.
ii. The scalpel was used to remove frog and pith when spine was severed from the head
iii. The incision was made on the abdominal.
iv. Ovary lobe containing oocytes were removed into OR-2 buffer solution without Ca^{2+} in a petri dish.
v. Ovary sac was torn to release the individual oocytes apart.
vi. Washing of the oocytes was done 2-3 times with OR-2 (without Ca^{2+})

vii. Drained Oocytes and split into two batches (one for collagenase treatment and the other for manual removal of collagenation.

Collagenase treatment

i. Oocytes were incubated in OR-2 buffer solution without calcium ion in collagenase containing 25 ml for 30 mins, in petri dish wrapped with aluminium foil to prevent light, and slightly shaking to facilitate the process.
ii. The medium was removed and another 25ml collagenase solution was added the second time for 30mins.
iii. Oocytes were washed extensively (4-6 times) after collagenase treatment in calcium-free OR-2 before transferring them to a calcium-containing the solution.
iv. Afterwards, oocytes rinsed 2-3times with another OR-2 buffer solution with calcium ion to inhibit collagenase activity.
v. Then oocytes were kept in an incubator and left overnight at 18-20°C.

Cell culture

The bacterial GLUT 14 plasmid construct of *E. coli* strain (JM109XL-Blue) was incorporated into bacterial cells cultured on LB Agar with 100 milliunits of penicillin and 100 µg of streptomycin/ml, so as to purify the medium from unwanted bacteria. Cells were split on the medium in a petri dish and kept under atmospheric condition. The broth was then taken to the centrifuge tube for centrifugation. After centrifuged, the cells were then taken to miniprep kit to harvest the plasmid. Afterwards, the plasmid (cDNA) was subjected to purification using PCR purification kit. GLUT-14 cDNA was excised from the plasmid on a promoter region known as T_7 by digestion with a restriction enzyme (lyase). In vitro transcription of cDNA to cRNA was done with the use of Ambion mMessage, mMachine kit or Hiscribe T_7 kit. Incubation of the oocytes was done for 30mins before cRNA injection.

The procedure for the microinjection of cRNA into Oocytes is outlined in section 3.4.13.3.

The procedure for the uptake of 2-deoxy-D-Glucose is outlined in section 3.4.13.4.

Results

In Figure 54, the result showed greater uptake of the 2-D -Glucose in GLUT-14 cRNA and ethanol extract okra fruit + GLUT 14 cRNA oocytes than water injected oocytes, which

showed little or no uptake of 2-DG. More importantly, bioregulator treated okra fruit extracted with ethanol showed the highest uptake of 2-D-Glucose when compared with only the GLUT-14 cRNA. Oocytes injected with GLUT-14 cRNA and ethanol extract okra fruit (AeTe) + GLUT-14 cRNA showed a mean of 2840.2 and 2666.2 pmols/oocyte/15 min, 2-D-Glucose uptake respectively.

Conclusion

The glucose uptake into the various tissues is mediated by the glucose transporter 14. In this experiment, we have characterized JM109XL-Blue GLUT-14 activity in *Xenopus lavis* oocytes for the intestinal glucose transporter activity under the influence of ethanol and aqueous extracts of okra fruits using *Xenopus lavis* Oocytes. Extracts of okra fruit used showed no inhibitory effect on JM109XL-Blue GLUT-14 but rather improved its activity. Thus, okra fruit extract could be used for prophylaxis and management of diabetes mellitus.

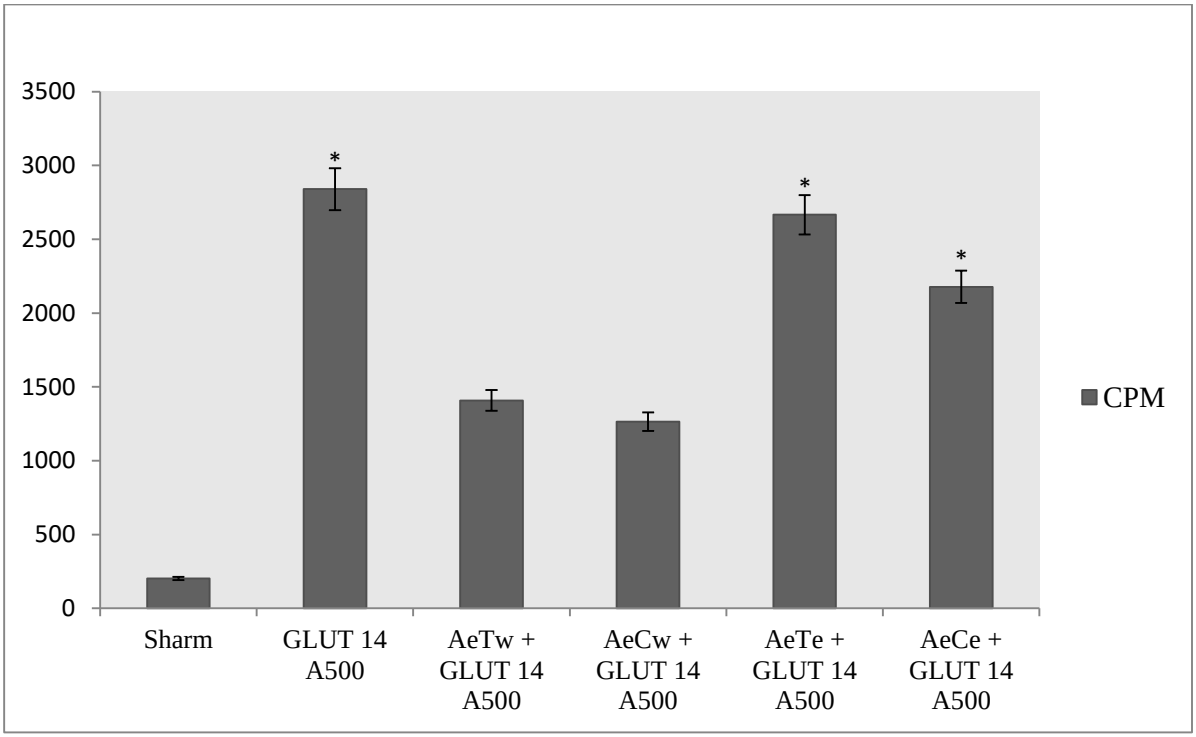


Fig. 54: Activity of the glucose transporter (GLUT)-14

Data: Means ± SEM for the 25 oocytes

*Significant at $\alpha_{0.05}$ relatives to the sham (water control)

Where sham = control (water control)

AeTw = water extract okra fruit treated with indole acetic acid

AeCw = water extract okra fruit without indole acetic acid

AeTe = ethanol extract okra fruit treated with indole acetic acid

AeCe = ethanol extract okra fruit without indole acetic acid

4.6 Experiment 6: Assessment of the antihyperglycemic effect of indole acetic acid-treated okra fruits in diabetic rats

Introduction

Diabetes mellitus complications and effects cut across all ages due to metabolic disruption of macromolecules (Georg and Ludvik, 2000). There is a need to regulate carbohydrate metabolic pathway in order to maintain normal glycemic status (Ashokkumar and Pari, 2005). Hyperglycemia complications are common in the two types of Diabetes mellitus (Chen *et al*., 2008). Cardiovascular complications in diabetes conditions are due to high lipid profiles (Yang *et al*., 2006). Currently, diabetes is managed with the use of chemical drugs, but these chemicals elicit one or more side effects, which call for the use of natural products from the plants, which might be less toxic compared to the chemical drugs coupled with diet and lifestyle modifications (Palsamy and Subramanian, 2008). Of recent, the focus is on ethnomedicine (alternative therapy) for diabetes (Chandramohan *et al*., 2008). Management of various disorders like ulcers, lung inflammation and diabetes have been involved with the use of okra (Sengkhamparn *et al*., 2009). However, there is dearth of information on the influence of okra extract on antioxidant enzymes, carbohydrate metabolic enzymes and non-enzymic antioxidant activity in the tissue of disease control animals, as well as how to improve the potency of okra for its hypoglycemic potential. Therefore, this study examined the antidiabetic potential of bioregulator induced okra fruit in diabetic rats.

Procedures

The okra fruit sample; indoleacetic acid treated okra sample and non-indole acetic acid treated okra sample were used for the experiment. A 250g of each sample was used. For aqueous extraction, 250g of indole acetic acid treated sample and non-indole acetic acid treated sample were dissolved into 2500ml of distilled water in ratio 1:10 respectively. Stirred until it viscous, it then heated and boiled for about 10 minutes, and allowed to cool before it subjected to centrifuge for 10 minutes at 15000 rpm. Then, the upper layer was put in -80°C before transferring into the freeze dryer machine in order to get soluble powder, which was stored for the experiment. For the ethanol extraction, 250g of indole acetic acid treated sample and non-indole acetic acid treated sample were dissolved in round bottom flask containing 75% ethanol in ratio 1:10 respectively i.e. 1875ml of ethanol to 250 ml of distilled H_2O, and

completed to 2500 ml. A 250 g to 2500 ml in 1:10 with the use of magnetic stirrer and temperature at 40°C for 3 hours. After which they were decanted, sieved and transferred into the rotary evaporator equipment for total removal of ethanol and to concentrate the solution, before transferring into the freeze dryer machine in order to get soluble powder, which was stored for the experiment.

The procedure for the induction of streptozotocin is outlined in section 3.4.14.

The procedure for the glucose level and serum preparation are described in sections 3.4.14.1-3.4.14.3.

Cholesterol and triglycerides contents were determined as outlined in sections 3.4.14.3.1-3.4.14.3.2.

Insulin content estimation in the pancreatic β-cell is described in section 3.4.15. Glycogen content in the tissues is described in section 3.4.16.

Tissue preparation was described in section 3.4.17.

Carbohydrate metabolizing enzymes were determined as described in sections 3.4.17.1 – 3.4.17.5.

Endogenous antioxidant enzymes assays were determined as outlined in sections 3.4.18 – 3.4.18.3.

The ascorbic acid and α- tocopherol contents determination procedures are outlined in sections 3.4.19 and 3.4.20 respectively.

Results

Figure 55 shows the body weight of streptozotocin-induced rats. The body weight of all experimental rats increased steadily at the point of STZ injection. Administration of glibenclamide and ethanol extract indole acetic acid okra fruit showed no noticeable increased in the weight of experimental rats' relative to the diabetes group.

In Figure 56 streptozotocin injection resulted to high blood glucose level in the blood of all experimental rats when compared with the control group, but after a week of treatment with okra fruit extract, a sharp decreased in the blood glucose level was noticed with the highest reduction in the ethanol okra fruit extract indole acetic acid treated group, but after 2-4 weeks post gavaging, level of plasma glucose increased steadily, and the blood glucose level was stable at 5^{th} week, but dropped at 6^{th} week post gavaging relative to diabetes group.

Cholesterol and triglycerides concentrations in the serum were shown in Figure 57. Cholesterol and triglycerides levels were increased in the diabetes group as well as ethanol okra fruit extract without indole acetic acid treated group as compared to the other groups.

Table 14 shows water and food intake in experimental rats. There was an increase in the feeding habit of the experimental rats' relative to the control group. But treatments with okra fruit extract and glibenclamide ameliorated this effect in the diabetic group.

Figure 58 shows the okra fruit extract effects on the weight of some tissues in diabetic rats. The result revealed that the extract has no effect on abdominal and peripheral fats weight. Whereas, reduction was observed in the weights of liver, kidney and pancreas in diabetic control group relative to other groups, especially the ethanol extract indole acetic acid okra fruit group (ETOHEE) and glibenclamide treated group.

Results of the photomicrograph taken from Pancreas showed a positive result for insulin in pancreatic β-cell as depicted in plate 1. Representative photomicrograph taken from Pancreas with a positive result for insulin was indicated with arrows. Normal Control Panel A (revealed intact pancreatic islet cells with normal structure), Diabetes Control Panel B (showed reduce islets size and extensively damaged of pancreatic β-cell, necrotic changes with fibrosis and atrophy), Okra water fraction (Panel C), Okra treated water extract (Panel D), Okra phenolic extract (Panel E), Okra treated phenolic extract (Panel F), and Glibenclamide (Panel G) showed some decreased histological alterations in the pancreatic islet cells. H&E, 100x. The red circle indicates the presence of positive cells for insulin.

Representative photomicrograph taken from Livers PAS stain positive for Glycogen was depicted in plate 2. In Normal Control Panel A, liver tissue is normal with normal architecture with perfectly arrange central vein (C.V) and hepatic cords with more positive cells stain for glycogen, Diabetes Control Panel B showing hepatocyte vacuolization and fatty changes, necrosis, dilation of hepatic sinusoids with little positive cells for glycogen, Okra water fraction (Panel C), Okra treated water extract (Panel D), Okra Phenolic extract (Panel E), Okra treated phenolic extract (Panel F), and Glibenclamide (Panel G) liver tissue is normal with normal architecture with perfectly arrange central vein (C.V) and hepatic cords, few necroses, less degenerative changes, and vacuolization. H&E, 100x.

Representative photomicrograph taken from skeletal muscle PAS stain positive for Glycogen was depicted in plate 3. Normal Control Panel A revealed normal skeletal muscle architecture with more positive cells stain for glycogen, Diabetes Control Panel B showed increase muscle wasting and structural proteins degradation with disturbance of the architecture pattern, Okra water fraction (Panel C), Okra treated water extract (Panel D), Okra Phenolic extract (Panel E), treated Okra phenolic extract (Panel F), and Glibenclamide (panel G) showed normal structure of skeletal muscle and less degradation of structural protein with more positive cells for glycogen.

Figure 59 reveals decreased in the antioxidant enzymes activities in diabetic control rats. Antioxidant enzymes activities increased after the administration of 100 mg/kg of ethanol extract of bioregulator-treated okra fruit and 5mg/kg glibenclamide repectively when compared with the diabetic control group.

Increased in the gluconeogenic enzymes activities with a decrease in glycolytic (hexokinase) and pentose phosphate enzymes activities were prevalent in the disease control group. However, this aberration was ameliorated in the experimental rats treated with glibenclamide and ethanol extract of okra fruit as indicated in groups V, VI and VII respectively. More also, okra fruit extracts administration improved glycogen synthase activity with a declined glycogen phosphorylase enzyme activity in all treated groups relative to diabetes control (Figure 60).

In Figure 61 reduced levels of vitamin C and E were observed in the diabetic control rats. However, Okra fruit extracts (especially ethanol extract of bioregulator treated okra) and glibenclamide administration ameliorated these effects in experimental rats' relative to the disease control group.

Conclusion

Okra fruit extracts administration in the experimental animals reduced the plasma glucose level during experimental period to some extent. This shows that the okra fruit extract has hypoglycemic potential. The observation of the results also suggests that the okra fruit extracts have the protective effect in hyperglycemic conditions by restoring the activities of glucose metabolizing enzymes.

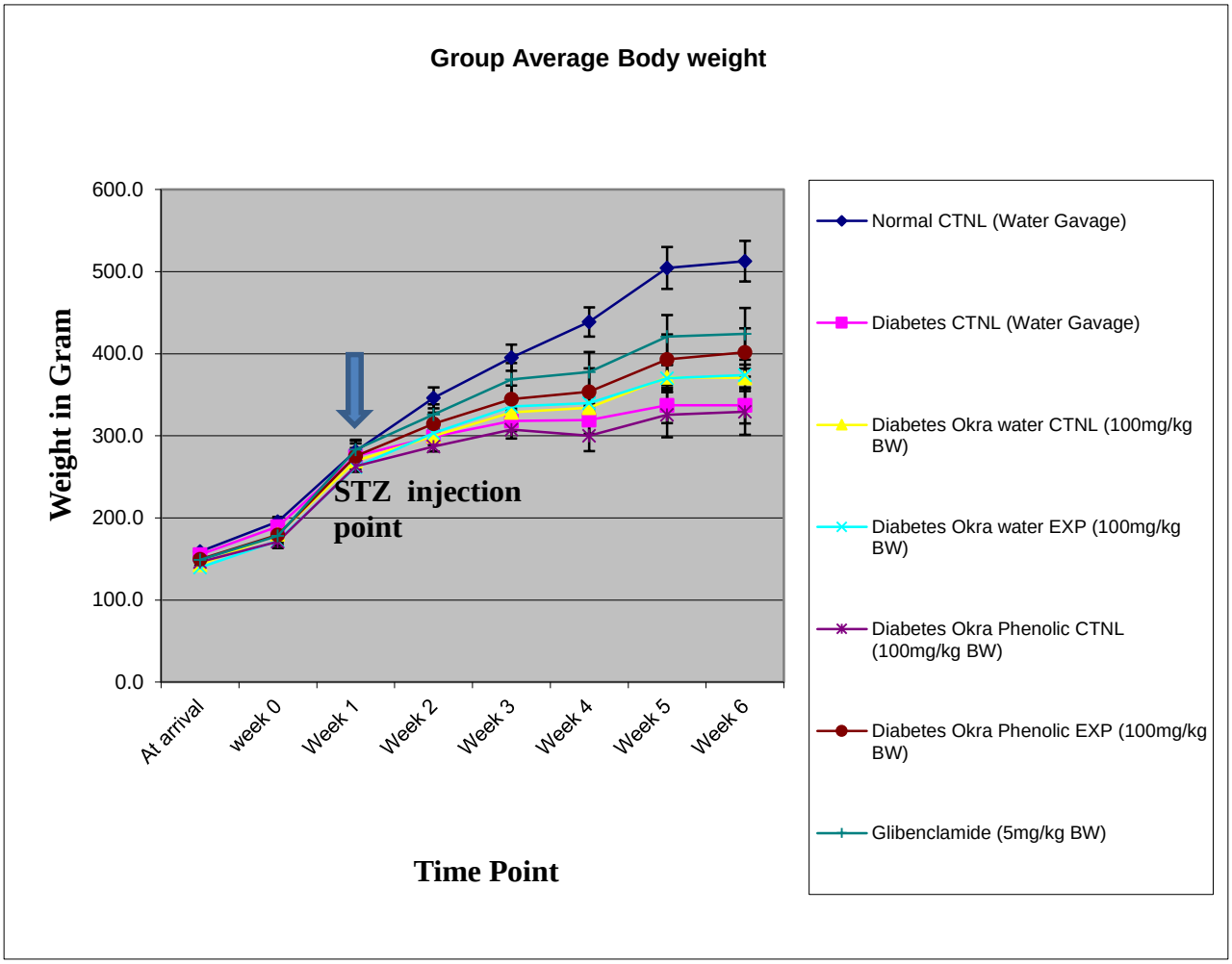


Fig. 55: Effect of okra extracts on diabetic rats weight

Data: Means ± SEM for the five rats

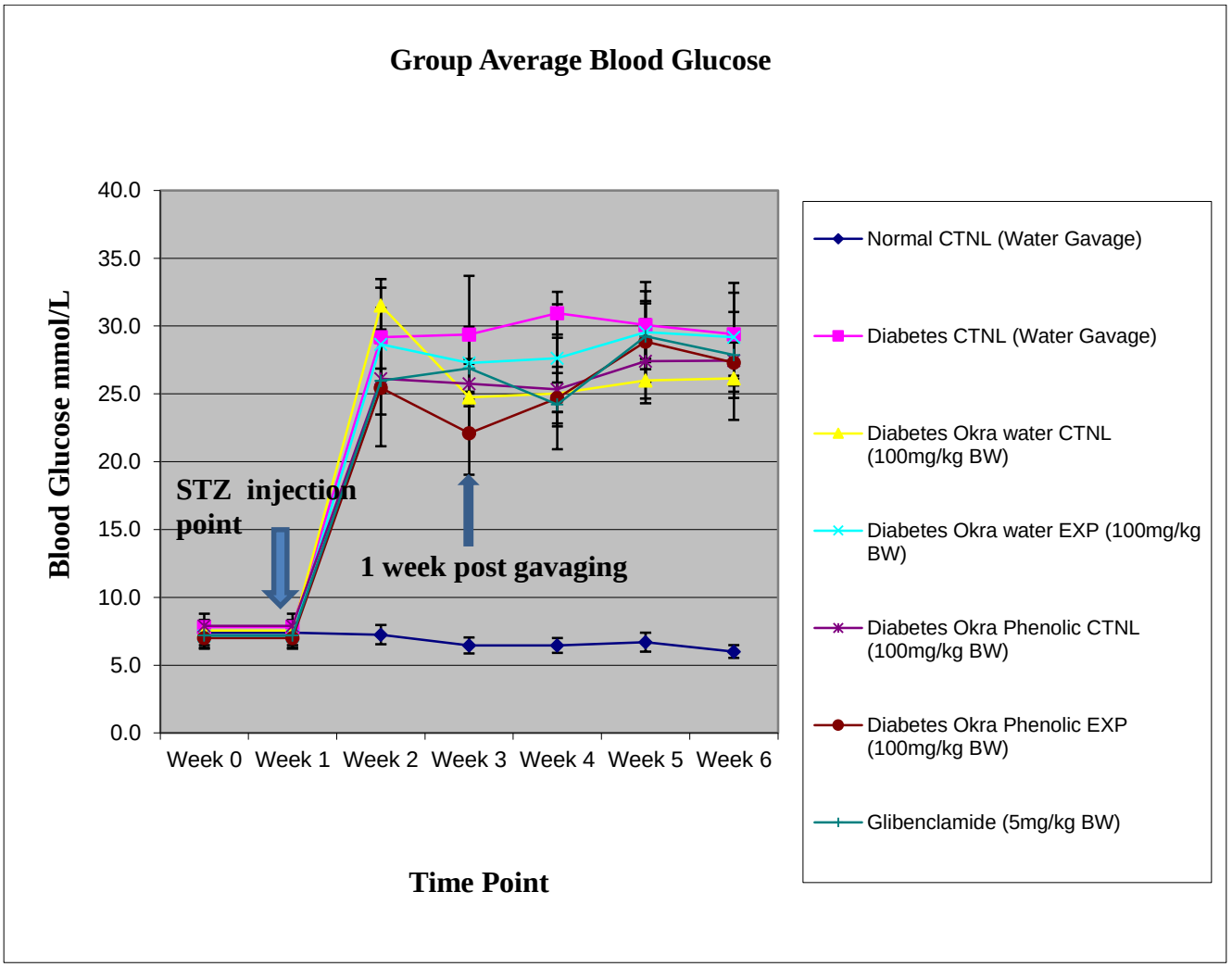


Fig. 56: Serum glucose levels in experimental rats under the influence of okra extracts

Data: Means ± SEM for the five rats

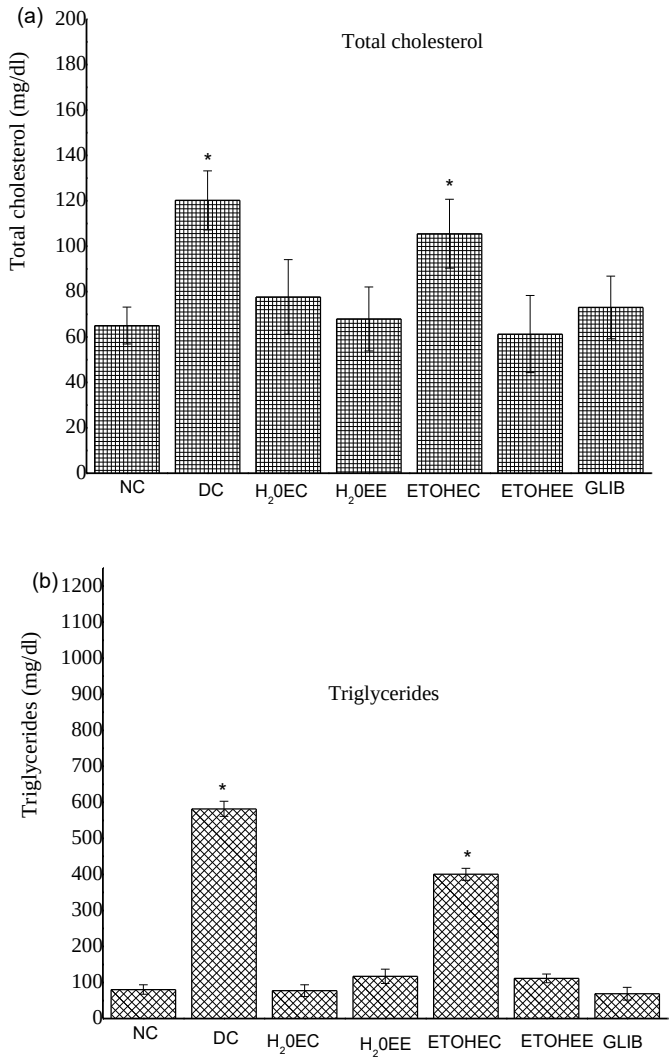


Fig. 57 (a, b): Effect of okra extracts on Total cholesterol and triglyceride levels in experimental animals

Data: Means ± SEM for the five rats.

*Significant at $\alpha_{0.05}$ relatives to the other groups

NC = Normal control, DC = Diabetes control

H_2OEC = Water extract okra fruit without indole acetic acid

H_2OEE = Water extract indole acetic acid-treated okra fruit

ETOHEC = Ethanol extract okra fruit without indole acetic acid

ETOHEE = Ethanol extract indole acetic acid-treated okra fruit, GLIB = Glibenclamide.

TABLE 14: Food (g/rat/day) and fluid (ml/rat/day) intake of experimental rats under the influence of okra extracts

Group	Food intake		Fluid intake	
	Before	**After**	**Before**	**After**
NC	35.5±1.22	37.3±1.33	84.65±4.90	90.05±8.90
DC	56.5±1.02	74.8±1.67*	76.89±5.00	170.73±20.67*
H_20EC	53.6±2.00	58.9±1.54	78.34±10.20	115.25±11.00
H_20EE	53.7±1.30	58.2±1.17	77.90±11.50	100.50±9.45
ETOHEC	53.7±1.15	53.7±1.76	85.55±5.99	119.60±8.76
ETOHEE	56.1±1.23	66.8±1.07*	88.12±6.80	114.05±10.00
GLIB	54.8±1.08	65.0±1.80*	86.85±3.47	100.25±15.10

Data: Means ± SEM for the five rats

*Significant at $\alpha_{0.05}$ relatives to the control group

Where; NC = Control

DC = Diabetes control

H_2OEC = Water extract okra fruit without indole acetic acid

H_2OEE = Water extract indole acetic acid-treated okra fruit

ETOHEC = Ethanol extract okra fruit without indole acetic acid

ETOHEE = Ethanol extract indole acetic acid-treated okra fruit

GLIB = Glibenclamide.

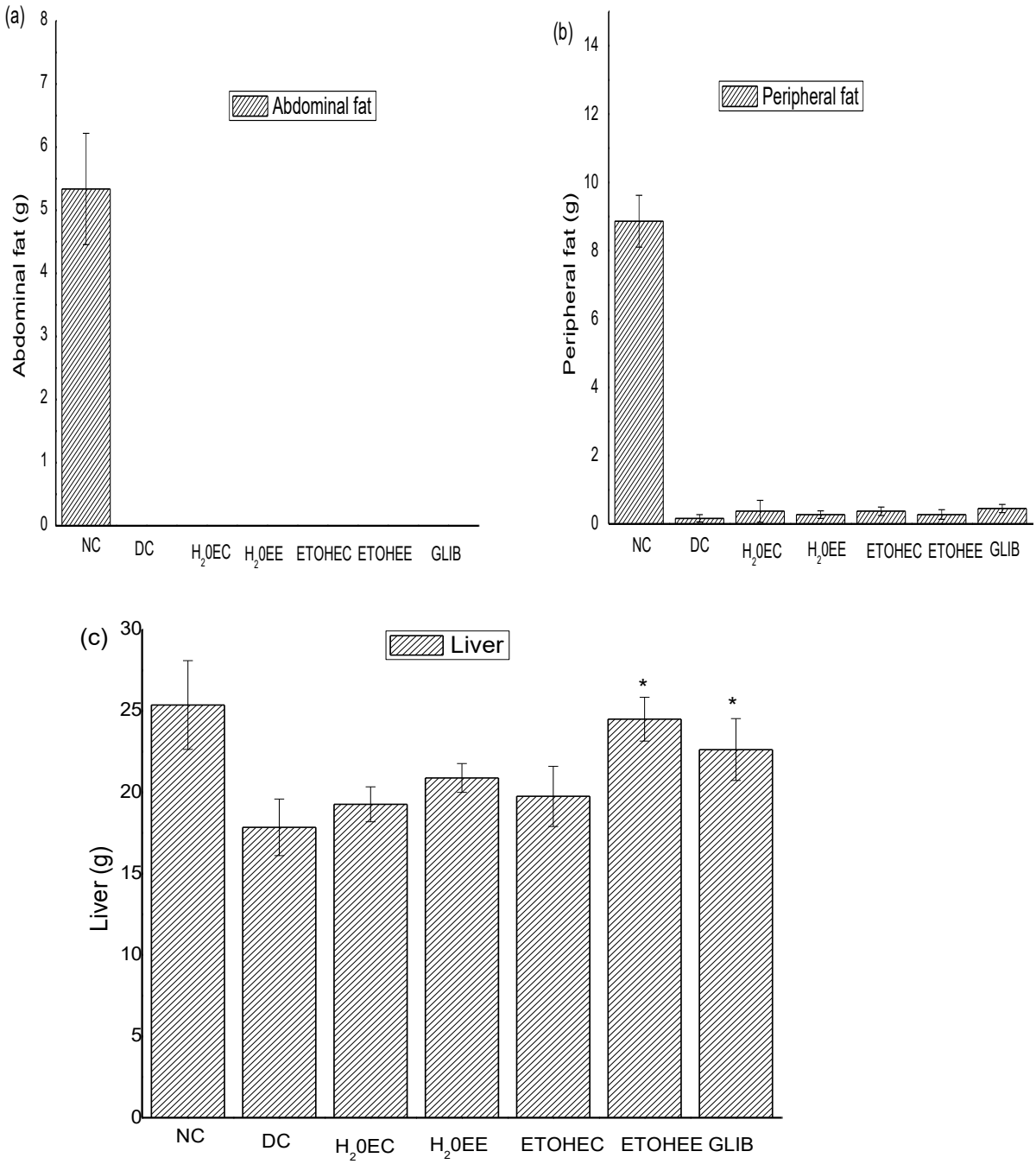
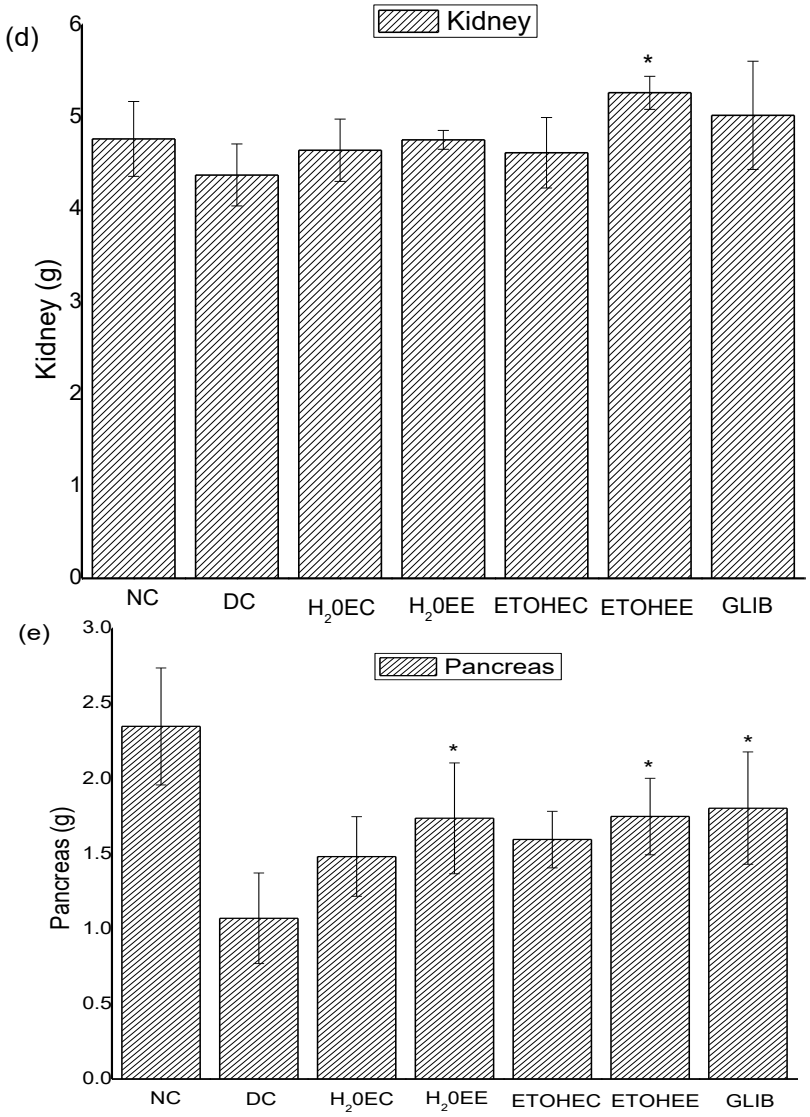


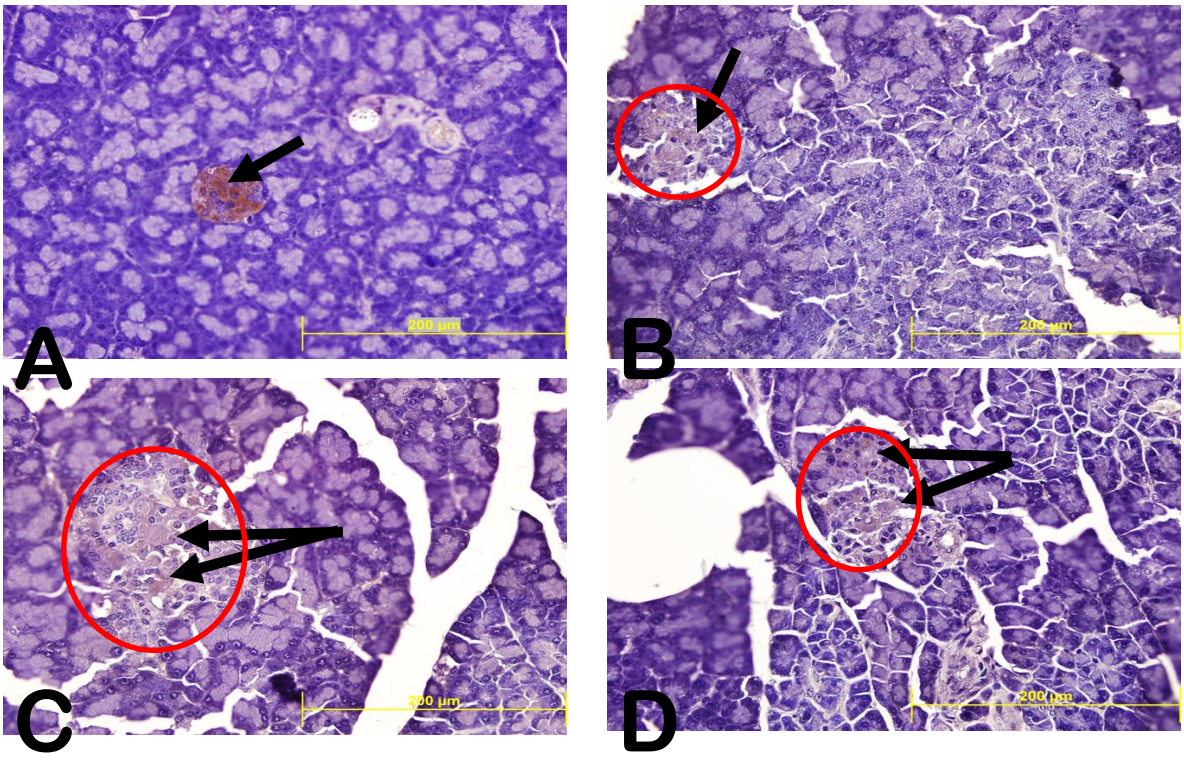
Fig. 58 (a-e): Effect of okra extract on some tissues in experimental animals

Data: Means ± SEM for the five rats

*Significant at $\alpha_{0.05}$ relatives to the diabetic animals

Where; NC = Control, DC = Diabetes control, H_2OEC = Water extract okra fruit without indole acetic acid, H_2OEE = Water extract indole acetic acid-treated okra fruit, ETOHEC = Ethanol extract okra fruit without indole acetic acid, ETOHEE = Ethanol extract indole acetic acid-treated okra fruit, GLIB = Glibenclamide


(d)
Kidney
Kidney (g)
0
1
2
3
4
5
6
*
NC
DC
H20EC
H20EE
ETOHEC
ETOHEE
GLIB
(e)
Pancreas
Pancreas (g)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
*
*
*
NC
DC
H20EC
H20EE
ETOHEC
ETOHEE
GLIB



Plat 1(a): Representative photomicrograph taken from Pancreas β-cell positive for Insulin using PAS stain.

Normal Control Panel A (revealed intact pancreatic islet cells with normal structure), Diabetes Control Panel B (showed reduce islets size and extensively damaged of pancreatic β-cell, necrotic changes with fibrosis and atrophy), Okra water fraction (Panel C), Okra treated water fraction (Panel D) showed a little decreased in the histological alterations in pancreatic islet cells, and the red circle indicates the presence of positive cells for insulin.
H&E, 100x.

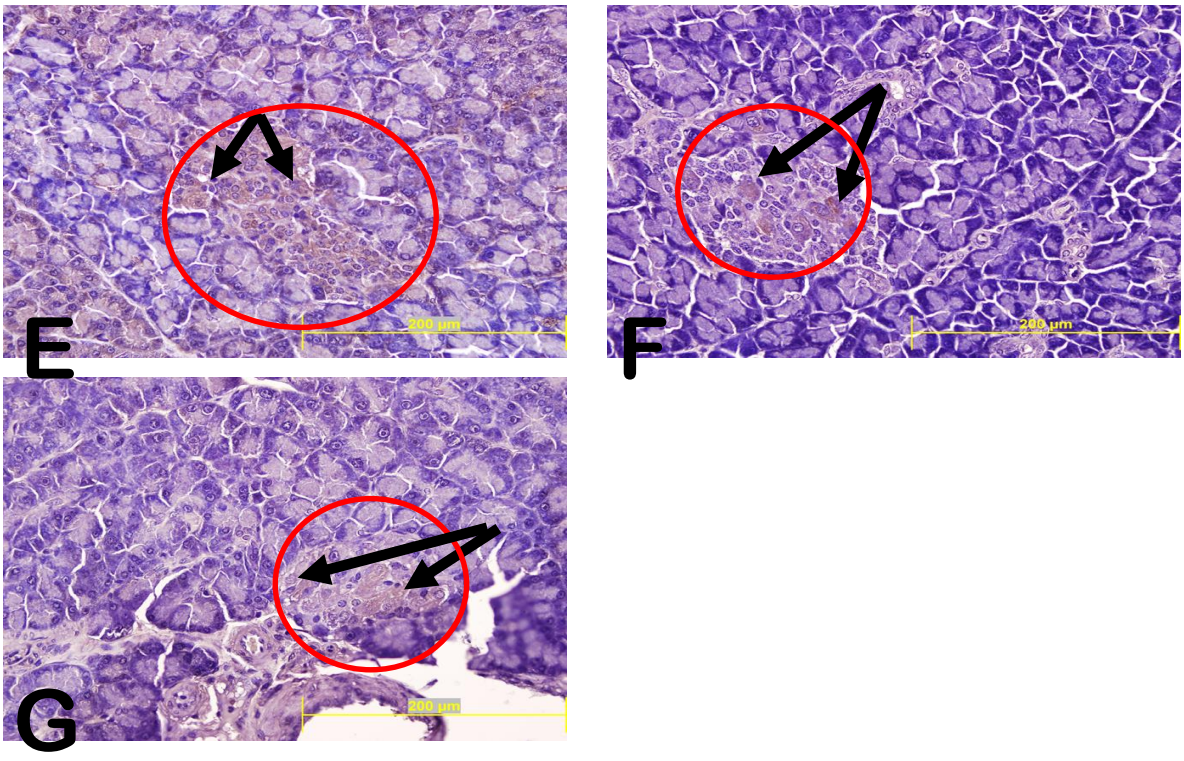


Plate 1(b): Representative photomicrograph was taken from Pancreas β-cell positive for Insulin using PAS stain

Okra Phenolic fraction (Panel E), Okra treated phenolic fraction (Panel F), and Glibenclamide (Panel G) showed a little reduction in the histological alterations of the pancreatic islet cells. H&E, 100x. The red circle indicates the presence of positive cells for insulin.

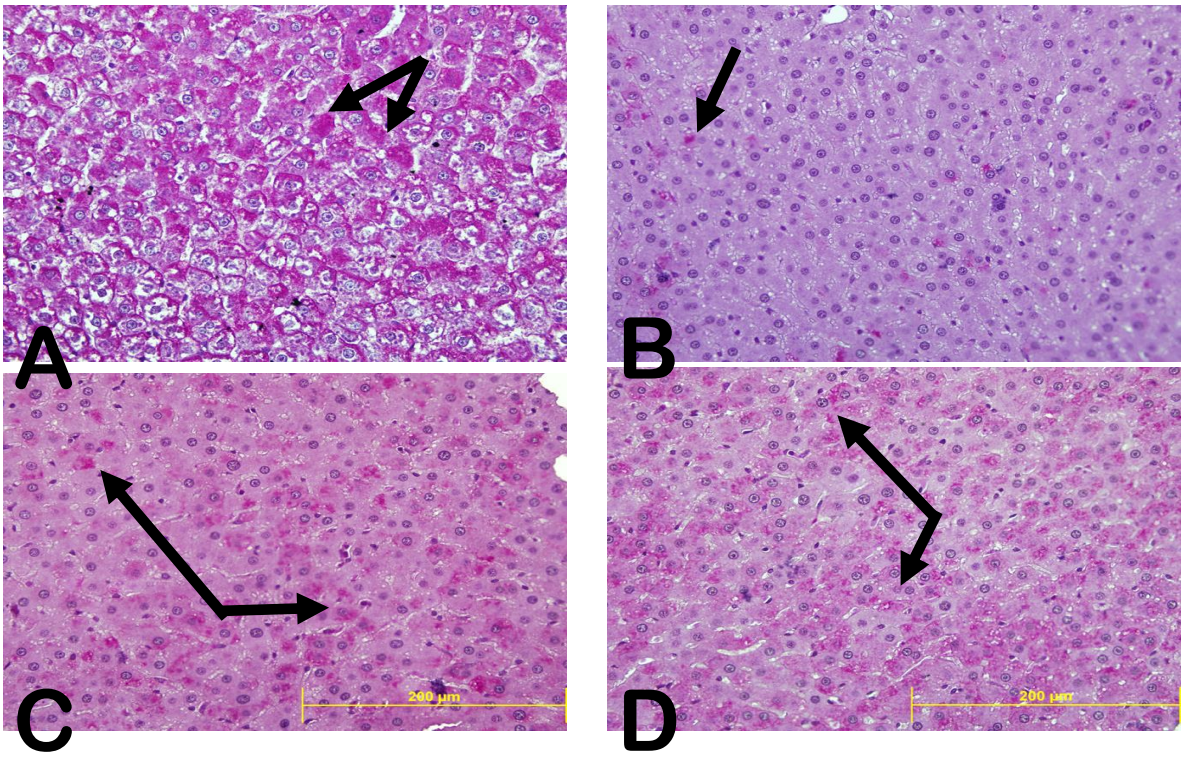


Plate 2(a): Representative photomicrograph taken from livers PAS stain positive for Glycogen

In Normal Control Panel A, liver tissue is normal with normal architecture with perfectly arrange central vein (C.V) and hepatic cords with more positive cells stain for glycogen. Diabetes Control Panel B showing hepatocyte vacuolization and fatty changes, necrosis, dilation of hepatic sinusoids with little positive cells for glycogen. Okra water fraction (Panel C), Okra treated water fraction (Panel D) liver tissue is normal with normal architecture with perfectly arrange central vein (C.V) and hepatic cords and few necrosis, less degenerative changes, and vacuolization. H&E, 100x.

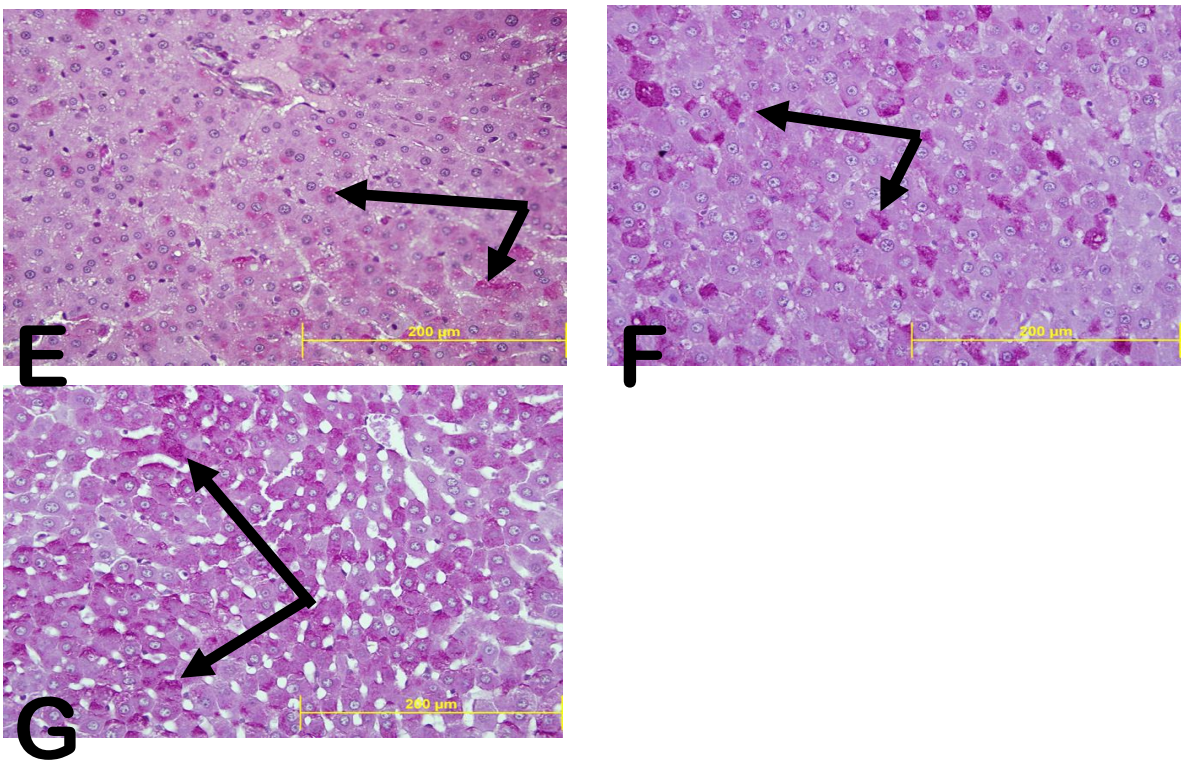


Plate 2(b): Representative photomicrograph was taken from livers PAS stain positive for Glycogen

Okra Phenolic fraction (Panel E), Okra treated phenolic fraction (Panel F), and Glibenclamide (Panel G) liver tissue is normal with normal architecture with perfectly arrange central vein (C.V) and hepatic cords with few necrosis, less degenerative changes, and vacuolization. H&E, 100x.

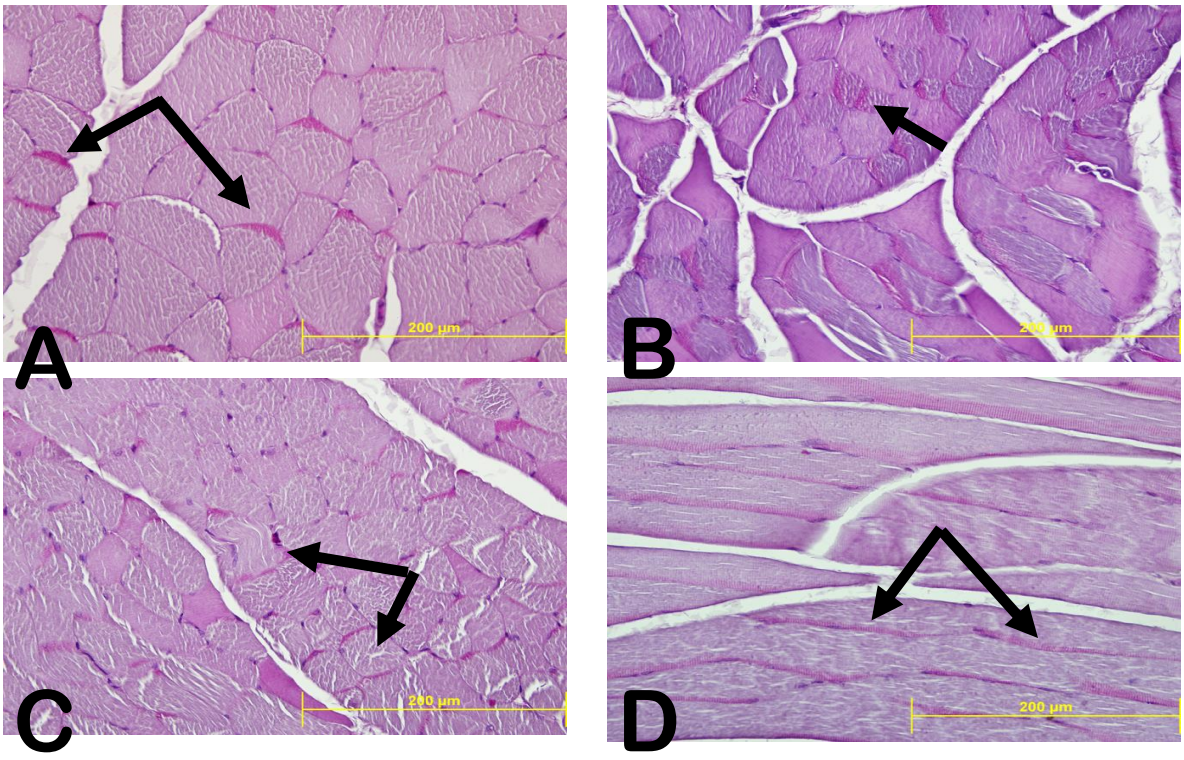


Plate 3(a): Representative photomicrograph taken from skeletal muscle PAS stain positive for Glycogen

Normal Control Panel A (revealed normal skeletal muscle architecture with more positive cells stain for glycogen), Diabetes Control Panel B showed increase muscle wasting and structural proteins degradation with disturbance of the architecture pattern. Okra water fraction (Panel C), treated Okra water fraction (Panel D) showed the normal structure of skeletal muscle and less degradation of structural protein with more positive cells for glycogen. H&E, 100x.

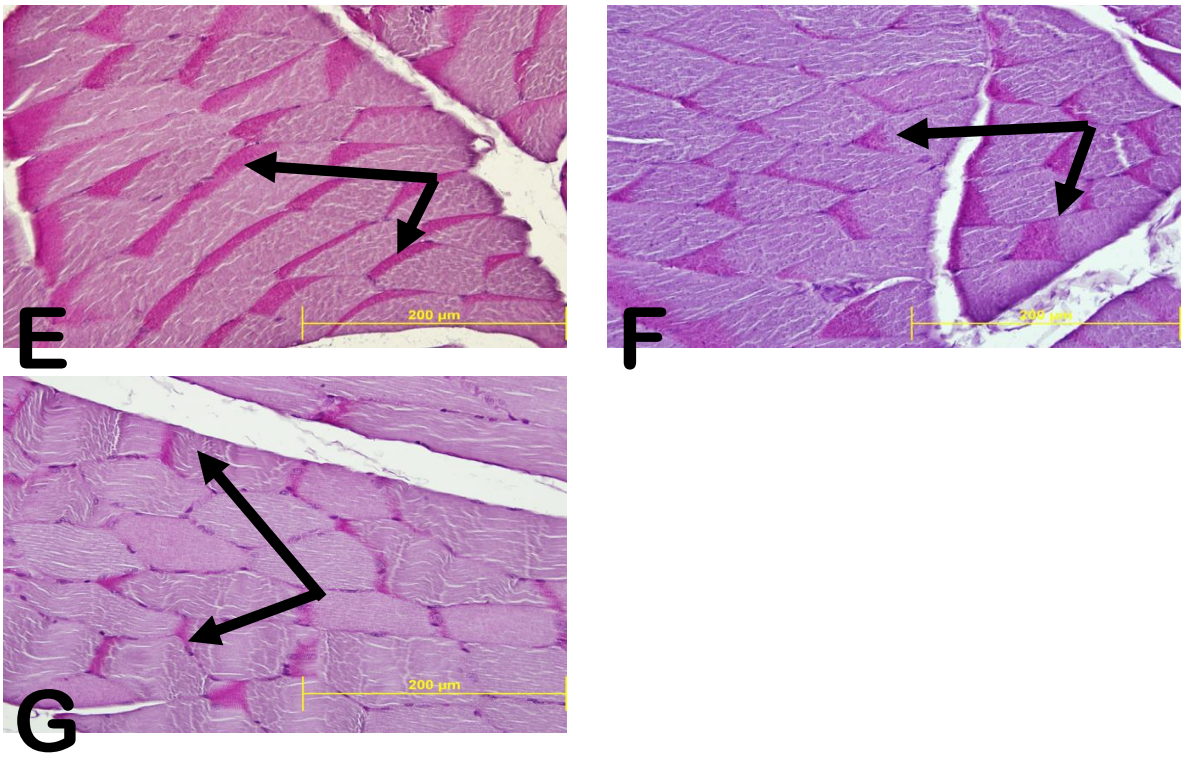


Plate 3(b): Representative photomicrograph taken from skeletal muscle PAS stain positive for Glycogen

Okra Phenolic fraction (Panel E), treated Okra phenolic fraction (Panel F), and Glibenclamide (panel G) showed the normal structure of skeletal muscle and less degradation of structural protein with more positive cells for glycogen. H&E, 100x.

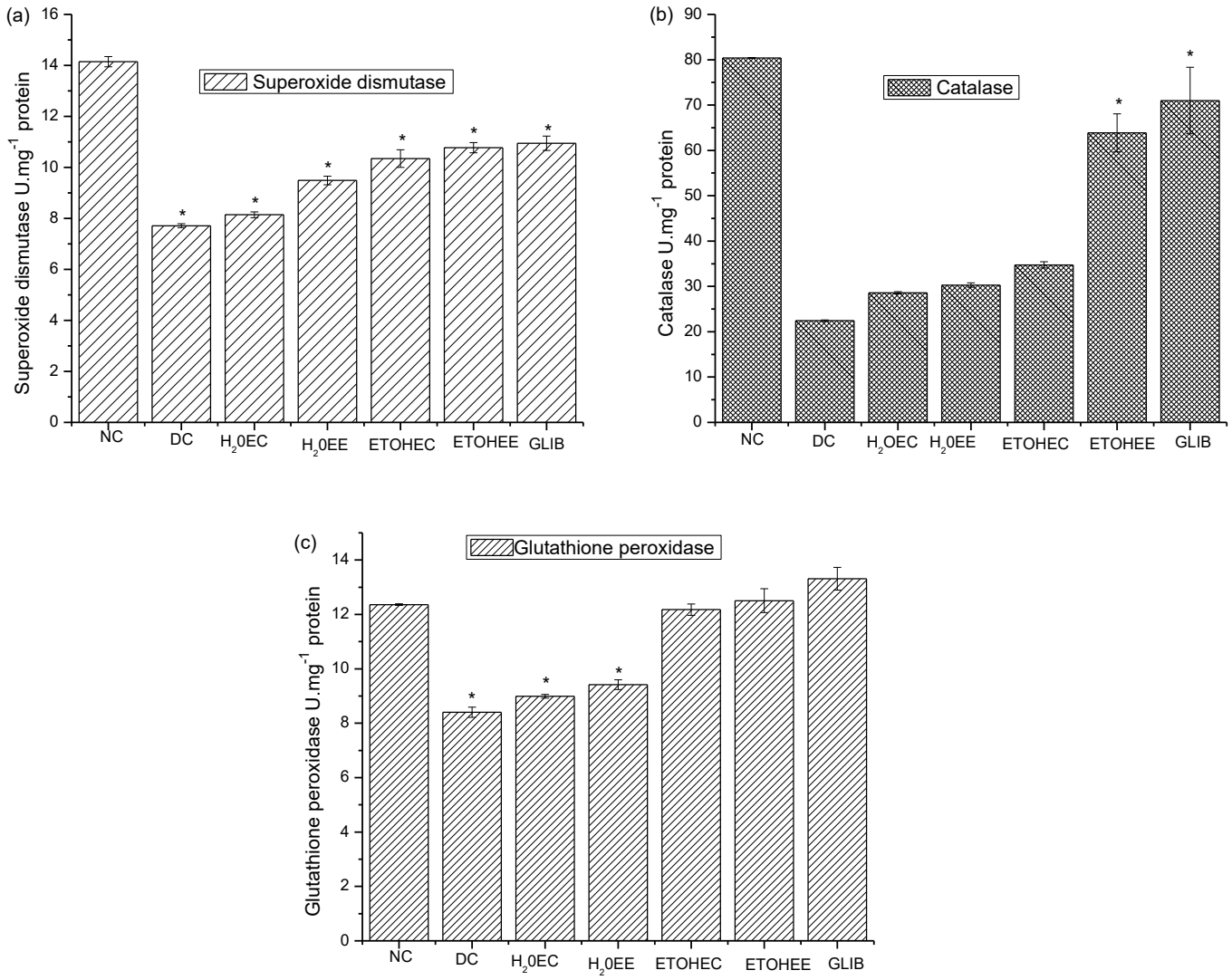


Fig. 59 (a-c): Effect of okra extracts on antioxidant enzymes in experimental animals

Data: Means ± SEM for the five rats

*Significant at $\alpha_{0.05}$ relatives to the control

NC = Control, DC = Diabetes control

H_2OEC = Water extract okra fruit without indole acetic acid

H_2OEE = Water extract indole acetic acid-treated okra fruit

ETOHEC = Ethanol extract okra fruit without indole acetic acid

ETOHEE = Ethanol extract indole acetic acid-treated okra fruit

GLIB = Glibenclamide.

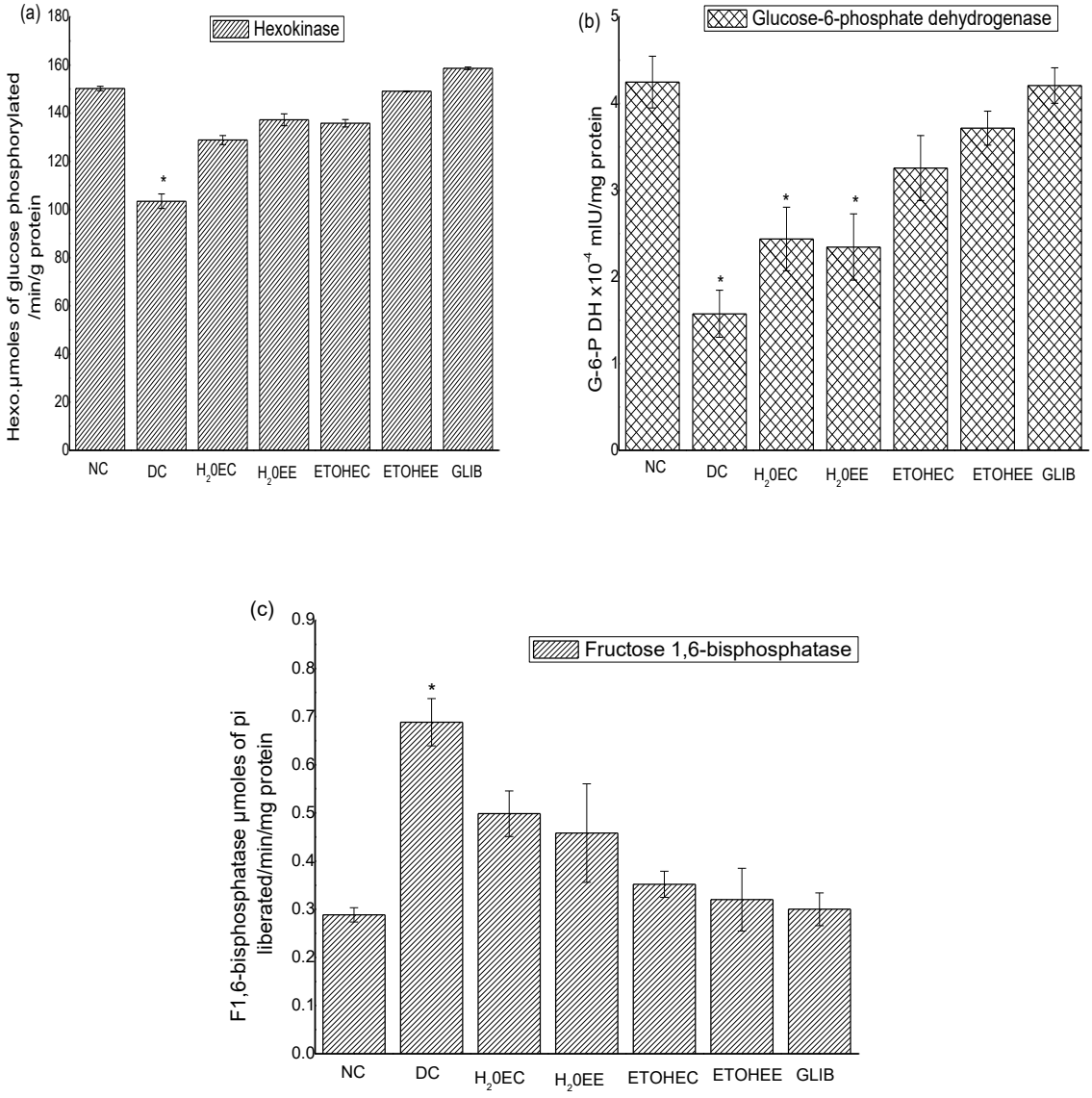
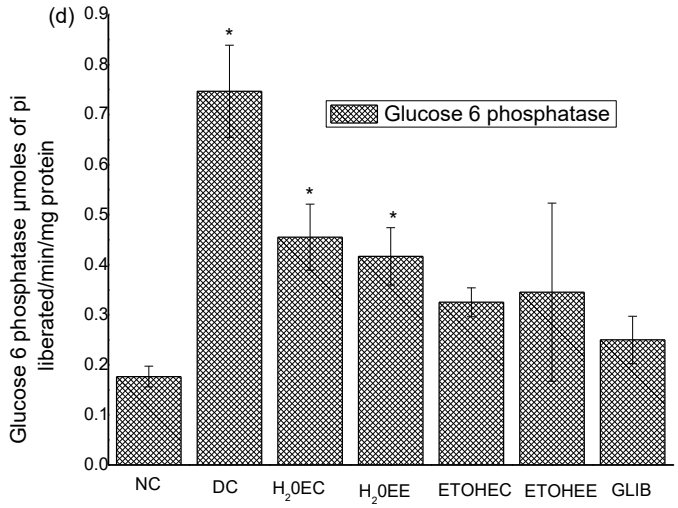


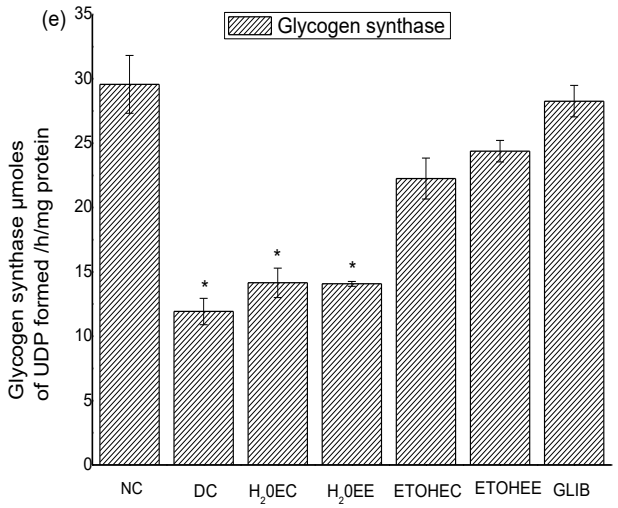
Fig. 60 (a-f): Effect of okra extracts on carbohydrate enzymes in experimental animals

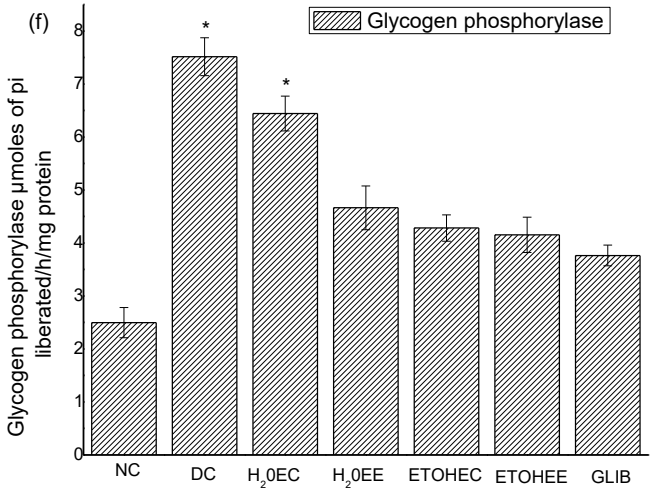
Data: Means ± SEM for the five rats

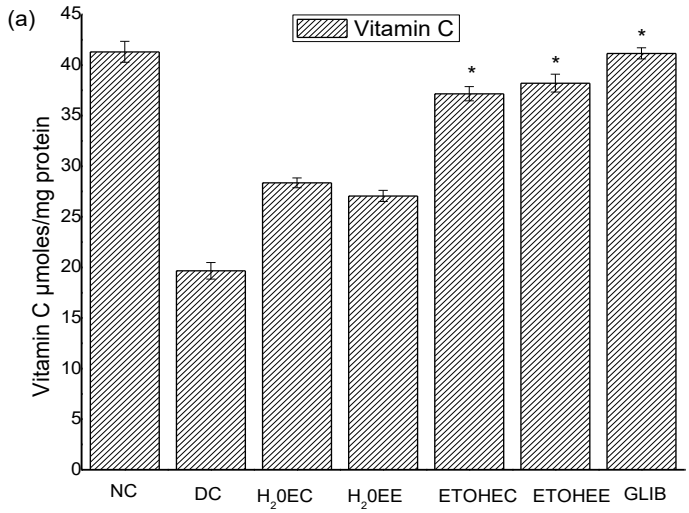
*Significant at $\alpha_{0.05}$ relatives to the control

Where; NC = Control, DC = Diabetes control, H_2OEC = Water extract okra fruit without indole acetic acid, H_2OEE = Water extract indole acetic acid-treated okra fruit, ETOHEC = Ethanol extract okra fruit without indole acetic acid, ETOHEE = Ethanol extract indole acetic acid-treated okra fruit, GLIB = Glibenclamide


(d)
Glucose 6 phosphatase
Glucose 6 phosphatase µmoles of pi liberated/min/mg protein
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
NC
DC
H20EC
H20EE
ETOHEC
ETOHEE
GLIB


(e)
Glycogen synthase
Glycogen synthase µmoles of UDP formed /h/mg protein
0
5
10
15
20
25
30
35
NC
DC
H20EC
H20EE
ETOHEC
ETOHEE
GLIB


(f)
Glycogen phosphorylase
Glycogen phosphorylase µmoles of pi liberated/h/mg protein
0
1
2
3
4
5
6
7
8
NC
DC
H20EC
H20EE
ETOHEC
ETOHEE
GLIB



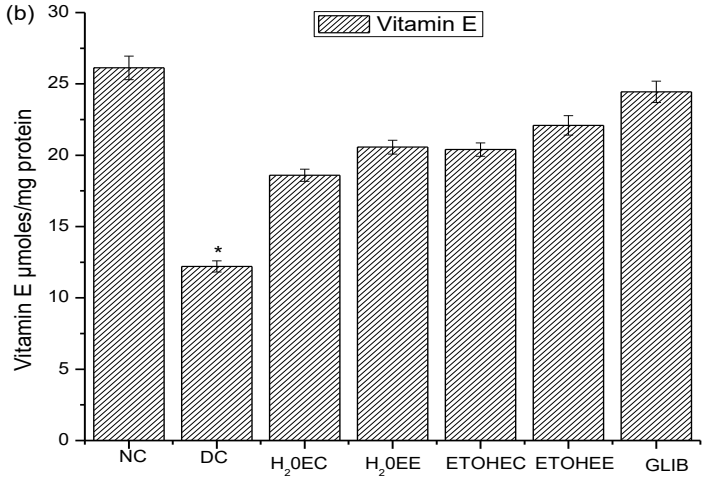


Fig. 61 (a, b): Effect of okra extracts on non-enzyme antioxidant enzymes in experimental animals

Data: Means ± SEM for the five rats.

*Significant at $\alpha_{0.05}$ relatives to the control and other groups.

Where; NC = Control, DC = Diabetes control, H_2OEC = Water extract okra fruit without indole acetic acid, H_2OEE = Water extract indole acetic acid-treated okra fruit, ETOHEC = Ethanol extract okra fruit without indole acetic acid, ETOHEE = Ethanol extract indole acetic acid-treated okra fruit, GLIB = Glibenclamide

4.7 Experiment 7: Assessment of the hypolipidemic effect of ethanol extract of indole acetic acid-treated okra fruits in triton-induced hyperlipidemia rats

Introduction

High lipid profiles causes coronary artery disease and cerebral vascular diseases (Shrivastava *et al*., 2009). Insulin resistance in obesity leads to the on set of Diabetes mellitus (Goedecke *et al*., 2009). Hayoz *et al*. (1998) observed high free radical's generation with a decrease in body defences antioxidant levels. Oxidative stress causes diabetic nephropathy during the initial stage of diabetes complications (Bonnnefont *et al*., 2000). Many chemical drugs have been used for controlling high serum lipid levels. However, based on the complications and cost implication, these drugs need to be avoided (Muscari *et al*., 2002). Okra is an edible vegetable and has been eaten in Nigeria because of its nourishing components. Traditionally, it is believed that the okra treats inflammation and various disorders and many more. More also, a number of previous studies have reported that okra possessed hypoglycemic effect (Liu *et al*., 2005). However, the use of bioregulators to improve its hypolipidemic potential is not yet unravelled. This study examined the hypolipidemic effect of indole acetic acid treated okra fruit in triton-induced hyperlipidemia rats.

Procedures

A 250 g sample of indole acetic acid treated okra and the non-indole acetic acid treated sample was dissolved in round bottom flask containing 75% ethanol in ratio 1:10 respectively i.e. 1875 ml of ethanol to 250 ml of distilled H_2O, and filled up to 2500 ml. A 250 g to 2500 ml in 1:10 was stir with the use of magnetic stirrer and temperature at $40^{\circ C}$ for 3 hours. After which they were decanted, sieved and transferred into the rotary evaporator equipment for total removal of ethanol and to concentrate the solution, before transferring into the freeze dryer machine in order to get soluble powder, which is stored for the experiment.

Treatment of rats with Triton-X-100 (100 mg/kg bw) to induce hyperlipidemia was outlined in section 3.4.21. Determination of glucose content was outlined in section 3.4.21.1.

The lipid profiles were measured as described in sections 3.4.21.3 – 3.4.21.6.

Relative body weight was determined as outlined in section 3.4.21.7.

Preparation of tissue homogenate for determination of malondialdehyde level, HMG-CoA reductase activity and total protein were described in sections 3.4.21.8 – 3.4.21.11.

Results

As shown in Figure 62, increased in blood glucose levels were observed in the diabetes control and 200 mg/kg of indole acetic acid-treated groups on the fourth week of okra fruit extract administration relative to the other experimental groups. However, in the other groups administered with okra fruit extract showed no increase in the serum glucose levels. The okra fruit extract administration was observed to be dose-dependent in serum glucose level reduction.

In Figure 63, body weights of experimental rats were observed to be increased after four weeks of treatment with extracts of okra fruit (200 and 400 mg/kg) and atorvastatin (30 mg/kg p.o.) respectively.

In Figure 64, Triton-X-100 induction increased the liver weight of the disease control group. However, no effect was observed on relative heart weight after four weeks of treatment relative to the control group. Administration of okra fruit extracts and atorvastatin on the second week of triton injection through fourth week of experiment decreased liver weight of experimental rats. However, no significant effect was observed in the heart weight.

Triton induction significantly ($p< 0.05$) increase serum lipid profile contents and atherogenic index in disease control relative to the control group, but administration of okra fruit extracts (especially the groups treated with indole acetic acid-treated fruit) and atorvastatin reduced the serum lipid profile contents and atherogenic index with an increase levels of high-density lipoproteins in all groups with little or no effect in group treated with atorvastatin relative to the control group (Figure 65).

In Figure 66, result showed that okra fruit extract has no effect on total protein. In Figure 67 there were increased levels of HMG and mevalonate after induction of the Triton-X-100 in the disease group, but the administration of okra fruit extracts reduced mevalonate and HMG levels in all experimental animals relative to normal control. However, after the administration of atorvastatin and 400 mg/kg indole acetic acid treated okra fruit extract, a reduction in HMG: Mevalonate ratio was observed relative to the disease control. This is an indication of low activity of HMG-CoA reductase.

Figure 68, shows the okra fruit extracts effect on malondialdehyde (MDA) level in the experimental rats. Triton induction raised the level of malondialdehyde in disease control and

200 mg/kg okra fruit extract without indole acetic acid treated groups. However, administration of 400 mg/kg indole acetic acid-treated okra fruit extract and 30 mg/kg atorvastatin showed a decrease in malondialdehyde levels relative to the disease control group (Figure 68).

Conclusion

Triton is a chemical used to induce hyperlipidemia through its cytotoxic effect on low-density lipoprotein (LDL) receptor protein. Hyperlipidemia causes an abnormal increase in cholesterol level in the blood, which results in insulin resistance a major cause of hyperglycemia. In conclusion, ethanol extract of indole acetic acid-treated okra fruit and administration at a dose of 400 mg/kg showed similar action with the standard drug (atorvastatin); hence this concentration is an effective dose for the okra fruit extract hypolipidemic potential.

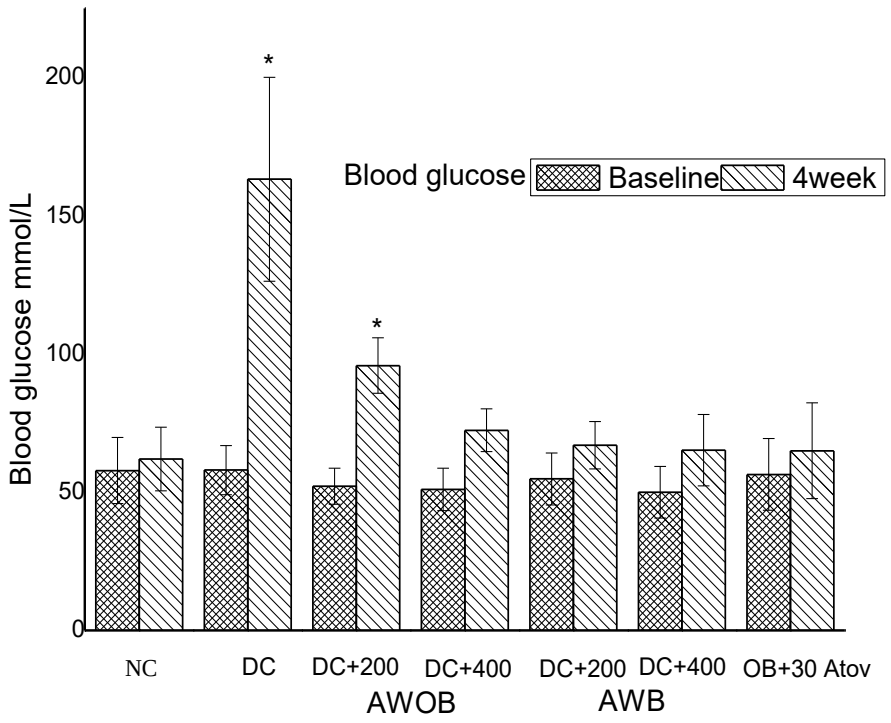


Fig. 62: Serum glucose level of experimental animals under the influence of okra extracts on

Data: Means ± SEM for the five rats

*Significant at $\alpha_{0.05}$ relatives to the control

Where NC = Control

DC = Disease control

DC + 200 = Disease control + 200mg/kg of the okra extract

DC + 400 = Disease control + 400mg/kg of the okra extract

DC + 30 Atov. = Disease control + 30mg/kg of atorvastatin

AWOB = Okra fruit extract without indole acetic acid

AWB = Okra fruit extract with indole acetic acid

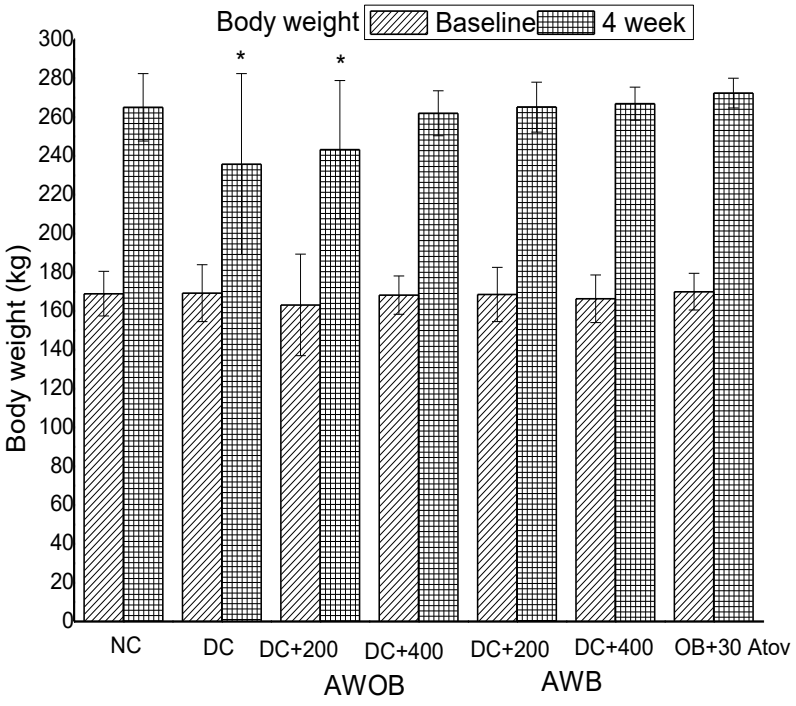


Fig. 63: Okra fruit extract effect on body weight of experimental animals

Data: Means ± SEM for the five rats

*Significant at $\alpha_{0.05}$ relatives to the control

Where NC = Control

DC = Disease control

DC + 200 = Disease control + 200 mg/kg okra extract

DC + 400 = Disease control + 400 mg/kg okra extract

DC + 30 Atov. = Disease control + 30 mg/kg of atorvastatin

AWOB = Okra fruit extract without indole acetic acid

AWB = Okra fruit extract with indole acetic acid

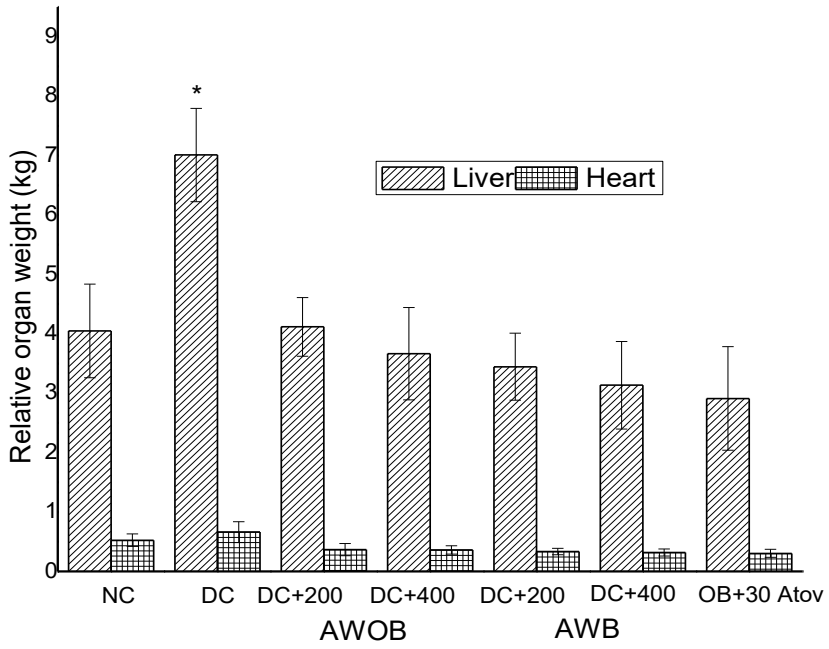


Fig. 64: Effect of okra extract on relative liver and heart weight of experimental animals

Data: Means ± SEM for the five rats

*Significant at $\alpha_{0.05}$ relatives to the control and other groups

Where NC = Control

DC = Disease control

DC + 200 = Disease control + 200 mg/kg okra extract

DC + 400 = Disease control + 400 mg/kg okra extract

DC + 30 Atov. = Disease control + 30 mg/kg of atorvastatin

AWOB = Okra fruit extract without indole acetic acid

AWB = Okra fruit extract with indole acetic acid

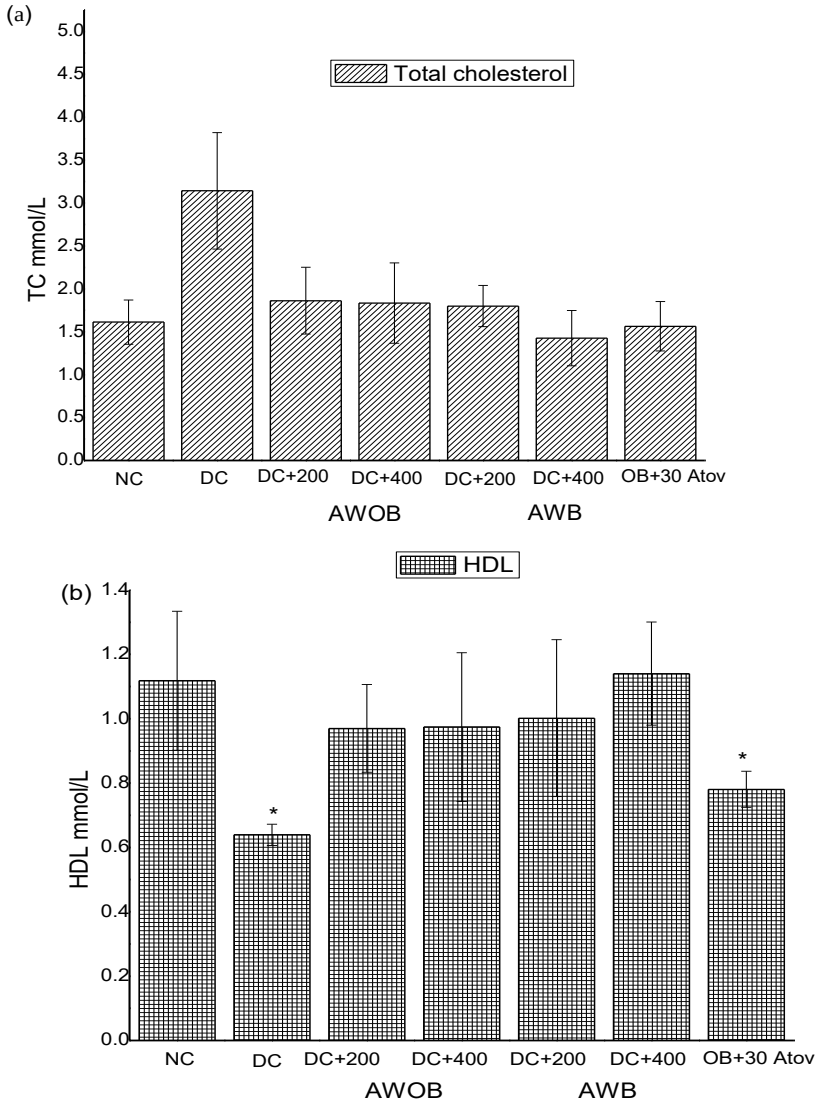
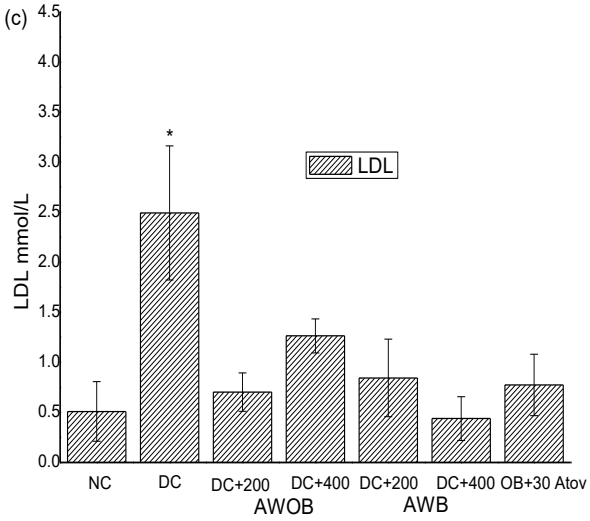


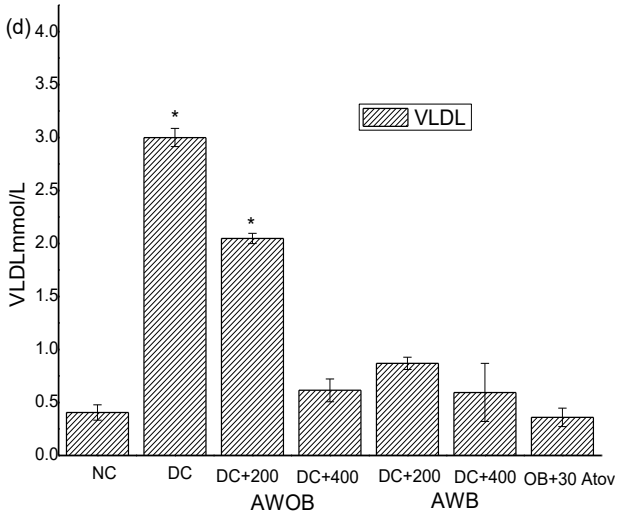
Fig. 65 (a-f): Effect of okra extract on experimental animals' lipid profiles

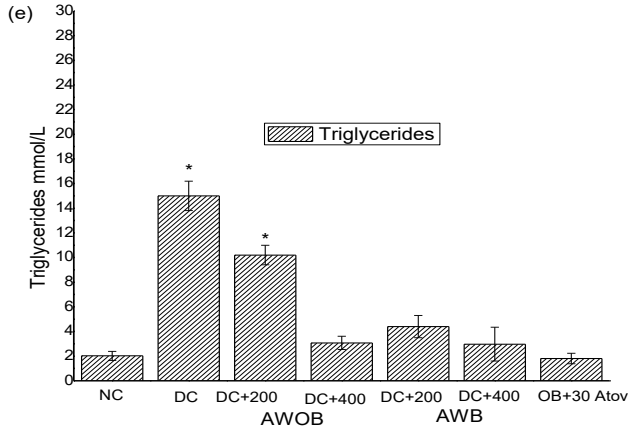
Data: Means ± SEM for the five rats.

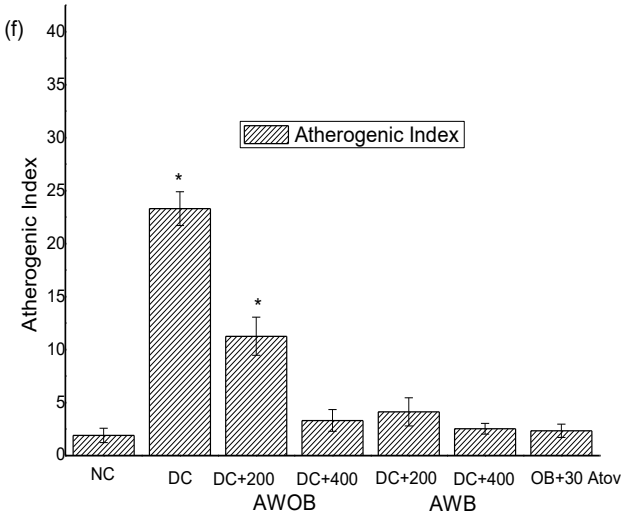
*Significant at $\alpha_{0.05}$ relatives to the control and other groups

Where; NC = Control, DC = Disease control, DC + 200 = Disease control + 200 mg/kg okra extract, DC + 400 = Disease control + 400 mg/kg okra extract, DC + 30 Atov. = Disease control + 30 mg/kg of atorvastatin, AWOB = Okra fruit extract without indole acetic acid, AWB = Okra fruit extract with indole acetic acid.


(c)
LDL mmol/L
LDL
NC
DC
DC+200
DC+400
DC+200
DC+400
OB+30 Atov
AWOB
AWB


(d)
VLDLmmol/L
VLDL
NC
DC
DC+200
DC+400
DC+200
DC+400
OB+30 Atov
AWOB
AWB


(e)
Triglycerides mmol/L
Triglycerides
NC
DC
DC+200
DC+400
DC+200
DC+400
OB+30 Atov
AWOB
AWB


(f)
Atherogenic Index
Atherogenic Index
NC
DC
DC+200
DC+400
DC+200
DC+400
OB+30 Atov
AWOB
AWB

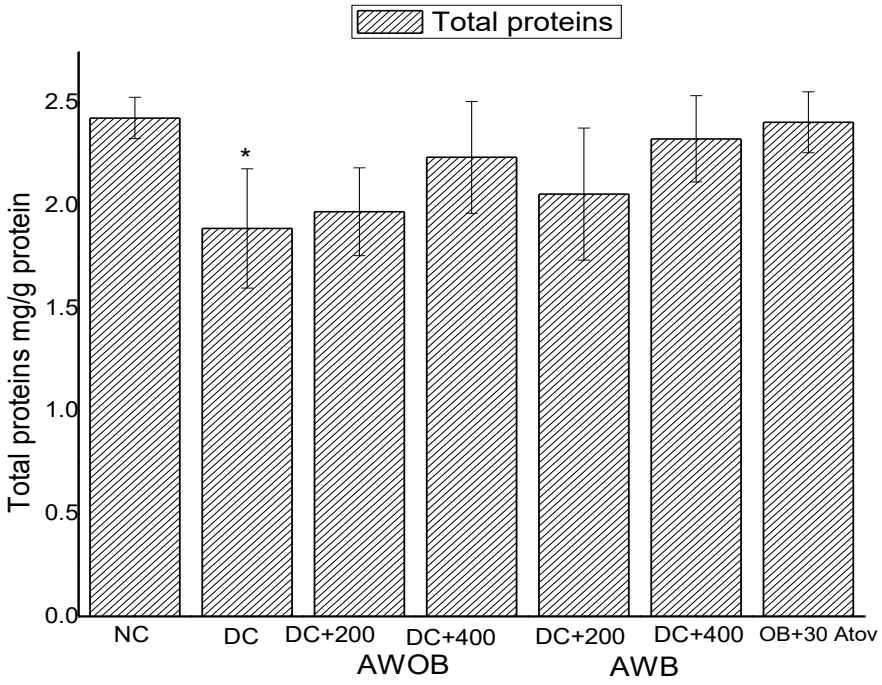


Fig. 66: Effect of okra extracts on total proteins of experimental animals

Data: Means ± SEM for the five rats

*Significant at $\alpha_{0.05}$ relatives to the control

Where NC = Control

DC = Disease control

DC + 200 = Disease control + 200 mg/kg okra extract

DC + 400 = Disease control + 400 mg/kg okra extract

DC + 30 Atov. = Disease control + 30 mg/kg of atorvastatin

AWOB = Okra fruit extract without indole acetic acid

AWB = Okra fruit extract with indole acetic acid

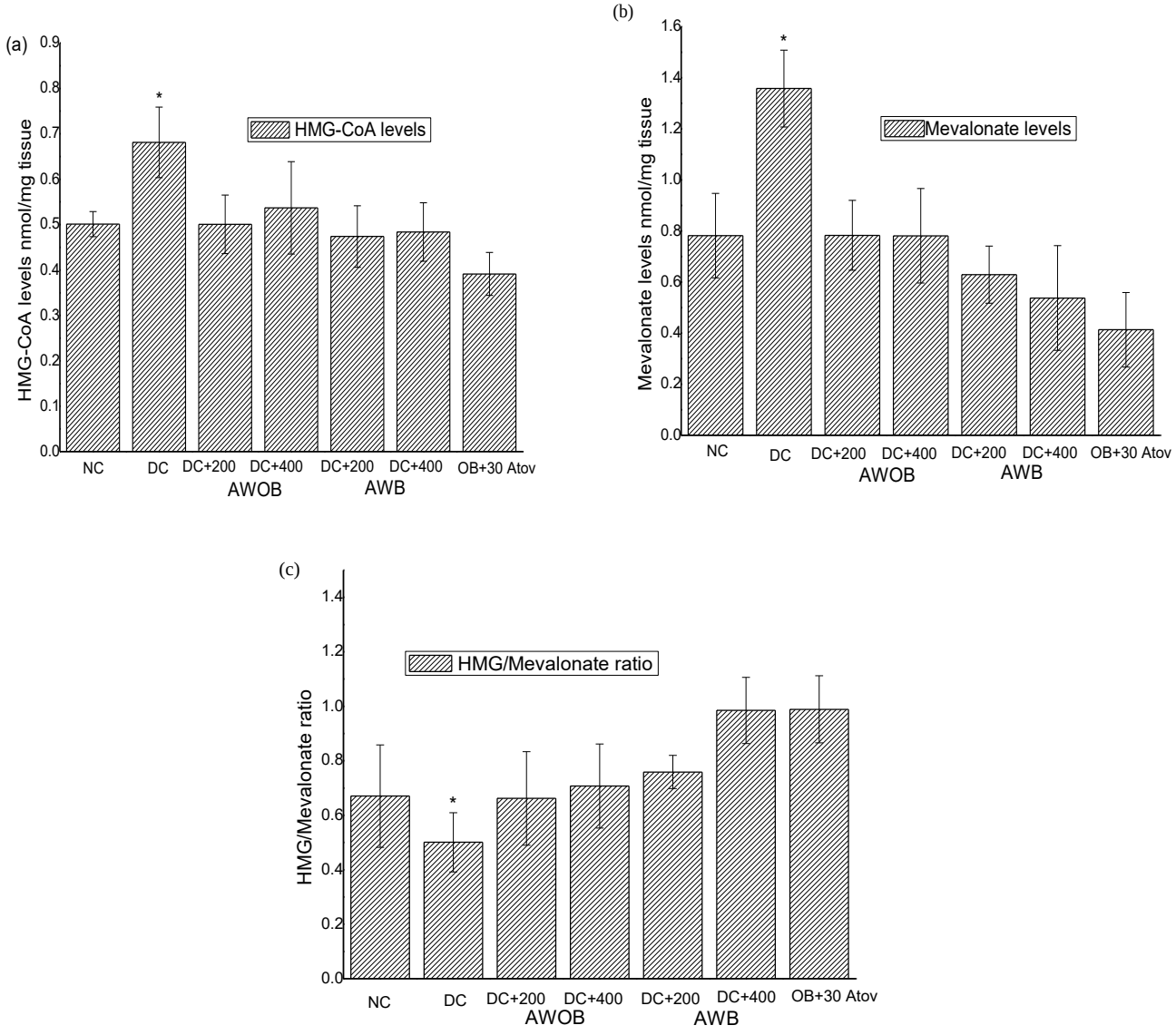


Fig. 67 (a-c): Effect okra extract on HMG-CoA reductase activity in experimental animals

Data: Means ± SEM for the five rats

*Significant at $\alpha_{0.05}$ relatives to the control and other groups

Where; NC = Control, DC = Disease control, DC + 200 = Disease control+ 200 mg/kg okra extract, DC + 400 = Disease control + 400 mg/kg okra extract, DC + 30 Atov. = Disease control + 30 mg/kg of atorvastatin, AWOB = Okra fruit extract without indole acetic acid, AWB = Okra fruit extract with indole acetic acid.

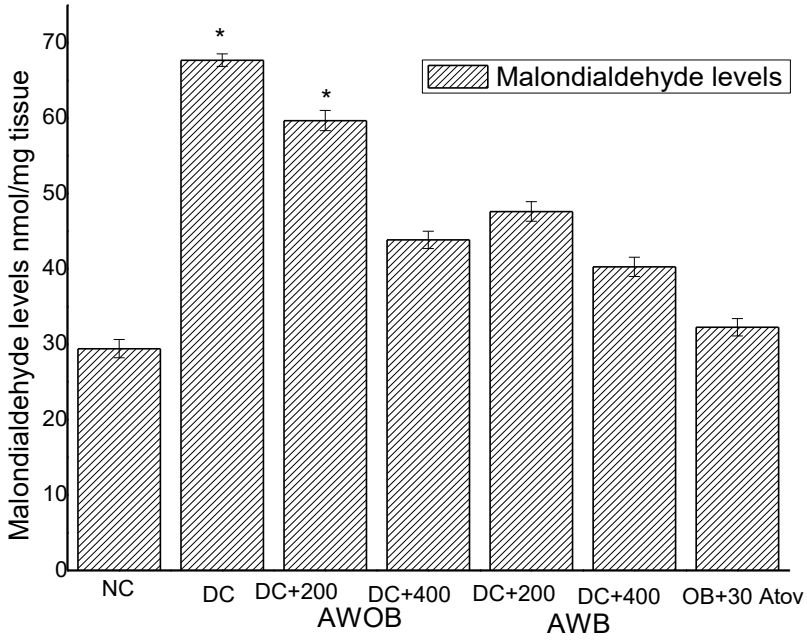


Fig 68: Malondialdehyde level under the influence of okra extract in experimental animals

Data: Means ± SEM for the five rats

*Significant at $\alpha_{0.05}$ relatives to the control and other groups

Where NC = Control

DC = Disease control

DC + 200 = Disease control + 200 mg/kg okra extract

DC + 400 = Disease control + 400 mg/kg okra extract

DC + 30 Atov. = Disease control + 30 mg/kg of atorvastatin

AWOB = Okra fruit extract without indole acetic acid

AWB = Okra fruit extract with indole acetic acid

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

This study examined the ameliorative potential of bioregulators in salt stressed okra plant genotypes (47-4 and LD 88). Salt stress hinders plant growth and development (Misra and Gupta 2006; Munns and Tester, 2008). Data presented in Figure 19 showed a decrease in photosynthetic pigments in salt-stressed groups, this may be possibly due to the salt toxicity on the photosynthesis pigments. This result was similar to the observation of Rao and Rao (1981). In Table 5 increased carotenoid levels under the salinity stress groups may represent the antioxidant role of carotenoids. Igbal *et al*. (2006) reported a similar result. Treatment with either indoleacetic acid or salicylic acid increased the osmotic pressure of cytoplasm which in turn increases the active solutes like soluble sugars and protein. Changes in these compatible solute contents may result to higher yield and increase mineral concentrations (Figures 23 and 24). It is noteworthy that indoleacetic acid or salicylic acid treatments in okra plant improved the accumulation of calcium, potassium and zinc contents in the root, shoot and leaf when compared with NaCl-treated control. This may suggest the osmoregulatory role of these minerals. Therefore, it is observed that both IAA and SA have an alleviating effects on salinity stress in plants and thus increase crop yield in terms of growth and biochemical parameters. Therefore, pretreatment of okra seeds (Genotypes 47-4 and LD 88) with either SA or IAA, in salt stress increase okra plants tolerance, through prevention of chloroplast degradation, and enhancement of photosynthetic pigments biosynthesis. Thus okra genotype 47-4 responds positively to IAA treatment than SA treatment, while genotype LD 88 responds to SA treatment than IAA treatment.

Free amino acid (proline) is generated in salt stressed plant. Proline is an active metabolite which reduces negative effects of salinity stress (Parviz and Satyawat, 2008). In Table 12 accumulation of proline in the leaves and the fruits of both genotypes 47-4 and LD 88, could be attributed to salinity tolerance in the genotypes. Amin *et al*. (2009) reported a similar result. Kishor *et al*. (2005) also observed increase proline biosynthetic enzymes in the salt-stressed plant. Data presented in Figure 28 revealed higher soluble sugar and protein accumulation in the fruits than the leaves of salt stress okra plant genotype 47-4. Richardson and McCree

(1985) reported similar result. Lower accumulation of the compatible solutes might be due to the salt stress effects on the genotypes, which was more in genotype LD 88 (Figure 38). Yordanov *et al*. (2003) and Hamdia, (2004) recorded similar results in their studies. Therefore, IAA and SA concentrations (10^{-2} and 10^{-4} mM SA and 0.4 and 0.5 mM IAA) increased tolerance of okra plant under salt stress.

Antioxidants scavenge and prevent reactive oxygen species when consumed in food ((Pieroni and Price, 2006). Data presented in Figures 41 and 42 revealed an increase in superoxide dismutase activities with increasing salinity stress level concentrations, especially at severe levels of salt stress. However, catalase and glutathione peroxidase at 200 mM NaCl showed low activities relative to the control group (0 mM NaCl). Meanwhile, concentrations of SA and IAA (10^{-4} mM and 0.4 mM) increased activities of catalase respectively (Figures 43 and 44). This observation was in concordant with the reports of Lee *et al*. (2001); Mittler, (2002); Mittova *et al*. (2003); Shingh and Prasad, (2009). Free radical scavenging activity of okra plant under the effects of bioregulators (IAA or SA) and salinity was observed to be enhanced especially in 10^{-2} mM and 10^{-4} mM SA concentrations at all the levels of salinity stress in the okra (47-4 and LD 88) genotypes (Figure 51). Data obtained in Figure 53b showed an increase levels of phytonutrients in indole acetic acid-treated okra plant. This shows that both indole acetic acid and salicylic acid improve tolerance of okra plant under salt stress.

Glucose transporter mechanism of action in the system greatly depends on facilitated glucose carriers in the cell membrane internal milieu (James *et al*., 1988). This experiment shows the inhibitory effects of okra fruit extract on intestinal glucose transporters using *Xenopus lavis* oocytes model and E. coli strain (JMI09XL-Blue) for the GLUT-14 variant of GLUT-2 and GLUT-4. Keller and Mueckler, (1990) reported *Xenopus lavis* oocyte as a model for the study of glucose transporters based on its low glucose transport activity. Result demonstrated that, the okra fruit extract posseses bioactive compound that improved the GLUT-14 activity in the oocytes (Figure 54). The low uptake of 2-deoxy –D-glucose observed in water-injected oocytes might be due to the low activity of endogenous glucose transporter GLUT-14. Keller and Mueckler, (1990) reported a similar observation. Suzawa et al., (2007) reported frog embryos GLUT-14 mediated the uptake of 2-deoxy –D-glucose. Therefore, based on the result obtained, okra extracts have no inhibitory effect on the activity of GLUT-14 cRNA when oocytes containing GLUT-14 cRNA were incubated in the okra fruit extracts. Thus, the

uptake of the 2-deoxy-D-glucose by water injected may be subjected to the remnants of the radioactivity on the surface of the oocytes and may not necessary be the effect of glucose transporter. This shows that okra fruit extract could be used for prophylaxis and management of diabetes mellitus.

Streptozotocin induces diabetic mellitus in experimental animals (Matteucci and Giampietro, 2008). Administration of streptozotocin (50 mg/kg bw i.v) causes pancreatic β-cells DNA fragmentation and thus decrease insulin secretion, which leads to hyperglycemia (Saini, 2010; Stephen *et al*., 2012). Coskun *et al*. (2005) reported regeneration of pancreatic β-cells under the influence of STZ with an antioxidants supplement.

Muscle wasting might be responsible for the reduction of weight observed in the experimental rats (Figure 55). Similar observation was reported by Shokeen *et al*., (2008). Result obtained in Figure 56 revealed that okra fruit extract caused a reduction in the level of plasma glucose during the experimental period to some extent. The diabetic control group blood glucose levels remained high throughout the experiment. In contrast, especially in the ethanol extract indole acetic acid treated okra fruit (ETOHEE) and glibenclamide groups, the blood glucose levels reduced throughout the experiment. Repeated administration of okra fruit extract prevented the elevation levels of lipid profile in the experimental rats (Figure 57). Since insulin resistance is resulted from prolonging increased in the blood level cholesterol which was observed in diabetic control. Decreased in the weights of some tissues in the experimental rats may be possibly due to the breakdown of glycogen and an impaired pancreatic β-cells respectively.

Concomitant depletion of abdominal and peripheral fats in all treated groups showed the response to oxidative stress exerted by the streptozotocin, which can also predispose the experimental animals to the risk of atherosclerosis (Figure 58). In Figure 59 reduction in the level of enzyme superoxide dismutase activities observed could be the effect of its glycation at specific lysine residues, which possibly leads to its inactivation. Maritim *et al*., (2003) observed similar result. A dose of 100 mg/kg okra fruit extracts of water extract of okra fruit without indole acetic acid (H_20EC), water extract indole acetic acid treated okra fruit (H_20EE), ethanol extract of okra fruit without indole acetic acid (ETOHEC) and ethanol extract indole acetic acid treated okra fruit (ETOHEE) revealed an increse in SOD, GPx and CAT levels (Figure 59). Restoration of antioxidant enzymes levels by a dose of 100 mg/kg okra fruit

extracts H_20EC, H_20EE, ETOHEC and ETOHEE could be as a result of decreased in H_2O_2 formation. Szaleczky *et al*. (1999) reported similar result. More also, reduction in the activity of hexokinase (Figure 60a) may be correlated to the deficient of insulin. This result is in tandem with the one reported by Ramachandran and Saravanan, (2013). However, improved hexokinase with decreased glucose-6-phosphate dehydrogenase activities in the liver (Figures 60 a and b) may suggest a reduction in the metabolism of glucose. Similar result was observed by Prasath, and Subramanian, (2011). Therefore, ethanol extract of indole acetic acid treated-okra fruit exhibits higher antihyperglycemic activities in STZ-induced rats.

Triton is a chemical used to induce hyperlipidemia effect on low-density lipoprotein (LDL) receptor protein (Chander *et al*., 2003). Hyperlipidemia causes an abnormal increase in cholesterol level in the blood, which results to insulin resistance, a major cause of hyperglycemia. In Figure 62 an excessive increased plasma glucose level in disease group may possibly due to insulin resistance. Observed loss in body weight of disease control group (Figure 63) could be attributed to muscle wasting. Also, high weight of the liver in the disease group (Figure 64) may be due to improper oxidation of fat in the liver. Increased serum lipids concentrations in disease control (Figure 65) could be attributed to free fatty acids mobilization from the peripheral deposit. A similar report was observed by Ahmed *et al*., (2001). Takahashi and Yoshimoto (2005) reported that hyperlipidemia promotes accumulation of oxidative low-density lipoproteins and thus promote endothelial dysfunction and eventually leads to onset of congestive heart diseases. In all the experimental rats, triton induced hyperlipidemia, as indicated by increased lipid profile levels in the serum. High level of HMG-CoA activity observed in the disease control rats (Figure 67) was as a result of continuous cholesterol synthesis. Result obtained in the Figure 68 showed a high level of malondialdehyde in the disease control rats. This indicates the extent of free radical generated in the group. The antihyperlipidemic activity of okra fruit extract (200 and 400 mg/kg) against disease control group (Triton -X-100) showed a decrease in atherogenic index, lipid profile, blood glucose level and HMG-CoA activity with an increase in HDL which depend on the dosage as compared with the standard drug (atorvastatin)-treated group. The extract of indole acetic acid treated okra fruit (400 mg/kg) showed maximum protective effect, and the result was similar with atorvastatin. Therefore, 400 mg/kg dose of okra fruit extract is an effective dose for its hypolipidemic potential.

5.2 Conclusion

Pre-sowing seed treatment with indole acetic acid or salicylic acid increased salt tolerance in okra plant through improved accumulation of compatible solutes and antioxidant enzymes in okra plant tissue. The concentrations of 0.4 and 0.5 mM IAA and 10^{-2} and 10^{-4} mM SA improved okra plant tolerance under the influence of salinity stress. Phytochemical constituents such as phenol and flavonoids in the okra fruit might be responsible for its antidiabetic effects. Moreover, bioactive compounds in the extracts of okra fruit used showed no inhibitory effect on JM109XL-Blue GLUT-14 activity, but rather improved its activity. Thus okra fruit extract could be used for prophylaxis and management of Diabetes mellitus. Furthermore, the okra fruit extract restored plasma glucose and insulin levels, glycogen deposit, hexokinase, fructose 1, 6-bisphosphatase and antioxidant enzymes activities in the experimental rat's tissues. Also, the extract of indole acetic acid-treated okra fruit (400 mg/kg) showed similar effects relative to the standard drug (atorvastatin) used for the treatment of hyperlipidemia.

5.3 Contributions to knowledge:

1. Okra seeds pretreated with indole acetic acid (IAA) and salicylic acid (SA) improved salt tolerance of okra plant through the accumulation of compatible solutes.
2. IAA and SA (0.4 and 0.5 mM) and (10^{-4} and 10^{-2} mM) concentrations respectively could be the promising concentrations for salt tolerance in okra plant.
3. Aqueous and ethanol extracts of okra fruits treated with indoleacetic acid improved glucose transporter activity. Therefore, this makes okra fruit extract useful for the prophylaxis and management of Diabetes mellitus.
4. Ethanol extract of IAA-treated okra fruits reduced the levels of blood glucose and lipid profile in streptozotocin-induced experimental rats. Therefore, indole acetic acid improved the potency of the okra fruit extract.
5. Ethanol extract of IAA-treated okra fruits reduced hyperlipidemia in the experimental rats in a dose dependent manner.
6. Ethanol extract of IAA-treated okra fruits (400 mg/kg bw) helps to reduce the HMG-CoA reductase activity in the same manner with the standard drug (atorvastatin), which in turn reduces the rate of cholesterol synthesis.

7. Indoleacetic acid or salicylic acid treatments improved the accumulation of calcium, potassium and zinc contents in the root, shoot and leaf of salt stressed okra plant.
8. Indoleacetic acid or salicylic acid treatments improved the flavonoids and phenolic contents in the fruits of okra plant.
9. Indoleacetic acid or salicylic acid treatments improved the activities of antioxidant components of salt stressed okra plant.

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