

TITLE OF ARTICLE:

THE ANTIOXIDANT AND ANTITOXICOLOGICAL EFFECT OF BENNI SEED  
OIL (Sesamum indicum LINN )

ON INDUCED HYPERCHOLESTEROLEMIA IN RATS

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## **ABSTRACT**

The study was designed to investigate the antioxidant and antitoxicological potential of *SesamumIndicum*Linn seed oil on hypercholesterolemic rat. Albino rats weighing between 120-130g were divided into four groups, group 1, was fed with normal rat diet, groups 2, 3 and 4 were fed with 1% cholesterol and 20% soya bean oil for 3 weeks to establish hypercholesterolemic state. Groups 3 and 4 were later fed with 5% and 10% *Sesamumindicum* L. seed oil incorporated in normal rat diet for another 6 weeks respectively. Significant ( $P<0.05$ ) increase in lipid peroxidation and reduction in superoxide dismutase (SOD) and catalase (CAT) activity 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 plasma level of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) of hypercholesterolemic rats.

Administering *Sesamumindicum* Linn seed oil significantly reduced ( $P<0.05$ ), plasma ALT, AST, ALP and lipid peroxidation, elevated the level of SOD and CAT in the liver of hypercholesterolemic rats (+ control).

These findings indicate that *Sesamumindicum* Linn seed oil can reduce hepatotoxicity 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 imbalance leads to oxidative stress that is being suggested as the root cause of ageing and various human diseases like arterosclerosis, stroke, diabetes, cancer and neurodegenerative disease, such as Alzheimer's and parkinsonism[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], benefits in Parkinsonism disease [9] and antitumor effect [10]. Sesamin, sesamol 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 *S. indicum* Linn seed oil contain high levels of unsaturated fatty acid and may therefore be useful for prophylaxis 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 plasma 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. It was 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 form 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. They were housed in the animal house (Faculty of Science, Ekiti State University). The rats were acclimated to standard laboratory conditions and were given diet and water *ad libitum* 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)

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.

Table 1: Diet composition (gkg<sup>-1</sup>)

	A	B	C	D
Corn starch	290	130	290	240
Soya meal	510	510	510	510
Sucrose	100	100	100	100
Vit-mineral mix	50	50	50	50
Soyabean oil	50(5%)	200(20%)	-	-
Benniseed oil	-	-	50(5%)	100(10%)
Cholesterol	-	10(1%)	-	-

- **Preparation of Plasma 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 into lithium-heparin bottles. It was centrifuged at 3000 rpm for 10 mins, the plasma was separated and kept until required for analysis. The liver was excised immediately and thoroughly washed in ice-cold saline. It was homogenized immediately after it was weighed with (1x4 w/v saline solution).

**Biochemical Analysis:**

Lipid peroxidation was estimated by measurement of thiobarbituric acid-reactive substances (TBARs) by the method of [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.1.1.1.6) was estimated by the procedure of [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 [15]. It is based on the inhibition of auto-oxidation of adrenaline to adrenochrome by SOD.

Activities of plasma AST and ALT were estimated by using commercially available kits by method of [16] The plasma ALP was estimated by the method of [17]

### Statistical analysis:

All the results obtained were expressed as mean $\pm$ SD of rats in each group. Statistical significance of difference of means was analyzed by students' t-test. The results were considered statistically significant at  $P < 0.05$ .

### Results:

Table 2: Effect of *Sesamum indicum* Linn seed oil supplementation on antioxidant enzymes in liver tissue of albino rat after 6 weeks.

Parameters	A	B	C	D
TBARs(M)	508173.08 $\pm$ 14.7540.19 <sup>b</sup>	657692.34 $\pm$ 874366.54 <sup>a</sup>	175961.54 $\pm$ 150941.90 <sup>b</sup>	108173.08 $\pm$ 99336 <sup>b</sup>
CAT(% inhibition)	0.49 $\pm$ 0.04 <sup>b</sup>	0.46 $\pm$ 0.00 <sup>a</sup>	0.49 $\pm$ 0.02 <sup>b</sup>	0.53 $\pm$ 0.04 <sup>b</sup>
SOD ( $\mu$ mol/mn )	2.56 $\pm$ 0.94 <sup>b</sup>	1.56 $\pm$ 1.10 <sup>a</sup>	2.56 $\pm$ 1.41 <sup>b</sup>	3.11 $\pm$ 0.94 <sup>b</sup>

Values are expressed as mean $\pm$ 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 The enzyme activities and lipid peroxidation (LPO) which is express in TBARs are shown in Table 2. A significant increase in the concentration of TBARs was observed in hypercholesterolemic rats compared to the control and test groups while the antioxidant enzymes showed significant decrease TBARs concentration reduced significantly while the activity of the antioxidant enzymes increased significantly ( $P < 0.05$ ).

Table 3: Effect of *Sesamum indicum* seed oil supplementation on plasma Alanine amino transferase (ALT) Aspartatetransaminase (AST) and Alkaline phosphatase (ALP) activities (TU/L) in the experimental rats.

Parameters	A	B	C	D
AST	175±60.0 <sup>a</sup>	296.7±152.7 <sup>b</sup>	205.0±35.12 <sup>a</sup>	163.3±54.87 <sup>a</sup>
ALT	87.3±9.13 <sup>a</sup>	171.5±33.5 <sup>b</sup>	127.3±9.13 <sup>a</sup>	81.00±4.00 <sup>a</sup>
ALP	240.0±900 <sup>a</sup>	385.0±88.1 <sup>b</sup>	288.3±41.6 <sup>a</sup>	168.0±47.6 <sup>a</sup>

The effect of *Sesamum indicum* L. seed oil supplementation on hepatoprotective enzyme activities are shown in Table 3. A significant increase in plasma ALT, AST and ALP concentration was observed in hypercholesterolemic rats compared to control and test groups while the plasma ALT, AST and ALP activities reduced significantly in the test groups.

## DISCUSSION

Freeradicals 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 antihepatotoxicity capacity of hypercholesterolemic

rats. Lipid peroxidation, consequence of free radical oxidation of LDL, protein and DNA, results in the formation of unstable hydroperoxide which breakdown to malondialdehyde (MDA), leading to cellular injury and damage [19]. The result of this study on the content of MDA in hypercholesterolemic rats (Table 2) suggests that hypercholesterolemia could enhance the process of lipid peroxidation. Sesame seed supplement of hypercholesterolemic diet resulted in a significant ( $P < 0.05$ ) decreased content of MDA in liver. The seed oil might be capable of lowering or slowing down oxidative related lipid peroxidation. Lipid peroxidation has been suggested as one of the molecular mechanism involved in organochlorine, carbamate or organophosphorous, pesticides. Large numbers of xenobiotics have been identified to have potential to generate free radicals in biological system [20].

Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics. A report indicated that additive and synergistic interactions may occur with combination of dietary antioxidants against free radical including damage in cells and tissues [21]. Under normal Physiological conditions, a delicate balance exists between the rate of formation of  $H_2O_2$  via dismutation of  $O_2^-$  by SOD activity and the rate of removal of  $H_2O_2$  by CAT. Therefore, any impairment in this pathway will affect the activities of other enzymes in the cascade [22]. 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 [23]. 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.

Oxidative stress is one of the causative factors that link with atherogenesis[24]. A cholesterol-rich diet brings about remarkable modifications in antioxidant defense mechanisms. 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 [25][26] and has resulted possibly in occasional harm[27]. 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 [28]. Furthermore, recent report showed that hypercholesterolemia affects the antioxidant defense system and decrease the activities of SOD and CAT; elevating the lipid peroxide content [29]. Table 2 shows an increase in lipid peroxidation (LPO) level of group B (hypercholesterolemic diet fed rats) compared to the test groups and the negative control group, also a decreased with the test groups and the control (-). MDA the product of lipid peroxidation is an index of the level of oxygen free radicals. A decrease in lipid peroxidation leads to a reduction of atherosclerosis caused by hypercholesterolemia[30]. In this study the treatment of hypercholesterolemic rats with sesame seed oil able to prevent the increase in TBARs level, may be due to its vitamin E content. 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 [31]

Other study has also suggested that sesamol and its metabolites sesamol, and sesamolol in the vivo system will strongly inhibit lipid peroxidation, hence preventing oxidative damage, DNA stress and contribute to the antioxidant properties of sesame lignans[32]. Catalase (CAT) is one of the most important antioxidant enzymes which convert the toxic  $H_2O_2$  into water ( $H_2O$ ). Decrease in the CAT activity in hypercholesterolemic rats in this study indicates possible damage to the cells of liver. The study of [33] also showed decrease in activity of catalase. Similarly, catalase is involved in the removal of toxic  $H_2O_2$  from the cell. It is found in the peroxisomes of liver. The decreased catalase activity observed in the liver of group B suggests possible damage to peroxisomes. The superoxide radicals are main reactive oxygen species in the cell and superoxide dismutase (SOD) plays a key antioxidant role. The SOD activity was significantly ( $P < 0.05$ ) increase in the test groups (Table 2). The SOD neutralizes superoxides anion very quickly. A decrease in SOD activity observed in the group B (Table 2) might mean greater reduction in neutralization of superoxide anions which might lead to increase in superoxide radicals.

Reduced activities of SOD and CAT in liver tissue was observed in the hypercholesterolemic rats group and this activity may result in a number of deleterious effects due to accumulation of superoxide radicals ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ). Supplementation with *Sesamum indicum* L seed oil increased the activities of SOD and CAT in hypercholesterolemic rats. The result of the SOD and CAT activity 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 [34]. Transaminases function in the first step in the catabolism of most L-amino acids. Once an amino acid have reached the liver, the transaminase specific for the substrate help remove its  $\alpha$ -amino

group, the amino group is transferred to the  $\alpha$ -carbon atom of  $\alpha$ -ketoglutarate, leaving behind the corresponding  $\alpha$ -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. Occlusion of coronary artery by myocardial infarction due to hypercholesterolemia can lead to local oxygen starvation and ultimately the degeneration of a localized portion of heart tissue, such a damage will cause the aminotransferase enzymes to leak out from the injured heart cells into the blood streams. Therefore, measurement of concentration of these aminotransferases- Alanineaminotransferases (ALT) and Aspartateaminotransferase (AST) can provide information regarding the severity and stage of damaged damage to the heart. ALT and AST tests are also important in determining whether people obsessed or hypercholesteremic have suffered liver damage. Liver degeneration cause by these diseases is accompanied by leakage of various enzymes from injured hepatocytes into the blood. Aminotransferases, because their activities can be detected in very small amount, are most useful in monitoring 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 [35].

ALP is found majorly in the bile duct of the liver. It is anhydrolase 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 [36] due to increase synthesis in the presence of increasing biliary pressure caused by peroxides formation induced by hypercholesterolemia.

Therefore, in this study, the elevated level of AST, ALT and ALP in the plasma of induced hypercholesterolemic rats (Table 3) suggest hepatocellular damage caused by cholesterol toxicity. This report is in agreement with [37], who reported increase in serum activity of AST, ALT and ALP in hypercholesterolemic albino rats. However, treatment with *Sesamum indicum* L. seed reduced the elevated levels of AST, ALP and ALT in the plasma of group 3 and 4 rats (Table 3). 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 hypercholesterolemic diet with sesame seed oil in rats modulates the antioxidant enzymes in a manner that favour the reduction of lipid peroxidation (LPO) and plasma Alanineaminotransferase (ALT), Aspartatetransaminase (AST), AlkalinePhosphatase(ALP) and suggest a possible adaptive mechanisms to counteract oxidative stress situations. This effect of sesame may be due to the presence of sesamin and sesaminol which are reported to increase hepatic mitochondria rate [38], also it was earlier stated that lecithin of sesame seed have hepatoprotective role in cellular level [39].

## **CONCLUSION**

Hypercholesterolemic diet supplemented with sesame seed oil exerts antioxidative and antihepatotoxic effect by decreasing lipid peroxidation, plasma Alanine amino transferase (ALT), Aspartatetransaminase (AST), Alkalinephosphatase (ALP) and maintaining normal levels of superoxide dismutase (SOD) and catalase (CAT) activities. Hence, consumption of sesame seed oil may help reduce hypercholesterolemia.

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