Original Research Article

Evaluation of the Effect of Diet Containing Sesame Seed (*Sesamum indicum*) on Some Biochemical Variables of Diabetic albino Wistar Rats

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ABSTRACT

Aim: To evaluate the effect of diet containing Sesame Seed (*Sesamum indicum*) on some biochemical parameters in Diabetic albino Wistar Rats

Study design: Experimental study.

Place and Duration of Study: Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria, between April and November, 2019.

Methodology: Forty (40) male rats weighing between 140 to 150g were used for this study. The rats were housed in animal cages in a well-ventilated experimental room and allowed to acclimatize for a period of 14 days before the commencement of the treatments. The animals were grouped into six (6) groups of 5 rats each. The group one (1) was the negative control and group two (2) positive control of induced group with streptozotocin without treatment. The remaining groups were also induced with streptozotocin and then treated with varying concentration of Sesame seed ranging from 4g to 16g daily for a period of six weeks. Rats were sacrificed by cardiac puncture, blood sample collected after six weeks of the experiment. The Samples were analysed for glucose, lipid profile, antioxidant capacity, urea, creatinine, aspartate amino transaminase (AST) and alanine amino transaminase (ALT). GraphPad prism version 5.0 was used to analyse data statistically and p values less than 0.05 ($p<.05$) were considered statistically significant.

Results: Result revealed a statistically significant decrease in fasting blood glucose of the various treatment groups when compared with the positive control. When positive control group was compared with treatment group 3 to 6, a statistically significant decrease was observed in their total cholesterols ($p<.05$), the triglyceride of group 3, 5 and 6, and statistically significant increase of high density lipoprotein of group 3, 4, 5and 6, also a statistically significant decrease of low density lipoprotein of group 3, 4, 5 and 6. Total antioxidant capacity and reduced glutathione levels comparison of group 2 with group 3, 4, and 6, demonstrated a significant increase ($p<.05$). The liver enzymes, AST and ALT of the control group 2 compared with the treatment group showed a mild decrease ($p<0.05$). Similar result was also observed with renal parameters using urea and creatinine as guides.

Conclusion: Based on the findings, this study has demonstrated that sesame seed seed has anti-diabetic, anti-lipidemic and antioxidant properties and also has gradual organ protective ability when used on a long period of time on a daily basis.

Keywords: Diet, Sesame Seed (Sesamum indicum), Biochemical Variables, Diabetic Wistar albino Rats.

1. INTRODUCTION

The use of Sesame (*Sesamum indicum L*.) has gained wide acceptance in food industry especially among the fast food vendors in and around the world. Sesame has been variously used to spice up or coat food products like meat pie, fish roll, and hamburger and so on.

Dating back to as early as 1600 BC, Sesame seeds are among the oldest condiments known to man. Sesame belongs to the family *Pedaliaceae* and is widely cultivated in the tropical parts of Africa and Asia and several species are said to be in existence [1]. Their wild species are located mainly in Africa and only a few in India [2]. The original home of this crop is known to be Ethiopia. Sesame seeds also known as benne seeds originated from Africa before spreading to Asia and other part of the world. Nigeria is one of the leading producers and consumers of Sesame seeds in Africa. In Nigeria, Sesame is grown in northern and western Nigerian. This could be due to its drought resistant nature, [3]. The major world Sesame producers are India, Myanmar, China and Sudan with 68% of the total world production. In the world, India ranks first in the production of Sesame seeds and is grown in different seasons covering practically all agro-ecological zones [4].

Oil extracted from Sesame is honoured as a rich food because of its high nutritive quality and stability. It has many uses and is markedly different from other vegetable oils due to its high nutritional and therapeutic values. Potential health benefits of Sesame include anti oxidative, anticancer, anti-hypersensitive and anti-immunoregulatory actions. The seeds are used for the production of oil, paste, salads and in various food formulations. From the chemical composition of Sesame, it is known that the seeds contain 50-60% oil, 18-25% protein, 13.5% carbohydrate and 5% ash [5]. Sesame ability to stabilise oxidation can be attributed to its endogenous anti-oxidant lignans along with tocopherols. Sesamin (0.4-1.1%), sesamolin (0.3-0.6%) and traces of sesamol contribute to the unique properties of Sesame oil. In the eastern parts of the world, Sesame has long been considered as a 'health food' that provides high energy and prevents ageing [6]. The oil is rich in unsaturated fatty acids (85%) and has a mild taste. It is said to be plant breeder's dream crop because of its great genetic diversity [4].

Sesame oil was more effective in lowering the enzymes level, the protective effect due to the antioxidant component and Sesame oil contain some powerful antioxidant such as (1p-6,phytate, lignans, pinoresionol, vitamins E, lecithin, myristic acids and lineolate) which may prevent free radicals formation and scavenge free radicals that are already formed. Sesame oil works on increase of secretion of bile salt in liver, ALT secretion from heart, liver and muscle and Sesame oil known as a polyunsaturated oil protects the heart work and lowers cholesterol in the blood because of antioxidant component like vitamins E, A, and flavonoids (sesamin, sesamol and sesamolin) which protect cell body from the damage of free radicals, also fatty acids containing non saturated and saturated lanolin which are important acid on action. AST because of flavonoids and antioxidant vitamins B6 transform to pyridol-5-phosphate PLP which work like co enzyme for amino translate and reduce glycogenesis and over antioxidant hydrogens for peptides. Sesame oil also activated Co a-oxidation and increase the active of Co a–oxidase cycle, even Sesame oil also contains amino acids which helps in protecting liver function and immunity (sesamol, sesamolionol and sesaminol) phytotochemicals are responsible for this protective response. High amount of sesamin and sesamol has been identified in Sesame and they are reported to increase the hepatic mitochondria and peroxismol fatty oxidation rate, also Sesame lignans have antioxidant and health promoting activities.

Diabetes mellitus (DM) is a global health issue affecting children, adolescent and adult population known to increase the risk of developing cardiovascular disease – CVD, which remains the main cause of mortality in these individuals. Not only is the risk of developing a first cardiovascular event increased in diabetes, these individuals also have a poorer prognosis following treatment compared to those without diabetes, regardless of the treatment given in the acute stage. With the number of patients with diabetes reaching 3 million in the UK alone in 2012 an increased understanding of the disease processes involved in DM and improved therapeutic strategies will be required to reverse this unnerving trend, This strong association between DM and CVD is multifactorial and is related to clustering of classical cardiovascular risk factors, including hyperglycaemia, obesity, hypertension, dyslipidaemia and oxidative stress culminating in some of the attendant complications.

Given the economic burden and other health complication arising from the issues of diabetes mellitus in conjunction with the complication associated with conventional medicine, the use of organic products becomes very necessary to support the individuals living with these conditions; in this case Sesame seed is prominent here.
Therefore, the aim of this study was to evaluate the effect of diet containing Sesame Seed (*Sesamum indicum*) on some biochemical parameters in Diabetic albino Wistar Rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Forty (40) male albino Wistar rats with weight between 140 and 150 g were obtained from the Experimental Animal Farm at the University of Port Harcourt, Nigeria. The albino Wistar rats were housed in animal cages in a well-ventilated experimental room. The rats were allowed to acclimatize for a period of 14 days before the commencement of treatments. Handling of animals was in accordance with relevant institutional and ethical guidelines as approved for scientific study. The rats were protected and controlled under the light regime (12 hrs light: 12 hrs dark), at room temperature (22±2°C) and humidity constant (55±5%). During the study**,** the rats were fed a diet of standard pellets and had free access to water.

2.2 Induction of Diabetes in Rats

Twenty five adult Wistar rats weigh 140-150 grams (75-90 days old) were used for inducing diabetes. The animals were injected with streptozotocin at the dose of 60 mg/kg of the body weight intraperitoneally. Diabetic animals and non-diabetic control group were kept in metabolic cages individually and separately and under feeding and metabolism control. Glucose in the blood of diabetic rats exceeded that of the non-diabetic control ones. Food consumption was measured in terms of grams (gm.), water consumption was measured in terms of (ml) on a daily basis while every 2 - 4 days in 6weeks, the levels of glucose in blood serum was measured, so that chemical diabetes was verified in rats injected with Streptozotocin.

2.3 Dose Calculation and Administration of Streptozotocin

Streptozotocin was administered through intraperitoneal route after appropriate calculations of doses were made.
Standard dose for diabetes induction= 60mg/kg

1. Average Weight of the rats = 145g

145mg/kg standard dose = 145mg is given to 1kg or 1000g rat
60mg = 1000g rat
Xmg = 145g rat

$$X = \frac{60 \times 145}{1000} = 8.7\text{mg/145g rat}$$

Therefore, 8.7mg of the streptozotocin was given to all rats in the group weighing 145g (this was done for all groups of animals taking their weights into consideration). Only rats with plasma glucose above 11.0mmol/l were considered diabetic.

2.4 Preparation of Sesame Seeds for Administration

About two kilograms (2kg) of Sesame seeds were bought from Mile 3 market, Port Harcourt, Rivers State, identified at Department of Animal and Environmental Biology, Rivers State University, Port Harcourt and grounded to marsh powder using a factory blender and stored in an air-tight jar to avoid moisture in preparing the food formula. Normal food powder of rats were collected and sieved and white Sesame seeds available in the market, were powdered with domestic mill, then the powder prepared from normal food of rat with ground Sesame seeds (4, 8, 12 & 16g) were mixed according to their groups dose regimen and turned to a pulp with water and became tubular shaped with the pastry cone then they were put in trays within a period of 2-3 days and then were dried in open air and given to rats.

2.5 Pilot Studies

After processing Sesame as feed, ten rats where used for the pilots studies of which grounded Sesame of 2g to 20g where used to feed each rats daily, in the following succession.

1 = 2g	6 =12g
2 = 4g	7 = 14g
3 = 6g	8 = 16g
4 = 8g	9 = 18g
5 = 10g	10 = 20g

They were fed in that order for two weeks daily and their levels of response were noted. In these two weeks exercise, the rats fed from above 16g were not able to finish their meal and those below 4g per day almost found the food insufficient. It was on this not a 4g to16g were used, in all no apparently signs of observable toxicity.

2.6 Experimental Design

Groups	Treatment	Dosage/Administration
Group 1	Negative control (no diabetes)	Saline + normal chow
Group 2	Positive control (Diabetes only)	No treatment
Group 3	Diabetes + Sesame seed powder	(4g/Sesame + 20g rat chow)/six weeks
Group 4	Diabetes + Sesame seed powder	(8g/Sesame + 20g rat chow)/six weeks
Group 5	Diabetes + Sesame seed powder	(12g/Sesame + 20g rat chow)/six weeks
Group 6	Diabetes + Sesame seed powder	(16g/Sesame + 20g rat chow)/six weeks

2.7 Assay Methods

2.7.1 Determination of Phytochemical Composition of Sesame Seed

2.7.1.1 Qualitative and quantitative Phytochemical Analysis

Standard methods were used to test for tannins, saponins, resins, alkaloids, glycosides, flavonoids, terpenoids, proteins and carbohydrates. All the various phytochemicals that tested positive were further prepared in accordance with ISO17025. Phytochemicals of interest were determined using the UV Visible via scan analysis with the wavelength range of 200-1100nm. At each wavelength, its adsorption were compared with the UV developed standard for phytochemicals to determine the phytochemicals Present and it quantification was done using Beer's law to get the actual concentration.

2.7.1.2 Determination of Total Antioxidant Capacity (T-AOC)

T-AOC reflect the total antioxidant capacity in the system. Many antioxidants in the body can reduce fe^{3+} to fe^{2+} and fe^{2+} can form stable complexes with phenanthroline substance. The antioxidant capacity (T-AOC) can be calculated by measuring the absorbance at 520nm.

2.7.1.3 Determination of Reduced Glutathione (GSH)

Reduced glutathione (GSH) can react with dithionitrobenzoic acid (DTNB) to thio-nitrobenzoic acid and glutathione disulphide. Nitromercaptobenzoic acid is a yellow compound which has the maximum absorption peak at 420nm. The GSH content can be calculated by measuring the absorbance at 420nm.

2.7.1.4 Determination of Serum Total Cholesterol

Serum total cholesterol concentration was assayed using the CHOD-PAP method of *Allain* et al. [7].

2.7.1.5 Serum Triglycerides

Serum triglycerides (TG) concentration was assayed using the GPO-PAP method of McGowan et al. [8].

2.7.1.6 Determination of Serum HDL-Cholesterol

Serum HDL-cholesterol was assayed using the precipitation/CHOD-PAP method of Burstein et al. [9]

2.7.1.7 Determination of Serum LDL-Cholesterol

Serum LDL-cholesterol (LDL-C) was calculated using the method of Friedewald equation [10].

2.7.1.8 Determination of Serum Alanine Aminotransferase (ALT) Activity

The serum alanine aminotransferase (ALT) activity was determined using the method of Reitman and Frankel, [11].

2.7.1.9 Serum Aspartate Aminotransferase (AST) Activity

The serum aspartate aminotransferase (AST) activity was determined using the method of Reitman and Frankel, [11].

2.7.1.10 Determination of Plasma Glucose

Plasma glucose was determined using the GOD-POD

2.7.1.11 Determination of Serum Urea

The serum urea concentration was estimated using the method of Weatherburn [12].

2.7.1.12 Determination of Serum Creatinine

The serum creatinine concentration was estimated using the modified Jaffe's method and modified by Vaishya et al. [13]

2.8 Statistical Analysis

The data obtained in this study were analysed using Graph Pad Prism 5.0 statistical software. Descriptive statistics to check for mean values and standard deviation, analysis of variance and Tukey's test of multiple comparison were used to check for significance between groups. $p < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

Table 1: Preliminary Qualitative and Quantitative (mg) Phytochemical Screening Results of the Sesame Seed

S/N	Phytochemicals	Qualitative	Quantitative
1	Alkaloids	+ve	32.8±0.15
2	Cardiac glycosides	-ve	
3	Flavonoids	+ve	5.20±0.15
4	Phenols	+ve	19.40±0.47
5	Phlobatanins	-ve	
6	Saponins	+ve	4.8±0.12
7	Sterols	-ve	
8	Tannins	+ve	17.01±0.12
9	Terpenoids	-ve	
10	Quinones	-ve	
11	Oxalate	-ve	
12	Diterpenes	-ve	

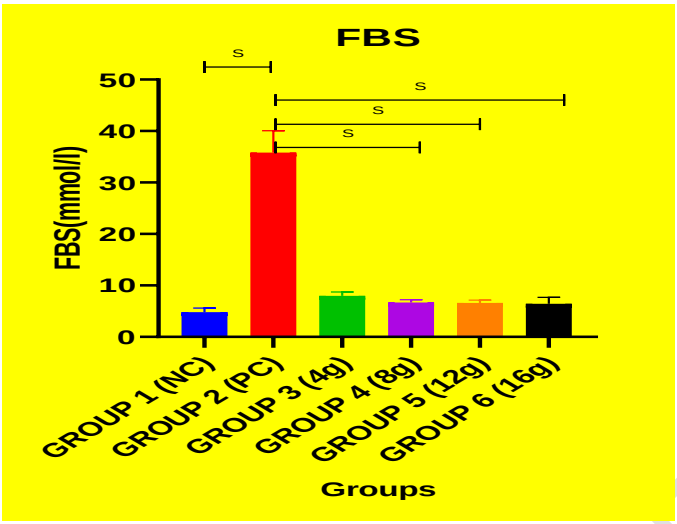

Fig. 1: Chart of Fasting blood glucose concentration for rats in all groups (S-significant at p<0.05)

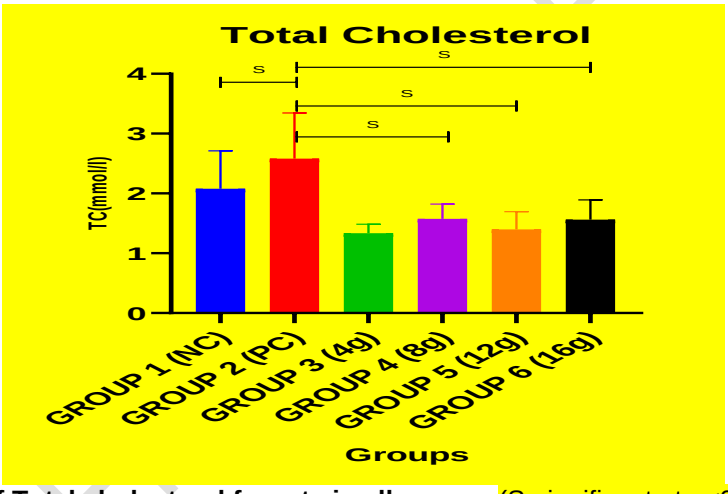

Fig. 2: Chart of Total cholesterol for rats in all groups (S-significant at p<0.05)

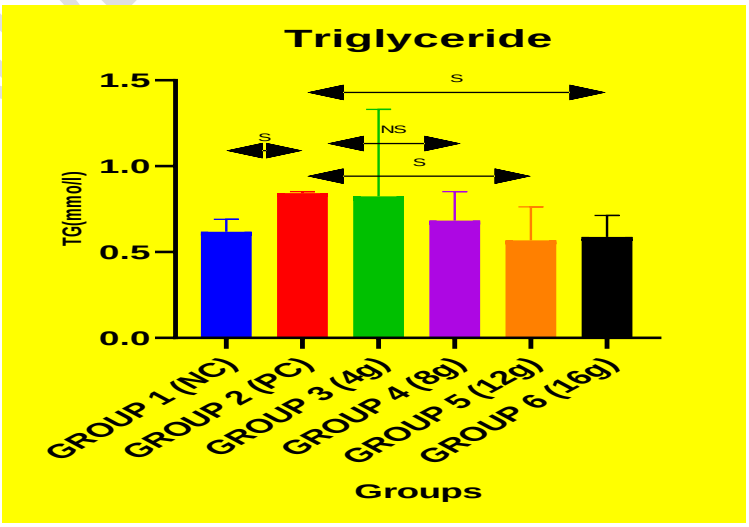

Fig. 3: Chart of Triglyceride for rats in all groups (S-significant, NS – non-significant at p<0.05)

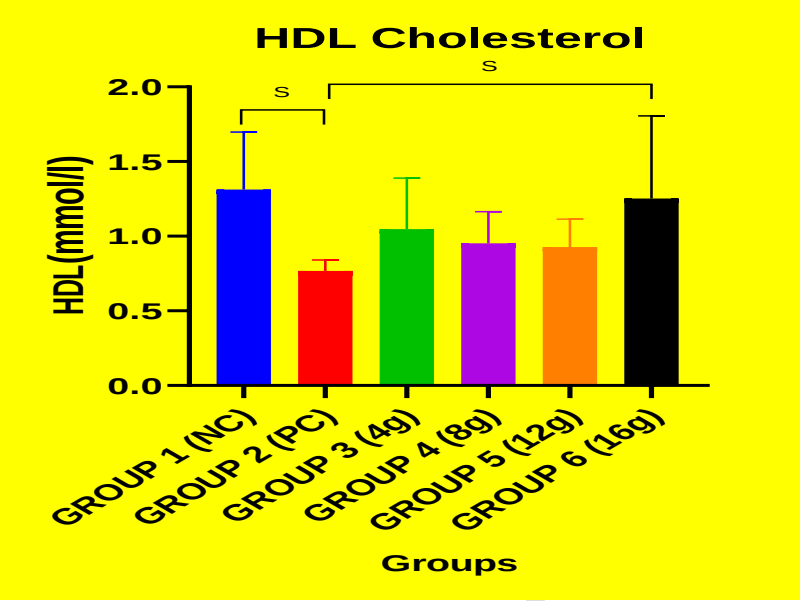

Fig. 4: Chart of HDL cholesterol for rats in all groups (S-significant at p<0.05)

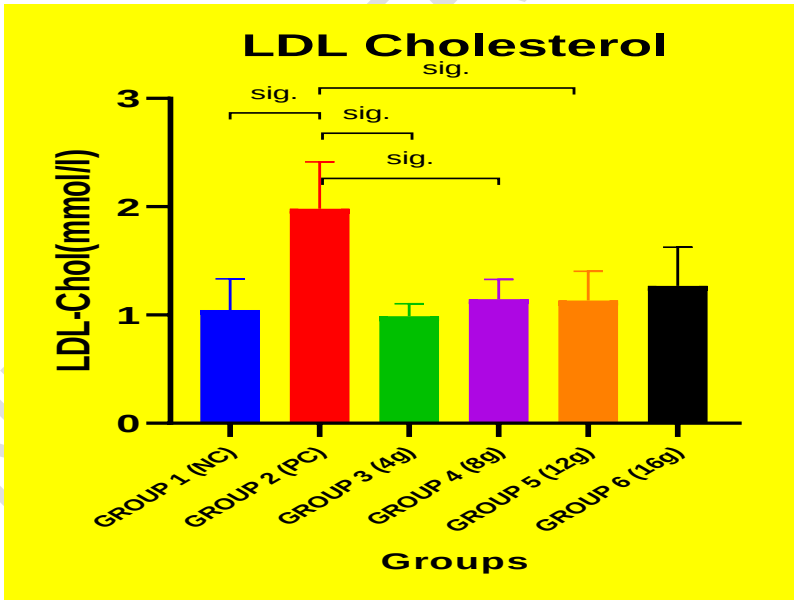

Fig. 5: Chart of LDL cholesterol for rats in all groups (sig-significant at p<0.05)

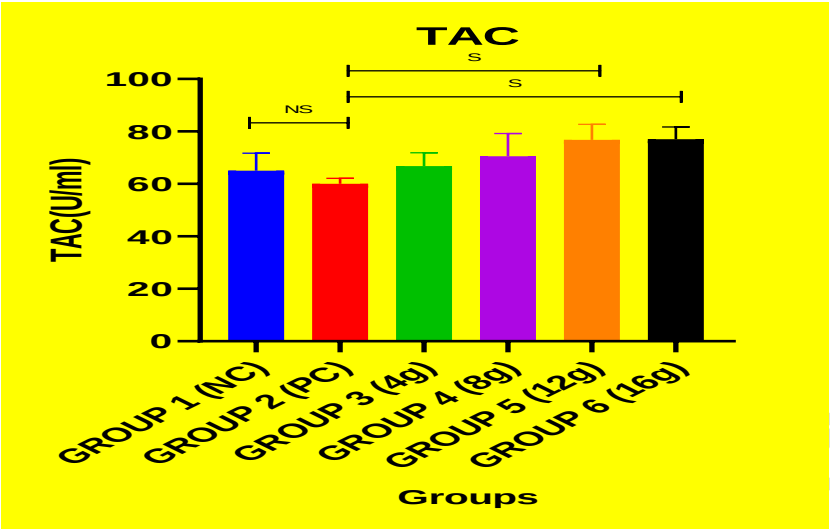

Fig. 6: Chart of Total antioxidant capacity for rats in all groups (S-significant, NS – non-significant at p<0.05)

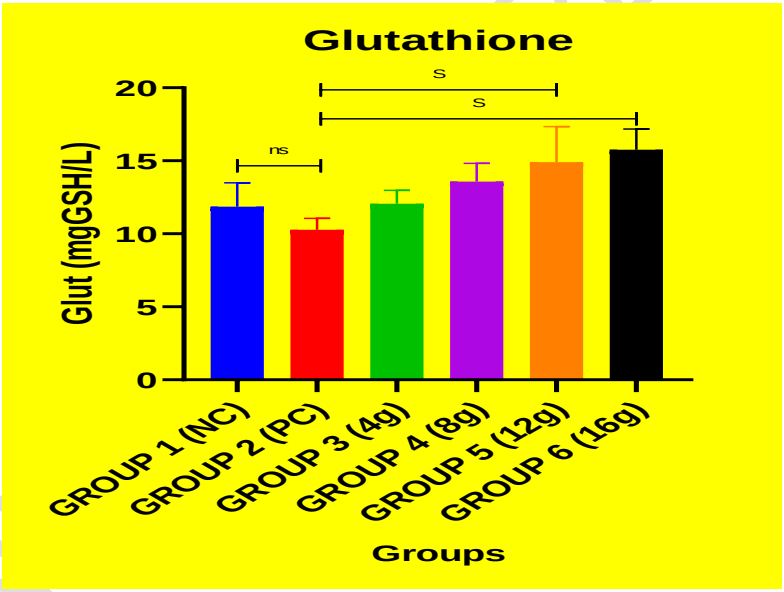

Fig. 7: Chart of Glutathione for rats in all groups (S-significant, ns – non-significant at p<0.05)

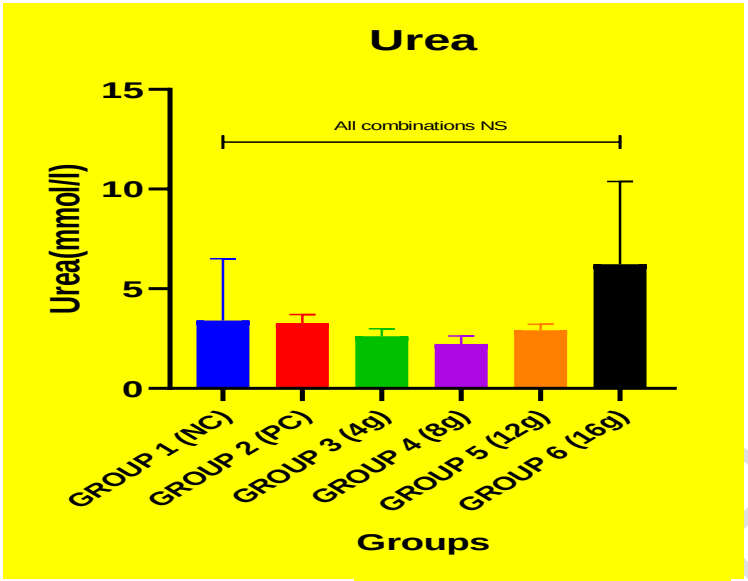

Fig. 8: Chart of Urea for rats in all groups (NS – non-significant at p<0.05)

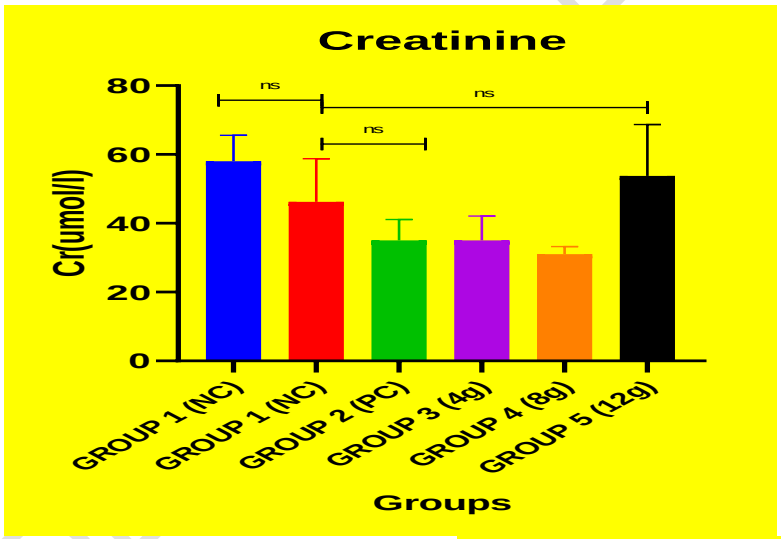

Fig. 9: Chart of Creatinine for rats in all groups (ns – non-significant at p<0.05)

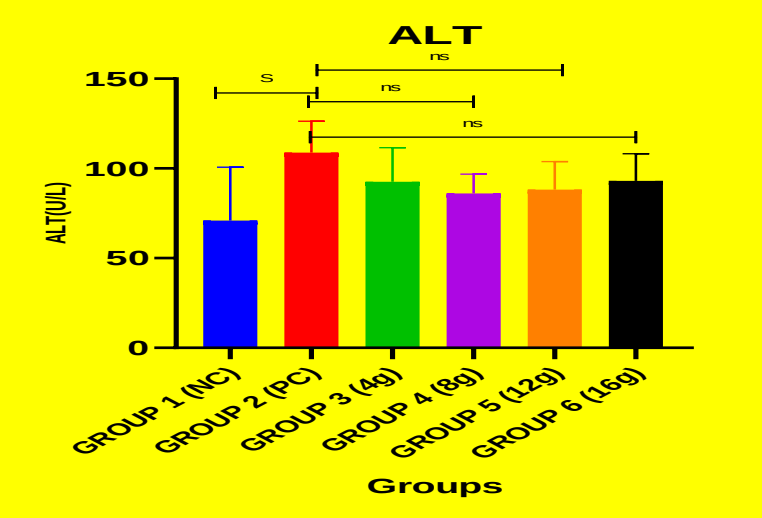

Fig. 10: Chart of Alanine amino transaminase for rats in all groups (S-significant, ns – non-significant at p<0.05)

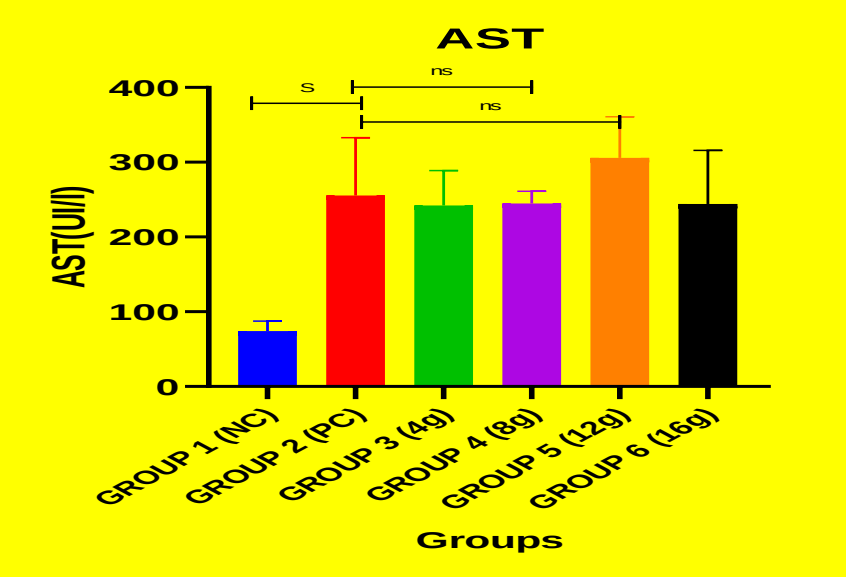

Fig. 11: Chart of Aspartate amino transaminase for rats in all groups (S-significant, ns – non-significant at p<0.05)

This study demonstrated that the glucose concentration of the rats showed a statistically significant increase in the mean value of the negative control (NC) group1 when compared with positive control (PC) group 2 *(p<.05)* (Fig. 1), confirming a successful induction and diabetic condition. Conversely, there was a statistically significant decrease in the values of rats in the treatment groups of varying Sesame concentrations, when compared with the positive control which is the induced diabetic non-treated group after treatment. This is in agreement with Baskar et al. [14] who stated in their work that a moderate intake of Sesame seeds on a day-to-day basis can go a long way in helping people living with diabetes. There was also a significant difference in the mean values of group 3 treatment group when

compared with other treatment groups (Fig. 1), supporting still the work of [14]. The reducing effect of Sesame seed is likened to its high magnesium content, as many studies have proven that magnesium can have a positive effect in controlling the symptoms of diabetes [14]. Magnesium is an essential element in the mechanism of glucose transport across the cell membrane and various enzymes important in carbohydrate oxidation requires magnesium as cofactor. Magnesium through this mechanism increases insulin sensitivity to cells, improving cells uptake of glucose which consequently reduces the plasma glucose level. Bearing in mind that diabetes is more a lifestyle disease condition; patients who deal with it from a healthier dietary routine to help them combat the disease are more likely to have good result than others. And Sesame used on a daily has been proven even in this study to be highly therapeutic.

A comparison of the lipid parameters of the negative control compared with positive control, gave a statistically significant increased difference in all the lipid variables (Fig. 2 to Fig. 5), demonstrating that diabetes has an increasing relationship with lipid parameters. However, when positive control were compared with the treated groups, a statistically significant decrease was observed in the total cholesterol of group 3, 4, 5 and 6 *($p<.05$)* ((Fig. 2), triglyceride of group 3, 5 and 6 (Fig. 3), supporting the work of Hamalatha et al. [15]. And statistically significant increase of high density lipoprotein (HDL) of group 4 and 5 (Fig. 4) and statistically significant decrease of low density lipoprotein (LDL) of group 3, 4, 5 and 6 (Fig. 5). This in tandem with the study of [15] and Matsumura et al. [16] whose result demonstrated that a remarkable major lignan sesamin, present in Sesame seeds is mainly related to lipid metabolism through a series of biochemical actions in both humans and animals. Increase in the concentration of the seed however did not show any statistically significant difference in high density lipoprotein of group 3 and 6, same seen in triglyceride of group 4. No statistically significant difference was observed in all the lipid parameters when the treated groups where compared with each other. This shows that the various grams of Sesame weighed per day and fed to the different groups of rats daily did not affect the rat that much to make any significant difference statistically, this means that Sesame even at increase dose did not adversely affect the rat under this dosage regimen used in this study.

The biochemical actions of Sesame lignand sesamin and episesamin of Sesame seed has shown significant increase in the gene expression of mitochondrial and peroxisomal fatty acid oxidation enzymes such as carnitine palmitoyltransferase, acyl-CoA dehydrogenase, acyl-CoA oxidase, 3-hydroxyacyl-CoA dehydrogenase, enoyl-CoA hydratase, and 3-ketoacyl-CoA thiolase thus increasing the hepatic activity of fatty acid oxidation which is due to enhanced ketone body production. This hepatic fatty acid metabolism accounts for lowering the serum lipid level [14].

Another study by Mensink et al. [17] also supports this result, which reported that the multiple components of Sesame oil could be responsible for the lipid-lowering effects. As it contains about 47% of oleic acid and 39% of linoleic acid thus rich in both monounsaturated fatty acid and polyunsaturated fatty acid which are known to reduce plasma lipids [17]. Moreover, Hirose et al. [18] and Ashakumary et al. [19] concluded that Sesame oil also contained lignands that are known to complex cholesterol from the gut and prevent cholesterol absorption.

A comparison between antioxidant capacity and reduced gluthatione level in various treated groups and the controls did not show any statistically significant in group 1 and group 2 (Fig. 6 and 7), same was observed in comparison with group 1 and group 3 and 4. But a comparison between group 1 and 5 and 6, showed statistically significant difference. This buttresses the fact that Sesame seed and oil has a great antioxidant ability on an increased dosage and when used on a very long period of time. It has ability to mop off free radicals when used in an increased dosage and on daily basis. Supporting the work of Miyahara et al. [20] that sesamolin as the second major lignan of Sesame oil, has significant number of biological activities that induces apoptosis of human lymphoid leukemia molt 4B cells, inhibit the growth of those cells and prevents it from mutagenic activity of hydrogen peroxide [21]. Sesamolin has also free radicals scavenging activity and provides protection against neuronal hypoxia [22].

A comparison of group 2 which is the positive control that is the induced group without treatment and the group 3 and 4 of the treated group showed no significant increase. This is as a result of lower dosage used on these groups for treatment. However when group 2 where compared with group 5 and 6 having higher dosing daily treatment, there was statistically increase in TAOC and glutathione (Fig. 6 and 7), which confirms the antioxidant capacity of Sesame seed when used on daily basis and on increased dosing range as against that used in group 3. Again, a statistically significant increase was observed in comparison between group 3 and group 5 and 6. This again further explains the quantity of Sesame seed used per meal on a daily and its positive antioxidant effect. As reported by Oliver et al. [23] in their study that, plant phenols, including simple phenolic acids, flavonoids, stilbenes, and a variety of other polyphenolic compounds, possess hydroxyl groups conjugated to an aromatic hydrocarbon group. Phenolic compounds are ubiquitous in plant foods with total daily intakes estimated at 500-1000 mg. The reduction in the risk of several chronic diseases associated with the consumption of plant phenols has been attributed to their array of bio-mechanisms, including antioxidation, anti-inflammation, carcinogen detoxification, and cholesterol reduction. This phenolic compounds are found in Sesame seed (Table 1) in the form of sesamolin, sesamin and sesamol and have proven to be the major reason for the increased antioxidant capacity of Sesame when consume on increased quantity and on a long term basis.

A comparison of renal functions using urea and creatinine as template. The controls, compared to group 2 showed a statistically non - significant decrease on both the urea and creatinine values at $p<.05$ (Fig. 8 and 9). The urea of group 1 were compared with other groups like 3, 4 , 5 and 6 all showed a no statistically significant decrease difference, same was also observed with that of urea of group 2 and other groups, likewise the comparison between the treated groups, all showed a statistically non-significant decreased difference. This however means that sesame seed has a gradual resuscitative effect and not immediate in its action. This the research data actually demonstrated a good regenerative effect on cells of the kidney and its biochemical parameters, but just that they are not statistically significant enough.

The creatinine values however demonstrated a statistically significant decrease when group 1 members were compared with group 3, 4, 5 and 6 (Fig. 9). However, a comparison between group 2 and group 3, 4, 5 and 6 showed no significant changes, same applicable with comparison among other treated groups. However, between groups 5 and 6, a significant increase in serum creatinine was observed. This shows that sesame has effect on body vital organs, which may not be not immediate, but on prolong usage could positively proffer a preservative protection on the body's vital organs and a good reversal effect on damaged tissues.

When liver parameters where compared, Aspartate transferases (AST) and alanine transferases (ALT) of group 1 and 2 comparison showed a significant increase (Fig. 10 and 11), indicating that there is an effect of inducing agent on the liver cells. However, when the group 2 where compared with group 3, 4, 5 and 6 treated groups, there was no statistically significant difference in the activity of the hepatic enzymes. This probably shows that the effect of Sesame seed, though effective, but could be slow in reversing the actions of mildly elevated liver enzymes. The same result was also seen when the treated groups were compared with each other, a statistically non-significant decrease were observed, showing also that an increased dosage administration of Sesame on daily bases has a strong potential in correcting elevated liver enzymes.

4. CONCLUSION

Based on the findings, this study has demonstrated that sesame seed seed has anti-diabetic, anti-lipidemic and antioxidant properties and also has gradual organ protective ability when used on a long period of time on a daily basis.

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Author NEO and BH designed the study, while the latter performed the statistical analysis. Author WC wrote the protocol, and wrote the first draft of the manuscript and managed the analyses and literature searches of the study. All authors read and approved the final manuscript.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

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