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A STUDY ON THE ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECTS OF *AFRAMOMUM SCEPTRUM* AND *PARINARI CONGENSIS* SEED EXTRACTS IN ALLOXAN -INDUCED DIABETIC WISTAR RATS

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ABSTRACT

Multiple biochemical pathways and reactions accompanying oxidative stress and subsequent release of free radicals have been suggested in the deleterious condition of hyperglycemic. These conditions trigger inflammatory responses as well. The extent at which hyperglycemia progresses to diabetes is rapidly increasing and requires continued life-long management and control to impede high morbidity and premature mortality rate arising from the disease. Plant products have long been utilized to handle high glucose because of little or no side-effects attached. Most of these plants have inherent antioxidant and anti-inflammatory potentials due to presence of potent bioactive components. Thus, antioxidant and anti-inflammatory potentials of combined seed extracts of Aframomum sceptrum and Parinari congensis in alloxan induced hyperglycemic rats were investigated. Acute toxicity studies showed no sign of toxicity and death at relatively high doses of 4000mg/kg B.W of aqueous and ethanol extracts of combined seeds administered. Thus, $1/10^{th}$ of this dose (400mg/kg B.W) was considered a fixed dose for this study. Hyperglycemia in experimental rats was induced by a single intraperitoneal administration of alloxan (100 mg/kg B.W) The rats were group and administered with aqueous and ethanol extracts of Aframomum sceptrum and Parinari congensis seeds (400mg/kg bwt) for 21 days. Metformin was used as reference drug. Liver index, Liver marker enzymes, oxidative stress and pro-inflammatory markers were determined by standard test methods and commercial bioassay kits procured from reputable firms. The seed extracts significantly ($p<0.05$) decreased liver marker enzymes alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). Antioxidant enzymes SOD, CAT and GSH increased significantly ($p<0.05$), while MDA, TNF-α and IL-6 decreased significantly ($p<0.05$).In addition, results obtained for ethanol seed extracts (ETASPC) treatment of alloxan-rats was found to be comparable to metformin, reference drug. From the study, it can be deduced that the combined seed of Aframomum sceptrum and Parinari congensis synergistically exhibited antioxidant and anti-inflammatory activity preventing progression to diabetes and can be effectively used to handle control hyperglycemia in diabetes and its related complications.

KEY WORDS: *Synergistic, antioxidant, anti-inflammatory, hyperglycemia, ethanol metformin*

1.0 INTRODUCTION

Free radicals have been closely connected to critical factors underlying the pathogenesis of a wide variety of diseases which include cancer, coronary heart diseases, atherosclerosis and diabetes as decrease in antioxidant activity reflects. Antioxidant refers to any substance whose availability, even in minute concentration can inhibit or delay the oxidation of molecules and prevent the occurrences of disease. There are several endogenous and exogenous molecules that play important role in antioxidant defense mechanism which may be considered as biomarkers of oxidative stress and inflammation (Ullah *et al*., 2016).

Diabetes mellitus (DBM) is a disease that is characterized by chronic hyperglycemia due to deficiency in production or action of insulin or a combination of both (Giacco and Brownlee, 2010).The disease requires continued life-long management and control to reduce the high morbidity and premature mortality rate associated arising from macrovascular and microvascular complications (King *et al*., 1998, Ullah *et al*., 2016). Prolonged hyperglycemia can cause oxidative stress and subsequent production of reactive oxygen species (ROS) which can trigger the production of inflammatory cytokines (Pandey *et al*., 2012). These processes have been considered as critical factors underlying the pathogenesis of diabetes mellitus (DM), leading to high risk of complications (Ceriello, 2006, Ullah *et al*., 2016). Multiple biochemical pathways in the deleterious effects of chronic hyperglycemic- induced oxidative stress include overproduction of ROS from respiratory chain (ETC). Also auto-oxidation of glucose and generation of related advanced glycation end products (AGE) are related mechanisms of hyperglycemic induced toxicity (Giacco and Brownlee, 2010, Ibrahim *et al*., 2012).

The incidence of diabetes mellitus (DM) is rapidly increasing around the world (Nasri *et al*., 2015). There are several lines of chemo therapies that are used to handle the disease. However, they are usually very expensive and may produce serious side effects (Varughese *et al*., 2013). Majority of the population in developing countries frequently use medicinal plants as first source of health care to fight infectious and non-fectious diseases (Odugbemi, 2006, Ayoola *et al*., 2008, Ujowundu *et al*., 2015).Some of these plants are believed to suppress oxidative stress and the inflammatory processes which are generated in diseased conditions (Dash *et al*., 2005).Several reports have addressed the possible participation of medicinal plants in antioxidant and anti-inflammatory activities (Kumaran and Karunakaran, 2006, Nasri *et al*., 2015).

Aframomum sceptrum (Oliv. & T. Hanb.) K. Schum belongs to the Zingiberacea family while *Parinaricongensis*F.Didr belongs to the Chrysobalanacea family. These plants are geographically wide spread in tropical regions of Africa (Christenhusz and Brying, 2016). These seeds are locally used as spices in Nigeria to enhance the flavor and aroma of soups as well as several alcoholic drinks (Ndukwu and Ben-Nwadibia, 2005, Ogunka-Nnoka and Mepba, 2008, Erukainure *et al*., 2011). Ethnopharmacological survey revealed that these seeds are commonly used in traditional herbal formulations used in management of malaria, post natal womb discomforts and infections (Erukainure *et al*., 2011, Feitosa e*t al*., 2012). Some species of these seeds have been reported for hypoglycemic potentials and antidiabetic effects (Ighodaro *et al*., 2012, Dokubo *et al*., 2013). This study assess the antioxidant and anti-inflammatory potentials of combined seed extracts *of Aframomum sceptrum* and *Parinari congensis* in alloxan induced diabetic wistar rats.

2.0 MATERIALS AND METHODS

2.1 Collection of the Plant Materials

The seeds of *Aframomum sceptrum* and *Parinari congensis* were obtained from Mile 3 Market, Diobu. Port Harcourt. For identification, the plant materials were taken to the taxonomy unit of the Department of Plant science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria

2.2 Reagents and Chemicals

Ellmans reagent, Alloxan monohydrate, Thiobarbituric acid and pyrogallol were procured from Sigma Company, St. Louis, USA. 500mg Metformin was obtained from Letco Medical, Decatur, Alabama.. All other reagents and Chemicals ere of analytical grade supplied by the Department of Biochemistry, University of Port-Harcourt, Nigeria and procured from reputable firms.

The seeds of *Aframomum sceptrum* and *Parinari congensis* were sorted and ground to fine powder using a mechanical grinder. 100g each of the powdered sample was combined and macerated in 1000ml of water and ethanol with intermittent shaking to facilitate extraction at room temperature for 72hours. The extracts were filtered using a Buchner funnel and Whatman no. 1 filter paper. The resulting filtrates were evaporated to dryness using a rotary evaporator and later oven dried at a temperature of 50^{o}C and later reconstituted separately in 20% tween 80.The volume of extracts were administered according to the body weight of animals as described by (Erhirhe *et al*., 2014).

2.4 Experimental Animals

Wistar rats weighing between 150-200 g were used for this study. They were obtained from the animal house of University of Port Harcourt and were allowed to acclimatize under laboratory conditions prior to experiment for seven (7) days. Food and water were given ad *libitum* prior to the conduct of experiment. The experiment was performed according to the University's ethical guidelines for use of laboratory animals.

2.5 Acute Toxicity

Acute toxicity test was conducted according to the OECD guide lines No. 425. 2001. Normal healthy rats were divided into three groups containing five (5) rats. Group A served as control and was given food and water, group B was administered with relatively high dose of 4000mg/kg body weight of aqueous extract of *Aframomum sceptrum* and *Parinari congensis* (AQASPC) seeds and group C was administered with 4000mg/kg body weight of ethanol extract of *Aframomum sceptrum* and *Parinari congensis* (ETASPC) seeds orally. The rats were then observed continuously for 14 days for behavioral, neurological, autonomic responses and death.

2.6 Induction of Diabetes in Rats

Diabetes was induced in the rats by injecting 100mg/kg body weight of alloxan monohydrate in 0.9% NaCl solution to rats that were fasted overnight intraperitoneally (i.p) using insulin syringes. The rats were kept for 24 hour on 10% glucose solution toprevent initial hypoglycemia associated with alloxan. After 48 hours of i.p injection, blood glucose level was determined using Accucheck Active glucometer. Rats with blood glucose levels equal to ≥ 200mh/dl were considered diabetic and used for this study (Bamidele *et al.*, 2014). The rats were further divided into four (4) groups containing five rats each. Group II received 100 mg/kg body weight of alloxan only, group III received 100 mg/kg of metformin only, and group IV received 400mg/kg body weight of aqueous seed extract of combined *Aframomum sceptrum* and *Parinari congensis* (AQASAP) while group IV received 400mg/kg body weight of ethanol seed extract of *Aframomum sceptrum* and *Parinari congensis* (ETASPC). Group I served as normal control and received 1ml of 20% tween 80.

2.7 Collection of blood samples and organs

After the last dose, the rats were fasted overnight and sacrificed under chloroform anesthesia. Blood samples were collected via cardiac puncture and transferred to appropriate sample bottles for biochemical analyses after 21 days of continuous treatment. Livers were excised from rats, washed in 0.05M ice-cold phosphate buffer saline (PBS) (pH 7.4) to remove excess blood and weighed. 1:10 w/v liver was homogenized in cold phosphate buffer. The homogenate was centrifuged at 10,000G for 10 mins to obtain the post-mitochondrial supernatant (PMS). The supernatant was again centrifuged at 15000 rpm for 1 hr at 4ºC. The supernatant of this fraction was collected for determination of oxidative stress enzymes and pro-inflammatory cytokines

2.8 Determination of liver weight and liver index of rats

The liver weight and liver index were estimatedy (Narayana *et al.*, 2013).

2.9 Determination of Biochemical Parameters

AST , ALT and ALP were assayed using Randox test kits (Randox Laboratories, Crumlin, England). as described by Reitman and Frankiel, (1957). Tumor necrosis factor- alpha (TNF-α) and interlukin-6(IL-6) were assayed based on sandwich enzyme linked immune sorbent assay technology (ELISA) as instructed in the kit manual (Ray Biotech, USA). SOD was determined by the auto-oxidation method of pyrogallol described by Marklund and Marklund (1974), Catalase was determined by the method of Shina (1972), GSH was determined by the method of Ellman's reagent (Ellman, 1959), LPO peroxidation determined by MDA a product of lipid perioxidation as described by Devasagayam e*t al.*,(2003).

2.10 Statistical Analysis of Data

Data obtained from this study were expressed as mean ± SEM followed by one-way analysis of the variance (ANOVA) and turkey post hoc test for the establishment of significance differences set at ($p<0.05$).

3.0 RESULTS

3.1 Effects of Combined Seed extracts of *A. sceptrum* and *P. congensis*Seed on Liver and Relative Liver Weight (Liver Index) of Rats

The result obtained for the effects on of Combined *A. sceptrum* and *P. congensison* Liver weight and Liver Index is presented in Figure 1. From the figure, the diabetic control group showed significant ($p<0.05$) increase in the liver weight and liver index compared to normal control group. This is an indication of pathological alteration. The result obtained for the extract treated groups (AQASPC and ETASPC) were not significantly ($p>0.05$) different when compared to the reference group and control group respectively. This is an indication of the safety profile of the extracts and had no adverse side effect.

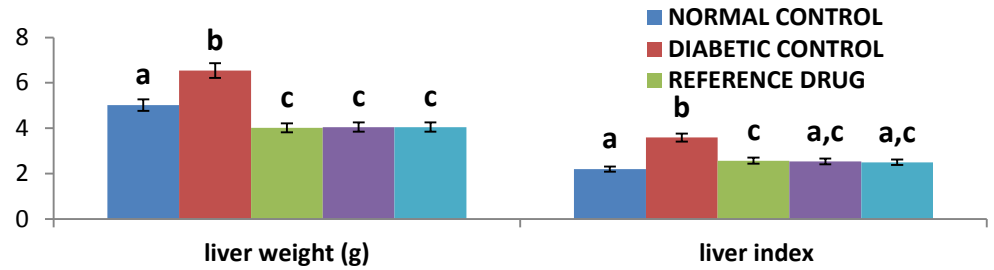

Figure 1: Effects of Aqueous and Ethanol Seeds Extract of Combined *A. sceptrum* and *P. congensis* on Rats Liver and Liver Index. Values are expressed as mean± S.E.M (n=5). Means with different superscript (a-d) are significantly different (Turkey HSD, $p<0.05$).

3.2 Effects of Combined Extracts of *A. sceptrum* and *P. congensis* Seedson Liver Function Enzymes (ALT, AST, and ALP)

The result obtained for the effect of combined *A. sceptrum* and *P. congensis*on liver marker enzymes is presented in Figure 2. From the figure, there was significant increase in ALT, AST, and ALP in the diabetic group compared to normal control. This is an indication of hepatocellular damage. There was Significant ($p<0.05$) decrease in the levels of these enzymes in the reference group AQASPC and ETASPC treated group compared to the diabetic control group. This is an indication of hepatoprotection.

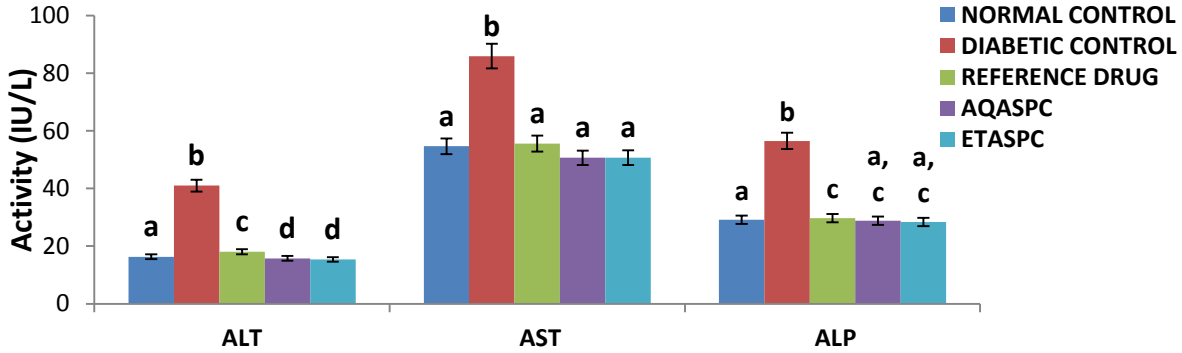

Figure 2: Effects of Aqueous and Ethanol Seed Extracts of Combined *A. sceptrum* and *P. congensis*on Liver Marker Enzymes. Values are expressed as mean± S.E.M (n=5). Means with different superscript (a-d) are significantly different (Turkey HSD, $p<0.05$).

3.3Effects of Combined Seed Extracts of *A. sceptrum* and *P. congensis* on Oxidative Stress Markers (SOD, CAT, GSH, MDA)

The result obtained for the Effect of combined *A. sceptrum* and *P. congensis*onoxidative stress markers is presented in Figure 3. From the figure, there was significant ($p<0.05$) decrease in SOD, CAT and GSH levels while significant increase ($p<0.05$) in MDA levels in the diabetic group compared to the normal control group. This result showed an imbalance in the antioxidant defense system. However reference group RD, AQASPC and ETASPC treated group showed significant ($p<0.05$) increase in SOD, CAT and GSH levels and significant decrease in the MDA levels compared to the diabetic control group.

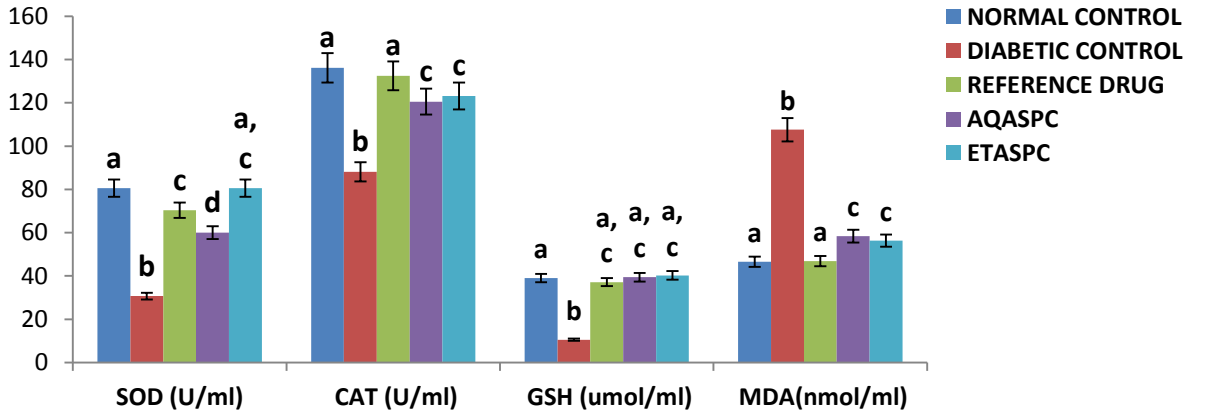

Figure 3: Effects of Aqueous and Ethanol Seed Extracts of Combined *A. sceptrum* and *P. congensis* on Oxidative Stress Levels. Values are expressed as mean± S.E.M (n=5). Means with different superscript (a-d) are significantly different (Turkey HSD, p<0.05).

3.4 Effects of Combined Seed Extracts of *A. sceptrum* and *P. congensison* Tumor Necrosis Factor-alpha (TNF-α) and Interlukin-6 (IL-6).

The result obtained for the effects on (TNF-α) and IL-6 is presented in Figure 4. There was significant increase (p<0.05) in the TNF -α and IL-6 levels in the diabetic group compared to control. In the reference group and extract treated groups, there was significant decrease (p<0.05) compared to the diabetic group. The decrease obtained in the AQASPC and ETASPC treated group were not significantly (p>0.05) different when compared and also with the reference and control group.

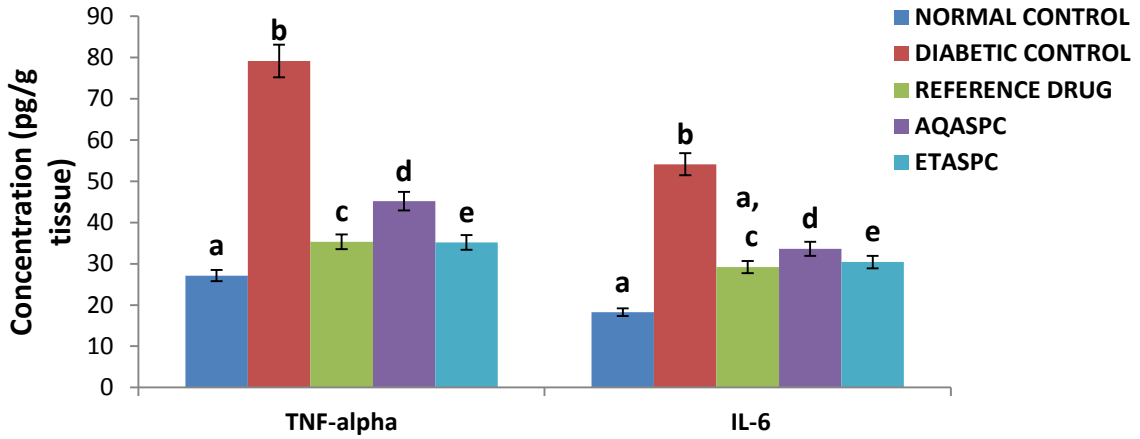

Figure 4: Effects of Aqueous and Ethanol Seed Extracts of Combined *A. sceptrum* and *P. congensis*on TNF-α. Values are expressed as mean± S.E.M (n=5). Means with differentsuperscript(a-e)are significantly different (Turkey HSD, p<0.05).

4.0 DISCUSSION

The liver is an organ that plays a major role of detoxification and regulation of various important metabolic functions. It depends on insulin for the homeostasis of glucose. It is severely affected during diabetes (Nidal and Adian, 2015). Thus assay for liver function parameters present an important tool for assessing the severity of hepatocellular damage. Significant increase in the liver function parameters (ALT, AST, ALP,) was observed in the diabetic control group. This result is similar to the findings of Aja *et al.*, 2015, Inyang *et al.*, 2015. This is an indication of hepatocellular damage. AST, ALT and ALP are liver function enzymes. AST is more ubiquitous in nature than ALT. These enzymes are released into blood circulation when the integrity of the liver is compromised Inyang *et al.*, 2015). Increased ALP is an indication of increase in biliary pressure. Bilirubin is transported to the liver bound to albumin. Increase in the level of this parameter is an indication of impaired hepatic secretory function. Reduction in the levels of these parameters by the extract is an indication of protection of structural integrity of hepatocyte cell membranes, improvement

of secretory function of hepatocytes and regenetation of liver cells against damage caused by alloxan (Aja et al., 2015).

Chronic hyperglycemia often leads to microvascular and macrovascular complications which have accounted for the rapid morbidity and mortality observed in patients who suffer the disease (King *et al.*, 1998, Giacco and Brownlee, 2010).Oxidative stress and inflammation have been involved in very important steps of the pathogenesis of disease and its related complications, both at the microvascular and macrovascular levels (Giacco and Brownlee, 2010, Pitocco *et al.*, 2013, Ullah *et al.*, 2016). Evidences supporting include free radical mechanism via non-enzymatic glucose autooxidation and oxidation of essential sulfhydryl group as well as increased release of peroxidation products. These processes often result to damage of enzymes, cellular membranes as well as insulin resistance (Maritim *et al.*, 2003).From this study, oxidative stress and increased markers of inflammation wereclearly observed to increase in rats treated with alloxan. Oxidative stress is a reflection of an imbalance between reactive oxygen species production (ROS) and antioxidant defense system ROS are characterized by their ability to cause oxidative damage which could lead to changes in the properties of biological membranes and trigger the pathogenesis of the diseases and its related complications such as cancer, atherosclerosis amongst others (Ceriello and Motz, 2004, Fearon and Faux, 2009, Tiwari *et al.*, 2013).Inflammation is a normal response to tissue injury, however, if not regulated, excessive inflammation may lead to several acute and chronic diseases. The mechanism of inflammation is linked to release of ROS which stimulate inflammation by the release of cytokines such as IL-1, TNF-αand interferon-γ which are responsible for the recruitment of additional neutrophils and macrophages (Sagnia *et al.*, 2014). A recent clinical study has suggested that acute hyperglycemia can result in elevated levels of circulating inflammatory cytokines (Jones and Persuad, 1998)

In diabetes condition, main sources of oxidative stress occur during oxidative metabolism in mitochondria, where oxygen is reduced to water and concomitant production of ATP. The remaining oxygen is transformed to oxygen free radical which is an important ROS that is converted to other reactive species (Rolo and Palmeira, 2006, Moussa, 2008, Bajaj *et al.*, 2012). In this study, decrease in hepatic content of biomarkers of oxidative stress (GSH, SOD and CAT) in animals treated with alloxan was observed. Also an increase in malondialdehyde (MDA), a product of lipid peroxidation was observed in diabetic group compared to the control. This is an indication of diminished antioxidant activity which cansubsequently make cell prone to oxidative stress as well as injury due to high level of glucose. Previous studies conducted have shown similar results in experimental animals (Atawodi *et al.*, 2014, Emeka *et al.*, 2014). Other in-vivo studies have also reported hyperglycemia in the generation of oxidative stress leading to endothelial dysfunction in blood vessels of diabetic patients impairment in antioxidant defense mechanism leading various biochemical transformation influx of enzymes that cause tissues and organs damage (Ceriello, 2006, Chikezie *et al.*,2015). SOD is a manganese containing enzyme that provides the first line of defense against ROS by catalyzing the dismutation of superoxide to molecular oxygenand hydrogen peroxide which are less toxic (Tiwari *et al.*, 2013). Catalase, on the other hand, is a peroxisomal heme protein that catalyzes the removal of hydrogen peroxide that diffuses out from the mitochondria to the cytosol into water and molecular oxygen (Ullah *et al.*, 2016) while GSH is a non enzymic antioxidant that interacts directly with free radicals and capable of preventing oxidative damage. It reduces the disulfide bonds thus acting as an electron donor (Pompella *et al.*, 2003). MDA is an end product of lipid peroxidation (LPO) reactive species used as a marker of oxidative stress. Increased lipid peroxidation correlates with high glucose levels and oxidative stress in alloxan treated groups. This is also an indication of decline in both enzymic and non enzymic antioxidant defense mechanisms (Tiwari *et al.*, 2013). Antioxidants protect the body against destructive effect of ROS. They act as scavengers helping to prevent cell and tissue damage as well as diseases (Reiter *et al.*, 2003). The body produces several of these antioxidants which include SOD, CAT and GSH. The increased levels of these enzymes in the extract treated groups are an indication of improved antioxidant and radical scavenging ability in preventing the progression to diabetes diseases. Antioxidants when available even in little amount can inhibit or delay the transformation of bio molecules and prevent the occurrences of disease

Inflammatory responses are mediated by a variety of secreted polypeptides known as cytokines. TNF-α and IL-6 are among cytokines that contributes in mediating inflammation reactions thus, implicated as better predictors of inflammatory related problems (Sagnia *et al.*, 2014). Dysregulation of these cytokines have been found to occur in many degenerative diaseases (Narkunaraja *et al.*, 2003,). Increased level of TNF-α and IL-6 observed in alloxan treated group is an indication of over expression or defective regulation of these polypeptides which can lead to systematic

inflammation and insulin resistance (Frankie *et al.*, 2004). Several studies have also reported (Pitocco *et al.*, 2013) TNF-α may impair insulin signaling transduction pathway while IL-6 may interact with IL-6 receptor alpha and glycoprotein to induce transcription of inflammatory gene products, susceptible to diabetes (Nakunaraya e*t al.*, 2003, Frankie *et al.*, 2004). The decreased levels of these cytokines observed in the extracted treated group showed anti-inflammatory effect of the extracts which may be modulated through binding to inflammatory receptors directly or indirectly thereby reducing the biological activities of pro-inflammation and subsequently reduce the possibility of developing diabetes and other related diseases.

4.1 CONCLUSION

In conclusion, free radicals are serious mediators sustaining inflammatory responses. Thus their neutralization of these radicals by antioxidants which are also scavengers can reduce inflammation and inhibit occurrences of the disease (Pandey *et al.*, 2012). From this study, aqueous and ethanol extracts of *A. sceptrum* and *P. congensis* exhibited antioxidant and anti-inflammatory effects. The ethanol seed extract of combined A. *sceptrum* and *P. congensis* exhibited better effect which can be compared to metformin a standard reference drug in scavenging the deleterious effects of these free radicals due to synergy. Thus, the seeds can be explored for potential therapeutic value they can serve as adjuvants to existing therapy which could provide the synergistic efficacy required to potentiate the body's antioxidant and anti-inflammatory defense mechanisms in management of diabetes.

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