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Oral acute and sub-chronic toxicity assessment of aqueous leaf extract of *Simarouba glauca* DC (Paradise tree)

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ABSTRACT

Simarouba glauca has been widely reported to be effective against a number of diseases and possesses medicinal benefits. Thus, the study was conducted to evaluate the toxic effect of aqueous leaf extract of *Simarouba glauca* (AESG) on relevant organs of male *Wistar* rats. The oral acute toxicity of AESG was evaluated according to the method described by Lorke. Sub-chronic toxicity of AESG was carried out in line with the guidelines of the Organization for Economic Co-operation and Development (OECD), using a total of twenty-four (24) male *Wistar* rats divided into four groups of six rats each. Test rats were orally administered AESG at doses of 500, 1000 and 2000 mg /kg body weight, respectively, daily for thirty (30) days. At the end of the study, rats were fasted overnight and sacrificed; the relevant biochemical and histopathology evaluation was carried out. Statistical analysis was conducted using the GraphPad Prism®, version 7. The data obtained indicated that the LD_{50} exceeded 5000 mg/kg. There were significant increases ($P < 0.05$) in percentage (%) body weight of test rats. There were no significant differences ($P < 0.05$) in mean liver, kidney, and heart weight/body weight (IOW/BWT) ratios. The AST activity was significantly lowered ($P < 0.05$) in rats administered AESG 2000 mg/kg. The ALP activities were significantly elevated ($P < 0.05$), while the GGT activities were significantly lowered ($P < 0.05$) in all groups of rats administered AESG. Plasma conjugated and unconjugated bilirubin were significantly lowered and elevated ($P < 0.05$), respectively in rats administered AESG 1000 and 2000 mg/kg. Plasma urea was significantly elevated ($P < 0.05$) in rats given AESG 1000 mg/kg. Test rats given AESG 2000 mg/kg recorded significant reduction ($P < 0.05$) in plasma sodium ions concentration. Rats given AESG 500 mg/kg recorded significant reduction ($P < 0.05$) in plasma bicarbonate ion levels. The findings suggest that AESG was not significantly toxic to the liver, kidney, and heart.

1. Introduction

The World Health Organization [1] describes traditional herbal medicines as natural occurring plant-derived substances with minimal or no industrial processing, used to prevent and treat illnesses within local or regional healing practices [2]. Traditional herbal medicines and their preparations have been widely used for thousands of years in developing and developed countries, owing to their natural origins and presumed less side effects [3]. Physiological and Pharmacological actions exhibited by a variety of plants can be attributed to chemical compounds synthesized by these plants. Modern-day synthetic pharmacological agents were hitherto prepared as crude drugs such as tinctures, teas, powders, and other herbal formulations [4]; and with several active drugs derived directly from plant sources. These include drugs such as aspirin (from willow bark), digitoxin (from foxglove), morphine (from the opium poppy), quinine (from cinchona bark), and pilocarpine (Jaborandi) [5]. However, huge concerns have been raised about the safety of herbal drugs, Thus, necessitated several studies aimed at emphazing the need to evaluate the toxicity of medicinal plants [6–8]. Although reports of injury or death arising from adverse reactions to plant supplements are scanty [9]. Several pharmacological compounds such as alkaloids, anthraquinone glycosides, pyrrolizidine alkaloids, amongst others, synthesized by various plants, have been implicated in toxicity and damage to vital organs [10–12]. These findings further underscore the need for thorough safety evaluation of herbal preparations.

Simarouba glauca, commonly known as “Paradise tree” or “Laxmitaru”, belongs to the family *Simaroubaceae*. The plant is also known by

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Fig. 1. Young Paradise Tree (*S. glauca*) growing in *Cercobela* Farms® (Osagie-Eweka Photo Library, 2015).

other names such as bitter ash, bitter damson, princess tree, and others [13]. The plant is native to the Amazon rainforest and other tropical areas of Mexico, Cuba, Haiti, Jamaica, and North and Central America [14]; exotic to India, Sri Lanka, Philippines, Myanmar, and Nigeria. In the year 2007, it was introduced to Nigeria, in Ubiaja, Esan South East Local Government Area of Edo State by Blessing Akele, Ph.D. and Osagie-Eweka; and was cultivated in *Cercobela* Farms®. *Simarouba glauca* has a long history of herbal medicine having many pharmacological properties that have been documented [14–21]. The major active groups of phytochemicals in *S. glauca* are the quassinoids, which belong to the triterpene chemical family. Ailanthinone, glaucarubinone, and holacanthone are considered some of the main active quassinoids in *Simarouba*. Other chemicals include benzoquinone, canthin, dehydroglaucarubinone, glaucarubine, glaucarubolone, melianone, simaroubidin, simarolide, simaroubin, simarubolide, sitosterol, and tirucalla [22]. Some of these active compounds have demonstrated *in-vitro* anti-tumour activity [23–26], *in-vitro* anti-viral activity [27], *in-vitro* anti-amoebic activity [28–30], *in-vitro* anti-bacterial activity [31], *in-vivo* anti-malaria activity [32–34], among others (Fig. 1).

In view of the renewed interest in *S. glauca* and its several biological activities, it has become imperative to assess the safety of the plant's leaves as a prelude to further therapeutic studies.

2. Materials and methods

2.1. Collection of S. glauca leaves and preparation of aqueous extract

Leaves of *S. glauca* were obtained (harvested) from *Cercobela* Farms®, 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 N0. UBH_S382. The leaves were rinsed with tap water and air-dried at the Department of Biochemistry's Laboratory for twenty-eight (28) days at room temperature. Leaves were pulverized and sifted off a mesh to obtain fine particles at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin. A 500-gram leaf-powder was soaked in a 2.5 mL distilled water and stirred at intervals for 24 h. The procedure was repeated for another 24 h to obtain filtrate that was freeze-dried to obtain dried-water extract as previously reported by Osagie-Eweka et al. [35].

2.2. Reagents test kits

Total cholesterol, alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin and direct bilirubin, total protein, albumin, urea, creatinine, calcium, sodium, chloride, potassium, and bicarbonate test kits were purchased from Randox Laboratory (United Kingdom).

2.3. Experimental animals

A total of 36 male *Wistar* rats weighing between 184 and 210 grammes were used for the study. The animals were housed in metabolic cages; were fed a normal commercial pelleted diet (Livestock Feeds®), watered *ad libitum,* and maintained under laboratory conditions of 12 h light/12 h dark cycle with a two-week acclimatization prior to commencement of studies. The research was conducted in accordance with the internationally acceptable guidelines for use of experimental animals.

2.4. Acute toxicity studies (in vivo)

Acute toxicity evaluation was conducted by the methods previously reported by Lorke [36] to determine LD_{50}. In Phase I, a total of nine (9) male *Wistar* rats were used after a two-week (2) acclimatization. *Wistar* rats were divided into three groups of $n = 3$ with each group receiving 10, 100, and 1000 mg/kg body weight, respectively, of AESG; and were observed for 24 h for signs of behavioural changes and (or) death. In the post-administration phase (II), a total of three (3) male rats were used and divided into three (3) groups of $n = 1$. Each group was administered doses of 1600, 2900 and 5000 mg/kg AESG, respectively, and were observed for another 24 h for signs of behavioural changes associated with toxicity and (or) mortality (Tables 1 and 2).

The lethal dose (LD_{50}) of AESG leaf was calculated as shown below:

$$LD_{50} = \sqrt{\frac{D_0 + D_{100}}{2}}$$

D_0 = Highest dose that resulted to no death; D_{100} = Lowest dose that resulted to death.

2.5. Sub-chronic toxicity studies (in-vivo)

The Sub-Chronic toxicity study was conducted as prescribed in the OECD [37], N0. 425 test guidelines; described by Route et al. [38] and Oliveira et al. [39]. 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

Table 1
Acute Toxicity Study of Aqueous Leaf Extract (AESG) Administered to Male *W*istar Rats (Lorke' Method Phase I).

GROUP	WEIGHT OF RATS (g)	DOSE	OBSERVATIONS			
			BEHAVIORAL CHANGE	EATING HABIT	SLEEP	MORTALITY
I	204.23 ± 3.0	AESG 10 mg/kg	NØ	NØ	NØ	NØ
II	195.33 ± 5.5	AESG 100 mg/kg	NØ	NØ	NØ	NØ
III	203.25 ± 4.0	AESG 1000 mg/kg	NØ	NØ	NØ	NØ

Weights are mean ± SD, n = 3, NØ = No Significant Observation, AESG (Aqueous Leaf Extract of *S. glauca*).

Table 2
Acute Toxicity Study of Aqueous Leaf Extract (AESG) Administered to Male *Wistar* Rats (Lorke' Method Phase II).

GROUP	WEIGHT OF RATS (g)	DOSE	OBSERVATIONS			
			BEHAVIORAL CHANGE	EATING HABIT	SLEEP	MORTALITY
I	199	AESG 1600 mg/kg	NØ	NØ	NØ	NØ
II	200	AESG 2900 mg/kg	NØ	NØ	NØ	NØ
III	210	AESG 5000 mg/kg	NØ	NØ	NØ	NØ

Weights are mean ± SD, n = 1, NØ = No Significant Observation, AESG (Aqueous Leaf Extract of *S. glauca*).

of $n = 6$. Test animals received 500, 1000, and 2000 mg/kg body weight, respectively, of AESG daily for thirty (30) days; while the control group received only rat pellets and water.

2.6. Collection of data and specimens

At the end of the study, the rats were fasted overnight; then 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 or thoracic aorta, using a 5 mL syringe; emptied into a 5 mL heparinized specimen bottle. The blood was then centrifuged at 3500 rpm for 15 min to obtain a clear supernatant (Plasma) that was stored at -18°c until required for biochemical analyses; conducted out within a few days. One-gramme portion of relevant organs was excised, cleared off connective tissues, and homogenized in a 5 mL normal saline. The homogenate was centrifuged at 3500 rpm/10 min to obtain a clear supernatant; relevant biochemical analyses were conducted. The liver, kidney, and heart organs were harvested and stored in formal saline solution (0.9 g of NaCl in 90 mL of distilled water and mixed with 10 mL of 40 % formalin to obtain a final volume of 100 mL) for histopathology evaluation.

2.7. Biochemical analyses

Plasma and tissue Total Cholesterol, High-Density Lipoprotein (HDL-C), Liver function and related heart function tests, which included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total proteins, albumin, and total and direct bilirubin, were respectively done using colorimetric methods as previously reported [40–45]. The kidney function tests which included urea, creatinine, calcium, sodium, chloride, potassium, and bicarbonate were respectively conducted according to the methods previously reported [46–52], and with the aid of commercially available test kits, products of Randox® Laboratories (United Kingdom)

2.8. Histopathology evaluation of sectioned liver tissue

The fixed, excised organs in formal-saline were trimmed into 5 mm thick and dehydrated with graded concentrations of ethanol (70, 95, and 99 %: absolute ethanol); cleared in xylene and embedded in paraffin wax. The embedded tissues (Liver, Kidney, and Heart) were sectioned at 6 μm thickness, stained with haematoxylin and eosin (H & E), and examined under the light microscope, according to the methods described by Gurr [53] and Windsor [54]. The sections were photographed at a magnification of x400 with the Vanox-T Olympus photographic microscope.

2.9. Statistical analyses

Data obtained from the study are expressed as mean and standard deviation (mean ± SD) where applicable. Statistical differences between means of test group were evaluated by paired *t*-test and 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®, version 7.

3. Results

3.1. Results of acute toxicity study of AESG in wistar rats

The data of the acute toxicity evaluation show that AESG administered to test rats was relatively safe; no death was recorded after phase I & II of the study. This suggests that the LD_{50} of AESG exceeded 5000 mg/kg (Tables 1 and 2).

3.2. Results of sub-chronic toxicity study of AESG in wistar rats

3.2.1. Effect on body weight changes

The data presented in Fig. 2a indicate that there were significant

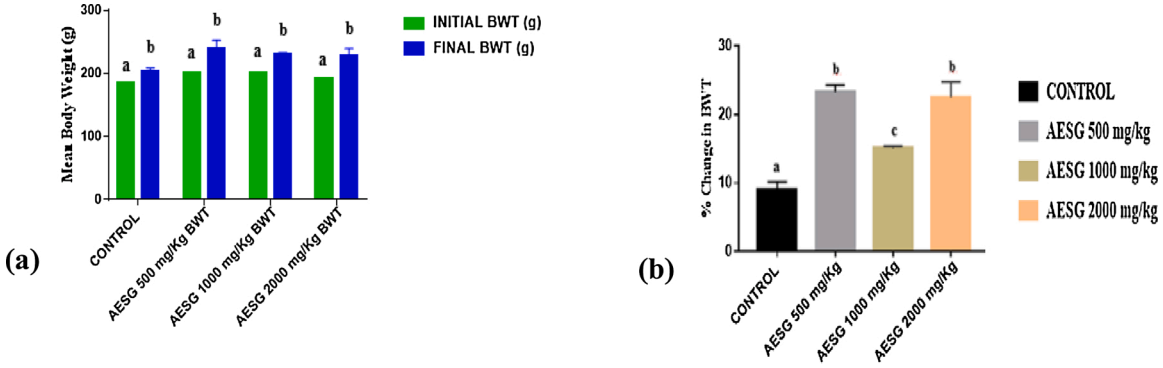


Fig. 2. a) Effect of varying doses of Aqueous Leaf Extract of *S. glauca* (AESG) on Body Weight (g) of Male *Wistar* Rats after 30 days. b) Percentage Changes in body weight of Male *Wistar* Rats administered Respective doses of AESG after 30 days. Data with similar lower-case alphabet are not significantly different ($p < 0.05$); data with different lower-case alphabets are significantly different ($p < 0.05$). Data are presented as Mean ± SD.

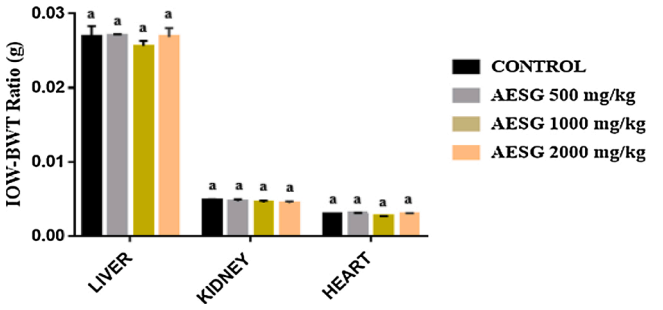


Fig. 3. Effect of varying doses of AESG on Internal Organ/Body Weight (IOW/BWT) Ratios of.
Male *Wistar* Rats after 30 days. Data with similar lower-case alphabets are not significantly different ($p < 0.05$); data with different lower-case alphabets are significantly different ($p < 0.05$). Data are Mean ± SD.

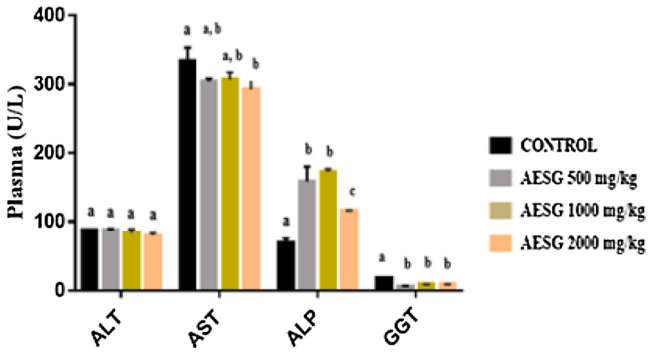


Fig. 4. Effect of varying doses of AESG on Plasma ALT, AST, ALP and GGT activities of Male *W*istar Rats. Data with similar lower-case alphabets are not significantly different ($P < 0.05$); data with different lower-case alphabets are significantly different ($P < 0.05$). Data are Mean ± SD.

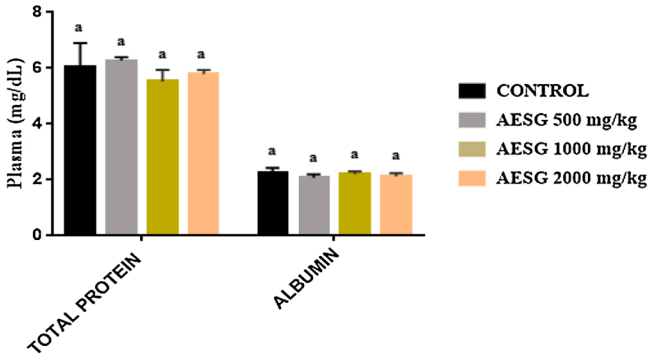


Fig. 5. Effect of varying doses of AESG on Plasma Total Proteins and Albumin levels of Male Wistar Rats. Data with similar lower-case alphabets are not significantly different ($P < 0.05$); data with different lower-case alphabets are significant different ($P < 0.05$). Data are Mean ± SD.

increases ($P < 0.05$) in final mean body weights of test animals administered AESG 500, 1000, and 2000 mg/kg, respectively; including the control after 30 days, when compared with their respective initial mean body weights, taken before commencement of AESG administration. Fig. 2b clearly shows percentage (%) weight gain; indicates that rats administered respective doses of AESG gained significant weight ($P < 0.05$) when compared to the control; in fact, rats administered AESG 500 and 2000 mg/kg, respectively, gained the highest weight. There were no significant differences in liver, kidney, and heart IOW/body weight ratios of test rats relative to the respective controls (Fig. 3).

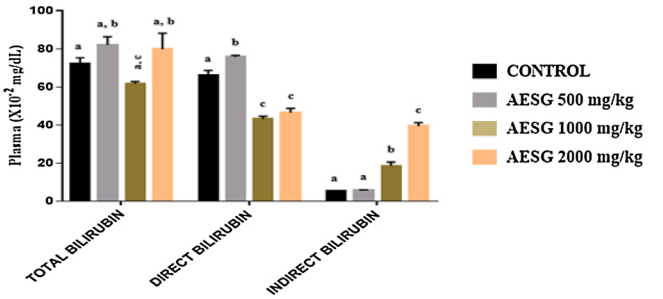


Fig. 6. Effect of varying doses of AESG on Plasma Total, Direct and Indirect Bilirubin of Male *Wistar* Rats after 30 days. Data with similar lower-case alphabets are not significantly different ($P < 0.05$); data with different lower-case alphabets are significantly different ($P < 0.05$). Data are Mean ± SD.

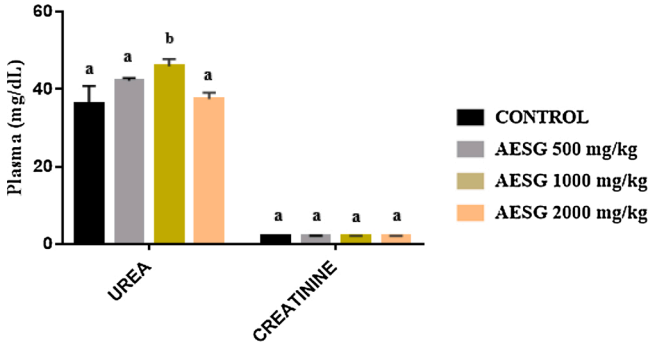


Fig. 7. Effect of varying doses of AESG on Plasma Urea and Creatinine Levels of Male *Wistar* Rats. Data with similar lower-case alphabets are not significantly different ($P < 0.05$); data with different lower-case alphabets are significantly different ($P < 0.05$). Data are Mean ± SD.

3.2.2. *Effect on liver function parameters and total proteins*

There were no significant differences ($P < 0.05$) in plasma ALT activities of *Wistar* rats administered respective doses of AESG, when compared with the plasma ALT activity of the control (Fig. 4). The plasma AST activities of test rats administered AESG 500 and 1000 mg/kg, respectively, was not significantly different ($P < 0.05$), although rats administered 2000 mg/kg recorded significant reduction ($P < 0.05$) in plasma AST activity relative to the plasma AST activity of the control (Fig. 4). Plasma ALP activities were significantly elevated ($P < 0.05$) in all groups of experimental rats given AESG; the highest ALP activity was recorded in the group of rats administered AESG 500 and 1000 mg/kg, respectively, relative to the control (Fig. 4). The plasma GGT activity of experimental rats administered respective doses of AESG was significantly lowered ($P < 0.05$) compared to the control (Fig. 4). Plasma total protein and albumin concentration of rats administered respective doses of AESG was not significantly different ($P < 0.05$) when compared to their respective controls (Fig. 5).

The plasma total bilirubin of experimental rats administered AESG was not significantly different ($P < 0.05$) compared to the plasma total bilirubin of the control after 30 days (Fig. 6). Plasma conjugated bilirubin was significantly elevated ($P < 0.05$) in experimental rats administered AESG 500 mg/kg; whereas significant reduction ($P < 0.05$) was observed in test rat administered AESG 1000 and 2000 mg/kg respectively compared to the plasma conjugated bilirubin of the control (Fig. 6) after 30 days. There was significant elevation ($P < 0.05$) in plasma unconjugated bilirubin levels of experimental rats administered AESG 1000 and 2000 mg/kg respectively; while experimental rats administered AESG 500 mg/kg was not significantly different ($P < 0.05$) compared to the plasma unconjugated bilirubin of the control (Fig. 6) after 30 days.

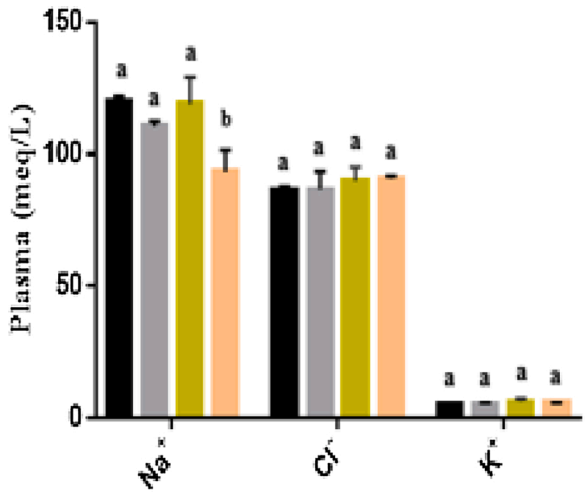


Fig. 8. Effect of varying doses of AESG on Plasma Sodium, Chloride and Potassium ion Concentration of Male *Wistar* Rats.

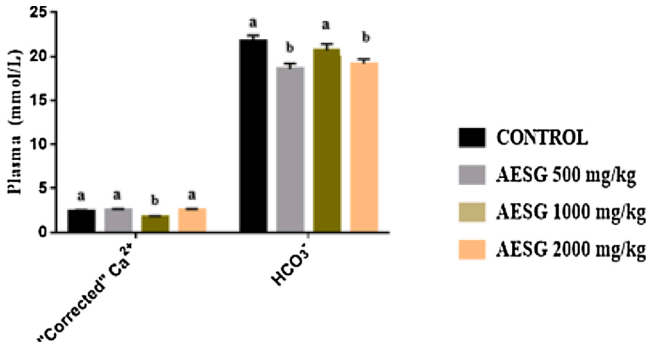


Fig. 9. Effect of Varying Doses of AESG on Plasma "Corrected" Ca^{2+} and HCO_3^- Concentration of Male *Wistar* Rats. Data with similar lower-case alphabets are not significantly different ($P < 0.05$); data with different lower-case alphabets are significantly different ($P < 0.05$). Data are Mean ± SD.

3.2.3. *Effect on kidney function parameter*

Test rats administered AESG 1000 mg/kg recorded significant increase ($P < 0.05$) in plasma urea concentration; whereas the plasma urea levels of rats given AESG 500 and 2000 mg/, respectively, were not significantly different ($P < 0.05$), relative to the plasma urea concentration of the control (Fig. 7). The plasma creatinine levels of rats administered AESG was not significantly different ($P < 0.05$) when compared to the control (Fig. 7). There was a significant reduction ($P < 0.05$) in plasma Na^+ concentration of test rats administered AESG 2000 mg/kg; whereas the plasma sodium ions of rats administered AESG 500 and 1000 mg/kg, respectively, were not significantly different ($P < 0.05$), relative to the control (Fig. 8). The plasma chloride and potassium ion concentrations of rats administered AESG were not significantly different ($P < 0.05$), relative to the control (Fig. 8). There were no significant differences ($P < 0.05$) in plasma "corrected" calcium of test animals given AESG 500 and 2000 mg/kg, respectively; whereas rats administered AESG 1000 mg/kg recorded significant reduction ($P < 0.05$) in plasma "corrected" calcium concentration, relative to the control (Fig. 9). Test animals administered AESG 500 mg/kg recorded a significant reduction ($P < 0.05$) in plasma bicarbonate ion concentration; whereas rats given AESG 1000 and 2000 mg/kg, respectively, were not significantly different ($P < 0.05$) compared to the control (Fig. 9).

4. Discussions

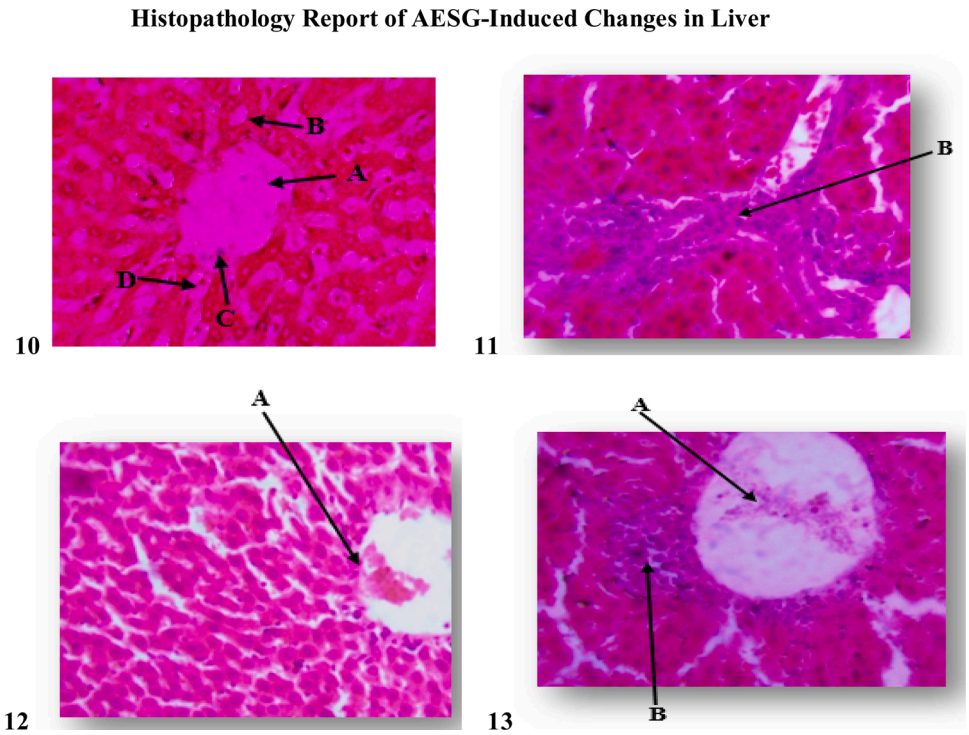
An oral acute toxicity study was carried out to evaluate the lethal dose (LD_{50}) and perhaps, the immediate side effects, as well as subchronic toxicity of AESG. A substance, if poisonous, would likely exhibit its effect within minutes; with the more poisonous substance eliciting toxic effect at relatively low doses [37]. According to the guidance document on acute oral toxicity testing, recommended by Organization for Economic Cooperation and Development [37], the aqueous leaf extracts of *S. glauca* were tested. Several studies on the acute toxicity of a number of related and unrelated plants have been reported [38,55,56]. Oliveira et al. [39] also reported that the stem-bark ethanol extract of *S. versicolor* (which belongs to the same family *Simaroubaceae*), administered to *Wistar* rats for 30 days did not result to any observable signs of toxicity or mortality. In the present study, oral acute administration of AESG to rats at doses up to 5000 mg/kg did not result to fatality; as such, AESG is relatively safe with little or no noticeable immediate toxic effect.

A significant decrease in body weight of animals on exposure to certain substance(s) over a period of time may be an indication of the harmful nature of that substance [57]. This is more so when there are observed deleterious changes in organ/body weight ratio of vital organs, such as the liver, kidney, and heart. In the present study, the significant increase in body weight of experimental rats indicated that AESG did not result to a loss in body weight of rats relative to the control (Fig. 2a and b); nor did it elicit significant lesions and tissue hyperplasia disproportionate to body weight (Fig. 3, Figs. 10–13). The findings of the present study are, therefore, consistent with the report of Rout et al. [38]. The presence of essential vitamins and minerals, in their right proportions, might stimulate appetite and increase in body weight. Therefore, the increase in body weight of rats given AESG might not be unconnected with the vitamin content (Vitamin B_1, B_2, and B_3) of *S. glauca* [58].

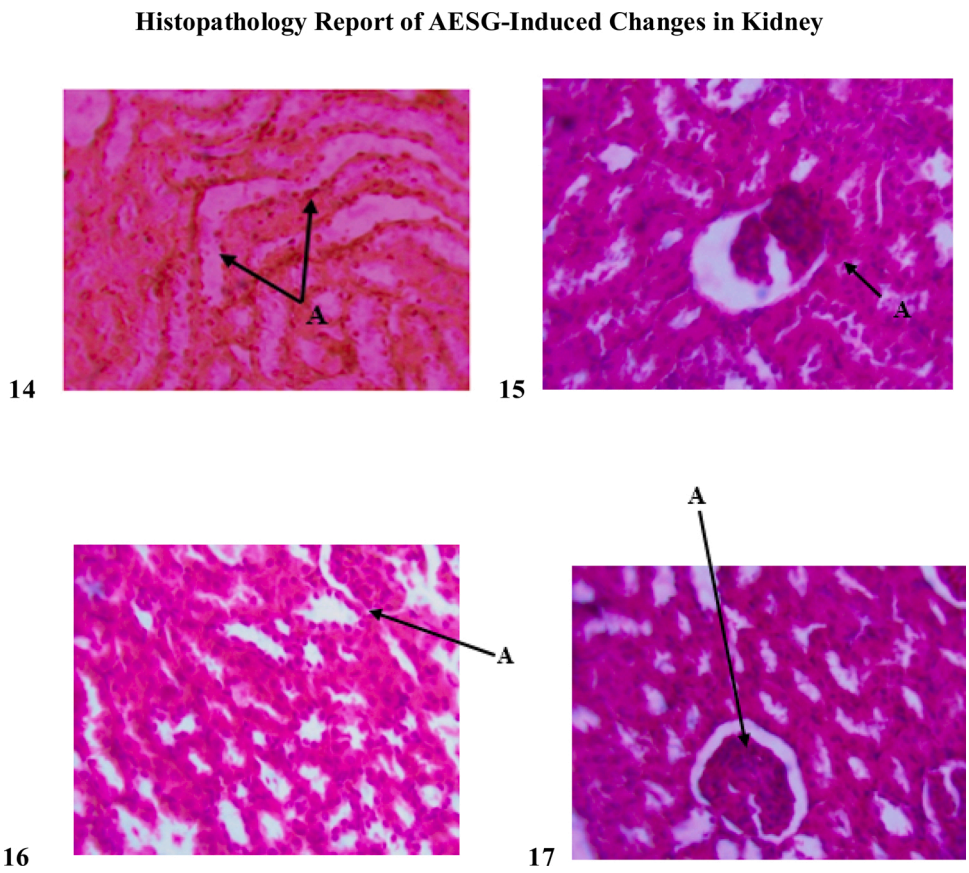
Alterations, particularly organ hyperplasia to body weight ratio, may indicate toxicity-induced organ damage [59]. Although, there was mild congestion in hepatic central vein and inflamed periportal cells of experimental rats administered AESG, this might be attributed to toxicity associated with the lengthy administration of extracts at rather very high doses.

The degree of liver damage induced by chemical substances or otherwise may be evaluated by determining the levels of specific biochemical markers of liver function, such as AST, ALT, ALP, and GGT [60]. In the present study, AESG did not cause any significant clinical damage to the liver (Figs. 11–13). This is further strengthened by the observation that there were no significant changes in plasma ALT levels of test rats administered AESG, relative to control (Fig. 4). There was no observed increase in the plasma AST and GGT activities (Fig. 4). The European document for ecotoxicology and toxicology had stated that the biological significance of the decrease in specific liver enzyme activity was unclear; as such, was typically dismissed as being of no toxicological importance [61]. Contrariwise, the plasma ALP activity was significantly elevated ($P < 0.05$) in all test rats. Several iso-enzymes of ALP exist in the liver, bones, placenta, kidneys, and intestines. The activity of this enzyme is increased in many clinical states, the most important being bone and liver diseases [62]. Significant elevation in plasma ALP activity without hepatic lesion has been reported, linked to cholestasis [63]. With the plasma levels of ALT remaining normal and GGT reduced, it is unlikely that the increase in plasma ALP could be of hepatic origin. The significant increase in plasma ALP activities observed in the current study might have been due to secretions from other tissues capable of synthesizing iso-enzymes of ALP (tissue nonspecific ALP, TNSALP) [64–66] (Fig. 4). This, therefore, suggests biliary duct obstruction [60]. It is also important to note that the prominent congestions observed in the liver (Figs. 11–13-IV) of rats administered AESG might have caused hepatobiliary obstruction, which is suggestive that the biliary duct ALP (TNSALP) could be responsible for the elevated ALP activity in the bloodstream (Fig. 4); although the magnitude of this contribution, nevertheless, remains uncertain.

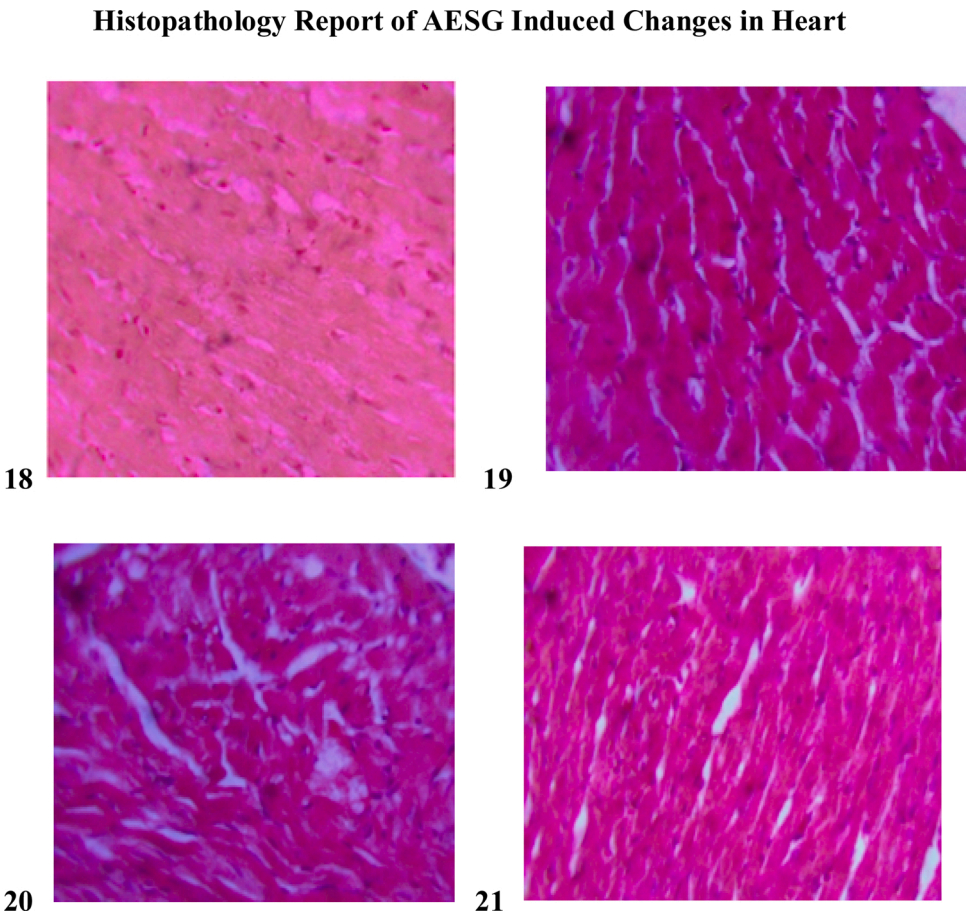
In the present study, no changes or alterations in plasma total proteins and albumin concentrations were recorded in the test rats



Figs. 10–13. Photomicrograph of sectioned liver of control rat with normal/clear central vein, CV (A), Normal Hepatic Artery, HA (B), Anastomosing plates of hepatocytes surround the portal tract (C), and hepatic sinusoids (D); normal lobular architecture. Fig. 11 Photomicrograph of sectioned liver of *Wistar* rats administered AESG 500 mg/kg indicates inflamed periportal spaces (B); normal lobular architecture. Fig. 12 Photomicrograph of sectioned liver of *Wistar* rats administered AESG 1000 mg/kg indicates partially congested central vein, CV (A); normal lobular architecture. Fig. 13 Photomicrograph of sectioned liver of *Wistar* rats administered AESG 2000 mg/kg indicates partially congested central vein, CV (A), and inflamed periportal space (B); normal lobular architecture.



Figs. 14–17. Sectioned Kidney of control *Wistar* rat shows normal tubules and normal glomerulus (A). Fig. 15 Indicates that sectioned kidney of test rats administered AESG 500 mg/kg with mildly atrophied glomerulus and tubules that appear normal (A). Fig. 16 Indicates sectioned kidney of rat administered AESG 1000 mg/kg shows normal tubules and glomerulus (A). Fig. 17 Shows sectioned kidney of rat administered AESG 2000 mg/kg with normal tubules and glomerulus (A).



Figs. 18–21. Photomicrograph of sectioned heart of control rat indicates normal myocardial fibril. Fig. 19. Sectioned heart of rat administered AESG 500 mg/kg indicates normal myocardial fibril. Fig. 20. Sectioned heart of rats administered AESG 1000 mg/kg also indicates normal myocardial fibril. Fig. 21 Sectioned heart of rat administered AESG 2000 mg/kg also indicates normal myocardial fibril.

administered AESG (Fig. 5); it obviously suggests that AESG did not impair the synthesizing function of the liver.

The residual circulating amount of conjugated bilirubin in the plasma of a healthy individual is very minimal and, as such, an increase in plasma conjugated bilirubin in an adult suggests impaired hepatocellular function; whereas, this is not the case with unconjugated bilirubin [67]. However, increase in unconjugated bilirubin ($\geq$ 90 %) is indicative of acute haemolysis of red blood cells or Gilbert syndrome [64]; and perhaps, an increased degradation of haem. Hepatotoxicity characterized by significant elevation in plasma unconjugated bilirubin has been reportedly linked to some pharmacological and phyto-therapeutic principles present in plants, such as alkaloids, tannins, flavonoids, among others; particularly, if administered at high doses [68,69]. In the present study, the plasma total bilirubin was obviously not significantly different (Fig. 6), indicating that AESG did not demonstrate a significant hepatotoxic effect. However, plasma conjugated bilirubin was significantly elevated ($P < 0.05$) in test rats administered AESG 500 mg/kg. Although the liver histopathology reports of test animals presented in plates II-IV show portal congestion, however, the significant elevation in plasma conjugated bilirubin observed in the group of rats administered AESG 500 mg/kg might have resulted from the severely impaired hepatobiliary flow caused by portal congestion (Fig. 11) [67], and supported by elevated plasma ALP levels. The significant reduction in plasma conjugated bilirubin observed in rats given higher doses of AESG 1000 and 2000 mg/kg, respectively, further supports the aforementioned claim that the liver function was not significantly compromised. The significantly elevated plasma unconjugated bilirubin observed in test rats administered AESG 1000 and 2000 mg/kg, respectively (Fig. 6), suggests plasma bilirubin overload, complicated by poor liver bilirubin conjugation capacity. Therefore, it is also likely that the significant elevation in plasma indirect bilirubin recorded in test rats administered AESG 1000 and 2000 mg/kg, respectively, might have been elicited by certain phyto-therapeutic compounds in medicinal plants, as earlier reported [68,69]. A previous study shows that *S. glauca* contains a significant number of alkaloids, tannins, flavonoids, amongst others [35]; which gives credence to the claim earlier reported by Hoffman and Manning [68] and Evans [69].

The Kidney's function is evaluated by its capacity to effectively remove toxic waste products from the blood, and to regulate plasma electrolytes. Estimated urea and creatinine levels are a reliable acute kidney marker and may assist in diagnosis of kidney impairment [70]. Urea is a product of protein and purine metabolism; it is regarded as toxic when it exceeds allowable limits. Creatinine is an endogenously synthesized compound from creatine and phosphocreatine in skeletal muscles; its excretion from the blood is entirely dependent on the kidney's filtration capacity and, thus, significant elevations in creatinine levels of serum or plasma may indicate glomerular dysfunction.

In the present study, there were no significant differences ($P < 0.05$) in the plasma creatinine levels (Fig. 7); although it was observed that rats administered AESG 1000 mg/kg recorded a significant elevation ($P < 0.05$) in plasma urea level (Fig. 7). The implication of the data obtained in the study is that the functional integrity of the kidney was not compromised by AESG (Figs. 14–17). Wasan et al. [71] had earlier reported that oral administration of leaf extracts of *S. glauca* to test animals was capable of stimulating increase in plasma urea concentration, likely due to the presence of chemotherapeutic agents inherent in the leaves of

S. glauca (quassinoids glaucarubinone and alianthinone) [13]. Therefore, the significant increase in plasma urea at AESG 1000 mg/kg might have resulted from an increase in amino acids metabolism elicited by the effect of therapeutic compounds present in the plant [13].

The data obtained from the present study also showed that AESG did not cause significant alterations ($P < 0.05$) in plasma sodium, chloride, and potassium ion concentrations, respectively (Fig. 8); that the electrolyte regulatory function of the kidneys was not compromised (Figs. 14–17). However, there was a marked reduction in plasma sodium ion concentration of test rats given AESG 2000 mg/kg. Plants capable of lowering sodium ion in the system are perceived as possible diuretic agents [72].

The significant reduction ($P < 0.05$) in plasma "corrected" calcium ion level at AESG 1000 mg/kg suggests that AESG might have repressed parathyroid functioning at that dose [73]. Moreso, poor proximal and distal tubule reabsorption of calcium, elicited by AESG, might have also resulted in hypoparathyroidism. The significant reduction ($P < 0.05$) in plasma bicarbonate ion level at AESG 500 and 2000 mg/kg suggests AESG-induced compensatory response to metabolic alkalosis [74] (Figs. 18–21).

5. Conclusion

The AESG orally administered to experimental rats was relatively safe with LD_{50} above 5000 mg/kg. AESG appeared to have elicited alterations in plasma ALP and bilirubin levels. Nonetheless, it did not result to elevations in specific liver enzymes and non-enzyme indicators; no hepatocellular, glomerular or myocardial damages were prominent, suggesting that AESG was neither significantly toxic nor resulted in injury to vital organs; may be safely administered at lower doses.

Author's statement

All suggestions by the reviewers have been duly implemented. The discussion can't be written in 2–3 pages, clearly due to the enormous data generated in the study. The robust discussion is necessary to accommodate the enormous data generated from the toxicity study. We acknowledge that the histology staining was poor, however the structures are visible enough for relevant indications. The toxicology report guidelines to authors did not indicate a particular reference style. It states that a chosen reference style is fine; that the reference must be consistent.

Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2021.01.008.

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