



## Effect of Nigerian propolis on glycemia, lipid profile, and oxidative stress markers in alloxan-induced diabetic rats

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### Abstract

Propolis is used by the Fulani Nomads in Nigeria to manage diabetes. This study was carried out to evaluate the effect of Nigerian propolis on hyperglycemia-induced oxidative stress and hyperlipidemia in diabetic rats. Diabetes was induced with Alloxan (100mg/Kg). Animals were divided into 6 groups (n=5); Grp. A & B were non-diabetic receiving normal saline and 200mg/Kg propolis respectively. Grp. C, D, E, & F were diabetic receiving normal saline, 150mg/Kg metformin, 200mg/Kg propolis, and 300mg/Kg propolis respectively for 28 days. Hyperglycemia, elevated serum levels of low density lipoprotein cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL-C), triglycerides (TG), total cholesterol (TC), aspartate aminotransferase (AST), alanine transferase (ALT), urea, malonaldehyde (MDA) and decreased levels of high density lipoprotein cholesterol (HDL-C), superoxide dismutase (SOD), and glutathione (GSH) were observed in the diabetic untreated animals. Diabetes had no effect on serum creatinine level. Propolis decreased blood glucose level and serum levels of LDL-C, TC, and elevated HDL-C. AST, ALT, and urea levels decreased. MDA level decreased with increase in SOD and GSH levels. These changes were significant ( $P < 0.05$ ). Propolis of Nigerian origin possesses hypoglycemic and antihyperlipidemic activities in addition to its ability to ameliorate oxidative-stress induced organ dysfunction.

Key words: Nigerian propolis, hyperglycemia, hyperlipidemia, oxidative stress.

## Introduction

Diabetes mellitus is a common metabolic disorder characterized primarily by its blood glucose “raising” activity which results from impaired secretion of insulin or insulin insensitivity<sup>[1]</sup>. This disorder is one of the world’s fastest growing metabolic disorders<sup>[2]</sup> affecting about 4% of the world’s population and is estimated to increase by 5.4% in 2025<sup>[3]</sup>. Diabetes results into metabolic imbalances, such as; hyperglycemia and hyperlipidemia and the deviation from the normal physiology of many tissues<sup>[4]</sup>. Reactive oxygen species (ROS) production increases in diabetes owing to hyperglycemia which induces autoxidative glycosylation of cell membranes, destruction of the antioxidant systems, lipid peroxidation, and tissue injury<sup>[5]</sup>. It is an established fact that hyperlipidemia is a major risk factor to the development of atherosclerosis and its complications<sup>[6]</sup>.

Organs such as the kidney and liver are affected physiologically and morphologically in diabetes. The ability of the kidney to keep the level of metabolites such as; uric acid, creatinine, urea, and ions at optimum level in order to maintain a stable internal environment confers on it a great homeostatic function, however, the level of these metabolites increases greatly due to renal impairment resulting from diabetes mellitus<sup>[7]</sup>. The level of markers of hepatic injury such as Alanine transferase (ALT) and aspartate aminotransferase (AST) are also elevated in diabetes.

Several attempts have been made to tackle hyperglycemia and comorbidities that come with increased blood glucose level. To this effect drugs like sulfonylureas, that stimulate insulin secretion by the islets and  $\alpha$ -glucosidase inhibitors that augment glucose utilization and suppress glucose production have been developed<sup>[8]</sup>. Despite the limited efficacy of these therapies, it is also not devoid of side effects, therefore necessitating the search for new classes of drugs to combat this disorder. To this effect, many substances from plant source have been found to possess anti-diabetic activity with minimal side effects and the search is on-going<sup>[9]</sup>.

The success of research on the effect of medicinal plants on diabetes is apparent in the discovery and synthesis of metformin, a sulfonylurea from *Galega officinalis*<sup>[10]</sup>.

“Propolis is a natural product derived from plant resins and collected by honeybees to be used as glue and as draught-extruder for beehives”<sup>[11]</sup>. It has been used for centuries in folk medicine and have been found to possess; antimicrobial<sup>[12,13,14]</sup> anti-protozoal and anti-parasitic<sup>[15]</sup>, anti-inflammatory<sup>[16]</sup>, anti-ulcer<sup>[17,18]</sup>, and anti-tumor activities<sup>[19,20]</sup> among others. Propolis has a complicated chemical composition and more than 300 compounds have been identified in propolis samples. These substances include; polyphenols, phenolic aldehydes, amino acids, coumarins, steroids, sesquiterpene quinines and inorganic compounds

The content of propolis sample is dependent on collecting location, time, and plant source, therefore the phytogeographical areas and time region causes great variation in the biological activities of propolis<sup>[11]</sup>. Several studies have been carried out to examine the effects of propolis from different geographical regions on experimentally induced diabetes and its complications, but sparse data is available on the effect of propolis of Nigerian origin. This study was undertaken to investigate the effect of Nigerian propolis on hyperglycemia, hyperlipidemia, anti-oxidant system, and biomarkers of hepatic injury and renal function in experimentally induced diabetes mellitus.

## Methods

### Drugs and reagents

Nigerian propolis was purchased from the Federal University of Agriculture, Abeokuta, Ogun state in Nigeria. The alloxan and metformin used were products of Sigma Aldrich. Kits used in evaluating Superoxide dismutase (SOD), Malonadehyde (MDA), creatinine, urea, blood glucose, ALT, AST and lipid profile were properties of the Animal science department, Faculty of Agriculture, University of Ibadan, Nigeria.

### **Extract preparation**

Raw propolis was obtained by scraping it off its hive frames. Ethanolic extract was prepared according to the method used by Ivan et al.<sup>[21]</sup>. In brief, One gram of raw propolis was extracted with 25 mL of 95% ethanol for 24 h at 37 °C and the filtrate was adjusted to 25 mL with 80% ethanol.

### **Animals**

Laboratory investigations on the animals were carried out in accordance with the ethical guidelines stipulated by the ethical committee of the College of Health Sciences, University of Ibadan, Nigeria. These guidelines were in accordance with the internationally accepted principles for laboratory animal use and care. Thirty adult male Wistar rats were used for this experiment. The animals weighed between 200-250g. The animals were kept and housed in the animal holdings of the Department of physiology, University of Ibadan, Nigeria, under standard laboratory conditions of temperature, light and humidity and were fed with rat pellets and water *Ad libitum*. The rats were randomly divided into six groups (A, B, C, D, E and F) of five animals each. Standard drug (metformin) and ethanolic extract of propolis were administered orally for 28 days.

### **Experimental design**

The rats were divided into six groups of 5 rats each.

Group A–Normal control (non-diabetic); receiving normal saline

Group B–Vehicle control (non-diabetic); receiving 200mg/kg of propolis

Group C–Diabetic control; receiving normal saline

Group D–Diabetic treated; receiving 150mg/kg of metformin

Group E–Diabetic experimental; receiving 200mg/kg of propolis

Group F–Diabetic experimental; receiving 300mg/kg of propolis

### **Induction of diabetes**

The animals were fasted overnight. Diabetes was induced by single intraperitoneal (i.p) injection of alloxan monohydrate (100 mg/kg) in sterile normal saline (0.9%). The diabetic state was determined 72 hours after alloxan administration through the tail, using the one touch ultra-glucometer. Weekly record of blood glucose level was taken afterwards.

### **Animal sacrifice**

After 4 weeks of treatment, animals were sacrificed. The animals were sacrificed by cervical dislocation after been anaesthetized and then dissected. Blood was obtained directly from the heart into plain centrifuge bottles.

### **Preparation of serum**

After allowing blood samples to stand for an hour, they were centrifuged at 3000r/min for 15 minutes to obtain the serum and analysed for urea, creatinine, ALT, AST, LDL-Chol, HDL-Chol, TC, TG, VLDL, SOD and MDA.

### **Biochemical assays**

The serum samples were used for biochemical analysis of AST and ALT activities using Rietman-Frankel's method<sup>[22]</sup>. Triglycerides, cholesterol, SOD and MDA levels were analysed in the serum using commercial diagnostic kits (Randox).

VLDL-C and LDL-C lipoproteins were precipitated by addition of phosphotungstic acid and magnesium chloride to determine the level of HDL-C.

Supernatant containing HDL-C fraction was assayed for cholesterol. LDL-C level was determined using the method of Friedwald et al<sup>[23]</sup>. Serum urea and creatinine were determined using the methods of Talke & Schubert<sup>[24]</sup> and Jaffe et al.<sup>[25]</sup> respectively.

### **Statistical analysis**

Data were presented as mean  $\pm$  standard error of mean and analysed by one-way analysis of variance, followed by Waller-Duncan post-hoc test. Statistical significance was accepted at  $P \leq 0.05$ .

## Results

### *Effect of Propolis on Body Weight*

Table 1 shows the initial weights and final weights of animals in all groups after the 28 day experiment. Body weight of animals in the diabetic untreated group decreased after 28 days while there was significant ( $P < 0.05$ ) increase in the body weights of animals in other groups.

see Table 1.

### *Hypoglycemic Effect of Propolis*

Figure 1 shows and compares weekly changes in the blood glucose levels in different groups. Alloxan administration increased blood glucose level until the first week in both treated and untreated groups. Blood glucose level was significantly ( $P < 0.05$ ) reduced in the metformin and propolis (both doses) treated groups from the second week onwards when compared to the diabetic control group. There was no significant change in the level of blood glucose between the Non-diabetic control and vehicle control groups all through the experimental period. There was also no significant difference between the hypoglycemic activity of propolis at both doses (200mg/Kg and 300mg/Kg).

### *Effect of Propolis on Lipid Profile*

Figure 2 shows serum levels of VLDL-C, LDL-C, HDL-C, TC, and TG in all the groups. Diabetes elevated serum levels of VLDL-C, LDL-C, TC and TG and decreased the level of HDL-C. The administration of metformin and the different doses of propolis produced no significant ( $P < 0.05$ ) decrease in the elevated levels of VLDL-C and triglycerides when compare to the diabetic untreated group. Elevated serum level of LDL-C was significantly reduced after treatment with metformin and propolis. Elevated serum level of TC was significantly decrease by both doses of propolis only and not metformin. The two doses of propolis produced significant increase in the serum level of HDL-Chol while metformin produced no effect on the decreased serum HDL-Chol when compared to the diabetic untreated

group. There was no significant difference in the activities of the two doses of propolis on lipid profile. There was also no difference in the lipid profiles of the normal and vehicle control groups.

see Fig. 1

see Fig. 2

VLDL- Very Low Density Lipoprotein, LDL- Low Density Lipoprotein, HDL- High Density Lipoprotein, CHOL- Total Cholesterol, TG- Triglyceride

### *Effect on Serum Levels of MDA, SOD, and GSH*

Figure 3 shows serum levels of MDA while Figure 4 shows serum levels of SOD, and GSH. The level of MDA was elevated, while SOD and GSH were decreased significantly ( $P < 0.05$ ) in the diabetic untreated group compared to the normal and vehicle control groups. Treatment with metformin and propolis significantly decreased the elevated MDA level when compared to the diabetic untreated groups. The decreased SOD and GSH levels in diabetic group were significantly increased after treatment with propolis and metformin. Treatment of vehicle control group with propolis and diabetic group with 300mg/Kg propolis also produced significant increase in SOD levels when compared to the normal untreated group.

see Fig. 3

see Fig. 4

### *Effect on Serum ALT and AST*

Table 2 shows the serum levels of AST and ALT in all groups. Serum levels of ALT and AST were elevated significantly ( $P < 0.05$ ) in the diabetic untreated group when compared to the normal control group. Administration of metformin and propolis significantly decreased the elevated level of AST when compared to the diabetic untreated group. Metformin had no significant effect on the elevated level of ALT while propolis significantly reduced the level of ALT when compared to the diabetic untreated group.

### Effect on Serum Creatinine and Urea

Table 2 shows the serum levels of creatinine and urea in all groups. The serum level of urea was significantly ( $P < 0.05$ ) increased in the diabetic untreated group compared to the normal control group. Administration of propolis (at both doses) and metformin significantly reduced the elevated level of urea to a level not significantly different from that in the normal control group. There was no significant difference in the serum levels of creatinine in all groups.

see Table 2.

### Discussion

Since ancient times, plants have been a primary source of medicine for treating several ailments<sup>[26]</sup>. Traditional plants have also been used in different parts of the world, especially in developing countries owing to meagre income of the populace to treat diabetes<sup>[27,26]</sup>. More than 350 plants have been implicated with hypoglycemic activity<sup>[28]</sup>. A notable milestone was the discovery and synthesis of metformin, a sulfonylurea from *Galega officinalis*<sup>[10]</sup>.

One of the potent methods employed in inducing diabetes in experimental animals is by the use of alloxan<sup>[29]</sup>, as it has been found to selectively destroy the insulin-producing  $\beta$ -cells of the pancreas by oxidation of essential sulphhydryl (-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis<sup>[30,31,32]</sup>. Hyperglycemia-induced increase in the level of reactive oxygen species causes autoxidative glycosylation of cell membranes, destruction of the antioxidant systems, lipid peroxidation, and tissue injury<sup>[5]</sup>. The use of alloxan have been found to mimic the oxidative stress status experienced by diabetic patients.

Propolis of different geographical regions have been found to possess several biological activities, among which is its oxygen radical scavenging activity<sup>[33]</sup>. Previous studies have been carried out on propolis of Croatian, Brazilian, Chinese, Egyptian

origin among others and have been found to possess hypoglycemic activity<sup>[34,35,36]</sup>. Much research effort has been made on the biological activities of honey from Nigeria with little effort geared towards studying the biological activities of its propolis.

In the present study, intraperitoneal administration of alloxan produced hyperglycemia, hyperlipidemia, elevated serum level of markers of hepatic injury and renal dysfunction, and also depressed serum antioxidant capacity. This is evident in the significant rise in blood glucose level, the significant ( $P < 0.05$ ) rise in the serum level of LDL-C, VLDL-C, total cholesterol, triglycerides, and lowered level of HDL-C, significant rise in serum level of AST and ALT, significant rise in serum level of urea, and the significant rise in the level of serum MDA and lowered levels of SOD and GSH of animals in the diabetic untreated group when compared to the normal non-diabetic animals.

Administration of propolis at the doses of 200mg/Kg and 300mg/Kg to diabetic rats produced significant decrease in the blood glucose level from the first week of administration onward and there was no significant difference in the hypoglycemic activity of propolis at both doses. This result conforms to the findings of Wang and Li<sup>[37]</sup> and Murata et al.<sup>[38]</sup>. The significant hypoglycemic activity of propolis may suggest that propolis exerts this activity by direct and indirect mechanism in rats<sup>[39]</sup>. If propolis had acted only as an indirect hypoglycemic agent, no effect would have been observed when administered to alloxan treated rats due to the severe destructive effect of alloxan on the  $\beta$ -cells of the pancreas<sup>[40]</sup>. Propolis may also have acted indirectly by stimulating the few surviving  $\beta$ -cells to secrete more insulin rather than aiding the regeneration of necrotic  $\beta$ -cells of the pancreas.

The observed increase in serum levels of LDL-C, VLDL-C, TC, TG, and decreased level of HDL-C in diabetic untreated groups is in accordance with the findings of Douillet et al.<sup>[41]</sup> and Naziroglu et al.<sup>[42]</sup>. Administration of propolis to diabetic animals at doses of 200mg/Kg and 300mg/Kg significantly

lowered the level of LDL-C and total cholesterol and also significantly elevated the level of HDL-C when compared to the diabetic untreated animals. Propolis administration at both doses produced no significant effect on the elevated levels of triglycerides and VLDL-C. This result does not conform to that of Michelle et al.<sup>[43]</sup> who reported that Brazilian propolis had no effect on elevated level of serum LDL-C, VLDL-C, TC, TG, and decreased HDL-C in diabetes after 28 days of treatment. El-Sayed et al.<sup>[36]</sup> also reported propolis of Egyptian origin of having antihyperlipidemic effect by significantly ameliorating the elevated level of LDL-C, TC, TG, and decreased HDL-C in diabetes. Administration of propolis at the dose of 200mg/Kg to non-diabetic animals produced no significant difference in the lipid profile of the normal non-diabetic control and the vehicle control group that received propolis. This result is in agreement with the findings of Mani et al.<sup>[44]</sup> who observed that long term administration of propolis to normal rats produced no alteration to the serum lipid profile. The lowering of TC, LDL-C and the concomitant increase in the serum level of HDL-C confers a cardio-protective function on propolis thereby decreasing the risk of microvascular/macrovacular disease and related complications characteristic of diabetes<sup>[45]</sup>. Hypoglycemic activity of propolis, as evident from this study may be responsible for the amelioration of alterations in lipid and lipoprotein profiles characteristic of diabetes.

Serum activities of ALT and AST are indicators of hepatotoxicity and are also used as biomarkers for early acute hepatic damage<sup>[46]</sup>. Increased levels of these enzymes indicate cellular infiltration and disturbance in the functioning of the hepatic cell membranes<sup>[47]</sup>. Serum levels of AST and ALT were significantly elevated in the diabetic untreated group when compared to the normal untreated group, signifying hepatotoxicity and acute hepatic damage. Administration of propolis (at both doses) and metformin significantly decreased the serum level of AST when compared to the diabetic untreated group. Propolis, at both doses also significantly decreased serum level of ALT, while metformin had

no significant effect on the elevated serum level of ALT compared to the diabetic untreated group. The lowering of the activities of these enzymes to a normal serum level following administration of propolis may be due in part to the ability of propolis to prevent or correct cellular infiltration characteristic of disturbance in the functional and morphological state of the liver in diabetes.

Serum levels of creatinine, a metabolic by-product of creatine that supplies energy for muscle contraction and urea, generated in the liver by metabolised protein are markers of optimal renal function. Elevated levels of these metabolites signify impairment in kidney(s) function<sup>[7]</sup>. In the diabetic untreated group, serum level of urea was significantly elevated compared to the normal control group. Treatment with metformin and the two doses of propolis extract significantly ameliorated the clearance of this metabolite by the kidney, thus restoring the serum level of urea to normal. This is in agreement with the findings of Yamabe et al.<sup>[48]</sup>. From this study, the induced-diabetes had no significant effect on the serum level of creatinine, thus no significant difference was found in the serum level of creatinine between all the groups. The increased level of serum urea rightly points to poor clearance by the kidney. Muscle wasting is characteristic of diabetes and muscle mass is one of the factors that determine the amount of creatinine generated. Muscle mass is directly proportional to the amount of creatinine generated<sup>[49]</sup>. Thus, the loss of weight after diabetes induction in the untreated rats may be responsible for low generation of creatinine, which failed to raise serum level of creatinine despite the impairment in renal function.

Insufficiency of antioxidant defence system leads to elevation in the levels of free radicals. Elevated level of free radicals may lead to disruption in cellular functions, oxidative damages to membranes and enhanced susceptibility to lipid peroxidation<sup>[5]</sup>. Serum level of MDA was elevated, while SOD and GHS levels in the diabetic untreated rats were depressed, indicative of oxidative stress and depression of the antioxidant defence system. This result

shows that the complications observed may be due to depression of the defence system in the rats. Administration of the two doses of propolis and metformin significantly decreased the serum level of MDA and elevated the serum levels of SOD and GSH when compared to the diabetic untreated rats. This also suggests that propolis may have carried out its antidiabetic effect by augmenting the depressed antioxidant defence system in the rats.

## Conclusion

Propolis of Nigerian origin possesses hypoglycemic and antihyperlipidemic activities in addition to its ability to ameliorate oxidative-stress induced organ dysfunction.

## Acknowledgments

Nil.

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Group	A	B	C	D	E	F
Initial BW (g)	225± 7.5	210 ± 9.1	242±6.3	235±8.4	247±11.5	235±7.8
Final mass BW (g)	270±10.2*	258±10.2*	229±10.3	262±9.4*	278±8.9*	260±8.5*

Table 1: Effect of Propolis on Body Weight (BW)

\*P&lt;0.05 significantly different from diabetic control group

A – Non-diabetic control, B – Non-diabetic (200mg/kg propolis), C –Diabetic control, D – Diabetic treated (150mg/kg metformin), E – Diabetic experimental (200mg/kg propolis), F– Diabetic experimental (300mg/kg propolis).

HEPATIC AND RENAL FUNCTION MARKERS (mg/dL)				
Group	ALT	AST	UREA	CREATININE
A	31.17 ± 2.69*	111.99 ± 4.10*	45.10 ± 3.84*	0.97 ± 0.01
B	29.47 ± 2.01*	113.22 ± 5.71*	41.72 ± 2.92*	0.94 ± 0.01
C	128.56 ± 6.93	271.11 ± 9.12	64.25 ± 5.12	1.01 ± 0.04
D	125.27 ± 5.18	112.31 ± 5.82*	39.38 ± 2.18*	0.98 ± 0.02
E	32.45 ± 1.67*	114.47 ± 6.75*	44.77 ± 4.29*	0.96 ± 0.06
F	34.46 ± 2.43*	99.53 ± 4.52*	37.90 ± 3.39*	0.94 ± 0.05

Table 2: Effect of propolis on serum markers of hepatic and renal functions

\*P&lt;0.05 significantly different from diabetic control group

A – Non-diabetic control, B – Non-diabetic (200mg/kg propolis), C –Diabetic control, D – Diabetic treated (150mg/kg metformin), E – Diabetic experimental (200mg/kg propolis), F– Diabetic experimental (300mg/kg propolis).

ALT- Alanine transferase, AST- Aspartate aminotransferase.

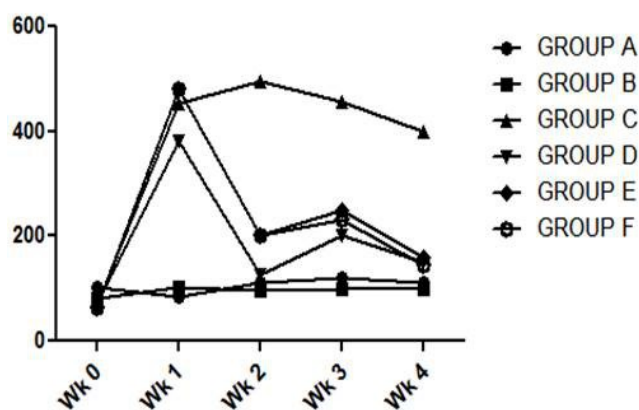


Figure 1: Showing and comparing weekly changes in the blood glucose level of animals in all groups

A – Non-diabetic control, B – Non-diabetic (200mg/kg propolis), C –Diabetic control, D – Diabetic treated (150mg/kg metformin), E – Diabetic experimental (200mg/kg propolis), F– Diabetic experimental (300mg/kg propolis).

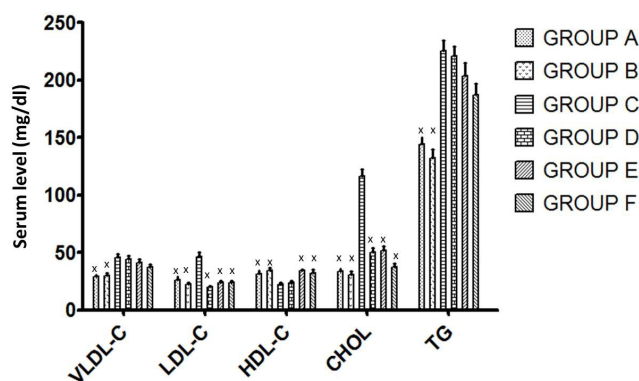


Figure 2: Showing and comparing lipid profiles between all groups and the diabetic untreated group. \* $P < 0.05$  significantly different from diabetic control group  
 A – Non-diabetic control, B – Non-diabetic (200mg/kg propolis), C –Diabetic control, D – Diabetic treated (150mg/kg metformin), E – Diabetic experimental (200mg/kg propolis), F– Diabetic experimental (300mg/kg propolis).

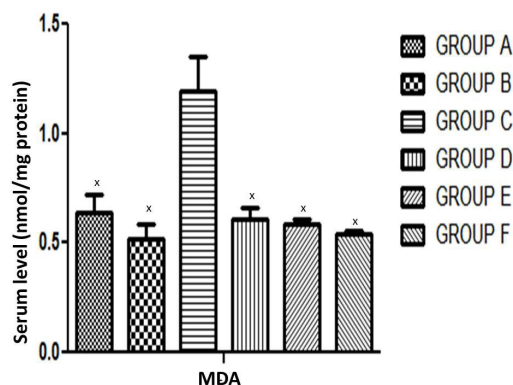


Figure 3: Serum level of MDA in all groups  
 \* $P < 0.05$  significantly different from diabetic control group  
 A – Non-diabetic control, B – Non-diabetic (200mg/kg propolis), C –Diabetic control, D – Diabetic treated (150mg/kg metformin), E – Diabetic experimental (200mg/kg propolis), F– Diabetic experimental (300mg/kg propolis). MDA- Malonaldehyde

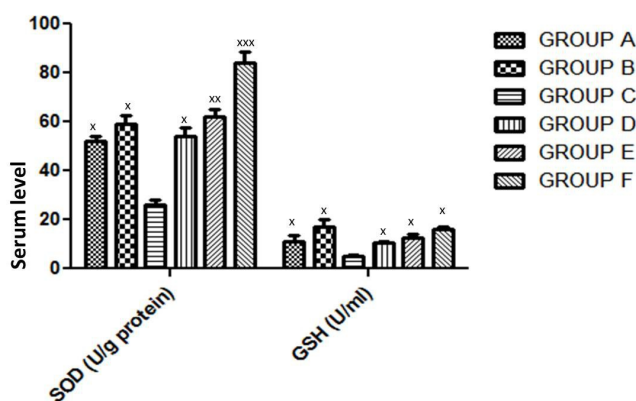


Figure 4: Showing SOD and GSH levels in all groups  
 \* $P < 0.05$  significantly different from diabetic control group  
 \*\* $P < 0.05$  significantly different from diabetic control group and normal control  
 A – Non-diabetic control, B – Non-diabetic (200mg/kg propolis), C –Diabetic control, D – Diabetic treated (150mg/kg metformin), E – Diabetic experimental (200mg/kg propolis), F– Diabetic experimental (300mg/kg propolis). SOD- Superoxide dismutase, GSH- Glutathione