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Hepatoprotective and Nephroprotective Effects of *Garcinia kola* Heckel Stem Bark Extract and Triterpenoid Fraction Against Sodium Arsenite-Induced Toxicity in Rat Models

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Abstract: *Garcinia kola* Heckel (Clusiaceae) has been widely used in ethnomedicine practice as a therapy against numerous disorders. Therefore, this study was designed to evaluate the protective effects of orally administered *G. kola* stem bark ethanolic extract (EEGK) and triterpenoid fraction (TFGK) against sodium arsenite-induced hepatotoxicity and nephrotoxicity using rat models for 14 days. Assays for plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, urea, and creatinine, liver, kidney, and plasma superoxide dismutase (SOD), glutathione peroxidase (GPx) and reduced glutathione (GSH) activities were carried out using spectrophotometric methods. Gas chromatography-mass spectrometry analytical method was used to identify the bioactive compounds present in TFGK and EEGK. Hematological parameters were assayed using autoanalyzer. Data showed that TFGK reduced liver function markers viz. ALT, AST, ALP, and total bilirubin, while EEGK reduced kidney function markers viz. plasma creatinine and urea. Furthermore, EEGK elevated plasma, liver and kidney SOD, GPx, and GSH while TFGK modulated hematological markers. Findings from this study showed that TFGK substantially protected against sodium arsenite-induced hepatotoxicity while EEGK protected against sodium arsenite-induced nephrotoxicity.

Key words: Antioxidant; kidney; liver; protection; stem bark.

Introduction

The use of stem, leaf and root of medicinal plants in the treatment of ailments is prominent in many developing countries. Perhaps, this could be attributed to the presence of therapeutic phyto-compounds present in these plants and their perceived fewer side effects [1]. In sub-Saharan Africa, *Garcinia kola* Heckel of the family of Clusiaceae, commonly known as bitter kola is used in herbal formulations with potential therapeutic benefits [2]. *G. kola* is a perennial plant located mainly in West and Central Africa [3,4].

G. kola seeds phytochemicals are known to possess antiviral, hepatoprotective, antioxidant and antidiabetic properties [2,5,6]. The leaf extracts of *G. kola* have been shown to possess bactericidal property [7]. *G. kola* stem bark is used in folklore remedy to dress fresh wounds [8]. In Nigeria, a decoction of *G. kola* stem bark is used as therapy against dysmenorrhoea and inflammation. Despite the use of *G. kola* stem bark in the treatment of some ailments, the medicinal properties of *G. kola* stem bark and the scientific verification have not been well researched. However, preliminary phytochemical analysis of *G. kola* stem bark had shown much promising values [9].

Sodium arsenite ($NaAsO_2$) is a toxic metallic pollutant of public health concern that is present in contaminated ground water due to agricultural runoff and mining processes [10,11]. It has been re-

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ported that sodium arsenite is sixty times more potent than sodium arsenate [12]. Molecularly, arsenic is known to induce toxicity and carcinogenicity through the generation of oxidative stress and cellular responses resulting from binding of arsenic to thiol (SH) substituents groups of macromolecules [13]. This binding results in alteration of several enzyme activities and propagation of deleterious reactive oxygen species (ROS) leading to a wide range of metal toxicities in human health [14]. More so, the primary targets of sodium arsenite induced toxicities are liver and kidneys. The most applied therapy against arsenite toxicity has been metal chelation therapy which forms metal complexes with the attendant removal of excess arsenite from the body system [12]. This type of therapy has been associated with adverse effects to the biochemical system. However, the use of plant extracts as a therapy against arsenite toxicity with minimal or no adverse effect could also be considered and scientifically validated.

Therefore, this study was designed to investigate the hepatoprotective and nephroprotective effects of *G. kola* stem bark ethanolic extract (EEGK) and triterpenoid fraction (TFGK) against arsenite-induced toxicities in rat models.

Materials and methods

Collection and identification

Stem barks of *G. kola* were collected from a farmland in Ilishan Remo, Ogun state and authenticated at the Herbarium of the Forestry Research Institute of Nigeria, Jericho, Ibadan, Oyo State with voucher number FHI/110398.

Plant processing and extraction

Stem barks of *G. kola* were thoroughly washed with distilled water to remove debris, chopped and oven-dried at 40°C for 7 days. The dried stem barks were pulverized using a mechanical blender.

Ethanolic extract preparation

Pulverised *G. kola* stem bark (200 g) was soaked with 1.6 L 70 % ethanol and mixed intermittently for 48 h at room temperature. The suspension was filtered using Whatman No. 1 filter paper and filtrate obtained was concentrated using a rotary evaporator (Buchi Rotavapor RE, Switzerland) at 40°C and stored in a refrigerator at 4°C until further use.

Triterpenoid fraction preparation

G. kola stem bark triterpenoid fraction was prepared according to the method of Pramod *et al*. [15]. Ground *G. kola* stem bark (250 g) was soaked with 95 % ethanol and the suspension was mixed intermittently for 7 days at room temperature. Subsequently, the extract was filtered using Whatman No.1 filter paper. The filtrate obtained was concentrated using a rotary evaporator (Buchi Rotavapor RE, Switzerland) at 40°C to obtain an ethanolic extract. The ethanolic extract was further partitioned between ethyl acetate and water. The obtained ethyl acetate fraction was further partitioned using n-hexane to isolate triterpenoid fraction and stored in a refrigerator at 4°C until further use.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of *G. kola* stem bark ethanolic extract and triterpenoid fraction was carried out at Industrial and Environmental Technical Department, National Research Institute of Chemical Technology, Zaria by using Agilent technology model 7890A GC-MS, MSD = 5975C (detector), Injector: 7683B Series, Initial temperature = 100°C held for 2 min, final temperature = 270°C at the rate of 10°C/min, 1 µL *G. kola* stem bark ethanolic extract/ triterpenoid fraction was injected. Temperature of heater was 250°C, pressure was 3.2652 psi, mode type slitless, column type (HP 5MS: 30 m × 320 µm × 0.25 µm) and carrier gas (Helium, 99.9999 % purity, flow rate = 1.4963 ml/min; average velocity = 45.618 cm/s). The constituent compounds in test samples were determined by comparing their retention times and mass weights with those of authentic samples obtained by GC as well as the mass spectra of National Institute of Science and Technology (NIST) version 2.0 MS database and literature.

Animal

Forty-five male albino rats (Wistar strain) weighing between 150 - 200 g were purchased from an inbred colony in Babcock University

Animal Facility. Animals were allowed to acclimatize in aerated cages under a natural light condition at room temperature and were fed with commercial pelleted diet and water *ad libitum* for two weeks. All animal experiments and protocols conformed to the guidelines of National Institute of Health for laboratory animal care and use [16]. Ethical clearance with certificate number BUHREC227/16 was obtained from the Babcock University Health Research Ethics Committee.

Experimental design

Hepatoprotective and nephroprotective studies of *G. kola* stem bark against sodium arsenite-induced toxicity in rats were carried out according to the experimental design described below.

Animals were randomly distributed into nine groups of five rats per group and were orally administered with various test agents using 1 mL 0.5 % carboxyl methylcellulose (CMC) as vehicle viz. Group 1 indicates normal rats administered with 1 mL 0.5 % CMC, Group 2: control rats administered with 5 mg/kg b.w. sodium arsenite, Group 3: standard rats were administered with 5 mg/kg b.w. sodium arsenite + 10 mg/kg b.w. silymarin, Group 4: rats were administered with 5 mg/kg b.w. sodium arsenite + 100 mg/kg b.w. ethanolic extract of *G. kola* stem bark, Group 5: rats were administered with 5 mg/kg b.w. sodium arsenite + 200 mg/kg b.w. ethanolic extract of *G. kola* stem bark, Group 6: rats were administered with 5 mg/kg b.w. sodium arsenite + 300 mg/kg b.w. ethanolic extract of *G. kola* stem bark, Group 7: rats were administered with 5 mg/kg b.w. sodium arsenite + 100 mg/kg b.w. triterpenoid fraction of *G. kola* stem bark, Group 8: rats were administered with 5 mg/kg b.w. sodium arsenite + 200 mg/kg b.w. triterpenoid fraction of *G. kola* stem bark, and Group 9: rats were administered with 5 mg/kg b.w. sodium arsenite + 300 mg/kg b.w. triterpenoid fraction of *G. kola* stem bark.

Different doses of EEGK and TFGK were given daily to respective animals for 14 days, while sodium arsenite was administered on the 7th and 14th day. Twenty-four hours after the end of treatment period, the animals were euthanized using chloroform and sacrificed. Whole blood samples were collected by cardiac puncture using 2 mL hypodermic syringes into ethylene diamine tetraacetic acid (EDTA) and heparinized bottles. Blood samples collected into EDTA bottles were used for hematological analysis while heparinized bottles were immediately centrifuged at 3000 rpm for 10 min to obtain plasma. Liver and kidney samples were excised from sacrificed animals and washed with 1.15 % ice-cold KCl. The wet liver and kidney tissues were weighed and homogenized in 0.1 M Tris-HCl buffer, pH 7 at 4^{o}C. The homogenate was centrifuged at 3000 rpm for 10 min at 4°C to obtain supernatant using a refrigerated centrifuge. Plasma and tissue supernatants were used to measure the effects of different doses of EEGK and TFGK on liver and kidney functions markers. The following hematological parameters: white blood cell (WBC), red blood cell (RBC), platelet, hemoglobin, hematocrit, neutrophils, lymphocytes, eosinophil, monocytes and basophil (EMB) were analysed at the Hematological Unit, Babcock University Teaching Hospital using an auto-analyser (Swelab Alfa 3- Part Hematology Analyser by Boule Medicals).

Determination of G. kola stem bark extract/ fraction effects on liver function markers

Effects of varying doses of EEGK and TFGK on liver function markers were performed using plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities as provided by the Randox diagnostic kits in accordance with the principle described by Reitman & Frankel [17]. Plasma total bilirubin was carried out using the procedure provided by the Randox kit in accordance with the determined described by Malloy & Evelyn [18].

Determination of G. kola stem bark extract/ fraction effects on kidney function markers

Effects of varying doses of EEGK and TFGK on kidney function markers were performed using the colorimetric estimations of plasma creatinine concentration in accordance with Jaffe's alkaline picrate method as described by Wen-Sheng *et al.* [19]. Plasma urea concentration was determined using colorimetric method as described by Randox diagnostic kit.

***In vivo* antioxidant bioassays**

Effects of varying doses of EEGK and TFGK on selected plasma, liver and kidney antioxidant biomarkers *in vivo* were performed using spectrophotometric determination of reduced glutathione (GSH) as described by Sedlak & Lindsay [20] and modified by Jollow *et al.* [21], superoxide dismutase (SOD) activity as described by Mistra & Fridovich [22], glutathione peroxidase (GPx) activity as described by Rotruck *et al.*[23] and lipid peroxidation assay as described by Stocks & Dormandy [24].

Statistical analysis

Statistical analysis was carried out with the aid of Statistical Package for Social Sciences (SPSS) for Windows: SPSS Inc., Chicago, Standard version 17.0 to determine the difference between means using One-Way Analysis of Variance (ANOVA). Data were reported as mean ± standard error of mean of triplicate readings and the significance level was set at P< 0.05.

Results

Aspartate aminotransferase (AST) assay

Data in Fig. 1 showed that 10 mg/kg b.w. silymarin (28.87 ± 0.6 U/L), 100 mg/kg b.w. EEGK (44.93 ± 3.14 U/L), 200 mg/kg b.w. EEGK (37.87 ± 2.03 U/L), 300 mg/kg b.w. EEGK (21.80 ± 0.8 U/L), 100 mg/kg b.w. TFGK (49.27 ± 3.00 U/L), 200 mg/kg b.w. TFGK (30.67 ± 0.98 U/L) and 300 mg/kg b.w. TFGK (18.8 ± 0.6 U/L) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) reduced plasma AST activities when compared with untreated control group (51.47 ± 0.20 U/L). Furthermore 300 mg/kg b.w. TFGK treated group had a significantly (P<0.05) reduced AST activity compared with 10 mg/kg b.w. silymarin and EEGK treated groups.

Alanine aminotransferase (ALT) assay

Data in Fig. 1 showed that 10 mg/kg b.w. silymarin (19.53 ± 3.88 U/L), 100 mg/kg b.w. EEGK (24.07 ± 3.17 U/L), 200 mg/kg b.w. EEGK (16.93 ± 2.27 U/L), 300 mg/kg b.w. EEGK (12.40 ± 0.92 U/L), 100 mg/kg b.w. TFGK (29.33 ± 3.40 U/L), 200 mg/kg b.w. TFGK (14.00 ± 3.11 U/L) and 300 mg/kg b.w. TFGK (10.60 ± 0.20 U/L) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) reduced plasma ALT activities when compared with untreated control group (36.33 ± 5.83 U/L). Furthermore 300 mg/kg b.w. TFGK treated group had significantly (P<0.05) reduced ALT activities compared

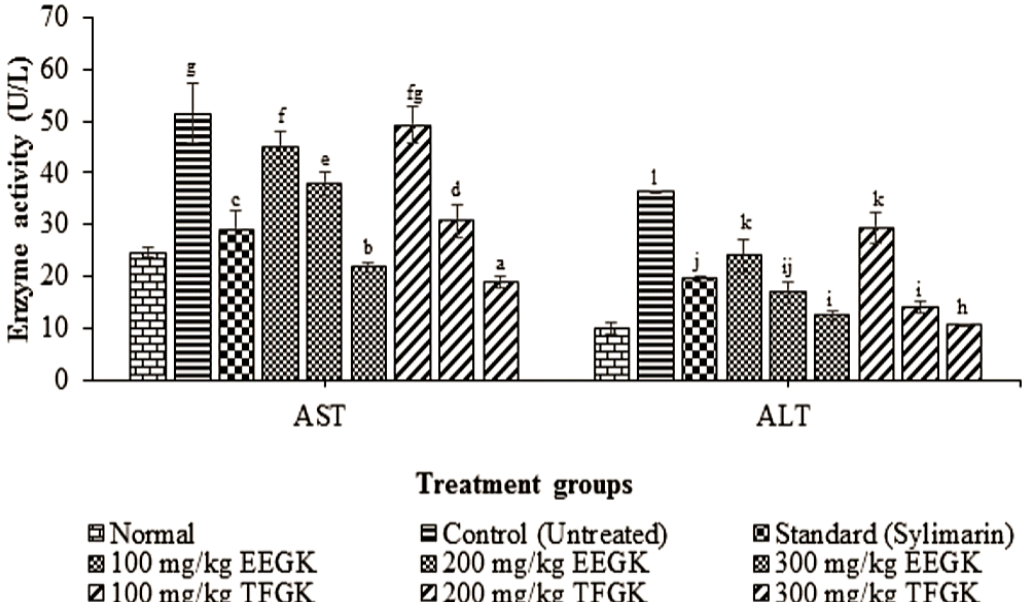

Fig. 1. Effects of different doses of *Garcinia kola* stem bark ethanolic extract (EEGK) and triterpenoid fraction (TFGK) on plasma AST and ALT in rats induced with hepatic damage by sodium arsenite. Different letters indicate significantly different from each other at P<0.05

with 10 mg/kg b.w. silymarin and EEGK treated groups.

Alkaline phosphatase (ALP) assay

Data in Fig. 2 showed that 10 mg/kg b.w. silymarin (98.87 ± 0.37 U/L), 100 mg/kg b.w. EEGK (124.32 ± 0.55 U/L), 200 mg/kg b.w. EEGK (107.76 ± 1.07 U/L), 300 mg/kg b.w. EEGK (95.5 ± 1.73 U/L), 100 mg/kg b.w. TFGK (122.11 ± 1.19 U/L), 200 mg/kg b.w. TFGK (110.6 ± 1.80 U/L) and 300 mg/kg b.w. TFGK (89.80 ± 1.81 U/L) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) reduced plasma ALP activities when compared with untreated control group (138.55 ± 0.29 U/L). Furthermore, 300 mg/kg b.w. TFGK hexane fraction had a significantly (P<0.05) reduced ALP activities compared with 10 mg/kg b.w. silymarin and EEGK treated groups.

Total bilirubin assay

Data in Fig. 3 showed that 10 mg/kg b.w. silymarin (1.30 ± 0.04 mg/dL), 100 mg/kg b.w. EEGK (2.19 ± 0.04 mg/dL), 200 mg/kg b.w. EEGK (1.83 ± 0.02 mg/dL), 300 mg/kg b.w. EEGK (1.79 ± 0.06 mg/dL), 100 mg/kg b.w. TFGK (1.93 ± 0.03 mg/dL), 200 mg/kg b.w. TFGK (1.61 ± 0.04 mg/dL), and 300 mg/kg b.w. TFGK (1.63 ± 0.03 mg/dL) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) reduced plasma bilirubin level when compared with untreated control group (2.43 ± 0.08 mg/dL). Furthermore, 300 mg/kg b.w. TFGK treated group had significantly (P<0.05) reduced plasma total bilirubin when compared with EEGK treated groups.

Urea assay

Data in Fig. 4 showed that 10 mg/kg b.w. silymarin (26.08 ± 0.75 mg/dL), 100 mg/kg b.w. EEGK (29.98 ± 0.24 mg/dL), 200 mg/kg b.w. EEGK (27.83 ± 0.24 mg/dL), 300 mg/kg b.w. EEGK (26.11 ± 0.17 mg/dL), 100 mg/kg b.w. TFGK (30.88 ± 0.20 mg/dL), 200 mg/kg b.w. TFGK (28.91 ± 0.28 mg/dL) and 300 mg/kg b.w. TFGK (27.72 ± 0.28 mg/dL) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) reduced plasma urea levels when compared with untreated control group (35.12 ± 0.24 mg/dL). Furthermore, 300 mg/kg b.w. EEGK had a significantly (P<0.05) reduced plasma urea level compared with TFGK treated groups. In addition, there was no significant difference (P>0.05) between 300 mg/kg b.w.

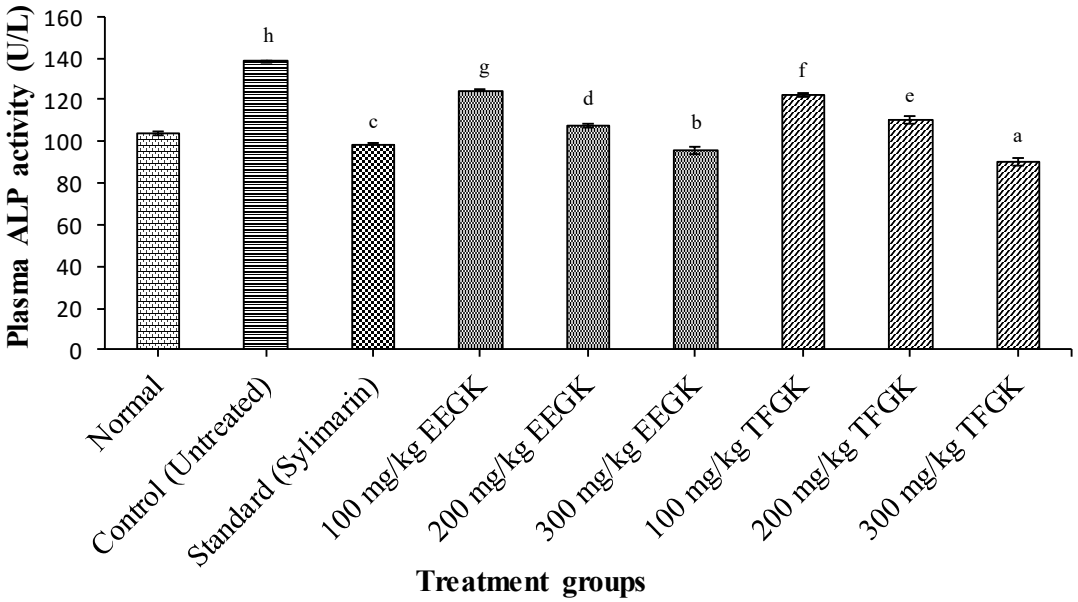

Fig. 2. Effects of different doses of *Garcinia kola* stem bark ethanolic extract (EEGK) and triterpenoid fraction (TFGK) on plasma ALP in rats induced with hepatic damage by sodium arsenite. Different letters indicate significantly different from each other at P<0.05

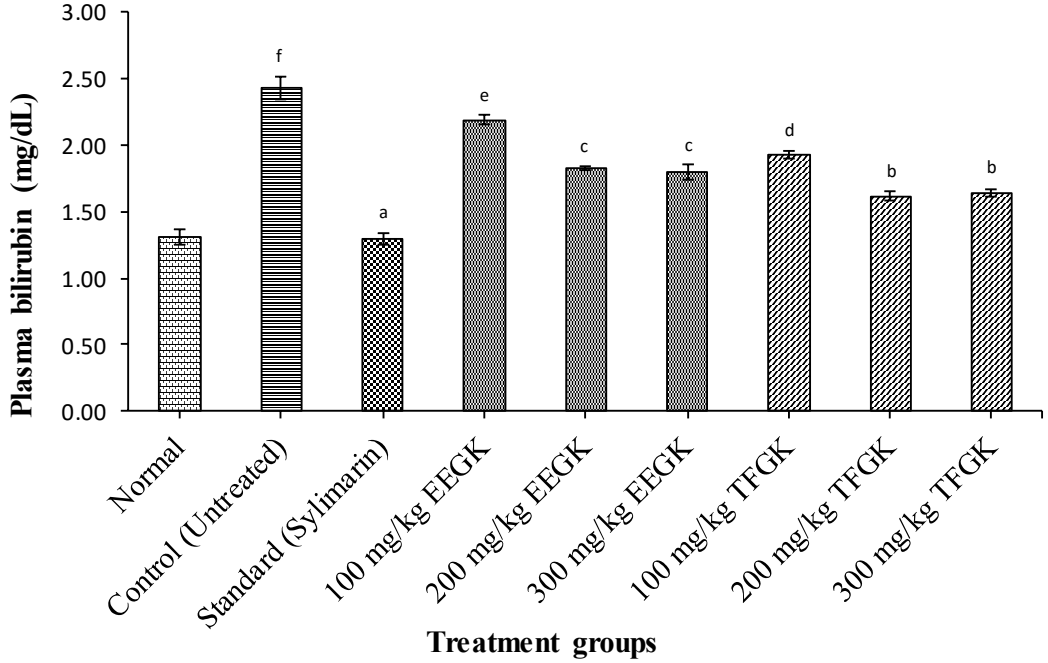

Fig. 3. Effects of different doses of *Garcinia kola* stem bark ethanolic extract (EEGK) and triterpenoid fraction (TFGK) on plasma bilirubin in rats induced with hepatic damage by sodium arsenite. Different letters indicate significantly different from each other at P<0.05

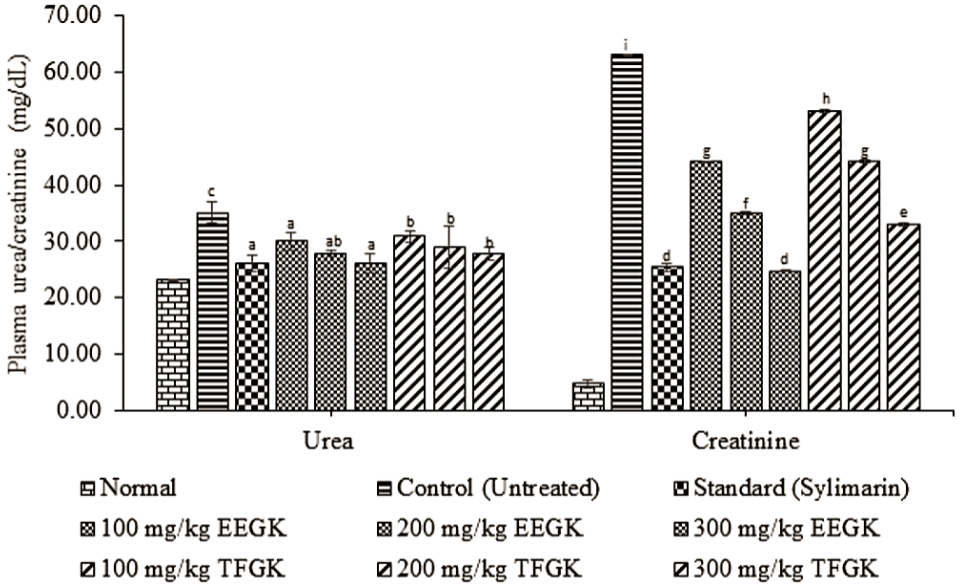

Fig. 4. Effects of different doses of *Garcinia kola* stem bark ethanolic extract (EEGK) and triterpenoid fraction (TFGK) on plasma urea/creatinine levels in rats induced with hepatic damage by sodium arsenite. Different letters indicate significantly different from each other at P<0.05

EEGK and 10 mg/kg b.w. silymarin treated groups.

Creatinine assay

In addition, Fig. 4 showed that 10 mg/kg b.w. silymarin (25.38 ± 1.51 mg/dL), 100 mg/kg b.w. EEGK (44.06 ± 1.62 mg/dL), 200 mg/kg b.w. EEGK (35.03 ± 0.69 mg/dL), 300 mg/kg b.w. EEGK (24.78 ± 1.68 mg/dL), 100 mg/kg b.w. TFGK (53.03 ± 0.99 mg/dL), 200 mg/kg b.w.

TFGK (44.25 ± 3.75 mg/dL) and 300 mg/kg b.w. TFGK (32.94 ± 1.18 mg/dL) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) reduced plasma creatinine levels when compared with untreated control group (63.00 ± 1.88 mg/dL). Furthermore, 300 mg/kg b.w. EEGK had a significantly (P<0.05) reduced plasma creatinine level compared with TFGK treated groups. In addition, there was no significant difference (P>0.05) between 300 mg/kg b.w. EEGK and 10 mg/kg b.w. silymarin treated groups.

Reduced glutathione (GSH) assay

Data in Table 1 showed that 10 mg/kg b.w. silymarin (2.72 ± 0.07 units/mg protein), 200 mg/kg b.w. EEGK (2.09 ± 0.02 units/mg protein), 300 mg/kg b.w. EEGK (2.25 ± 0.08 units/mg protein), 200 mg/kg b.w. TFGK (2.19 ± 0.04 units/mg protein) and 300 mg/kg b.w. TFGK (1.97 ± 0.05 units/mg protein) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) elevated plasma GSH concentrations when compared with untreated control group (1.73 ± 0.17 units/mg protein). Furthermore, 300 mg/kg EEGK treated animals had significantly (P<0.05) elevated plasma GSH concentrations when compared with 300 mg/kg b.w. TFGK treated animals.

In addition, Table 1 showed that 10 mg/kg b.w. silymarin (4.23 ± 0.05 units/mg protein), 100 mg/kg b.w. EEGK (3.41 ± 0.14 units/mg protein), 200 mg/kg b.w. EEGK (3.37 ± 0.05 units/mg protein), 300 mg/kg b.w. EEGK (4.95 ± 0.07 units/mg protein) and 300 mg/kg b.w. TFGK (3.21 ± 0.05 units/mg protein) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) elevated liver GSH concentrations when compared with untreated control group (2.84 ± 0.04 units/mg protein). Liver GSH concentrations in 300 mg/kg b.w. EEGK treated groups was significantly (P<0.05) elevated when compared with standard drug treated groups. In addition, the liver GSH concentrations in EEGK treated groups were significantly (P<0.05) elevated when compared with TFGK treated groups.

Furthermore, Table 1 showed that 10 mg/kg b.w. silymarin (3.89 ± 0.27 units/mg protein), 100 mg/kg b.w. EEGK (2.33 ± 0.22 units/mg protein), 200 mg/kg b.w. EEGK (2.17 ± 0.03 units/mg protein), 300 mg/kg b.w. EEGK (3.61 ± 0.09 units/mg protein), 100 mg/kg b.w. TFGK (1.97 ± 0.08 units/mg protein), 200 mg/kg b.w. TFGK (1.90 ± 0.02 units/mg protein) and 300 mg/kg b.w. TFGK (2.62 ± 0.06 units/mg protein) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) elevated kidney GSH concentrations when compared with untreated control group (1.43 ± 0.05 units/mg protein). Kidney GSH level of 300 mg/kg b.w. EEGK treated group was not significantly (P>0.05) different when compared to 10 mg/kg b.w. silymarin treated group. However, the kidney GSH concentrations of EEGK treated groups was significantly (P<0.05) elevated when compared with TFGK treated groups.

Glutathione peroxidase (GPx) assay

Data in Table 1 showed that 10 mg/kg b.w. silymarin (1.75 ± 0.02 µg/min/mg protein), 100 mg/kg b.w. EEGK (1.52 ± 0.01 µg/min/mg protein), 200 mg/kg b.w. EEGK (1.54 ± 0.01 µg/min/mg protein), 300 mg/kg b.w. EEGK (1.73 ± 0.02 µg/min/mg protein) , 100 mg/kg b.w. TFGK (1.47 ± 0.02 µg/min/mg protein), 200 mg/kg b.w. TFGK (1.52 ± 0.01 µg/min/mg protein), and 300 mg/kg b.w. TFGK (1.63 ± 0.02 µg/min/mg protein) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) elevated plasma GPx activity when compared with untreated control group (1.38 ± 0.01 µg/min/mg protein). Plasma GPx activity of 10 mg/kg b.w. silymarin treated group was not significantly different (P>0.05) when compared to 300 mg/kg b.w. EEGK treated group. However, the plasma GPx activity of 300 mg/kg b.w. EEGK treated group was significantly (P<0.05) elevated when compared with 300 mg/kg b.w. TFGK.

Furthermore, Table 1 showed that 10 mg/kg b.w. silymarin (2.20 ± 0.02 µg/min/mg protein), 100 mg/kg b.w. EEGK (1.67 ± 0.01 µg/min/mg protein), 200 mg/kg b.w. EEGK (1.74 ± 0.02 µg/min/mg protein), 300 mg/kg b.w. EEGK (1.86 ± 0.02 µg/min/mg protein), 100 mg/kg b.w. TFGK (1.55 ± 0.02 µg/min/mg protein), 200 mg/kg b.w. TFGK (1.74 ± 0.01 µg/min/mg protein) and 300 mg/kg b.w. TFGK (1.78 ± 0.01 µg/min/mg protein)

Table 1. Effects of different doses of *Garcinia kola* stem bark ethanolic extract and triterpenoid fraction on plasma, liver and kidney antioxidants in rats induced with toxicity by sodium arsenite

Parameters	Tissues	Normal group	Control group	Standard group	100 mg/kg EEGK	200 mg/kg EEGK	300 mg/kg EEGK	100 mg/kg TFGK	200 mg/kg TFGK	300 mg/kg TFGK
GSH (units/ mg protein)	Plasma	2.56±0.05	1.73±0.17^{a}	2.72±0.07^{e}	1.61±0.06^{a}	2.09±0.02^{c}	2.25±0.08^{d}	1.59±0.08^{a}	2.19±0.04^{d}	1.97±0.05^{b}
	Liver	5.57±0.18	2.84±0.04^{b}	4.23±0.05^{e}	3.41±0.14^{d}	3.37±0.05^{d}	4.95±0.07^{f}	2.81±0.05^{b}	2.50±0.04^{a}	3.21±0.05^{c}
	Kidney	3.42±0.19	1.43±0.05^{a}	3.89±0.27^{e}	2.33±0.22^{c}	2.17±0.03^{c}	3.61±0.09^{e}	1.97±0.08^{b}	1.90±0.02^{b}	2.62±0.06^{d}
GPx (µg/min/ mg protein)	Plasma	1.62±0.03	1.38±0.01^{a}	1.75±0.02^{e}	1.52±0.01^{c}	1.54±0.01^{c}	1.73±0.02^{e}	1.47±0.02^{b}	1.52±0.01^{c}	1.63±0.02^{d}
	Liver	1.84±0.03	1.42±0.01^{a}	2.20±0.02^{g}	1.67±0.01^{c}	1.74±0.02^{d}	1.86±0.02^{f}	1.55±0.02^{b}	1.74±0.01^{d}	1.78±0.01^{e}
	Kidney	1.73±0.00	1.72±0.00	1.73±0.00	1.72±0.00	1.73±0.00	1.73±0.00	1.72±0.00	1.72±0.00	1.73±0.00
SOD (units/ mg protein)	Plasma	10.16±0.30	6.20±0.26^{a}	11.17±0.31^{d}	6.32±0.10^{a}	10.01±0.06^{c}	12.13±0.07^{e}	6.58±0.13^{a}	7.19±0.03^{b}	9.79±0.22^{c}
	Liver	10.01±0.56	4.82±0.08^{a}	6.60±0.05^{d}	4.54±0.13^{a}	6.08±0.17^{c}	7.23±0.11^{e}	4.23±0.51^{a}	4.72±0.09^{a}	5.69±0.09^{b}
	Kidney	28.79±1.02	13.02±0.46^{b}	19.28±0.55^{e}	13.03±0.65^{b}	12.93±0.18^{b}	15.99±0.28^{d}	12.85±0.48^{b}	11.89±0.35^{a}	12.60±0.50^{b}
Malondi-aldehyde (µmol/mg protein)	Plasma	14.68±1.23	38.42±3.68^{f}	13.93±0.65^{a}	27.89±1.90^{e}	21.21±0.83^{c}	27.08±0.19^{e}	23.43±0.28^{d}	21.35±0.48^{c}	19.15±0.17^{b}
	Liver	0.00±0.00	4.83±0.11^{h}	1.14±0.10^{a}	3.64±0.01^{g}	2.99±0.01^{e}	2.23±0.13^{c}	3.38±0.01^{f}	2.51±0.00^{d}	2.11±0.00^{b}
	Kidney	1.04±0.01	2.28±0.00^{h}	1.03±0.00^{a}	1.97±0.00^{f}	1.70±0.01^{c}	1.24±0.00^{b}	2.12±0.01^{g}	1.95±0.00^{e}	1.75±0.00^{d}

EEGK indicates ethanolic extract of *G. kola* stem bark
TFGK indicates triterpenoid fraction of *G. kola* stem bark
Different letters indicates significantly different at $P<0.05$
n= 5 rats per group

treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) elevated liver GPx activities when compared with untreated control group (1.42 ± 0.01 µg/min/mg protein). Liver GPx activity of standard drug treated group was significantly (P<0.05) elevated when compared with test extracts treated groups. However, the liver GPx activity of 300 mg/kg b.w. EEGK treated group was significantly (P<0.05) elevated when compared with 300 mg/kg b.w. TFGK treated group.

In addition, data in Table 1 **s**howed that 10 mg/kg b.w. silymarin (1.73 ± 0.00 µg/min/mg protein), 200 mg/kg b.w. EEGK (1.73 ± 0.00 µg/min/mg protein), 300 mg/kg b.w. EEGK (1.73 ± 0.00 µg/min/mg protein) and 300 mg/kg b.w. TFGK (1.73 ± 0.00 µg/min/mg protein) treated animals induced with toxicity using sodium arsenite had slightly elevated kidney GPx activity when compared with untreated control group (1.72 ± 0.00 µg/min/mg protein). There were no significant difference (P>0.05) when 10 mg/kg b.w. silymarin treated animal's kidney GPx activity was compared with GPx activities of 300 mg/kg b.w. EEGK and TFGK treated groups.

Superoxide dismutase (SOD) assay

Data in **Table 1** showed that the 10 mg/kg b.w. silymarin treated group (11.17 ± 0.31 units/mg protein), 200 mg/kg b.w. EEGK (10.01 ± 0.06 units/mg protein), 300 mg/kg b.w. EEGK (12.13 ± 0.07 units/mg protein), 200 mg/kg b.w. TFGK (7.19 ± 0.03 units/mg protein) and 300 mg/kg b.w. TFGK (9.79 ± 0.22 units/mg protein) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) elevated plasma SOD activities when compared with untreated control group (6.20 ± 0.26 units/mg protein). Furthermore, plasma SOD activity of 300 mg/kg b.w. EEGK treated group was significantly (P<0.05) elevated when compared with 10 mg/kg b.w. silymarin and TFGK treated groups.

Table 1 showed that 10 mg/kg b.w. silymarin treated group (6.60 ± 0.05 units/mg protein), 200 mg/kg b.w. EEGK (6.08 ± 0.17 units/mg protein), 300 mg/kg b.w. EEGK (7.23 ± 0.11 units/mg protein), and 300 mg/kg b.w. TFGK (5.69 ± 0.09 units/mg protein) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) elevated liver SOD activities when compared with untreated control group (4.82 ± 0.08 units/mg protein). Furthermore, liver SOD activity of 300 mg/kg b.w. EEGK treated group was significantly (P<0.05) elevated when compared with 10 mg/kg b.w. silymarin and 300 mg/kg TFGK treated groups.

Data in Table 1 also showed that 10 mg/kg b.w. silymarin treated group (19.28 ± 0.55 units/mg protein), and 300 mg/kg b.w. EEGK (15.99 ± 0.28 units/mg protein) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) increased kidney SOD activity when compared with untreated control group (13.02 ± 0.46 units/mg protein). Furthermore, kidney SOD activity of 300 mg/kg b.w. EEGK treated group was significantly (P<0.05) elevated when compared with TFGK treated groups.

Lipid peroxidation (LPO) assay

Data in Table 1 indicated that malondialdehyde levels in plasma, liver and kidney were significantly (P<0.05) suppressed in 10 mg/kg b.w. silymarin treated group (13.93 ± 0.65, 1.14 ± 0.10 and 1.03 ± 0.00 mmol/mg protein), 100 mg/kg b.w. EEGK (27.89 ± 1.90, 3.64 ± 0.01 and 1.97 ± 0.00), 200 mg/kg b.w. EEGK (21.21 ± 0.83, 2.99 ± 0.01 and 1.70±0.01 mmol/mg protein), 300 mg/kg b.w. EEGK (27.08 ± 0.19, 2.23 ± 0.13 and 1.24 ± 0.00 mmol/mg protein), 100 mg/kg b.w. TFGK (23.43 ± 0.28, 3.38 ± 0.01 and 2.12 ± 0.01mmol/mg protein), 200 mg/kg b.w. TFGK (21.35 ± 0.48, 2.51 ± 0.00 and 1.95 ± 0.00 mmol/mg protein) and 300 mg/kg b.w. TFGK (19.15 ± 0.17, 2.11 ± 0.00, and 1.75 ± 0.00 mmol/mg protein) treated groups when compared with untreated control group (38.42 ± 3.68, 4.83 ± 0.11 and 2.28 ± 0.00 mmol/mg protein) respectively. In addition, 300 mg/kg b.w. TFGK treated group significantly (P<0.05) reduced plasma and liver malondialdehyde levels than EEGK treated groups whereas 300 mg/kg b.w. EEGK treated group significantly (P<0.05) reduced kidney malondialdehyde levels than TFGK treated groups.

Hematological parameters assessment

White blood cell (WBC) counts

Data in Table 2 showed that 10 mg/kg b.w.

silymarin treated group (7.62 ± 0.54 ×10^9/L), 100 mg/kg b.w. EEGK (12.18 ± 0.78×10^9/L), 200 mg/kg b.w. EEGK (9.50 ± 0.36×10^9/L), 300 mg/kg b.w. EEGK (6.45 ± 0.5 ×10^9/L), 100 mg/kg b.w. TFGK (7.23 ± 0.32×10^9/L), 200 mg/kg b.w. TFGK (5.83 ± 0.38 ×10^9/L) and 300 mg/kg b.w. TFGK (5.46 ± 0.46×10^9/L) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) reduced WBC counts when compared with untreated control group (18.6 ± 0.88×10^9/L).

Furthermore, WBC counts for 300 mg/kg b.w. EEGK, 200 mg/kg b.w. TFGK and 300 mg/kg b.w. TFGK were significantly (P<0.05) reduced when compared with 10 mg/kg b.w. silymarin treated group. However, 200 mg/kg b.w. and 300 mg/kg b.w. TFGK treated groups were significantly (P<0.05) reduced when compared with EEGK treated groups.

Red blood cell (RBC) counts

Data in Table 2 showed that 10 mg/kg b.w. silymarin treated group (7.05 ± 0.77 × 10 ^ 12 /L), 100 mg/kg b.w. EEGK (3.57 ± 0.17 × 10 ^ 12 /L), 200 mg/kg b.w. EEGK (3.58 ± 0.28 × 10 ^ 12 /L), 300 mg/kg b.w. EEGK (5.46 ± 0.29 × 10 ^ 12 /L), 100 mg/kg b.w. TFGK (4.51 ± 0.55 × 10 ^ 12 /L), 200 mg/kg b.w. TFGK (4.95 ± 0.43 × 10 ^ 12 /L) and 300 mg/kg b.w. TFGK (6.08 ± 0.26 ×10 ^ 12 /L) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) elevated RBC counts when compared with untreated control group (2.90 ± 0.32 × 10 ^ 12 / L). 10 mg/kg b.w. silymarin and 300 mg/kg b.w. TFGK treated groups were significantly (P<0.05) elevated when compared to EEGK treated groups.

Platelet counts

Data in Table 2 showed that 10 mg/kg b.w. silymarin treated group (606.4 ± 66.66 × 10^9/L), 200 mg/kg b.w. EEGK (780.60 ± 71.47 ×10^9/L), 300 mg/kg b.w. EEGK (252.80 ± 38.70 ×10^9/L), 100 mg/kg b.w. TFGK (762.80 ± 60.95 ×10^9/L), 200 mg/kg b.w. TFGK (411.2 ± 137.54 ×10^9/L), 300 mg/kg b.w. TFGK (416.60 ± 27.40 ×10^9/L) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) reduced platelet count when compared with untreated control group (1022.80 ± 165.95 ×10^9/L). Furthermore, platelet counts of 300 mg/kg b.w. EEGK, 200 and 300 mg/kg b.w. TFGK treated groups were significantly (P<0.05) reduced when compared with 10 mg/kg b.w. silymarin treated group.

Hemoglobin levels

Data in Table 2 showed that 10 mg/kg b.w. silymarin treated group (11.32 ± 0.51 g/dL), 200 mg/kg b.w. EEGK (10.46 ± 0.40 g/dL), 300 mg/kg b.w. EEGK (11.46 ± 0.38 g/dL), 100 mg/kg b.w. TFGK (10.26 ± 0.63 g/dL) and 300 mg/kg b.w. TFGK (11.86 ± 0.36 g/dL) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) elevated hemoglobin levels when compared with untreated control group (9.38 ± 1.08 g/dL). Furthermore, there were no significant difference (P>0.05) in the hemoglobin levels of 10 mg/kg b.w. silymarin, 300 mg/kg b.w. EEGK and TFGK treated groups.

Hematocrit counts

Data in Table 2 showed that 10 mg/kg b.w. silymarin treated group (40.86 ± 4.23 %), 100 mg/kg b.w. EEGK (34.28 ± 0.96 %), 200 mg/kg b.w. EEGK (35.18 ± 1.56 %), 300 mg/kg b.w. EEGK (33.06 ± 2.08 %), 100 mg/kg b.w. TFGK (31.94 ± 3.26 %), 200 mg/kg b.w. TFGK (31.30 ± 5.81 %) and 300 mg/kg b.w. TFGK (36.60 ± 1.28 %) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) elevated hematocrit levels when compared with untreated control group (17.64 ± 1.50 %). Furthermore, standard drug and 300 mg/kg TFGK treated groups had significantly (P<0.05) elevated hematocrit count than EEGK treated groups.

Neutrophil counts

Data in Table 2 showed that 10 mg/kg b.w. silymarin treated group (51.28 ± 2.81 ×10^9/L), 100 mg/kg b.w. EEGK (56.82 ± 3.75 ×10^9/L), 200 mg/kg b.w EEGK (46.76 ± 4.17 ×10^9/L), 300 mg/kg b.w. EEGK (41.52 ± 4.55 ×10^9/L), 100 mg/kg b.w. TFGK (55.88 ± 4.46 ×10^9/L), 200 mg/kg b.w. TFGK (41.76 ± 4.94 ×10^9/L) and 300 mg/kg b.w. TFGK (32.54 ± 3.53 ×10^9/L) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) reduced

Table 2. Effects of different doses of *Garcinia kola* stem bark ethanolic extract and triterpenoid fraction on haematological indices in rats induced with toxicity by sodium arsenite

Haematological parameters	Normal group	Control group	Standard group	100 mg/kg EEGK	200 mg/kg EEGK	300 mg/kg EEGK	100 mg/kg TFGK	200 mg/kg TFGK	300 mg/kg TFGK
White blood cell (×10^9/L)	7.10±0.27	18.60±0.88^{f}	7.62±0.54^{b}	12.18±0.78^{e}	9.50±0.36^{d}	6.45±0.50^{b}	7.23±0.32^{bc}	5.83±0.38^{a}	5.46±0.46^{a}
Red blood cell (×10^12/L)	5.93±0.26	2.90±0.32^{a}	7.05±0.77^{d}	3.57±0.17^{b}	3.58±0.28^{b}	5.46±0.29^{c}	4.51±0.55^{c}	4.95±0.43^{c}	6.08±0.26^{d}
Platelets (×10^9/L)	475±41.81	1022.80±165.95^{e}	606.40±66.66^{c}	935.40±45.76^{e}	780.60±71.47^{d}	252.80±38.70^{a}	762.80±60.95^{d}	411.20±137.54^{a}	416.60±27.40^{b}
Hemoglobin (g/dL)	11.30±0.53	9.38±1.08^{a}	11.32±0.51^{bc}	9.94±0.27^{ab}	10.46±0.40^{b}	11.46±0.38^{c}	10.26±0.63^{b}	8.82±0.61^{a}	11.86±0.36^{c}
Hematocrit (%)	36.60±1.76	17.64±1.50^{a}	40.86±4.23^{c}	34.28±0.96^{b}	35.18±1.56^{b}	33.06±2.08^{b}	31.94±3.26^{b}	31.30±5.81^{b}	36.60±1.28^{bc}
Neutrophils (×10^9/L)	46.05±2.00	72.62±3.02^{d}	51.28±2.81^{bc}	56.82±3.75^{c}	46.76±4.17^{b}	41.52±4.55^{a}	55.88±4.46^{c}	41.76±4.94^{ab}	32.54±3.53^{a}
Lymphocytes (×10^9/L)	36.80±4.54	68.94±4.51^{c}	51.02±2.68^{a}	69.66±5.63^{c}	58.82±3.99^{ac}	51.26±3.74^{a}	64.22±6.44^{c}	63.24±6.20^{c}	55.18±3.76^{a}
EMB (%)	0.11±0.01	0.24±0.07^{b}	0.06±0.03^{a}	0.15±0.07^{b}	0.10±0.04^{ab}	0.07±0.04^{a}	0.15±0.08^{b}	0.11±0.06^{b}	0.05±0.02^{a}

EEGK indicates ethanolic extract of *G. kola* stem bark
TFGK indicates triterpenoid fraction of *G. kola* stem bark
Different letters indicates significantly different at $P<0.05$
n=5 rats per group

neutrophil counts when compared with untreated control group (72.62 ± 3.02 ×10^9/L). Furthermore, neutrophil counts of 300 mg/kg b.w. TFGK treated groups significantly (P<0.05) reduced when compared with 10 mg/kg b.w. silymarin and EEGK treated groups.

Lymphocyte counts

Data in Table 2 showed that 10 mg/kg b.w. silymarin treated group (51.02 ± 2.68 ×10^9/L), 200 mg/kg b.w. EEGK (58.82 ± 3.99 ×10^9/L), 300 mg/kg b.w. ethanolic extract (51.26 ± 3.74 ×10^9/L), and 300 mg/kg b.w. TFGK (55.18 ± 3.76 ×10^9/L) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) reduced lymphocytes counts when compared with untreated control group (68.94 ± 4.51 ×10^9/L). Furthermore, there was no significant difference (P>0.05) in lymphocytes counts of standard drug, 100 mg/kg b.w. and 200 mg/kg b.w. EEGK and 300 mg/kg TFGK treated groups.

Eosinophil, monocyte and basophil counts

Data in Table 2 showed that 10 mg/kg b.w. silymarin treated group (0.06 ± 0.03 %), 200 mg/kg b.w. EEGK (0.10 ± 0.04 %), 300 mg/kg b.w. EEGK (0.07 ± 0.04 %), and 300 mg/kg b.w. TFGK (0.05 ± 0.02 %) treated animals induced with toxicity using sodium arsenite had significantly (P<0.05) reduced EMB counts when compared with untreated control group (0.24 ± 0.07 %). Furthermore, there were no significantly difference (P>0.05) in EMB counts of 10 mg/kg b.w. silymarin, 100 mg/kg b.w. and 200 mg/kg b.w. EEGK and 300 mg/kg TFGK treated groups.

Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography-mass spectrometry analytical data in Fig. 5 and 6 showed 14 and 15 peaks which indicated the presence of 14 and 15 bioactive compounds in EEGK and TFGK respectively. Bioactive compounds in EEGK included hexadecanoic acid, erucic acid, and oleic acid while TFGK included 3,4-Dimethyl-2,5-dihydrofuran, trans-farnesol, brassidic acid and hexadecanoic acid (Table 3 and 4).

Discussion

Bioactive compounds present in different parts of medicinal plants has led to the continuous quest by plant medicinal scientists to investigate their potential pharmacological effects on biochemical systems particularly in neutralizing the toxic effects of heavy metals [25]. Ingested arsenite has been reported to induce toxicity at the point of absorption through gastrointestinal tract causing lesions, enhanced permeability of blood vessels and binding to hemoglobin before its entrance to the liver inducing hepatotoxicity and to the kidney eliciting nephrotoxicity [26].

In this present study, data showed that 100 -

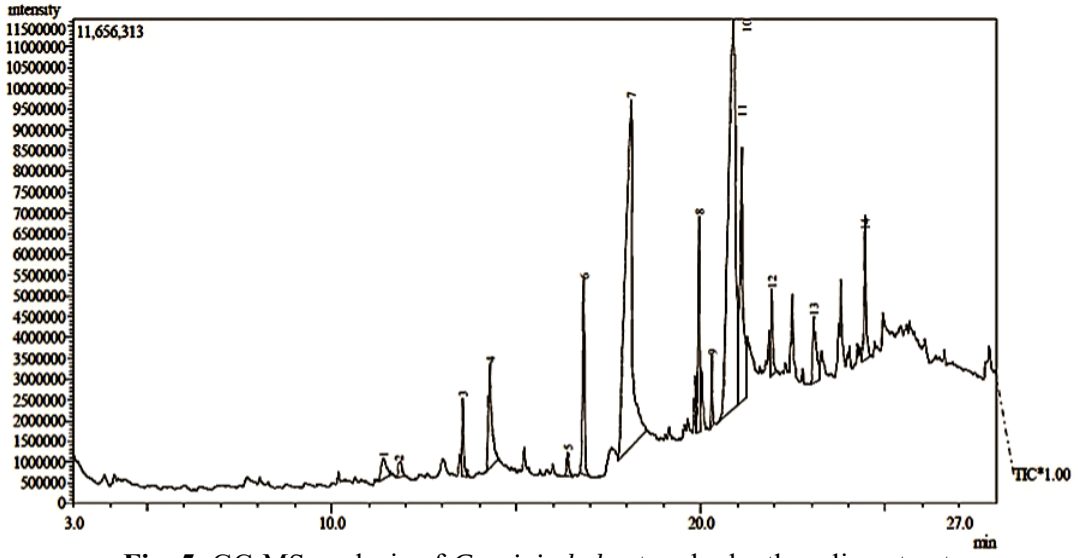

Fig. 5. GC-MS analysis of *Garcinia kola* stem bark ethanolic extract

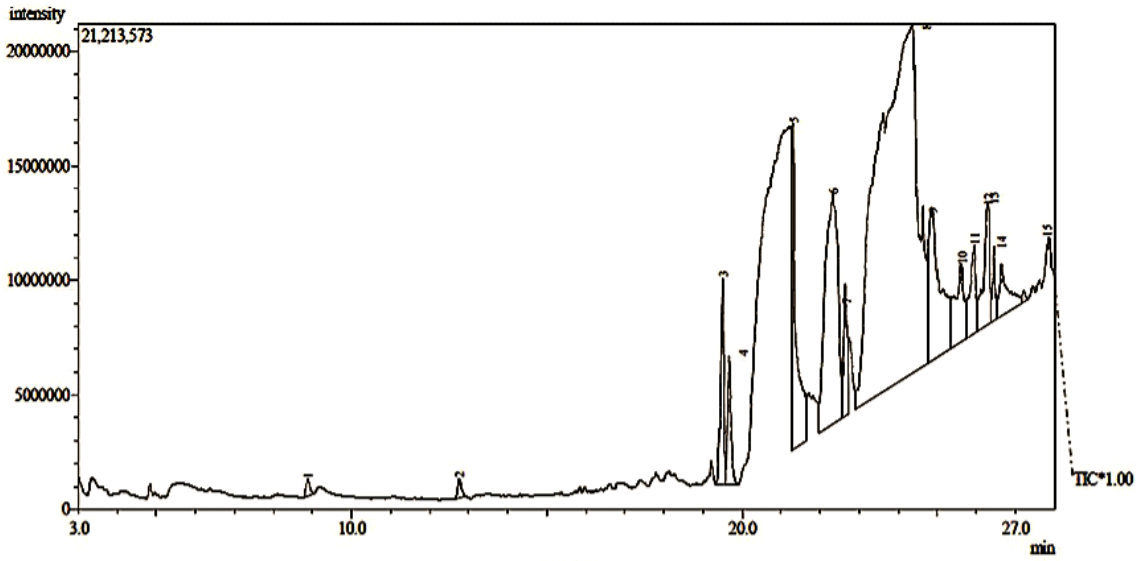

Fig. 6. GC-MS analysis of *Garcinia kola* stem bark triterpenoid fraction

Table 3. GC-MS analysis of *Garcinia kola* stem bark ethanolic extract

Mass peak	Retention time (min)	Library ID	Area (%)	Reported biological activity
1	11.40	3,4 Altrosan	1.29	Bacteriostat fungicide (Varsha et al., 2014)
2	11.86	Decanoic acid	0.70	Antioxidant, antifungal, antibacterial (Lalitharani et al., 2009)
3	13.55	Decanoic acid	1.47	Antioxidant, antifungal, antibacterial (Lalitharani et al., 2009)
4	14.29	Hexadecanoic acid	4.42	Anti-inflammatory, antioxidant, nephroprotective, hypocholesterolemic (Henry et al., 2002)
5	16.39	9-Hexadecenoic acid	0.66	Antioxidant, nephroprotective (Henry et al., 2002)
6	16.83	Pentadecanoic acid	4.90	Antiproliferative, antioxidant (Henry et al., 2002)
7	18.11	Hexadecanoic acid	29.09	Anti-inflammatory, antioxidant, nephroprotective, hypocholesterolemic (Henry et al., 2002)
8	19.96	11-Octadecenoic acid	4.41	Nephroprotective, antioxidant, hepatoprotective, hypocholesterolemic (Henry et al., 2002)
9	20.30	Octadecanoic acid	1.22	Antioxidant (Praveen et al., 2010)
10	20.88	Oleic acid	33.96	Antioxidant, nephroprotective, hepatoprotective (Henry et al., 2002)
11	21.11	Octadecanoic acid	9.92	Hepatoprotective, anticholestatic, neproprotective, antioxidant (Henry et al., 2002)

table 3. (Contined).

Mass peak	Retention time (min)	Library ID	Area (%)	Reported biological activity
12	21.92	Oxalic acid	2.00	Hepatoprotective, antiarthritic, antioxidant (Henry et al., 2002)
13	23.06	Erucic acid	2.93	Antioxidant, hepatoprotective, nephroprotective (WIPO, 2016)
14	24.46	13-docosenoic acid	3.02	Antioxidant, nephroprotective (Henry et al., 2002)

Table 4. GC-MS analysis of *Garcinia kola* stem bark triterpenoid fraction

No.	Mass peak	Retention time (min)	Library ID	Area(%)	Reported biological activity
1	1	8.88	3,4-Dimethyl-2,5-dihydrofuran	0.28	Antioxidant, hepatoprotective, nephroprotective (Revathi et al., 2014)
2	2	12.76	Cyclododeca (b)furan-3-carbonitrile	0.31	Hypolipidemic activity (Revathi et al., 2014)
3	3	19.51	Pentadecanoic acid	2.65	Antioxidant, nephroprotective, anti-inflammatory (Henry et al., 2002)
4	4	19.67	Hexadecanoic acid	1.89	Anti- inflammatory, Antioxidant, nephroprotective (Henry et al., 2002)
5	5	21.31	n-Hexadecanoic acid	5.67	Anti- inflammatory, Antioxidant, nephroprotective (Henry et al., 2002)
6	6.	22.33	9,15-Octadecadienoic acid	12.18	Hepatoprotective, neproprotective, antioxidant (Henry et al., 2002)
7	7.	22.65	Methyltetradecanoate	1.80	Antioxidant (Henry et al., 2002)
8	8.	24.36	Oleic acid	57.26	Antioxidant, nephroprotective, hepatoprotective (Henry et al., 2002)
9	9.	24.86	5,6-Dimethylundecane	6.95	Antioxidant, nephroprotective, hepatoprotective (Revathi et al., 2014)
10	10	25.62	*trans*-Farnesol	2.95	Hepatoprotective and anti-inflammation (Vinholes, et al., 2014)
11	11	25.95	Oleic acid	2.27	Antioxidant, nephroprotective, hepatoprotective, antitumor (Henry et al., 2002)
12	12	26.29	Brassidic acid	3.01	Nephroprotection (WIPO, 2016)
13	13	26.46	1-Decanol	0.80	Antiulcer, antioxidant, anti-cytotoxic (Henry et al., 2002)
14	14	26.66	Hexadecanoic acid	1.85	Anti- inflammatory, antioxidant, nephroprotective (Henry et al., 2002)
15	15	27.23	9,12-Octadecadienal	0.14	Hepatoprotective, anti-inflammatory Henry et al., 2002)

300 mg/kg b.w ethanolic extract and triterpenoid fraction of *G. kola* stem bark reduced the activities of plasma AST, ALT and ALP in rats induced with toxicity using sodium arsenite when compared with untreated control animals. This suggested that the test samples possess hepatoprotective activities against sodium arsenite-induced toxicity in rats. Data from previous study had shown that liver is a major organ of arsenite toxicity [27]. Abdel-Kader *et al*.[25] reported that plant extracts that suppress the leakage of liver cytoplasmic AST, ALT, and ALP to the plasma as a result of metal toxicity could possess hepatoprotective property.

Hence, the observed higher hepatoprotective effect exhibited by 300 mg/kg b.w. TFGK when compared with silymarin and EEGK could be attributed to the phytochemicals present in it. This is because some phytochemicals have been shown to reduce sodium arsenite-induced oxidative tissue damage, particularly the polyphenols [28]. Hepatobiliary function assay also showed that 300 mg/kg b.w.

TFGK treated animals had reduced the plasma total bilirubin when compared with animals treated with EEGK. This further strengthens the previous observation that TFGK possesses substantial hepatoprotective property than EEGK. Previous study had shown that plasma total bilirubin is a useful clinical parameter to assess hepatic necrosis and its accumulation in blood could serve as an indicator of reduced detoxifying capacity of damaged hepatic cells [29].

However, 300 mg/kg b.w. EEGK treated animals induced with toxicity using sodium arsenite had greater reduced plasma urea and creatinine concentrations when compared with TFGK treated animals. This result was comparable to silmarin treated animals. This suggested that EEGK contain nephroprotective property which might act by interfering with the mechanism of reabsorption and inhibition of urea and creatinine in nephrons. Previous research had shown that some plant extracts possess phytochemicals exhibiting nephroprotective effects [30]. It is also possible that the antioxidant compounds in EEGK and TFGK could have counteracted the toxicity of sodium arsenite-induced kidney and liver injuries thereby suppressing the deterioration in renal and hepatic functions.

In vivo antioxidant study showed that the sodium arsenite-administered animals treated with EEGK and TFGK had elevated plasma, liver and kidney, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and reduced glutathione (GSH) levels when compared with the untreated control group. This indicated that experimental animals treated with test samples had higher antioxidant protective system against oxidative stress elicited tissue damage as a result of sodium arsenite toxicity. Furthermore, it could be due to the presence of phytochemicals such as polyphenols in the test samples with the capacity to up-regulate plasma and liver SOD, GPx and GSH synthesis or enhanced SOD and GPX activities.

Previous findings had shown that sodium arsenite-induced toxicity is associated with enhanced generation of ROS which subsequently could disrupt cellular function and eventually results in apoptosis [28]. SOD and GPx are among the first line of defense while GSH contributes to the second line of defense endogenously against the cytotoxic effects of ROS in the animal system. One of the ROS generated by arsenite-toxicity is superoxide which is converted to hydrogen peroxide by SOD. Hydrogen peroxide is in turn, dismutated to water and molecular oxygen by peroxidases [31].

In addition, GPX can reduce lipid peroxides and other cytotoxic hydroperoxides to their corresponding hydroxyl compounds using reduced glutathione as a donor [32]. In this present study, the unchanged activities of kidney GPx in the experimental animals when compared with untreated control and normal groups also supports the claims that antioxidant enzyme activity and gene expression do not change in the same direction at all times during disease development in chronic renal failure [33].

Ram *et al.* [32] also reported that GPx activity do not change significantly during renal insufficiency when compared with normal animals. It also indicates that kidney GPx may have protected kidney against arsenite-induced renal toxicity under reduced oxidative stress. In addition, previous study had shown that the toxic effect of arsenite could significantly decrease the activities of these antioxidants [34]. In overall, the sodium arsenite-

administered animals treated with EEGK had an antioxidant defense system comparable to the group treated with silymarin.

Lipid peroxidation assay showed that the experimental group treated with TFGK had substantially reduced malondialdehyde (MDA) concentrations than EEGK treated groups while MDA concentrations in untreated control animals were elevated. This further supports the observation that *G. kola* stem bark possess the capacity to counteract the deleterious effect of oxidative stress by-products generated by arsenite toxicity. MDA is a metabolic end product of lipid peroxidation as a result of oxidative damage of membrane lipids [35]. Furthermore, polyphenolic compounds present in *G. kola* stem bark could serve as terminators of harmful lipid peroxidation chain reactions in the body system as reported by Vivek *et al.*[36] and Motamed *et al.*[37].

Investigation of the hematological parameters in sodium arsenite administered animals treated with EEGK and TFGK showed elevated red blood cell (RBC), hemoglobin, and hematocrit counts when compared with untreated control group. This indicates that the EEGK and TFGK possess compound(s) which could confer protection against the hematoxic effects of arsenite on hematopoietic cells. Greenberger *et al.*[38] had previously reported that antioxidant could protect hematopoietic cells against deleterious agents. In addition, an elevated hematocrit count has been shown to correspond to the concentration of hemoglobin as well as increased RBC [39]. More so, the observed increase in RBC, hemoglobin and hematocrit counts may be attributed to the presence of erythropoiesis capacities of EEGK and TFGK. Furthermore, test animals treated with TFGK had suppressed white blood cell (WBC), lymphocytes, neutrophils, eosinophils, monocytes and basophil counts when compared with EEGK treated group while these parameters were elevated in the untreated control group. This suggested that TFGK had higher immunomodulatory properties than EEGK. Previous report had shown that elevated WBC, lymphocytes, neutrophils, eosinophils, monocytes and basophils counts in the body could be an indication of acute infection, inflammation or reduced immunity in response to toxic stress [40]. This also supports the view that extract of *Ageratum conyzoides* protected against the alteration of hematological indices by sodium arsenite administration to rats [41].

Gas chromatographic-mass spectrometry (GC-MS) analysis of EEGK revealed metabolites previously reported to be relevant for the management of hepatotoxicity and nephrotoxicity as decanoic acid, hexadecanoic acid , 9-hexadecanoic acid, pentadecanoic acid, 11-octadecanoic acid, octadecanoic acid, oleic acid, stearic acid, oxalic acid, erucic acid, 13-docosenoic acid [42,43,44]. Furthermore, GC-MS analysis of TFGK detected triterpenoids previously reported to be relevant for the management of hepatotoxicity and nephrotoxicity as 3,4-dimethyl-2,5-dihydrofuran; penta-decanoic acid, n-hexadecanoic acid; 9,15-octadecadienoic acid, oleic acid, 9,12-octadecadienal, transfarnesol and brassidic acid [42,45,46,47].

Conclusion

The findings from this study have shown that *G. kola* stem bark possesses substantial hepatoprotective and nephroprotective actions against sodium arsenite-induced toxicity in rats. Furthermore, TFGK exhibited substantial hepato-protective and immunomodulatory activities while EEGK showed substantial nephroprotective and antioxidant activities against sodium arsenite-induced toxicity in rats. It is therefore recommended that the triterpenoid fraction and ethanolic extract of *G. kola* stem bark be channeled towards pharmaceutical drug development to be harnessed in the production of chemoprotective drug(s) for use in the management of arsenicosis.

Disclosure of interest statement

The authors declare no conflict of interest and are solely responsible for the writing and content of this work.

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