Effects of Aqueous Leaf Extract of *Simarouba glauca* DC (*Simaroubaceae*) on Lipoprotein Homeostasis and Oxidative Stress Biomarkers

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**ABSTRACT**

**Background and Purpose:** *Simarouba glauca* is widely reported to contain a number of biologically active compounds with potentials in the treatment of numerous diseases. The study was conducted to evaluate the sub-acute effects of the aqueous leaf extract of *Simarouba glauca* (AESG) on lipoproteins and oxidative stress biomarkers in male Wistar rats.

**Methods:** Oral administration of AESG was carried out in line with the guidelines of the Organization for Economic Co-operation and Development (OECD), No. 425 using a total of 24 male Wistar rats allotted to four groups (n=6); given distilled water, 500, 1000, and 2000 mg/kg/day of AESG respectively for 30 days.

**Results:** In plasma, there was a significant reduction (*P*<0.05) in HDL-cholesterol; elevated (*P*<0.05) triglycerides (TG) at 1000 and 2000 mg/kg/day; elevated (*P*<0.05), and LDL-cholesterol at 500 and 1000 mg/kg/day, relative to the control. While the level of liver total cholesterol (TC) reduced significantly, it increased in the heart. Catalase (CAT) activity in the liver increased significantly (*P*<0.05) at all doses. The dose of 1000 mg/kg/day significantly (*P*<0.05) elevated kidney CAT activity. The activities of superoxide dismutase (SOD) in liver and heart reduced (*P*<0.05) at 500 mg/kg/day. At all doses, the levels of reduced glutathione (GSH) in plasma, liver and heart were comparable with the control. Although, there were no significant changes in plasma and liver glutathione peroxidase (GSH-PX) activity at all doses, animals given 500 mg/kg had reduction (*P*<0.05) in the heart GSH-PX activity compared to the control.

**Conclusion:** Oral sub-acute AESG at high doses altered lipid homeostasis in plasma and heart without lipid peroxidation or oxidative stress. The extract has the potential to cause hyperlipidemia.

**KEYWORDS**

*Simarouba glauca*; Lipid Profile; Dyslipidemia; Oxidative Stress.

**ARTICLE HISTORY**

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INTRODUCTION

Lipids form an integral part of the cell composition with several support to the existence and survival of cells. Lipids play important roles in the human body as hormones or hormone precursors, aiding digestibility, providing energy, storage and metabolic fuels (Chung, 2021). They also act as functional and structural components of bio-membranes, form insulation to enable nerve conduction and prevent heat loss (Chung, 2021); amongst others. High-density lipoprotein cholesterol (HDLC) otherwise regarded as good lipoprotein cholesterol has been reported to function in the removal of cholesterol from peripheral cells and systemic circulation to the liver where it is converted to bile acids and excreted into the intestine (Wang and Briggs, 2004). Plasma low density lipoprotein cholesterol (LDLC) are a class of plasma lipoproteins that is regarded as bad cholesterol due to its strong pathological correlation with cardiovascular complications (Cesare et al., 2005). It has also been reported that oxidized LDLC resulting from oxidative stress can be injurious to vascular endothelium rendering it dysfunctional (Steinberg, 1997). Epidemiological studies have also implicated elevated triglycerides (TG) and reduced HDLC in cardiovascular diseases (Hokanson and Austin, 1996; Nordestgaard, 2014). Lipids form a component of plant cell membrane in varying proportions depending on taxonomical classification and varieties of plants exhibit medicinal properties. Several toxicological studies have shown that a number of medicinal plant supplements affect lipid metabolism ranging from hypolipidemia to severe dyslipidemia (Perez et al., 1999; Adebayo et al., 2006; Ogbonnia et al., 2010; Patrick-Iwuanyanwu et al., 2012; Zaza et al., 2016). Although herbal medicinal supplements are generally considered safe, some are known to be toxic at high doses, while others may be potentially toxic after prolonged use (Patrick-Iwuanyanwu et al., 2012).

The antioxidative effect of phytotherapeutic principles inherent in medicinal plants have been exhaustively researched and reported (Patel et al., 2011; Afagnini et al., 2017; Akindele et al., 2018; Kale et al., 2019; Shehu and Yabagi, 2019). The pro-oxidant action of phenoxy radicals generated from the electron-donating action of phenolic compounds has been strongly implicated in inducing oxidative stress (Sakihama et al., 2002). This suggests that medicinal herbal supplements that contain phenolics may generate phenoxy radicals as the primary oxidizing agents (Sakihama et al., 2002) capable of initiating lipid peroxidation.

Herbal medicinal preparations have been in use for thousands of years in developing and developed countries owing to their natural origin and presumed safety (Kamboj, 2012). Several plant metabolites such as alkaloids, anthraquinone glycosides, pyrrolizidine alkaloids amongst others have been implicated in toxicity (Rowin and Lewis, 1996; Becker et al., 1996; George, 2011). Therefore, the present study evaluates the safety of evaluation of the leaf extract of Simarouba glauca with respect to lipoprotein metabolism and oxidative stress.

Simarouba glauca, commonly known as “Paradise tree” or “Laxmitaru” belongs to the family Simaroubaceae (Patil and Gaikwad, 2011). S. glauca has a long history of herbal medicine application giving its many pharmacological properties that have been documented (Patil and Gaikwad, 2011). The stem-bark and leaf of S. glauca contain triterpenes useful in treating 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). The major active groups of phytochemicals in S. glauca are the quassinoids, which belong to the triterpene chemical family. Ailanthinone, glaucarubinone and holocanthon are considered as some of the main active quassinoids in genus Simarouba. Other chemicals include benzoquinone, canthin, dehydroglaucarubinone, glaucarubine, glaucarubolone, melianone, simaroubidin, simarolide, simaroubin, simarubolide, sitosterol and tirucalla (Technical Data Report for Simarouba (Simarouba amara), 2002).

MATERIALS AND METHODS

Collection of S. glauca Leaves and Preparation of Aqueous Extract

Leaves of S. glauca were collected from Cercobela Farms®, Ubiaja, Esan South East Local Government Area of Edo State, Nigeria. The plant was authenticated and at the Department of Plant Biology and Biotechnology, University of Benin where a herbarium voucher specimen (NO. UBH382) has been deposited. The leaves were rinsed with tap water and air-dried at room temperature at the Department of Biochemistry, University of Benin, for twenty-eight (28) days. Leaves were pulverized and sieved at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, to obtain fine a fine powder. A quantity (500 g) of the leaf powder was soaked in 2.5 L of distilled water and stirred at intervals for 24 h, and filtered. The left over material was reextracted in another portion of 2.5 L of distilled water and stirred at intervals for another 24 h. Both filtrate portions were pooled and freeze-dried (Osagie-Eweka et al., 2016). The yield of extraction was 6% w/w.

Chemicals and Reagents Test Kits

Randox® total cholesterol kit (Pipes buffer, 4-aminoantipyrine, phenol, peroxidase, cholesterol esterase
and cholesterol oxidase), Randox® TG (Pipes buffer, 4-chloro-phenol, Mg²⁺, 4-aminophenazone, ATP, lipase, glycerol-kinase, glyceral-3-phosphate oxidase, peroxidase), Randox® HDL-cholesterol (cholesterol oxidase, phosphotungstic acid and MgCl₂); were all purchased from Randox Laboratory (United Kingdom). Thiobarbituric acid (TBA), glacial acetic acid, phosphate buffer, H₂SO₄, KMNO₄, carbonate buffer, 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), potassium phosphate buffer, H₂O₂, and pyrogallol were obtained from reputable manufacturers. All reagent solutions were freshly prepared.

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 with normal commercial pellets (Livestock Feeds®) and drank water ad libitum. They were maintained under laboratory conditions of 12 h light/ 12 h dark cycle and were acclimatized for two weeks prior to commencement of studies. All experiments were conducted in accordance with the internationally accepted guidelines for laboratory animal use. The protocols were approved by the Faculty of Pharmacy, University of Benin Ethics Committee with reference number EC/FP/021/11.

Oral Administration of AESG
The study was conducted as prescribed in the OECD No. 425 test guidelines (Organisation for Economic Co-operation and Development, 2008) as described by Rout et al. (2014) and Oliveira et al. (2016). The rats were randomly allotted into four (4) groups (n=6). Test animals received oral doses of 500, 1000, and 2000 mg/kg body weight respectively of AESG daily for thirty (30) days while the control group received only water ad libitum.

Collection of Samples and Specimens
On the 30th day the rats were fasted overnight. The following day, they were anesthetized in a chloroform-saturated chamber and sacrificed. Samples of blood were withdrawn from the thoracic aorta into heparinized specimen bottles. The blood samples were centrifuged at 3,500 rpm for 10 min to obtain plasma samples which were stored at −18°C but were used for biochemical analyses within a few days. Kidney, liver, and heart were excised from each rat, cleared off connective tissues and homogenized in 5 mL normal saline using a refrigerated homogenizer (Model 153.02, California, USA). The homogenate was centrifuged at 3500 rpm for 15 min to obtain a clear supernatant which was stored and used as described for plasma samples.

Biochemical Analyses
Lipid profile tests which include total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) were done using colorimetric methods and calculative method described by Roeschlaw et al. (1974), Jacobs and Van Denmark (1960), and Friedewald et al. (1972) respectively using commercial test kits (Randox Laboratories, United Kingdom). Stress related oxidative status was evaluated by determining the levels/activities of malondialdehyde (MDA), catalase (CAT), superoxide (SOD) reduced glutathione (GSH) and glutathione peroxidase (GSH-PX) according to the methods reported by Gutteridge and Wilkins (1982), Cohen et al. (1970), Misra and Fridovich (1972), Ellman (1959) and Chance and Maehly (1955) respectively.

Statistical Analysis
Data are expressed as mean ± SD (standard deviation). Differences between means of test groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. Differences were considered significant at P<0.05. All statistical analyses were conducted using GraphPad prism®, version 7.

RESULTS
Effect of Oral AESG on TC, TG and Lipoproteins in Wistar Rats
Figure 1 shows a significant (P<0.05) reduction in plasma HDL-C and no significant differences in plasma TC at all doses relative to the control. The Figure also shows a significant (P<0.05) increase in plasma TG at AESG doses of 1000 mg/kg/day and 2000 mg/kg/day respectively compared to the control. Likewise, plasma LDL-C increased significantly (P<0.05) at the doses of 500 mg/kg/day and 1000 mg/kg/day respectively compared to the control. Figure 2 shows significant (P<0.05) reduction in liver and increase in heart TC respectively relative to the controls.
In Figure 3, there were no significant differences in liver, kidney and heart malondialdehyde (MDA) levels at all doses compared to the respective control. Figure 4 shows significant ($P<0.05$) elevation in liver catalase (CAT) activity at all doses, significant increase in kidney CAT activity at 1000 mg/kg/day of AESG and no significant difference in heart CAT activity at all doses relative to the respective controls. Figure 5 shows significant reduction ($P<0.05$) in liver and heart superoxide dismutase (SOD) activity at 500 mg/kg/day of AESG; and no significant difference in kidney SOD activity at all doses relative to the control.

Figures 6 and 7 show that there were no significant differences in plasma, liver and heart glutathione (GSH) levels at all doses compared to the controls. Figure 8 shows that there was no significant difference in plasma glutathione peroxidase (GSH-PX) activity at all doses relative to the control. In Figure 9, there was no significant difference in liver GSH-PX activity at all doses but it shows a significant reduction ($P<0.05$) in heart GSH-PX activity at AESG 500 mg/kg/day compared to the controls.
Figure 3: Effect of varying doses of AESG on liver, kidney and heart malondialdehyde (MDA) levels of male Wistar rats after 30 days. Data with similar lower-case alphabets are not significantly different. Data are presented as Mean ± SD, n=6.

Figure 4: Effect of varying doses of AESG on liver, kidney and heart catalase activities of male Wistar rats after 30 days. Data with similar lower-case alphabets are not significantly different; data with different lower-case alphabets are significantly different (\( P < 0.05 \)). Data are presented as Mean ± SD, n=6.
Figure 5: Effect of varying doses of AESG on liver, kidney and heart SOD activities of male Wistar rats after 30 days. Results with similar lower-case alphabets are not significantly different; results with different lower-case alphabets are significantly different ($P<0.05$). Data are presented as Mean ± SD. n=6.

Figure 6: Effect of varying doses of AESG on plasma GSH level of male Wistar rats after 30 days. Results with similar lower-case alphabets are not significantly different. Data are presented as Mean ± SD. n=6.
Figure 7: Effect of varying doses of AESG on liver and heart GSH levels of male Wistar rats after 30 days. Data with similar lower-case alphabets are not significantly different. Data are presented as Mean ± SD. n=6.

Figure 8: Effect of varying doses of AESG on plasma GSH-PX activity of male Wistar rats after 30 days. Results with similar lower-case alphabets are not significantly different; results with different lower-case alphabets are significantly different (P<0.05). Data are presented as Mean ± SD. n=6
DISCUSSION

In the present study, oral administration of AESG did not elicit obvious alteration in plasma TC although it resulted in marked increases in heart TC, plasma TG and LDL-cholesterol at higher doses with corresponding reductions in liver TC and plasma HDL-cholesterol at all doses. Epidemiological and clinical studies have strongly implicated elevated triglycerides and reduced HDL in cardiovascular diseases (Hokanson and Austin, 1996; Nordestgaard, 2014). Therefore, elevated TC and LDL-C may suggest atherosclerosis-related cardiovascular diseases such as hypertension and myocardial ischemia. High doses of AESG used in this study have demonstrated the possibility of these cardiovascular diseases. The high TC and LDL-cholesterol associated with high doses of AEGS may be due to the high free fatty acid content of Simarouba glauca plant as reported by Jena et al. (2010). Pharmacological interventions are often driven towards developing agents capable of preventing, mitigating and (or) lowering cholesterol in the system to prevent cardiovascular diseases.

Studies on medicinal application and hypolipidemic effect of plants have been reported by Perez et al. (1999), Adebayo et al. (2006) and Ogbonnia et al. (2010). In attempts to apply these medicinal supplements for the treatment of certain diseases and conditions, there is the possibility that active compounds such as phenolics in these medicinal plants can release pro-oxidants capable of eliciting oxidative damage.

The evidence that the pro-oxidant action of phenoxy radicals generated from the electron-donating action of phenolics compounds has been reported by Sakihama et al. (2002) and are strongly implicated in the onset of oxidative stress. In this study, AESG did not initiate responses capable of causing lipid damage or peroxidation as the MDA levels were within normal range relative to the control. However, there were elevations in selected in vivo oxidative stress enzymes, perhaps due to increase in metabolic activity associated with the metabolism of the extract.

CONCLUSION

This study has shown that although the aqueous extract of S. glauca did not induce oxidative stress, it has the potential for causing derangement in lipid levels towards hyperlipidemia. This may be a caution or contraindication in its ethnomedicinal uses.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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