THE EFFECT OF BENNISEED OIL (*Sesamumindicum* Linn) ON INDUCED HYPERCHOLESTEROLEMIA IN ALBINO RATS.

ABSTRACT

The study was designed to investigate the antioxidant and antitoxicological potential of *Sesamum indicum* Linn seed(benni seed) oil on hypercholesterolemic rat. Albino rats weighing between 120-130g were divided into two groups, group 1, was fed with normal rat diet(normal control), groups 2 was fed 1% cholesterol and 20% soya bean oil for 3 weeks to induce hypercholesterolemic state. Group 2 was later divided into groups 2, 3 and 4 ,group 2 was untreated,groups 3 and 4 were later fed with 5% and 10% *Sesamum indicum* L. seed oil incorporated in normal rat diet for another 6 weeks respectively. Significant ($P<0.05$) increase in lipid peroxidation(TBARs) and reduction in superoxide dismutate (SOD) and catalase (CAT) was observed in the liver of the hypercholesterolemic rats as compared to the normal control. At the same time, the oxidative stress causes significant ($P<0.05$) increase in serum level of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) of hypercholesterolemic rats.

Administering *Sesamum indicum* Linn seed oil significantly reduced ($P<0.05$), serum ALT, AST, ALP and lipid peroxidation, elevated the level of SOD and CAT in the liver of *Sesamum* oil treated hypercholesterolemic rats.

These findings indicate that *Sesamum indicum* Linn seed oil show possible prevention of hepatic stress by high cholesterol and free radical mediated oxidative stress in cells of experimental hypercholesterolemic rats.

INTRODUCTION

Hyperphysiological burden of free radicals causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body. This inbalance leads to oxidative stress that is being suggested as the root cause of ageing and various human diseases such as arterosclerosis, stroke, diabetes, cancer and neurodegenerative disease, such as Alzheimer's and pankinsonism [1].

Reactive oxygen species (ROS) have been implicated in more than 100 diseases [2] including cardiovascular disease. ROS causes damage to the hepatocellular membrane thus, serum activities of cellular enzymes such as transaminases and alkaline phosphatase increases. With the increase in cellular membrane permeability, intracellular fluid transfers onto intercellular space resulting in muscle and liver cell toxicity [3].

Moreover, the body's defense mechanisms would play a role in the form of antioxidants and try to minimize the damage adapting itself to the above stressful situation. These Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their actions.

However, the natural antioxidant defense mechanisms can be insufficient and hence, dietary intake of antioxidant component is important and recommended [4].

Sesamum indicum Linn (Pedaliaceae) commonly known as sesame, is a perennial herb found in Africa, Asia and Australia. The seed consumption appear to increase plasma gammatocopherol and enhances vitamin E activity which is believed to prevent cancer and heart disease [5]. Sesame oil has been used as an antibacterial

mouth wash and for relieving anxiety and insomnia [6]. In addition, sesame oil contains large amount of linoleate in triglyceride form which selectively inhibited malignant melanoma growth [7].

Sesamum indicum seed is reported to have antihypertensive effect [8],antitumor effect [9] and also provide benefits to patient with Parkinson's diseases [10]. Sesamin, sesamolin and myristic acid found in sesame have been found to possess antioxidant and health promoting activities [11]. In our previous studies, we have reported that the hypocholesterolemic potential of *S.indicum* Linn seed oil may in part be due to the high levels of unsaturated fatty acid in the oil and may therefore be useful for prophylasis and therapeutic treatment in clinical conditions associated with hyperlipidemia and hypercholesterolemia[12].

The aim of the present study was to access the antioxidant effect of *Sesamum indicum* Linn seed oil on cholesterol induced hepatotoxicity by examining its effect on serum liver function and oxidative stress biomarkers of liver tissue of control and hypercholesterolemic rats.

MATERIALS AND METHODS

- **Plant material-** Beniseed *(Sesamum indicum* Linn) was purchased from Oja-oba local market in Ado-Ekiti. It was cleaned of stones, sand, and other particles (such as leaves, stalks) washed and sundried and identified by the Department of plant Science, Ekiti State University (EKSU), Ado-Ekiti, Nigeria.
- **Chemicals-** All chemicals used in this study were of analytical grade. They were products of PROLABO International SAS, France.
- **Experimental Design-** Cholesterol was incorporated into normal rat diet to produce hypercholesterolemic diet with 20% oil source and 1% cholesterol. The antioxidant effect of feeding the rats with the oil from sesame seed was observed on liver of the rats for six weeks.

- **Animal Treatment**- Twenty four (24) female white albino rats (*Rattus norvegicus*) weighing between 120 — 130g were obtained from the animal house of Department of Biochemistry University of Ilorin, Nigeria. The animals were cleared by the ethical committee ,Ekiti State University Ado Ekiti,Ekiti State.They were housed in the animal house (Faculty of Science, Ekiti State University). The rats were acclimatized to standard laboratory conditions and were given diet and water *adlibtum* for six weeks.

  The rats were grouped as follows:

  Group A: Negative control (Normal rat diet).

  Group B: Positive control (Normal rat diet + 1% cholesterol+20% soyabean oil) (Hypercholesterolemia)

  Group C: Fed with group B diet for 3 weeks and subsequently supplemented with 5% beniseed oil for 6weeks.

  Group D: Fed with group B diet for 3 weeks and subsequently supplemented with 10% beniseed oil for 6weeks.

  The animals (rats) were exposed to normal day-night circle and were fasted overnight and then followed by cervical dislocation and immediately dissected.

The diet composition is according to the method of Ajayi *et al* ,2012 [12].

- **Preparation of serum and tissue homogenate**

  After the experimental regimen, the rats were fasted over night, sacrificed by cervical dislocation and blood samples were collected by cardiac puncture. It was centrifuged at 3000 rpm for 10 mins, the serum was separated and kept until required for analysis. The liver was excised immediately thoroughly washed, weighed and homogenized with (1x4 w/v saline solution).

Biochemical Analysis:

Lipid peroxidation of the organs were estimated by measurement of thiobarbituric acid-reactine substances according to the method of Varshney and Kale , 1990 [13]. The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was measured at 532nm.
The activity of catalase (CAT, EC.1.l 1.1.6) was estimated by the procedure of Sinha ,1972 [14], based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured at 570nm. Superoxide dismutase (SOD, EC. 1.15.1.1) activity was estimated by the method of Misra and Fridovirch ,1972 [15]. It is based on the inhibition of auto-oxidation of adrenaline to adrenochrome by SOD.
Activities of serum AST and ALT were estimated by using commercially available kits by method of Reitman and Frankel,1957 [16]. The serum ALP was estimated by the method of King and Armstrong,1988 [17].

Statistical analysis:

All the results obtained were expressed as mean±SD of rats in each group. Analysis of Variance was used to test for differences in the groups . The results were considered statistically significant at $P<0.05$.

Results: -

Table 1: Effect of *Sesamum indicum Linn* seed oil supplementation on lipid peroxidation (TBARSs), liver tissue antioxidant enzymes ,serum Alanine amino transferase (ALT), Aspartate transferase (AST) and Alkaline phosphatase (ALP) activities of albino rat after 6 weeks.

Parameters	A	B	C	D
TBARs(nmol/mg)	1.02±0.02[a]	5.22±0.20[b]	1.84±0.04[a]	1.01±0.01[a]
CAT(umol/min)	28.49±0.04 [b]	21.20±0.00[a]	25.49±0.02[b]	29.53±0.04[b]
SOD (U/ml)	12 2.56±0.94[b]	115 .60±1.10[a]	125.56±1.41[b]	1 31.1±0.94[b]
AST (Tu/L)	25.00±0.60[a]	36.7±2.07[b]	25.0±0.12[a]	28.23±0.27[a]
ALT (Tu/L)	34.43±0.13[a]	57.25±2.05[b]	37.43±1.13[ab]	31.00±0.04[a]
ALP (Tu/L)	25.20±0.90[a]	35.0±0.40[b]	28.3.±0.20 [a]	26.0±0.06[a]

Values are expressed as mean±SD. Mean with different superscripts in a row are statistically significant (P<0.05).

Effects of *Sesamum indicum L* seed oil supplementation on hepatic antioxidant enzyme activities and serum ALT,AST,ALP are shown in Table 1. A significant dencrease in the concentration of TBARs, serum ALT,AST,ALP and increase in antioxidant enzymes were observed in the control and test groups compared to .hypercholesterolemic rats. Group A: Negative control (Normal rat diet).Group B: Hypercholesterolemia untreated),Group C: Hypercholesterolemia treated with with 5% beniseed oil supplemented diet for 6weeks, Group D: Hypercholesterolemia treated with 10% beniseed oil supplemented diet for 6weeks.

Discussion

Free radicals react with lipids and causes peroxidative changes that result in enhanced lipid peroxidation which can be detected by the presence of peroxidation products [18]. The purpose of this study is to determine the effect of 5% and 10% of sesame oil supplemented diet on antioxidant and antitoxicological capacity of hypercholesterolemic rats. Lipid peroxidation, consequence of free radical oxidation of Low density lipoprotein, (LDL) and DNA, results in the formation of unstable hydroperoxide which breakdown to thiobabituric acid reactive substances (TBARs), leading to cellular injury and damage [19]. Under normal Physiological conditions, a delicate balance exists between the rate of formation of H_20_2 via dismutation of O^{-2} by SOD activity and the rate of removal of H_20_2 by CAT. Therefore, any impairment in this pathway will affect the activities of other enzymes in the cascade [20]. Free radicals result in the consumption of antioxidant defenses which may lead to disruption of cellular functions and oxidative damage to membranes and enhance susceptibility to lipid peroxidation [21]. Some of these free radicals interact with various tissue components resulting in dysfunction and hence the questions of whether oxidative stress is a major cause of injury remain equivocal.

Manipulation of oxidation in humans for the purpose of preventing cardiovascular disease has received substantial attention and effort. Administration of antioxidants, such as Vitamin E has generally lessened arterial lesions in animal models of atherosclerosis but has had no consistent benefit [22][23] and has resu1ted possibly in occasional harm[24]. Thus a search for antioxidants that either do not enter the liver or affect hepatic lipid peroxidation might be needed. In addition, agents that could transport oxidized or oxidizable materials from the arterial wall, where it may be harmful to the liver, may be beneficial and desirable [25]. Furthermore, a report showed that hypercholesterolemia affects the antioxidant defense system and decrease the activities of SOD and CAT; elevating the lipid peroxide content [26]. Table 1. Shows the effect of *Sesamum indicum Linn* seed oil supplementation on lipid peroxidation (TBARs), liver tissue antioxidant enzymes ,serum Alanine amino transferase (ALT), Aspartate transferase (AST) and Alkaline phosphatase (ALP) activities of albino rat after 6 weeks. In this study the treatment of hypercholesterolemic rats with sesame seed oil is able to reduce the increase in serum ALT,AST and ALP to the normal level. The protective effect conferred by this oil does not appear to be due to the Vit. E content or due to alteration in the absorption or distribution of sesamin alone. It

has been demonstrated that an increase in monounsaturated fatty acids or a reduction in PUFA in lipid membrane, decrease the susceptibility of membranes to oxidant attack [28].

Other study has also suggested that sesamolin and its metabolites sesamol, and sesamolinol in the vivo system will strongly inhibit lipid peroxidation, hence preventing oxidative damage, DNA stress and contribute to the antioxidant properties of sesame lignans [29]. Catalase (CAT) is one of the most important antioxidant enzymes which convert the toxic H_20_2 into water (H_20). Decrease in the CAT activity in hypercholesterolemic rats in this study indicates possible damage to the cells of liver. Lenzi et.al [30] in their study showed decrease in activity of catalase. Similarly, catalase is involved in the removal of toxic H_20_2 from the cell. It is found in the peroxisomes of liver thus the decreased catalase activity observed in the liver of group B suggests possible damage to peroxisomes. The superoxide dismutase (SOD) radicals are main reactive oxygen species in the cell and SOD plays a key antioxidant role. The SOD activity was significantly ($P<0.05$) increase in the test groups (Table 1). The SOD neutralizes superoxides anion very quickly. A decrease in SOD activity observed in the group B (Table 1) might mean greater reduction in neutralization of superoxide anions which might lead to increase in superoxide radicals.The result of the SOD and CAT activity in the test groups clearly shows that *Sesamum indicum* L possess a free radical scavenging activity, which could exert a beneficial action against pathological alteration caused by the presence of O^{-2} and OH^{-} This action could involve mechanism related to scavenging activity.

Indication of hepatocellular integrity most commonly measured in clinical toxicology studies are the enzymes AST, ALT and ALP levels[31]. They function in the first step in the catabolism of most L-amino acids once they have reached the liver, in removal of the α-amino groups, catalyzed by enzymes called aminotransferases or transaminoses in these transminationrxns, α-amino group is transferred to the α-carbon atom of α-ketoglutarate, leaving behind the corresponding α keto acid analog of the amino acid (with the aid of the coenzyme pyridoxal phosphate). The effect of transamination reactions is to collect the amino groups from

many different amino acids in the form of L-glutamate. The glutamate then functions as an amino group donor for biosynthetic pathways or for excretion pathways that lead to the elimination of nitrogenous waste products. ALT and AST tests are important in determining whether people obessed or hypercholestoremic have suffered liver damage. Liver degeneration cause by these diseases is accompanied by leakage of various enzymes from injured hepatocytes into the blood. Aminotranferase because their activity can be detected in very low amount are most useful in the monitoring of people suffering from these diseases since these enzymes activities are high in liver and thus are likely to be among the proteins leaked from damaged hepatocytes.[32]

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ALP is found majorly in the bile duct of the liver. It is an hydrolase enzymes responsible for removing phosphate groups from many types of molecules including nucleotides, proteins and alkaloids. This process is known as dephosphorylation. Increase in ALP activities may show that the bile ducts are blocked [33]due to increase, synthesis in the presence of increasing biliary pressure.

Therefore, in this study, the elevated level of AST, ALT and ALP in the serum of induced hypercholesterole rats (Table 1) suggest hepatocellular damage caused by cholesterol toxicity. This report is in agreement with Osfor et.al; 2013 [34] who reported increase in serum activity of AST, ALT and ALP in hypercholesterolemic albino rats. However, treatment with *Sesamumindicum* L. seed reduced the elevated levels of AST, ALP and ALT in the serum of hypercholesterolemic rats (Table 1). This shows that *sesamum indicum* exert antitoxicity to the liver cells in hypercholesterolemic rats, reducing the leakage of the above enzymes into the blood. The present study demonstrates that supplementation of hypercholesterolmic diet with sesame seed oil in rats modulates the antioxidant enzymes in a manner that favour the reduction of lipid peroxidation (LPO) and serum Alanine aminotransferase (ALT), Aspartate transaminase (AST), Alkaline Phosphatase (ALP) and suggest a possible adaptive mechanisms to counteract oxidative sress situations.This effect of sesame may be due to the

presence of sesamin and sesaminol which are reported to increase hepatic mitochondria rate [35], also it was earlier stated that lecithin of sesame seed have hepatoprotective role in cellular level [36].

CONCLUSION

Hypercholesterolemic diet supplemented to the sesame seed oil exerts antioxidative and antihepatotoxic effect by decreasing lipid peroxidation,serum Alanine amino transferase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and maintaining normal levels of superoxide dismutase (SOD) and catalase (CAT) activities. Hence, consumption of sesame seed oil may help reduce the effect of hypercholesterolemia.

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