

## METHANOL LEAF EXTRACT OF *SIMAROUBA GLAUCA* INDUCED DYSLIPIDEMIA-LINKED CARDIOVASCULAR DISEASE INDICATORS AND ITS EFFECT ON ANTIOXIDANT PROTEINS

Osagie-Eweka<sup>a\*</sup>, S.D.E, Orhue<sup>a</sup>, N.E.J, Omogbai<sup>b</sup> E.K.I and Moke<sup>c</sup> E.G.

<sup>a</sup>Department of Biochemistry, Faculty of Life Sciences, University of Benin. Benin City. Nigeria.

<sup>b</sup>Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin.

<sup>c</sup>Department of Pharmacology, Faculty of Basic Medical Sciences, Delta State University, Abraka

\*Corresponding Author: E-mail: [davies.osagie-eweke@uniben.edu](mailto:davies.osagie-eweke@uniben.edu).

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### ABSTRACT

The study focused on the effect of methanol leaf extract of *Simarouba glauca* (MESG) on lipoproteins, cholesterol and oxidative stress biomarkers in male Wistar rat. Toxicological inquest of MESG was evaluated as prescribed in the guidelines of organization for economic co-operation and development (OECD), No. 425. A total of twenty-four male Wistar rats were used; divided into four groups of six rats each, including the control. Test rats were given MESG 500, 1000 and 2000 mg/kg body weight respectively, daily for thirty (30) days. At the end of the study, the rats were fasted overnight and sacrificed and biochemical indicators were evaluated. Results showed marked increase ( $p < 0.05$ ) in Total Cholesterol at MESG 2000 mg/kg; a reduction and increase ( $p < 0.05$ ) in High-Density Lipoprotein Cholesterol and Triglycerides respectively, at all doses; an increase ( $p < 0.05$ ) in Low-Density Lipoprotein Cholesterol at MESG 2000 mg/kg. Additional data indicated no changes ( $p > 0.05$ ) in Malondialdehyde levels; liver Catalase was significantly ( $p < 0.05$ ) expressed at MESG 500 and 2000 mg/kg, kidney CAT was significantly ( $P < 0.05$ ) expressed at all doses. The liver Superoxide Dismutase (SOD) was significantly ( $p < 0.05$ ) expressed at MESG 1000 and 2000 mg/kg; the kidney and heart SOD were also significantly ( $P < 0.05$ ) expressed at MESG 500 and 2000 mg/kg respectively. The plasma GSH-PX was significantly ( $P < 0.05$ ) expressed at MESG 1000 mg/kg; while the liver and heart GSH-PX were significantly ( $P < 0.05$ ) expressed and repressed at 500 mg/kg respectively. Conclusively, MESG elicited obvious dyslipidemia; accompanied by marked alterations in selected endogenous oxidative stress biomarkers.

**Keywords:** *Simarouba glauca*; Toxicity, Dyslipidemia, Antioxidant Proteins.

### INTRODUCTION

Several plant metabolites such as alkaloids, anthraquinone glycosides, pyrrolizidine; amongst others have been implicated in toxicological studies (Rowin and Lewis, 1996; Becker *et al.*, 1996; George, 2011); a fall in the antioxidant defense system, arising from toxicity of ingested plants supplement may further consequently contribute to oxidative injury. The prooxidant actions of phenoxyl radical generated from the electron-donating action of phenolic compounds have been

strongly implicated in inducing oxidative stress in a related study (Sakihama *et al.*, 2002). Oxidative stress-linked endothelial dysfunction relatively propagated by oxidized Low-Density Lipoprotein Cholesterol (LDL-C) has been reported to play a significant role in vascular damage (Steinberg, 1997) arising from the formation of toxic lipid peroxides, platelet aggregation and activation of macrophages (Kawada, 2012). Although, the February 10, 2015 Washington Post reported that there are strong indications that

Cholesterol may not be associated with the complications of cardiovascular diseases (<https://www.washingtonpost.com/news/wonk/wp/2015/02/10/feds-poises-to-withdraw-longstanding-warnings-about-dietary-cholesterol/>).

Phenolics present in medicinal herbal supplements are capable of generating phenoxyl radicals as their primary oxidized product (Sakihama *et al.*, 2002), which can initiate the propagation of reactive molecule that may result to oxidative damage to tissues. Furthermore, the toxicological effects associated with the metabolism of phenolic compounds inherent in medicinal plants; a resulting xenobiotic process may in turn impact on lipid homeostasis which may to cause dyslipidemia.

Cardiovascular complications etiology is not unconnected with patho-physiological events associated with elevated plasma LDL-C (Cesare *et al.*, 2005). The high poly-unsaturated fatty acid content of LDL lipoproteins makes it easily susceptible to oxidation; whereas, otherwise is the case with the native LDL. Oxidized LDLs have affinity for macrophages via the scavenger receptor; thereby generating the well-known “foam cells” that forms atherosclerotic plaques. Notably, elevated triglycerides and reduced HDL-C have also been reportedly implicated in cardiovascular diseases (Hokanson and Austin, 1996; Nordestgaard and Varbo, 2014).

The toxicological implications of varying doses of medicinal supplements have been reported to range from severe dyslipidemia to hypolipidemia (Perez *et al.*, 1999; Adebayo *et al.*, 2006; Ogbonna *et al.*, 2010; Patrick-Iwuanyanwu *et al.*, 2012; Haza *et al.*, 2016;).

*Simarouba glauca*, Paradise tree or “Laxmitaru” belongs to the family *Simaroubaceae* (Patil and Gaikwad, 2011). *S.*

*glauca* has a long history of herbal medicine application considering its many documented evidence of pharmacological potentials (Patil and Gaikwad, 2011). The stem-bark and leaf of *S. glauca* contain triterpenes are useful in curing amoebiasis, diarrhea and malaria. Chemicals present in leaf, fruit, pulp and seed of *S. glauca* have been reported to possess analgesic, antimicrobial, antiviral, astringent, emmenagogue, stomachic, tonic, vermifuge properties (Joshi and Joshi, 2002).

Therefore, the study focused on whether the oral administration of MESHG elicited lipid peroxidation associated with dyslipidemia; capable of causing damage to cell membrane and toxicity at cellular levels.

## MATERIALS AND METHODS

### Collection of *S. glauca* Leaves and Preparation of Ethanol Extract

Leaves of *S. glauca* as shown in Plate 1, were harvested from *Cercobela Farms*<sup>®</sup>, Ubiaja, Esan South East Local Government Area of Edo State, Nigeria. A fresh plant specimen was authenticated and a voucher specimen deposited at the Department of Plant Biology and Biotechnology Herbarium, University of Benin, Benin City, Nigeria with voucher No. UBHS382. The leaves were rinsed with tap water and air-dried at the Department of Biochemistry’ Laboratory for twenty-eight (28) days at room temperature. Leaves were pulverized and sieved off a mesh to obtain fine particles at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin. A 500 g of the leaf powder was soaked in a 2.5 L methanol (99.5 % purity and analytical grade) and stirred at intervals for 24 hours. The extraction procedure was repeated by macerating the residue for another 48 hours to obtain filtrate that was freeze-dried to obtain dried methanol extract of 51.1 g yield with a

10.22 % yield; as previously reported by Osagie-Eweka *et al.* (2016).



**Plate. 1.** Paradise Tree (*S. glauca*) growing in *Cercobela Farms*<sup>®</sup> (Osagie-Eweka Photo Library, 2016).

### Reagents Test kits

Distilled H<sub>2</sub>O, Deionized H<sub>2</sub>O, Total Cholesterol Randox<sup>®</sup> (Pipes buffer, 4-aminoantipyrine, phenol, Peroxidase, cholesterol esterase and cholesterol oxidase), TG Randox<sup>®</sup> (Pipes buffer, 4-chloro-phenol, Mg<sup>2+</sup>, 4-aminophenazone, ATP, Lipase, glycerol-kinase, glycerol-3-phosphate oxidase, peroxidase), HDL-Cholesterol Randox<sup>®</sup> (Cholesterol Oxidase, Phosphotungstic Acid and MgCl); all from Randox Laboratory (United Kingdom). Thiobarbituric acid (TBA), glacial acetic acid, Phosphate buffer, H<sub>2</sub>SO<sub>4</sub>, KMnO<sub>4</sub>, Carbonate buffer, 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), Potassium Phosphate buffer, H<sub>2</sub>O<sub>2</sub> and pyrogallol were Sigma Aldrich, Germany.

### Experimental Animals

A total of 24 male *Wistar* rats weighing between 184 and 200 g were used for the study. The animals were housed in metabolic cages; fed a normal commercial pelleted diet (Livestock Feeds<sup>®</sup>), had access to water *ad libitum* and maintained under laboratory conditions of 12 h light/12 dark cycle with a two-week acclimatization prior to

commencement of studies. The research was conducted in accordance with the internationally accepted guidelines for laboratory animal use.

### Oral Administration of MESHG (*In-Vivo*)

The study was conducted as provided in the OECD (2008), NO. 425 test guidelines; earlier described by Rout *et al.* (2014) and Oliveira *et al.* (2016). A total of twenty-four (24) male *Wistar* rats were utilized in this phase of the study and were allowed access to food and drinking water *ad libitum*. The rats were distributed into four (4) groups of  $n = 6$ . Test animals received 500, 1000 and 2000 mg/kg body weight respectively of MESHG daily for thirty (30) days, while the control group received only rat pellets and water *ad libitum*.

### Collection of Data and Specimen

At the end of the study, the rats were fasted overnight, anesthetized using a chloroform saturated chamber and sacrificed. The thoracic and abdominal regions were opened up and blood was withdrawn from the hepatic portal vein and (or) thoracic aorta using a 5ml syringe; emptied into a 5 mL heparinized specimen bottles. The blood was then

centrifuged at 3,500 rpm for 10 minutes to obtain a clear supernatant (plasma) that was stored at  $-18^{\circ}\text{C}$  until required for relevant biochemical analyses; conducted within a few days.

### Biochemical Analyses

Lipid profile tests of Total Cholesterol (TC), High-Density Lipoprotein Cholesterol (HDL-C), Triglycerides (TG) and Low-Density Lipoprotein Cholesterol (LDL-C) were conducted using colorimetric methods described by Roeschlaw *et al.* (1974), Jacobs and Van Denmark (1960) and Friedewald *et al.* (1972) respectively with the aid of commercially available test kits, product of Randox<sup>®</sup> Laboratories (United Kingdom). The oxidative stress status was evaluated by testing the levels of the following which include Malondialdehyde (MDA), Catalase (CAT) and Superoxide Dismutase (SOD) activities, Reduced Glutathione (GSH) and Glutathione Peroxidase (GSHPX) according to the methods reported by Gutteridge and Wilkins (1982), Cohen *et al.* (1970) Misra and Fridovich (1972), Ellman (1959); Chance and Maehly (1955) respectively.

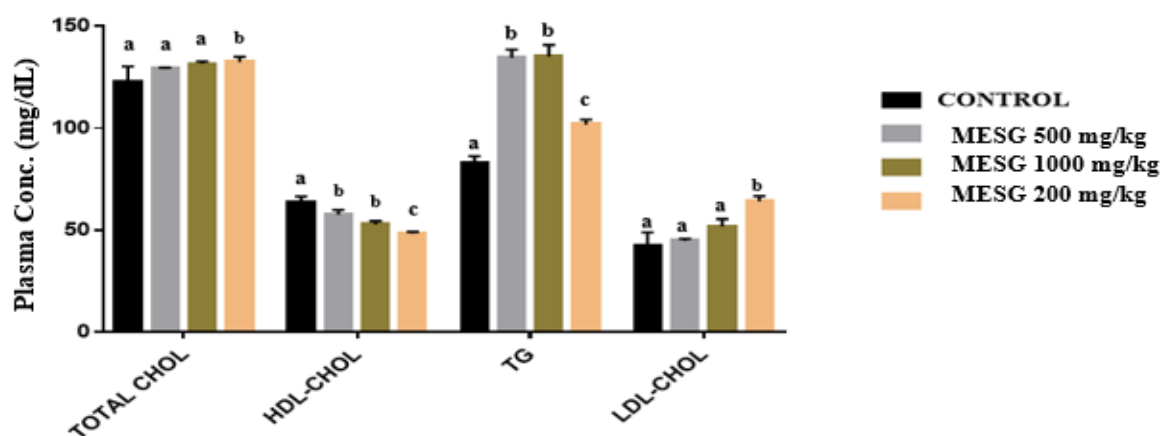
### Statistical Analyses

Data obtained from the study are expressed as mean and standard deviation (mean  $\pm$  SD) where applicable. Statistical differences between means of test groups were evaluated by one-way analysis of variance (ANOVA); while the post-hoc comparison tests were carried out using the Tukey's multiple comparison test. Differences in means were considered significant at  $p < 0.05$  and not significant at  $p > 0.05$ . All statistical analyses were conducted using *GraphPad prism*<sup>®</sup>, version 7.

### RESULTS

#### Effect of Oral Administration of MESH on TC, TG and Lipoproteins in Wistar Rat

The data in **Fig. 1** shows significant ( $p < 0.05$ ) increase and reduction in plasma triglycerides (TG) and HDL-C respectively at all doses; marked elevation of plasma total cholesterol (TC) and LDL-C at MESH 2000 mg/kg relative to the control.

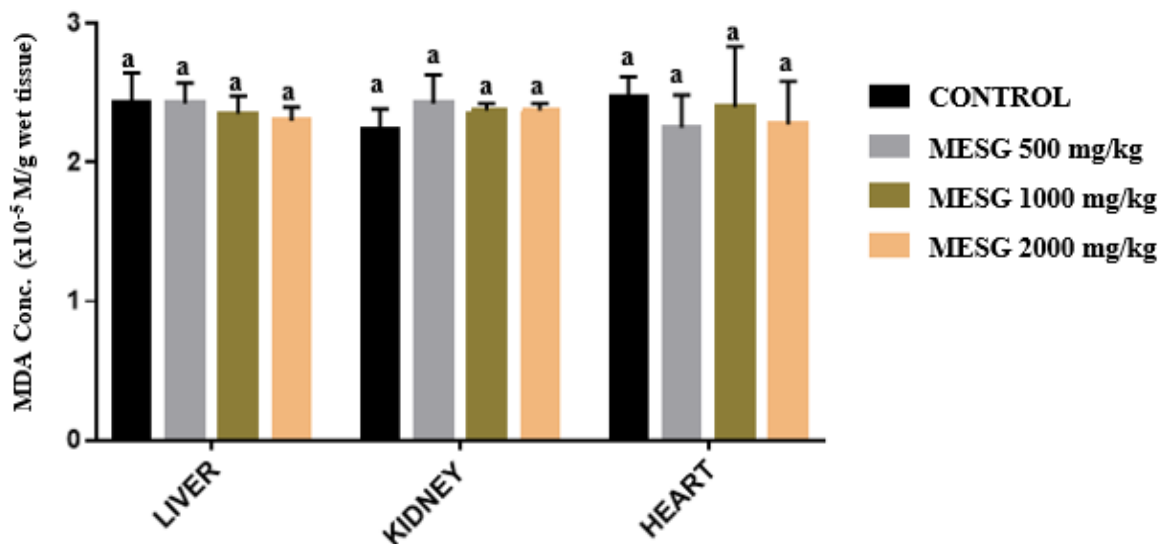


**Fig. 1.** Effect of varying doses of Methanol Leaf Extract of *S. glauca* (MESH) on Plasma TC, HDL-C, TG and LDL-C of Male Wistar Rats after 30 days. Data with similar lower-case letters are not significantly different ( $p > 0.05$ ); data with different lower-case letters are significantly different ( $p < 0.05$ ). Data are presented as Mean  $\pm$  SD.

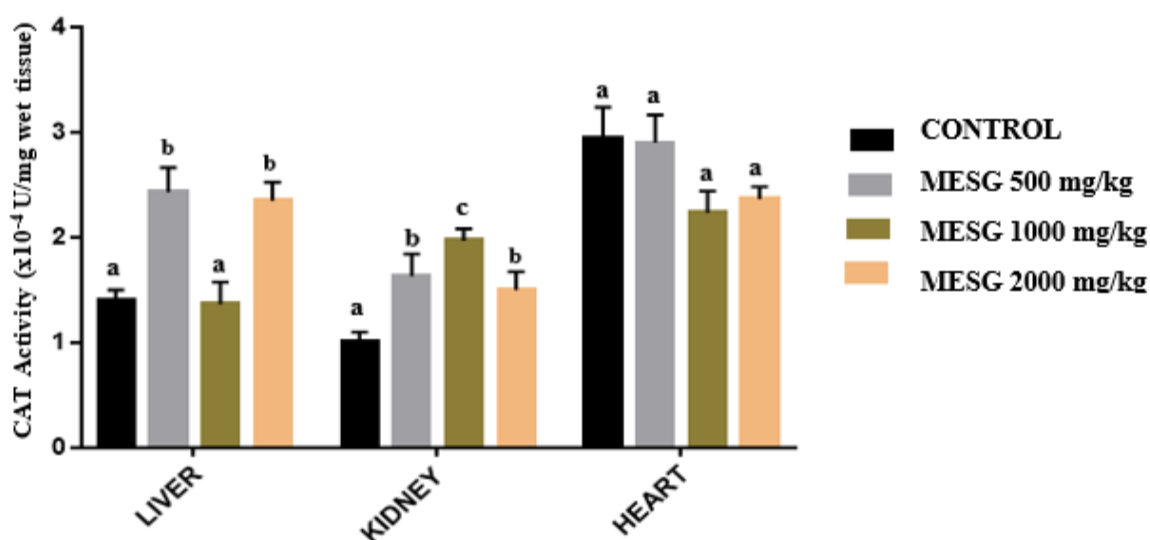
### Effect of MESG on Oxidative Stress Biomarkers in Male Wistar Rats

The data presented in **Fig. 2** indicated that there were no significant differences ( $p > 0.05$ ) in liver, kidney and Heart malondialdehyde (MDA) levels at all doses compared to the control. The data presented in **Fig. 3** show marked elevation ( $P < 0.05$ ) in liver catalase (CAT) activity at MESG 500 and 2000 mg/kg respectively; significant increase ( $p < 0.05$ ) in kidney CAT activity at all doses; no significant ( $p > 0.05$ ) changes in heart CAT activity at all doses relative to the control. The **Fig. 4** data indicate significant ( $P < 0.05$ ) elevation in liver superoxide dismutase (SOD) activity at MESG 1000 and 2000 mg/kg

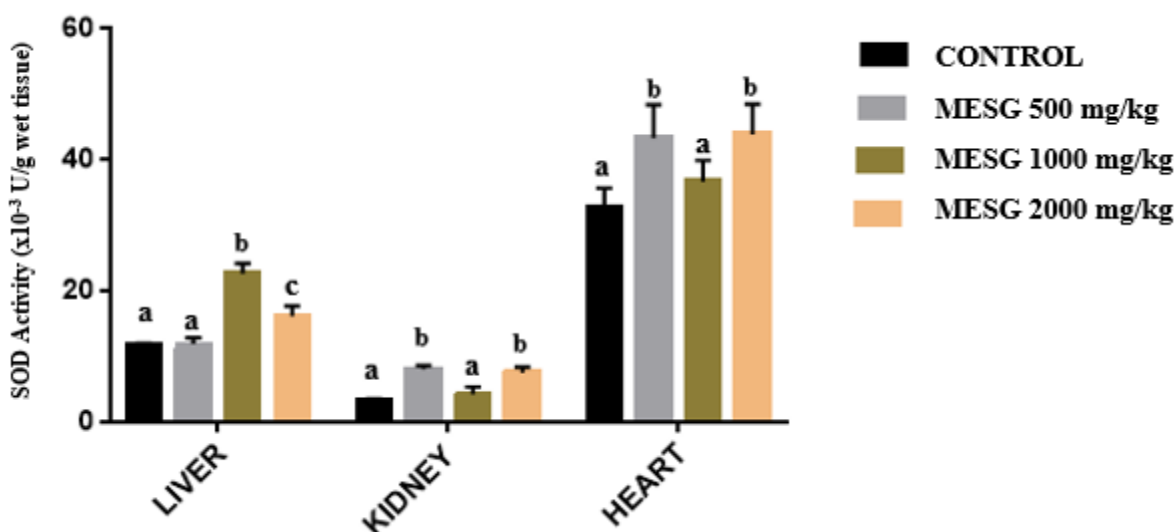
respectively; elevation ( $P < 0.05$ ) in kidney and heart SOD activities at MESG 500 and 2000 mg/kg respectively compared to the control. The **Figs 5a & b** show no significant alteration ( $P > 0.05$ ) in plasma, liver and heart glutathione (GSH) levels respectively at all doses relative to the control group. **Fig. 6a** reveals significant increase ( $p < 0.05$ ) in plasma glutathione peroxidase (GSH-PX) activity at MESG 1000 mg/kg body weight; whereas, others were not affected relative to the control. **Fig. 6b** data show marked expression and inhibition ( $p < 0.05$ ) in liver GSH-PX activity at MESG 500 and 2000 mg/kg respectively; there was significant ( $p < 0.05$ ) inhibitory activity in the heart tissue at MESG 500 mg/kg compared to the controls.



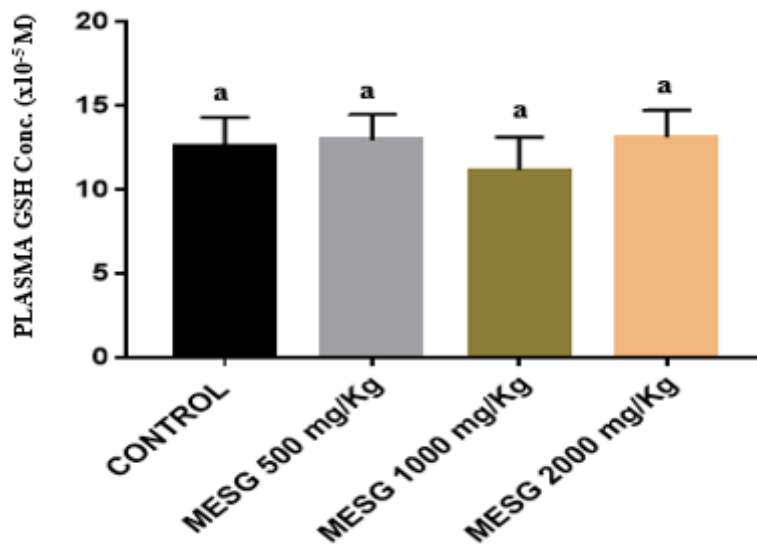
**Fig. 2.** Effect of varying doses of MESG on liver, kidney and heart MDA levels of Male Wistar Rats after 30 days. Data with similar lower-case letters are not significantly different ( $p > 0.05$ ); data with different lower- case letters are significantly different ( $p < 0.05$ ). Data are presented as Mean  $\pm$  SD.



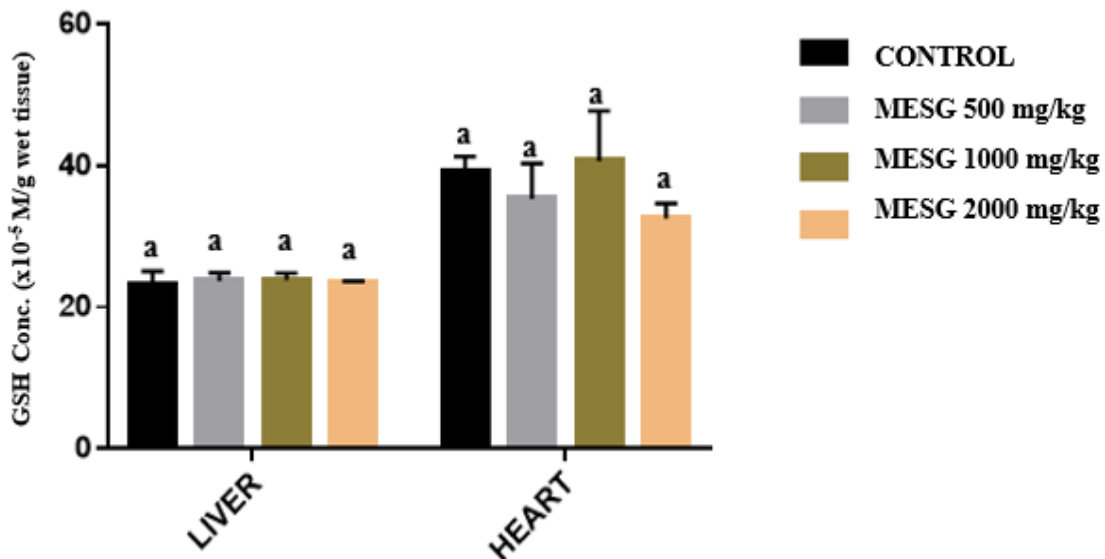
**Fig. 3.** Effect of varying doses of MESH on liver, kidney and heart CAT activity of Male *Wistar* Rats after 30 days. Data with similar lower-case letters are not significantly different ( $p > 0.05$ ); data with different lower- case letters are significantly different ( $p < 0.05$ ). Data are presented as Mean  $\pm$  SD.



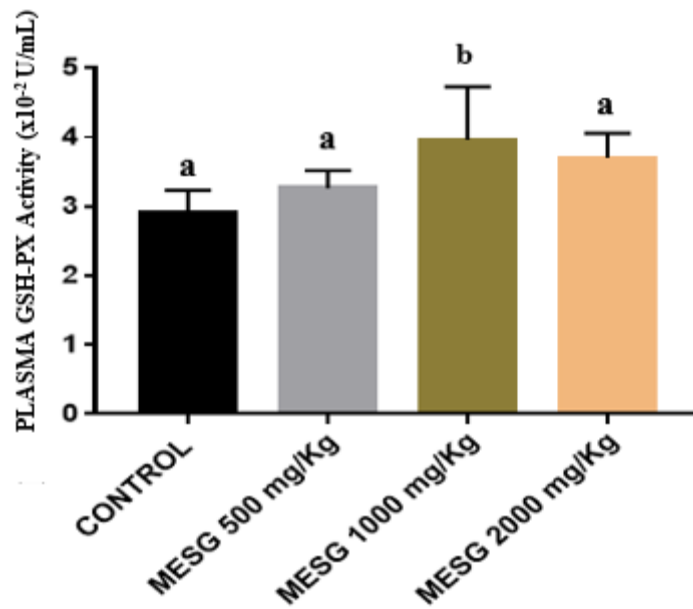
**Fig. 4.** Effect of varying doses of MESH on liver, kidney and heart SOD activity of Male *Wistar* Rats after 30 days. Data with similar lower-case letters are not significantly different ( $p > 0.05$ ); data with different lower- case letters are significantly different ( $p < 0.05$ ). Data are presented as Mean  $\pm$  SD.



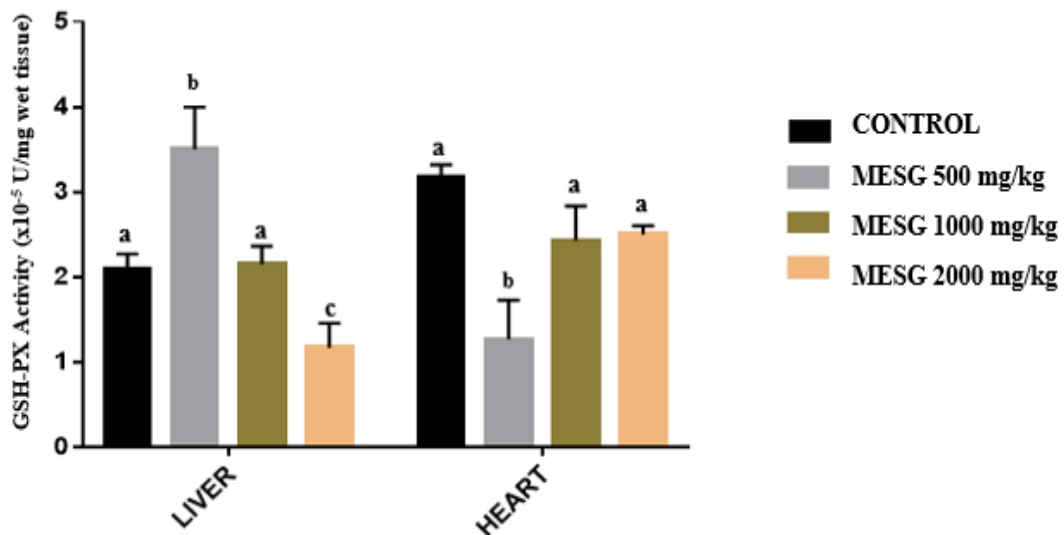
**Fig. 5a.** Effect of varying doses of EESG on plasma GSH level of Male *Wistar* Rats after 30 days. Data with similar lower-case alphabets are not significantly different ( $p > 0.05$ ); data with different lower- case alphabets are significantly different ( $p < 0.05$ ). Data are presented as Mean  $\pm$  SD.



**Fig. 5b.** Effect of varying doses of MESG on liver and heart GSH levels of Male *Wistar* Rats after 30 days. Data with similar lower-case letters are not significantly different ( $p > 0.05$ ); data with different lower- case letters are significantly different ( $p < 0.05$ ). Data are presented as Mean  $\pm$  SD.



**Fig. 6a.** Effect of varying doses of MESG on plasma GSH-PX activity of Male *Wistar* Rats after 30 days. Data with similar lower-case letters are not significantly different ( $p > 0.05$ ); data with different lower- case letters are significantly different ( $p < 0.05$ ). Data are presented as Mean  $\pm$  SD.



**Fig. 6b.** Effect of varying doses of EESG on liver and heart GSH-PX activities of Male *Wistar* Rats after 30 days. Data with similar lower-case letters are not significantly different ( $p > 0.05$ ); data with different lower- case letters are significantly different ( $p < 0.05$ ). Data are presented as Mean  $\pm$  SD.

## DISCUSSIONS

Lipid peroxidation processes have been reportedly proven to consist of a series of molecular mechanisms resulting from toxicity of plant metabolites, oxidative stress; damage

to membrane components of cells and cell death (Dianzani and Barrera, 2008).

Lipid oxidation, particularly the oxidized LDL-cholesterol elicits plaque on vascular tissues endothelium which results to oxidative damage; endothelium dysfunction and nitric



oxide insufficiency. Witztum and Steinberg (1991) have reported that oxidized lipids activate dyslipidemia and (or) elevated lipid, signaling the on-set of cardiovascular disease and related conditions.

Studies relating to the application of medicinal plants have reported findings on the interference of these active compounds on the liver lipid metabolism, circulatory system and at the modulatory intracellular signaling pathways and transcriptional activities (Kun-Ho *et al.*, 2016; Koo and Noh, 2007; Ahmida and Abuzogaya, 2009).

In the present study, the observed dyslipidemia (Fig. 1) resulting from the administration of MESH did not elicit significant lipid peroxidation as indicated by the measured malondialdehyde levels (Fig. 2). Although, an earlier study reported by Yang *et al.* (2008) indicated that malondialdehyde (MDA) correlated positively with elevated triglycerides (TG), total cholesterol (TC) and Low-Density Lipoprotein Cholesterol in the experimental subjects. Yang *et al.* (2008) also reported that SOD and GSH levels were significantly lowered in the subjects with dyslipidemia; whereas, the findings of the present study revealed increased expression of SOD activities in the liver, kidney and heart tissue respectively of experimental rat. However, the plasma reduced glutathione were within the normal levels. The normal MDA level of the present study may be attributed to the poor elevations in TC and LDL-C concentrations which obviously did not elicit oxidized LDL-Cholesterol-linked lipid peroxidation. Therefore, the outcome of the present study is at variance with that of Yang *et al.* (2008).

Nordestgaard and Varbo (2014) epidemiological study reported that marked increase in plasma TG and marked reduction

in HDL-cholesterol strongly indicate a link with cardiovascular disease.

There are speculations that the application of medicinal plants and their active compounds such as phenolics may release prooxidants capable of eliciting oxidative stress (Sakihama *et al.*, 2002); such action may consequently lead to proliferation of free pro-oxidant radical which may further activate elevations in endogenous anti-oxidative stress enzymes in response to radical overload. The report of Ugbogu *et al.* (2020) reveals that treatment with varying doses (200, 400 and 800 mg/kg body weight respectively) of aqueous fermented seed extract of *Pentaclethra Macrophylla* strongly indicated dyslipidemia with elevations in TC, HDL-C, TG and LDL-C; which further lays credence to our present findings. Although, Ugbogu *et al.* (2020) did not report oxidative stress status and lipid peroxidation of the experimental rats treated with *P. Macrophilla*; the plant type and doses differ compared to our present study. On the other hand, medicinal plants have been reported to enhance these antioxidant enzymes (Al-Sa'aidi *et al.*, 2012). In the present study, the observed increased expression in liver and kidney catalase (CAT) activity may not be unconnected to the effect of pro-oxidants released from the metabolism of phenolics compounds present in *S. glauca* (Osagie-Eweka *et al.*, 2016).

Conclusively, the data presented in Fig. 1 clearly reveal dyslipidemia that may be associated with cardiovascular complications when the MESH is applied at higher doses; with particular references to the TC, TG and LDL-C. However, the elevations in plasma TC and LDL-C at MESH 2000 mg/kg did not result to a corresponding increase in MDA levels suggesting that alterations of TC and LDL-C at the aforementioned doses did not

elicit lipid peroxidation; as such, the increase in the expression of CAT and SOD activities may indicate an attempt to mop-up prooxidants released during the metabolism of the phenolics inherent in MESHG; perhaps, linked to activation of the xenobiotic metabolic pathway. Furthermore, up-regulation of the NADPH-oxidase pathway may have also elicited increase in reactive oxygen species (ROS) in the liver and kidney being the major organs responsible for drug metabolism; this may also account for the increase in the expression of the liver and kidney CAT and SOD activities.

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