Clinical Phytoscience

ORIGINAL CONTRIBUTION

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Irvingia gabonensis leaf extract scavenges nitric oxide and hydrogen peroxide in vitro and modulates arsenic-induced hepatic oxidative stress in wistar rats

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Abstract

Background: Arsenic is a carcinogenic heavy metal that contaminates the environment, predisposing the exposed populace to its detrimental health effects. This study investigated the liver protective effect of ethanol leaf extract of *Irvingia gabonensis* (ELEIG) in sodium arsenite (SA)-exposed Wistar rats and its nitric oxide ($NO^{\cdot}$) and hydrogen peroxide (H_2O_2)-scavenging properties in vitro.

Methods: Eleven experimental groups made up of five (5) rats each (weight range 100 - 161 g) were used in this study. Group 1 (normal control) had normal rat chow and water. Group 2 received 4.1 mg/kg body weight (kgbw) of SA. Groups 3–8 received SA and graded doses of ELEIG and groups 9-11 had varied doses of ELEIG. Treatment, which spanned 14 days, was by oral gavage. Concentrations of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA) as well as activities of liver enzymes (AST, ALT, ALP, and GGT) and concentrations of total bilirubin (TBIL) and direct bilirubin (DBIL) were determined using standard procedures. Standard methods were also used to determine the in vitro $NO^{\cdot}$ and H_2O_2-scavenging properties of the extract.

Results: Exposure to SA orchestrated significant ($p < 0.05$) increases in CAT, MDA, AST, ALT, ALP and GGT and significant ($p < 0.05$) decreases in SOD and GPx, relative to control. There were insignificant ($p > 0.05$) differences in TBIL and DBIL concentrations, compared with control. Simultaneous and post-treatment with ELEIG at graded doses, alleviated the noxious effects of SA. In addition, ELEIG scavenged $NO^{\cdot}$ and H_2O_2 in concentration-dependent manner.

Conclusion: The results suggest that ELEIG possesses potent antioxidant property and combats SA-induced hepatic oxidative stress/toxicity in Wistar rats.

Keywords: *Irvingia gabonensis*, Arsenic, Sodium arsenite, Oxidative stress, Liver enzymes, Environmental pollutant, Antioxidants

Background

Environmental pollution is a global menace that negatively impacts the health of a myriad of individuals, resulting in the development of various disease conditions. Arsenic is an environmental pollutant and a carcinogen, that pollutes the environment naturally and by human activities [1]. Arsenic poisoning could occur by the consumption of arsenic-contaminated food and drinking water [1, 2]. High levels of arsenic in groundwater, has been detected in some countries like Nigeria [3, 4]. It is on record that an estimated 200 million people Worldwide are susceptible to health impairment due to high levels of arsenic in drinking water [5]. Arsenic has been reported to orchestrate hepatotoxicity via the

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induction of oxidative stress [6]. Furthermore, studies have shown that, liver function is compromised in different animal species by exposure to sodium arsenite [7].

The use of plants as medicine has been embraced by most Locals for several reasons, mostly because of the very high cost of orthodox drugs, and the recent re-emergence and dependence on medicinal plants in the treatment of disease conditions. Different parts of *Irvingia gabonensis* (edible bush mango) are utilized as medicines by Locals in combating various ailments [8]. The leaves are used as anti-poisons [9]. The Senegalese use the decoction of the stem bark in treating ailments such as gonorrhea, liver and gastrointestinal diseases and the root bark is used to treat wounds [9]. The stem bark has also been documented to possess bactericidal and fungicidal properties as well as in the treatment of yellow fever, hernias, dysentery and to mitigate poisons [10, 11]. In addition, the antitoxic effects of the leaves in Wistar rats have been documented by various researchers [12–15]. However, there is paucity of information on the antioxidant and medicinal effects of ethanol leaf extract of *Irvingia gabonensis* against arsenic-induced hepatic dysfunction.

Materials and methods

Leaf extract preparation

Fresh leaves of *Irvingia gabonensis* were harvested from Itak Ikot Akap community in Akwa Ibom State, Nigeria. They were authenticated in the Department of Pharmacognosy and Natural Medicine, University of Uyo with the voucher number: JAMES DANIEL UUH 042116 (Uyo). The leaves were washed with clean water to decontaminate them, air-dried for 7 days and pulverized using a manual grinder. The pulverized leaves were then macerated in absolute ethanol for 72 hours, with intermittent stirring to achieve adequate extraction of the bioactive phytoconstituents. The mixture was filtered repeatedly with the aid of a clean muslin cloth and the filtrate was concentrated at 45 °C, using a water bath. The dried extract was stored at 4 °C prior to analyses.

Determination of in vitro nitric oxide and hydrogen peroxide scavenging properties of ELEIG

Nitric oxide ($NO^{\cdot}$) radical-scavenging property was investigated as described by Green et al. [16]. Hydrogen Peroxide (H_2O_2)-scavenging property was determined according to the method of Ruch et al. [17].

Experimental animals and design

A total of 55 healthy and non-pregnant female Wistar albino rats (100 g – 161 g), were obtained from the animal house of Faculty of Basic Medical Sciences, University of Uyo, Uyo, and used for this study. They were acclimatized for seven (7) days in the same facility under standard conditions in a well-ventilated room, with free access to feed and water. After 7 days of acclimatization, the experimental animals were assigned to eleven (11) groups of five rats each in standard animal cages and their initial body weights were obtained using a digital weighing balance (Camry electronic scale EK5350, China) after overnight fast. The experimental design is shown in Table 1.

Blood sample collection and excision of liver tissues for analyses

At the end of the treatment period, the animals were anesthetized with chloroform and sacrificed by lower abdominal incision. Blood samples were collected by cardiac puncture using sterile syringes and needles into plain sample bottles for analyses and spun at 3000 rpm for 15 min using a table top centrifuge (Model 800-1, Zeny Inc. Salt Lake, USA) to obtain sera used for analyses. Liver tissues were harvested and rinsed with 1.15%

Table 1 Experimental Design

Groups	Treatment
1. Normal control	Normal feed and water ad libitum
2. SA only	4.1 mg/kgbw SA for 14 days
3. PT	4.1 mg/kgbw SA for 14 days, followed by 100 mg/kgbw ELEIG for another 14 days
4. PT	4.1 mg/kgbw SA for 14 days, followed by 200 mg/kgbw ELEIG for another 14 days
5. PT	4.1 mg/kgbw SA for 14 days, followed by 400 mg/kgbw ELEIG for another 14 days
6. ST	4.1 mg/kgbw SA + 100 mg/kgbw ELEIG simultaneously for 14 days
7. ST	4.1 mg/kgbw SA + 200 mg/kgbw ELEIG simultaneously for 14 days
8. ST	4.1 mg/kgbw SA + 400 mg/kgbw ELEIG simultaneously for 14 days
9. ELEIG only	100 mg/kgbw ELEIG only for 14 days
10. ELEIG only	200 mg/kgbw ELEIG only for 14 days
11. ELEIG only	400 mg/kgbw ELEIG only for 14 days

SA Sodium arsenite, *ELEIG* Ethanol leaf extract of *Irvingia gabonensis*, *mg/kgbw* Milligram per kilogram body weight, *PT* Post-treatment, *ST* Simultaneous treatment

ice-cold potassium chloride (KCl) solution to expunge traces of blood. Small portions of the liver tissues were fixed in 10% neutral buffered formalin for histological assessment. The remaining portions of the liver tissues were placed in sterile universal container and frozen for homogenization.

Homogenization of liver tissues

The liver tissues were homogenized, following the procedure reported by Ewere et al. [18].

Assessment of oxidative stress biomarkers

The concentrations of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and of malondialdehyde (MDA) in liver homogenates were determined by Enzyme-linked Immunosorbent Assay (ELISA) using Sunlong Biotech Co. (Zhejiang, China) assay kits according to manufacturer's protocol.

Determination of serum liver enzymes activities and serum total bilirubin (TBIL) and direct bilirubin (DBIL) concentrations

TECO diagnostic assay kits (Anahaema, USA) were used for the determination of serum liver enzymes activities and serum total bilirubin (TBIL) and direct bilirubin (DBIL) concentrations. Aspartate aminotransferase (AST) activity was determined according to the method of Young [19]. Alanine aminotransferase (ALT) activity was determined according to the method of Young et al. [20]. Alkaline phosphatase (ALP) activity was determined by the method of Kochmar and Moss [21] and the method of Young et al. [20] was used to determine the activity of gamma glutamyltransferase (GGT). Serum total bilirubin (TBIL) and direct bilirubin (DBIL) concentrations were determined by the method of Tietz [22].

Histological assessment of liver tissues excised from experimental rats

Histological assessment of the excised liver tissues was carried out, using the procedure reported by Ewere et al. [23]. The liver tissues were prepared for histological assessment using haematoxylin and eosin staining method (H&E). After the tissues were stained, sections were examined under a Leica DM500 microscope and results were reported by a Consultant Histopathologist. An attached Leica ICC50 digital camera was used to take photomicrographs.

Statistical analysis

For $NO^{\cdot}$ and H_2O_2 scavenging assays, the experiments were performed in triplicates and the data obtained were calculated and expressed as mean ± standard deviation (SD) with the aid Microsoft Excel. Data obtained from the animal study are presented as mean ± standard deviation (SD) and were analyzed using one-way analysis of variance (ANOVA). Post hoc analysis was carried out using least significant difference (LSD) with the aid of SPSS Software (IBM, version 20). Mean difference between groups were considered statistically significant at $p < 0.05$.

Results

Hydrogen peroxide (H_2O_2) scavenging properties of ELEIG

Results obtained revealed that ELEIG possesses potent H_2O_2 scavenging properties when compared with ascorbic acid standard. Percentage of H_2O_2 scavenged measured at concentrations of 10, 20, 40, 80 and 160 µg/mL of the extract were: 34.55 ± 1.39%, 37.70 ± 0.53%, 41.88 ± 0.53%, 42.93 ± 0.53%, 43.63 ± 0.30%, respectively, and 36.82 ± 0.30%, 38.22 ± 0.52%, 42.76 ± 1.68%, 50.43 ± 2.58%, 59.16 ± 2.77%, respectively, for ascorbic acid (standard). The $I.C_{50}$ of ELEIG and ascorbic acid (standard) were 258.47 µg/mL and 91.95 µg/mL, respectively. The results are shown in Fig. 1.

Nitric oxide ($NO^{\cdot}$) scavenging properties of ELEIG

Results obtained revealed that ELEIG possesses potent $NO^{\cdot}$ scavenging properties, relative to ascorbic acid standard. Percentage of $NO^{\cdot}$ scavenged measured at concentrations of 10, 20, 40, 80 and 160 µg/mL of ELEIG were: 23.55 ± 0.17%, 25.45 ± 0.08%, 28.26 ± 0.18%, 28.71 ± 0.10%, 30.44 ± 0.20%, respectively and 33.76 ± 0.18%, 35.17 ± 0.26%, 44.43 ± 0.07%, 48.50 ± 3.12%, 54.96 ± 0.07%, respectively for ascorbic acid (standard). The $I.C_{50}$ of ELEIG and ascorbic acid (standard) were 640.05 µg/mL and 109.72 µg/mL, respectively. The results are shown in Fig. 2.

Effect of ELEIG on hepatic function of SA-intoxicated experimental rats

Results obtained revealed that, intoxication with sodium arsenite caused significant ($p < 0.05$) increases in serum AST, ALT, ALP and GGT activities, relative to the control group. Post-treatment with ELEIG culminated in significant ($p < 0.05$) decreases in serum ALT, ALP and GGT activities, in dose-dependent fashion and non-significant ($p > 0.05$) decreases in serum AST activities in dose-dependent manner except group 5, whose serum AST activity was significantly ($p < 0.05$) decreased, when compared with group 2, administered sodium arsenite only. Simultaneous treatment with ELEIG followed the same trend as the post-treatment. Comparison of ELEIG exclusively-treated groups and the normal control group, showed no significant ($p > 0.05$) differences in serum activities of the liver enzymes, with the exception of ALP. However, ELEIG at the highest dose (400 mg/

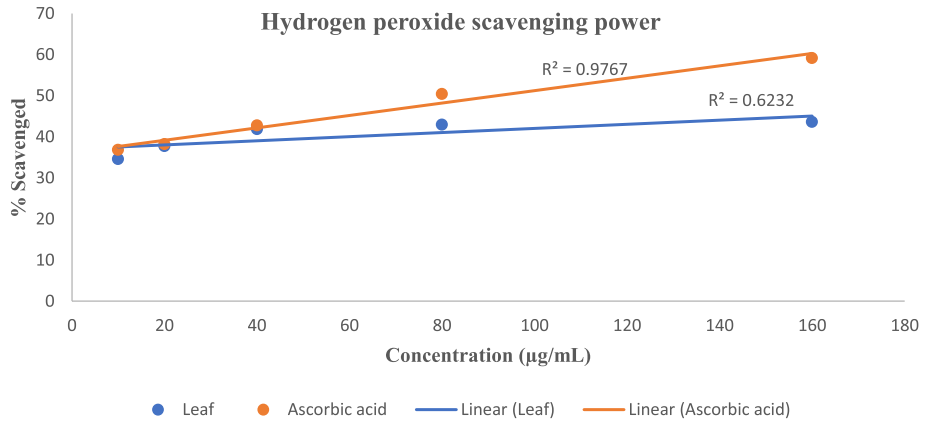

Fig. 1 Hydrogen peroxide scavenging properties of ELEIG in comparison to ascorbic acid standard

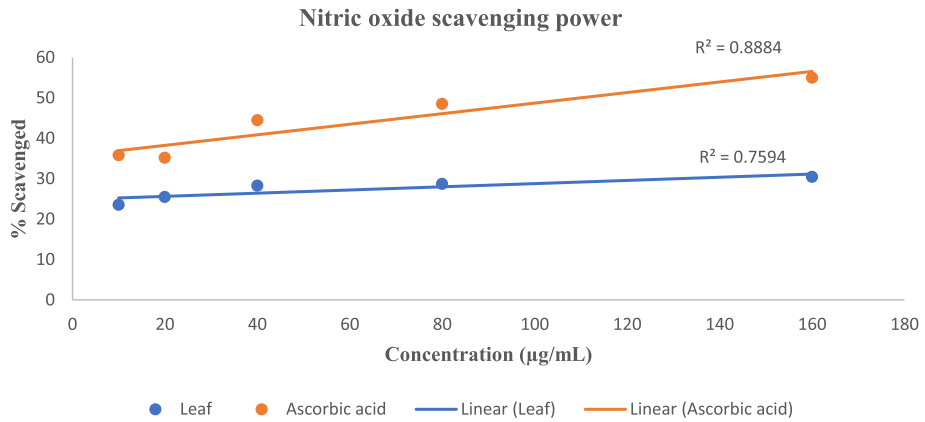

Fig. 2 Nitric oxide scavenging properties of ELEIG in comparison to ascorbic acid standard

kgbw), produced significant ($p < 0.05$) decreases in serum AST and ALT activities, relative to the normal control. Furthermore, there were no significant ($p > 0.05$) differences in serum TBIL and DBIL concentrations across all the treatment groups, after sodium arsenite intoxication. The results are shown in Table 2.

Effect of ELEIG on oxidative stress biomarkers of experimental rats

Results obtained showed that oral exposure of the experimental animals to sodium arsenite (group 2), culminated in significant ($p < 0.05$) decreases in hepatic SOD and GPx concentrations and significant ($p < 0.05$) increases in hepatic CAT and MDA concentrations, relative to the normal control. Post-treatment with ELEIG, at graded doses, led to significant ($p < 0.05$) increases in SOD and GPx concentrations as well as significant ($p < 0.05$) decreases in CAT and MDA concentrations, in dose-dependent and independent manner, relative to group 2. Similar trends were observed following simultaneous treatment with ELEIG. In addition, administration of ELEIG alone at various doses, produced similar results for the oxidative stress biomarkers with that of the control group. The results are presented in Table 3.

Effect of ELEIG on liver histology of experimental rats

Intoxication with sodium arsenite (group 2), induced severe vascular ulceration, portal congestion and periportal infiltrates of inflammatory cells (portal hepatitis) as well as severe microvesicular steatosis in the liver of the experimental rats. Simultaneous and post-treatment with graded doses (100, 200, and 400 mg/kgbw) of ELEIG, achieved dose-dependent ameliorative and therapeutic effects. The results are shown in the photomicrographs (Figs. 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13).

Discussion

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), when in excess, are generally known to orchestrate the induction of oxidative stress in vivo,

Table 2 Effect of ELEIG on hepatic function of SA-intoxicated experimental rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	TBIL (mg/dL)	DBIL (mg/dL)
1. Control	$130.80 \pm 64.39^{*}$	$39.20 \pm 5.26^{*}$	$73.00 \pm 6.20^{*}$	$0.54 \pm 0.10^{*}$	0.14 ± 0.06	0.04 ± 0.01
2. Arsenite only	$220.20 \pm 27.39^{\#}$	$83.40 \pm 11.22^{\#}$	$170.60 \pm 15.63^{\#}$	$1.19 \pm 0.36^{\#}$	0.24 ± 0.25	0.05 ± 0.02
3. PT at 100 mg/kgbw	$203.80 \pm 4.97^{\#}$	$47.80 \pm 3.70^{\#*}$	$157.20 \pm 6.76^{\#*}$	$0.67 \pm 0.06^{*}$	0.10 ± 0.02	0.04 ± 0.01
4. PT at 200 mg/kgbw	$194.50 \pm 4.65^{\#}$	$44.00 \pm 4.24^{*}$	$144.25 \pm 6.50^{\#*}$	$0.51 \pm 0.09^{*}$	0.07 ± 0.02	0.05 ± 0.01
5. PT at 400 mg/kgbw	$184.20 \pm 10.92^{\#*}$	$38.80 \pm 3.27^{*}$	$134.40 \pm 3.51^{\#*}$	$0.49 \pm 0.03^{*}$	0.10 ± 0.06	0.05 ± 0.02
6. ST at 100 mg/kgbw	$199.40 \pm 14.14^{\#}$	$35.80 \pm 6.30^{*}$	$124.80 \pm 4.21^{\#*}$	$0.52 \pm 0.07^{*}$	0.13 ± 0.14	0.04 ± 0.01
7. ST at 200 mg/kgbw	$191.50 \pm 5.20^{\#}$	$39.75 \pm 2.99^{*}$	$114.00 \pm 5.60^{\#*}$	$0.60 \pm 0.17^{*}$	0.11 ± 0.04	0.03 ± 0.02
8. ST at 400 mg/kgbw	$161.00 \pm 9.13^{*}$	$33.50 \pm 6.45^{*}$	$109.50 \pm 23.85^{\#*}$	$0.54 \pm 0.18^{*}$	0.09 ± 0.03	0.05 ± 0.01
9. ELEIG at 100 mg/kgbw	127.80 ± 10.96	35.20 ± 6.18	$94.40 \pm 9.13^{\#}$	0.62 ± 0.10	$0.60 \pm 0.50^{\#}$	0.06 ± 0.01
10. ELEIG at 200 mg/kgbw	101.80 ± 12.38	36.60 ± 2.30	$86.00 \pm 7.65^{\#}$	0.60 ± 0.16	0.10 ± 0.08	0.04 ± 0.01
11. ELEIG at 400 mg/kgbw	$85.80 \pm 14.34^{\#}$	$31.40 \pm 7.37^{\#}$	$86.20 \pm 2.39^{\#}$	0.64 ± 0.11	0.11 ± 0.06	0.05 ± 0.01

Data are expressed as mean ± SD ($n = 5$); # $p < 0.05$ compared with group 1; * $p < 0.05$ compared with group 2; *ELEIG E*thanol leaf extract of *Irvingia gabonensis*; *mg / kgbw* Milligram per kilogram body weight, *PT P*ost-treatment, *ST S*imultaneous treatment.

Table 3 Effect of ELEIG on oxidative stress biomarkers of experimental rats

Groups	SOD (ng/mL)	CAT (ng/mL)	GPx (ng/mL)	MDA (ng/mL)
1. Control	$5.25 \pm 0.22^{*}$	$0.45 \pm 0.33^{*}$	$4.38 \pm 0.51^{*}$	$2.55 \pm 0.27^{*}$
2. Arsenite only	$1.18 \pm 0.49^{\#}$	$2.91 \pm 0.42^{\#}$	$0.14 \pm 0.04^{\#}$	$6.96 \pm 0.69^{\#}$
3. PT at 100 mg/kgbw	$2.09 \pm 0.05^{\#*}$	$0.71 \pm 0.46^{*}$	$1.29 \pm 0.78^{\#*}$	$6.21 \pm 0.20^{\#*}$
4. PT at 200 mg/kgbw	$2.48 \pm 0.14^{\#*}$	$1.05 \pm 0.13^{\#*}$	$2.23 \pm 0.14^{\#*}$	$6.08 \pm 0.21^{\#*}$
5. PT at 400 mg/kgbw	$3.03 \pm 0.18^{\#*}$	$1.39 \pm 0.46^{\#*}$	$3.17 \pm 0.74^{\#*}$	$5.50 \pm 0.29^{\#*}$
6. ST at 100 mg/kgbw	$2.30 \pm 0.28^{\#*}$	$1.60 \pm 0.32^{\#*}$	$3.01 \pm 0.26^{\#*}$	$5.82 \pm 0.47^{\#*}$
7. ST 200 mg/kgbw	$2.94 \pm 0.07^{\#*}$	$1.00 \pm 0.08^{\#*}$	$3.53 \pm 0.43^{\#*}$	$4.50 \pm 0.35^{\#*}$
8. ST at 400 mg/kgbw	$3.12 \pm 0.08^{\#*}$	$0.95 \pm 0.06^{\#*}$	$3.21 \pm 0.22^{\#*}$	$4.23 \pm 0.82^{\#*}$
9. ELEIG at 100 mg/kgbw	$3.73 \pm 0.19^{\#}$	$0.93 \pm 0.12^{\#}$	$2.77 \pm 0.94^{\#}$	$4.50 \pm 0.73^{\#}$
10. ELEIG at 200 mg/kgbw	$3.92 \pm 0.30^{\#}$	$0.91 \pm 0.08^{\#}$	4.05 ± 0.13	$3.56 \pm 0.57^{\#}$
11. ELEIG at 400 mg/kgbw	$4.14 \pm 0.24^{\#}$	$0.88 \pm 0.12^{\#}$	4.52 ± 0.45	2.62 ± 0.44

Data are expressed as mean ± SD ($n = 5$); # $p < 0.05$ compared with group 1; * $p < 0.05$ compared with group 2; *ELEIG E*thanol leaf extract of *Irvingia gabonensis*, *mg / kgbw* Milligram per kilogram body weight, *PT* Post-treatment, *ST* Simultaneous treatment.

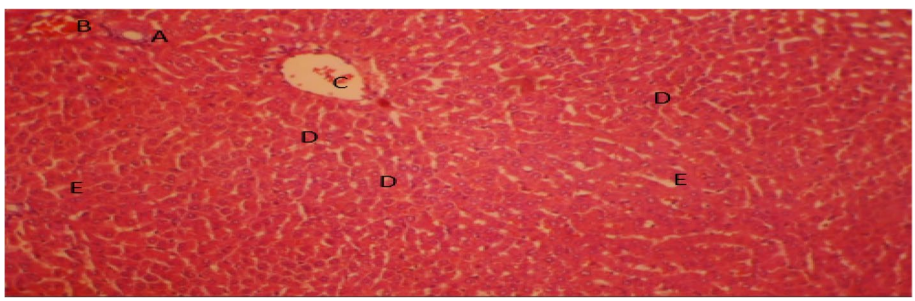

Fig. 3 Liver section of Control (Group 1) composed of: **A**, bile duct, **B**, portal vein, **C**, central vein, **D**, hepatocytes and **E**, sinusoids (H&E x 100)

which underlies the etiologies of various disease conditions [24]. Bioactive agents, especially those of plant origin, that have the capacity to mitigate the noxious effects of ROS, are of promise in combating the plethora of diseases, linked to oxidative stress [25]. Enzymes called nitric oxide synthases, are involved in the production of the free radical, nitric oxide ($NO^{\cdot}$) in biological tissues [26]. The enzymes catalyze the conversion of the amino

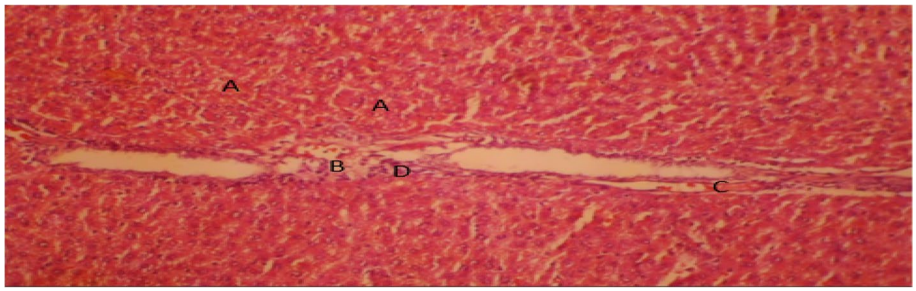

Fig. 4 Liver section of rat given SA only for 14 days (Group 2) showing: **A**, severe microvesicular steatosis, **B**, severe vascular ulceration, **C**, mild vascular congestion and **D**, mild periportal infiltrates of inflammatory cells (H&E x 100)

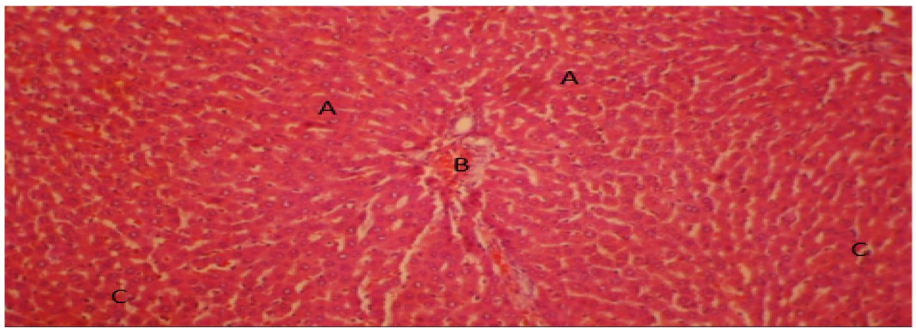

Fig. 5 Liver section of rat given SA for 14 days followed by 100mg/kg leaf extract for 14 days (Group 3) showing: **A**, normal hepatocytes, **B**, normal portal vascular architecture and **C**, moderate Kupffer cell activation (H&E x 100)

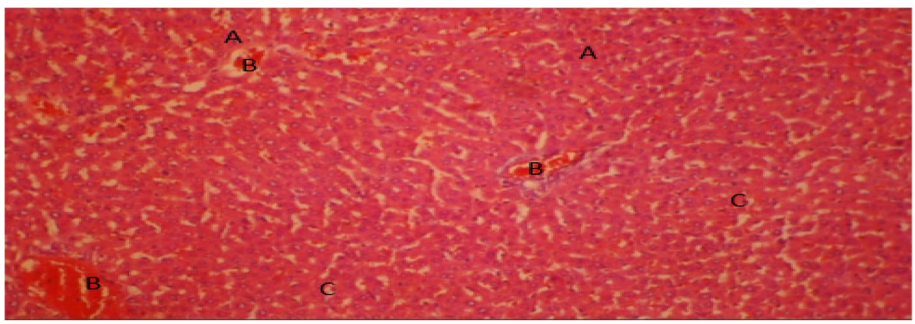

Fig. 6 Liver section of rat given SA for 14 days followed by 200mg/kg leaf extract for 14 days (Group 4) showing: **A**, normal hepatocytes, **B**, moderate active congestion and **C**, moderate Kupffer cell activation (H&E x 100)

acid, arginine to citrulline, while forming $NO^{\cdot}$ via a five-electron oxidative reaction [27]. Some negative health conditions such as dizziness, blurred vision, unusual bleeding, confusion, headache, and rapid heart rate, have been linked to excessive production of $NO^{\cdot}$. Hydrogen peroxide (H_2O_2) is a reactive oxygen species that is capable of decomposing rapidly into water and oxygen, which may ultimately lead to the generation of hydroxyl radical ($OH^{\cdot}$) that could initiate lipid peroxidation and DNA damage. Exposure to H_2O_2 could be through eye/skin contact or by the inhalation of vapour or mist [26]. In the present study, ELEIG scavenged $NO^{\cdot}$ and H_2O_2 in a concentration-dependent manner, comparable to ascorbic acid (standard). This implies the potent antioxidant property of the extract which may not be unconnected with its inherent phytochemicals that have been reported to possess high antioxidant properties [28].

The liver is a rich source of the aminotransferases, AST and ALT [29]. These enzymes are involved in transamination reactions [30]. In hepatocellular damage (which

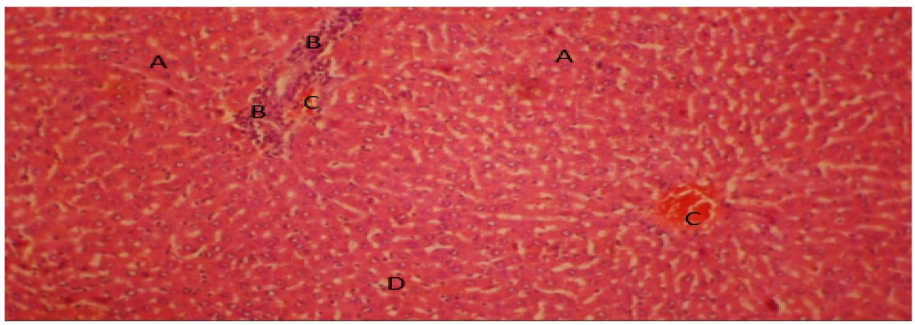

Fig. 7 Liver section of rat given SA for 14 days followed by 400mg/kg leaf extract for 14 days (Group 5) showing: **A**, normal hepatocytes, **B**, mild periportal infiltrates of inflammatory cells, **C**, mild vascular congestion and **D**, moderate Kupffer cell activation (H&E x 100)

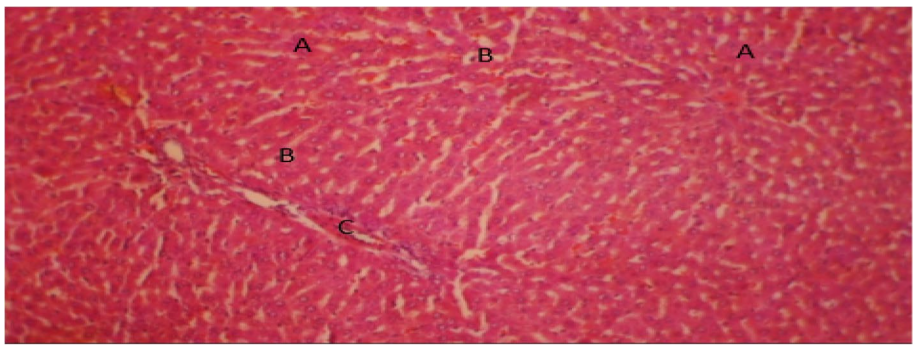

Fig. 8 Liver section of rat co-administered SA and 100mg/kg leaf extract for 14 days (Group 6) showing: **A**, normal hepatocytes, **B**, mild Kupffer cell activation and **C**, normal vascular architecture (H&E x100)

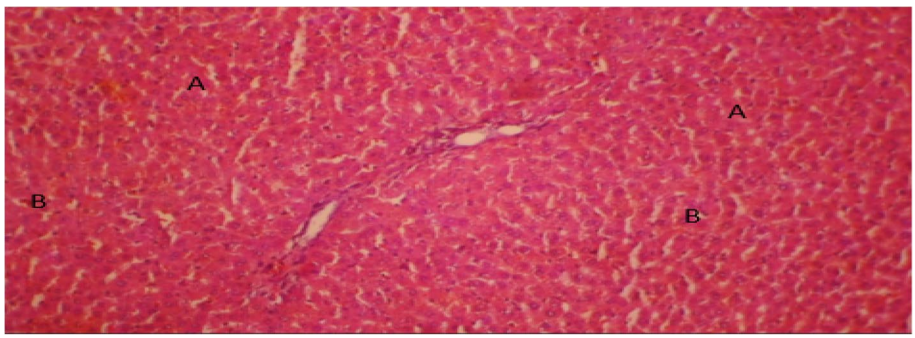

Fig. 9 Liver section of rat co-administered SA and 200mg/kg leaf extract for 14 days (Group 7) showing: **A**, normal hepatocytes and **B**, mild Kupffer cell activation (H&E x 100)

could by exposure to toxic elements), the blood levels of these enzymes are elevated. Their blood levels directly reveal the extent of tissue damage [31].

In this study, administration of sodium arsenite (SA) culminated in significant increases in serum levels of AST and ALT, relative to the normal control. This implies hepatocellular damage by SA, leading to the leakage of the enzymes into the blood stream [32]. This is corroborated by findings from previous studies [33, 34]. Treatment with ELEIG (concomitant and post-treatment), achieved significant decreases in AST levels at the highest dose of 400 mg/kgbw when compared group 2 (administered sodium arsenite only). In the same vein, treatment with ELEIG produced significant decreases in ALT levels

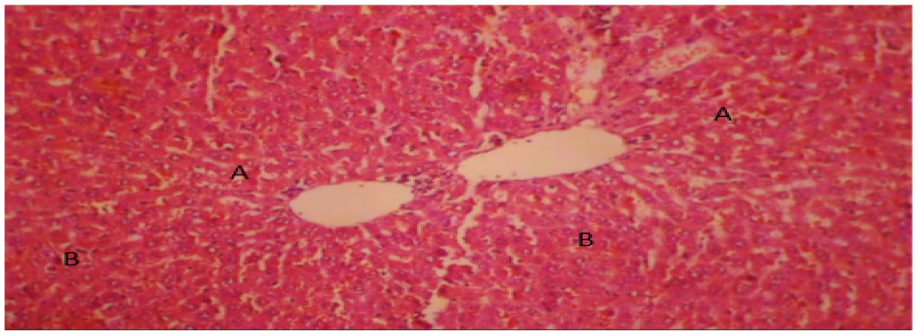

Fig. 10 Liver section of rat co-administered SA and 400mg/kg leaf extract for 14 days (Group 8) showing: **A**, mild haemorrhagic necrosis and **B**, moderate Kupffer cell activation (H&E x 100)

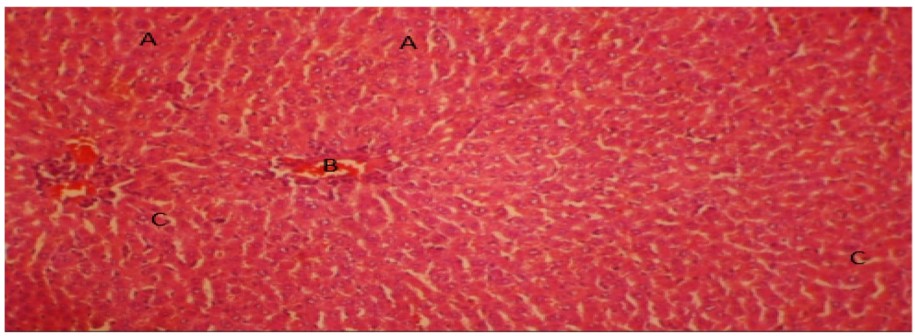

Fig. 11 Liver section of rat given 100mg/kg leaf extract only for 14 days (Group 9) showing: **A**, normal hepatocytes, **B**, portal vein and **C**, moderate Kupffer cell activation (H&E x 100)

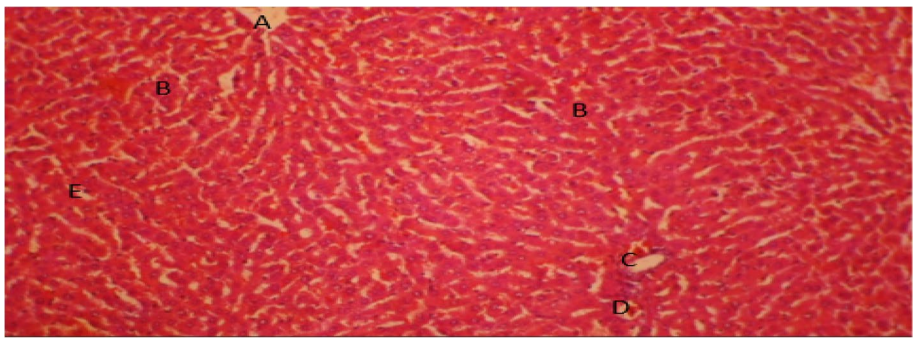

Fig. 12 Liver section of rat given 200mg/kg leaf extract only for 14 days (Group 10) showing: **A**, central vein, **B**, normal hepatocytes, **C**, bile duct, **D**, portal vein and **E**, mild Kupffer cell activation (H&E x 100)

in dose-dependent manner when administered after 2 weeks of SA intoxication (post-treatment) and in dose-independent manner when administered concomitantly, compared with group 2 (administered SA only). This connotes that the extract could be ameliorative or curative. Administration of graded doses of ELEIG alone produced non-significant differences in AST and ALT levels, but significant decreases at the highest dose of 400 mg/kgbw, when compared with the normal control. The observed positive effect of the ethanol leaf extract may not be unconnected to its constituent antioxidant phytochemicals [28], since sodium arsenite / arsenic has been reported to elicit its negative health effects by induction of oxidative stress, among others [6].

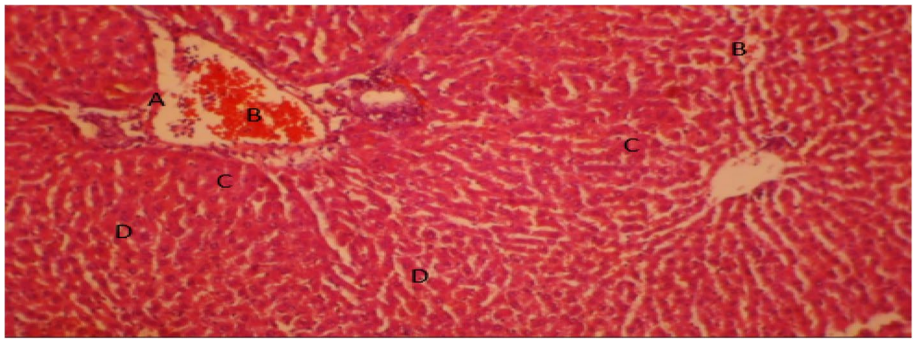

Fig. 13 Liver section of rat given 400mg/kg leaf extract only for 14 days (Group 10) showing **A**, marked portal vascular dilatation and **B**, mild active vascular congestion, **C**, normal hepatocytes and **D**, mild Kupffer cell activation (H&E x 100)

Alkaline phosphatases (ALPs), catalyze hydrolysis of organic phosphate in proteins, nucleotides and alkaloids at alkaline pH [35]. They are found in several tissues but high concentrations occur in liver, bone and kidney tissues as well as placenta and intestinal wall [36]. An increase in serum ALP activity connotes occurrence of liver and bone diseases [37]. It is also indicative of obstruction of bile ducts [36]. Xenobiotic-induced hepatotoxicity, may also increase serum ALP activity [38]. The degradation of glutathione in mammalian cells is catalyzed by gamma-glutamyltransferase (GGT), a cell-surface protein found in several tissues [39]. However, a higher percentage of serum GGT is derived from the liver [40]. Diseases of the liver, biliary system and pancreas, are associated with increased serum GGT activity. High blood level of GGT is closely related to hepatic steatosis [41].

In this study, oral exposure of the experimental rats to sodium arsenite caused significant increases in serum ALP and GGT activities, relative to the normal control. However, treatment with graded doses of ELEIG (concomitant and post-treatment), led to significant decreases in serum ALP levels in dose-dependent manner compared with group 2 (administered sodium arsenite only). In the same vein, concomitant and post-treatment with graded doses of ELEIG also led to significant decreases in serum GGT levels in dose-independent manner compared with group 2 (administered sodium arsenite only). Furthermore, administration of graded doses of ELEIG alone produced non-significant differences in GGT levels and significant increases in ALP levels compared with control. This is therefore suggestive of the hepatoprotective effect of ethanol leaf extract of *Irvingia gabonensis* which may have been influenced by its inherent antioxidant phytochemicals [28]. Previous studies had also reported the hepatoprotective effects of ethanol leaf extract of *Irvingia gabonensis* under conditions of induced toxicity [13, 14].

Bilirubin, the end product of haemoglobin catabolism [32], is a biomarker of hepatic and blood disorders. An increase in serum bilirubin level indicates the occurrence of liver disease. Increase in blood levels of conjugated (direct) bilirubin, occurs in diseases such as hepatocellular damage, toxic or ischemic liver injury and viral hepatitis [32, 42]. In this study, oral exposure of the experimental animals to sodium arsenite caused non-significant increases in serum total bilirubin (TBIL) and direct bilirubin (DBIL) concentrations, relative to the control. This indicates the interference with the transport function of the liver by sodium arsenite [32]. However, there were no significant differences in serum TBIL and DBIL concentrations on treatment with ELEIG. Administration of ELEIG alone also produced non-significant differences in serum TBIL and DBIL concentrations as compared with control.

Arsenic, a heavy metal, induces oxidative stress by generating free radicals / ROS [43] that culminate in cellular damage via depletion of enzyme activities through lipid peroxidation and reaction with nuclear proteins and DNA [44]. Inorganic arsenic compounds can also induce oxidative stress by inhibiting antioxidant enzymes via binding to their sulfhydryl (−SH) groups [45].

In this study, administration of sodium arsenite alone produced significant decreases in hepatic SOD and GPx levels and significant increases in CAT and MDA levels when compared with the control group. Endogenous enzymes act as the first line of cellular defense against reactive oxygen species [46]. Oxidative stress leads to a loss in the balance between ROS production and antioxidant defense systems due to the overwhelming power of prooxidants generated, which deregulates cellular functions that can lead to hepatic necrosis [47]. The observed significant increase in hepatic CAT level in this study

may be due to significant induction in catalase activity as an adaptive response for combating the induced oxidative stress. This is consistent with the findings of Gbadegesin et al. [13]. Catalase is the antioxidant enzyme that degrades hydrogen peroxide (a reactive oxygen species) produced during metabolism. It catalyzes the removal of hydrogen peroxide formed in the reaction catalyzed by superoxide dismutase [48]. Superoxide dismutase (SOD) enhances dismutation of superoxide radical to hydrogen peroxide that is then removed by catalase [49]. It may therefore serve as a primary defense and mitigates further production of free radicals. Glutathione peroxidases (GPx) facilitate the reduction of hydrogen peroxide and lipid hydroperoxides (products of lipid peroxidation) to their corresponding alcohols [50]. Malondialdehyde (MDA) is a product of lipid peroxidation whose concentration in increased in oxidative stress that can lead to the destabilization of membranes [34]. The mechanism of sodium arsenite-induced toxicity in this study may therefore be by its inhibition of antioxidant defense systems via the generation of ROS, culminating in the destabilization of cell membranes by lipid peroxidation. Other studies have also reported the induction of oxidative stress orchestrated by sodium arsenite which are consistent with the findings from this study [34].

Treatment with ELEIG (concomitant and post-treatment), produced significant increases in the levels of SOD and GPx in dose-dependent manner, and significant decreases in CAT and MDA concentrations in dose-dependent manner, when compared with group 2 administered sodium arsenite only. However, the significant decreases in CAT concentrations after post treatment with the extract were not dose-dependent. The significant decreases in CAT concentration by ELEIG, indicates an alleviation of oxidative damage by sodium arsenite which had led to induction of CAT level as an adaptive response. This connotes that the ethanol leaf extract could be ameliorative and curative. Administration of ELEIG alone at various doses also produced similar results for the oxidative stress biomarkers with that of the control group. It is therefore possible that the ethanol leaf extract mitigated sodium arsenite-induced toxicity / oxidative stress by enhancing the antioxidant defense systems in the treated rats, thereby enhancing the mopping up of the ROS generated. This may be due to the constituent antioxidant phytochemicals present in the extract. This is corroborated by the findings of Gbadegesin et al. [13].

There were no visible lesions in the liver tissues of the control group animals, following histological assessment. On the contrary, oral intoxication with sodium arsenite (group 2) caused severe vascular ulceration, portal congestion, infiltrates of inflammatory cells (portal hepatitis), and severe micro vesicular steatosis in the liver. Treatment with graded doses of ELEIG (concomitant and post-treatment), achieved dose-dependent ameliorative and therapeutic effects. Thus, the ethanol leaf extract proved effective in attenuating the sodium arsenite-induced steatohepatitis in the liver of the experimental animals. This is consistent with findings of Gbadegesin and co-workers [13].

Conclusion

On the premise of the results obtained from this study, it may be concluded that, ethanol leaf extract of *Irvingia gabonensis* possesses potent antioxidant property and has the capacity to mitigate sodium arsenite-induced hepatic oxidative stress and hepatotoxicity in Wistar rats, by enhancing the antioxidant defense systems in the intoxicated rats.

Abbreviations

ELEIG: Ethanol leaf extract of *Irvingia gabonensis*; SA: Sodium arsenite; TBIL: Total bilirubin; DBIL: Direct bilirubin; CAT: Catalase; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; MDA: Malondialdehyde; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT : Gamma glutamyltransferase; PT: Post-treatment; ST: Simultaneous treatment; mg/kgbw: Milligram per kilogram body weight; ROS: Reactive oxygen species; RNS: Reactive nitrogen species.

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Authors' contributions

EGE and NPO designed the study. EGE performed the statistical analysis and wrote the first draft of the manuscript. NPO supervised the study. EGE and SAO managed the analyses of the study. EGE managed experimental animal treatment and literature searches. JIN and NPO played major roles in writing the manuscript. GIE performed the histological assessments. All Authors approved the final manuscript.

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Availability of data and materials

All data obtained from this study are included in this article.

Declarations

Ethics approval and consent for publication

All authors hereby declare that the "Principles of laboratory animal care" (NIH publication No. 85–23, revised 1985) were followed as well as specific national laws, where applicable. All experiments were examined and approved by the appropriate Institutional Review Board.

Consent for publication

Not applicable.

Competing interests

There are none.

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