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Sapium ellipticum (Hochst) Pax leaf extract: Antioxidant potential in CCl_4-induced oxidative stress model

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ABSTRACT

Objective: The antioxidant potential of *Sapium ellipticum* (SE) extract was examined against CCl_4-generated reactive species in experimental rats, using Butylated hydroxytoluene (BHT) and L-Ascorbic acid (L-AA) as reference antioxidants.

Methods: Animals were assigned to six experimental groups (n = 6): Control, CCl_4-treated, CCl_4 + SE (400 mg dosage) treated, CCl_4 + SE (800 mg dosage) treated, CCl_4 + L-AA (100 mg dosage) treated and CCl_4 + BHT (100 mg dosage) treated. Single intraperitoneal injection of CCl_4 was used to induce oxidative stress whereas treatments with SE, LAA and BHT were done orally (p.o) for 28 days.

Results: SE elicited notable antioxidant capacity both in the liver and kidney, mainly through anti-peroxidation and promotion of superoxide dismutase (SOD) and catalase (CAT) activities. The extract at the two doses did not differ significantly in its antioxidant activities. Relative to CCl_4-untreated group, SE particularly at 800 mg dose significantly ($p < .05$) lowered the degree of peroxidation (69.74% and 47.75%) and improved the activities of SOD (45.12 and 51.2%) and CAT (30, 21 and 28.49%) in the liver and kidney respectively. The extract also increased reduced glutathione (GSH) level in the kidney but the change was not significant when compared with the CCl_4-untreated group.

Conclusion: SE extract appears to have the phyto-proficiency to protect against membrane peroxidation and improve the activities of some first line antioxidant enzymes such a SOD and CAT *in vivo*. This is probably due to its array of bioactive compounds (α-tocopherol, amentoflavone and lupeol) which we have previously identified and reported.

1. Introduction

The fundamental role of free radical induced-oxidative stress in the aetiology and progression of numerous diseases is well documented [1,2]. Under normal physiological conditions in all aerobic cells, free radicals exist in balance with cellular antioxidants [3]. They are partly generated through normal metabolic processes and provide some benefits to the body system. However, when present in excess amount, they inflict damage on cell membranes and molecules such as DNA, proteins and lipids [4]. On the contrary, antioxidants protect body cells against damaging effects of free radicals. Interestingly, several studies have reported the ability of plant extracts to promote the activity/level of cellular antioxidants such as catalase, superoxide dismutase, reduced glutathione, ascorbic acid, and alpha tocopherol to overcome induced-oxidative damage.

Sapium ellepticum (Hochst) Pax is used locally for treatment of a number of disease [5,6]. It belongs to the family *Euphorbiaceae* and is commonly referred to as jumping seed tree. *S. ellipticum* is widely distributed in eastern and tropical Africa. In southwest part of Nigeria, particularly among the Ilorin indigenes, the plant is popularly known as *aloko-ọgbọ*. The *in vitro* antioxidant properties of the stem bark extract of the plant has been reported by Adesegun et al. [7]. Cytotoxicity screening of selected Nigerian plants used in traditional cancer treatment on HT29 (colon cancer) and MCF-7 (breast cancer) cell lines (HeLa cervix adenocarcinoma cells) indicated that *Sapium ellipticum* leaf extract expressed the highest cytotoxic activity among other plants with anticancer potential, and the effect was comparable to that of ciplastin used as a reference drug [8]. Edimealem and colleagues [9] in their study demonstrated the presence of Lupeol, lupeol acetate and stigmasterol in the stem bark extract of *Sapium ellipticum*. We have also reported the presence of α-tocopherol, amentoflavone, lupeol and luteolin-7-O-glycosides in the leaf extract of the plant in a previous study

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[10]. Besides, we have reported the effects of SE extract on carbohydrate metabolizing enzymes such as glucokinase and glucose-6-phosphatase [11], α-amylase and α-glucosidase [12].

Generation of free reactive species by CCl_4 metabolism in tissues such as liver and kidney has been well documented and evidently associated with increased lipid peroxidation and oxidative stress [13,14], hence CCl_4 is often used as an efficient model for assessing the protective ability of compounds against free radical-induced oxidative stress. On the other hand, L-ascorbic acid (L-AA) and Butylated hydroxytoluene (BHT) are respectively natural and synthetic antioxidants that fulfil essential protective and metabolic roles in plants and animals. They readily scavenge reactive oxygen and nitrogen species to protect vital biomolecules against free radical damage and are often used as bench mark to evaluate the antioxidant potential of plant extracts. This present study therefore sought to assess the *in vivo* antioxidant potential of *Sapium ellipticum* leaf extract in CCl_4-free radical generating model using L-AA and BHT as reference antioxidants.

2. Materials and methods

2.1. Collection of Sapium ellipticum and Preparation of leaf extracts

Fresh *Sapium ellipcitum* leaves were harvested in the month of December 2012, from a forest in a suburb of Ibadan, southwest of Nigeria. The harvested leaves were taxonomically authenticated by a curator botanist (Mr. T.K. Odewo) at the Lagos University Herbarium (LUH), Nigeria, were a specimen was deposited to obtain a voucher specimen number LUH 5423. The plant material was freed of extraneous materials; air dried at room temperature and was milled into a fine powder with a milling machine. SE extract were prepared by macerating 50 g of the dried powdery sample in 1000 mL of the extracting solvent (absolute ethanol) at room temperature. The mixture was allowed to stand for 72 h and stirred intermittently with a glass rod to facilitate extraction. After which it was sieved first with a muslin cloth (maximum pore size 2 mm) and subsequently with Whatman filter paper (No. 42). The resulting volume on sieving was reduced with a rotary evaporator at 40 °C. Final solvent elimination and drying was done using a water bath at 40 °C. The crude extract was stored in sterile screwed (air-tight) bottle and aliquots were taken when required.

2.2. Collection and management of animals

Male adult albino rats of the Wistar strain were used for the study. They were obtained from the animal breeding unit of Institute for Advance Medical Research and Training (IMRAT), at the University College Hospital (UCH), Ibadan. All procedures for maintenance and sacrifice (care and use) of animals were carried out according to the criteria outlined by the National Academy of Science published by the National Institute of Health [15] and approved by Lead City University Ethical Committee on Animal Research (LCUECAR), The animals were handled humanely, kept in plastic suspended cages, placed in a well ventilated and hygienic rat house under suitable conditions of temperature and humidity. They were provided rat pellets (Ladokun feeds) and served water *ad libitum* and subjected to natural photoperiod of 12 h light and 12 h dark cycle. The animals were allowed two weeks of acclimatization prior to the commencement of study.

2.3. Experimental design

Thirty-six rats were randomly assigned to six (6) groups with six animals in each (n = 6). Oxidative stress was induced in the experimental animals by administration of a single intraperitoneal dose of 20% 2 mL/kg BW, carbon tetrachloride (CCl_4) dissolved in corn oil. SE at 400 and 800 mg/kg BW doses was used to challenge the induced oxidative stress. Butylated hydroxyl toluene (BHT) and L-Ascorbic Acid (L-AA) at a dose of 100 mg/kg BW were used as reference antioxidants. Treatments with SE, BHT and L-AA (p.o) was done twice daily at 8 h interval for a period of 28 days. All animals were allowed equal access to normal laboratory chow and water *ad libitum* throughout the study

Expt. group	Treatment/dose/route	Nomenclature
Group I	Rats were administered corn oil/0.5 mL/ p.o	CN
Group II	Rats were administered CCl_4/ 20% 2 mL/kg BW/i.p	CT
Group III	Rats were treated with SE (400 mg/kg BW/ p.o) after CCl_4 administration	CT4SE
Group IV	Rats were treated with SE (800 mg/kg BW/ p.o) after CCl_4 administration	CT8SE
Group V	Rats were treated with Butylated hydroxyl toluene (BHT)/100 mg/kg BW/p.o after CCl_4 administration	CTBHT
Group VI	Rats were treated with L-ascorbic acid (L-AA)/100 mg/kg BW/p.o after CCl_4 administration	CTLAA

2.4. Preparation of tissue homogenate and post-mitochondrial fraction (PMF)

At the end of 28 days of treatments, the rats were fasted over night (12 h) and sacrificed by cervical dislocation. The liver and kidney were harvested, rinsed with ice-cold 1.15% KCl solution, blotted and weighed. The organs were separately suspended in a homogenizing buffer (ice cold Tris-HCl buffer, 0.1 M, pH 7.4) and thereafter homogenized (organ-buffer ratio of 1:3 w/v) using a Potter Elvehjem type homogenizer. The homogenate was centrifuged at 10,500*g* for 30 min at 4 °C to obtain the post mitochondrial fraction (PMF) which was used for different biochemical assays.

2.5. Biochemical assays

Protein concentration of the liver and kidney post mitochondrial-fractions was estimated by the procedure of Lowry et al. [16] using bovine serum albumin (BSA) as standard. Catalase activity was determined according to the method of Sinha [17]. **S**OD activity was determined by the method of Misra and Fridovich [18]. Thiobarbituric acid reactive substance (TBARS) which is a breakdown product of lipid peroxidation was measured by the method of Buege and Aust [19]. The cytosolic glutathione–S–transferase activity was determined spectrophotometrically at 37 °C according to the procedure of Habig et al. [20]. The level of reduced glutathione (GSH) was determined by the method of Jollow et al. [21].

2.6. Statistical analysis of data

Data analysis was performed using statistical software, Prism graphpad, version 6.4. The statistical significance of difference between groups was analyzed using the one-way analysis of variance (ANOVA) followed by independent-sample t test. The level of significance was set at $p < .05$. The results are presented as the mean ± SEM (n = 6).

3. Results

3.1. Effects of SE extract on tissue protein concentration

Fig. 1 represents the effect of SE extract on tissue protein concentration. It shows that CCl_4 administration caused significant reduction in liver protein by 25.84% relative to the control group. It however did not significantly affect the level of protein in the kidney (2.50% reduction). Co-treatments of rats with SE extract particularly at a dose

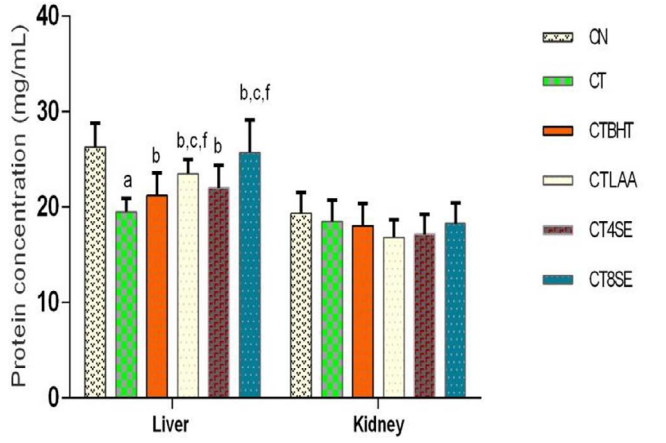

Fig. 1. . Effects of SE, BHT & L-AA on Protein concentration in liver and kidney of CCl_4-treated rats.

of 800 mg/kg BW showed greater sign of protein resuscitation (31.77%) when compared with the effects of BHT (8.71%) and L-AA (20.50%).

3.2. Effects of SE extract on SOD activities in the liver and kidney of Rats

SOD activities in the liver and kidney of rats treated with CCl_4 alone decreased significantly by 47.09 and 19.62% respectively compared with the control animals (CN). Whereas co-administration of SE extract at 400 and 800 mg dosage significantly improved hepatic SOD activity by 45.12 and 51.21% respectively compared with the toxicant group (CT). However, this boosting effect was significantly ($p < .05$) lower than that of BHT (65.85%) and L-AA (73.17%). Similar trend was observed in the kidney (Fig. 2).

3.3. Effects of SE extract on CAT activities in the liver and kidney of Rats

As shown in Fig. 3, CCl_4. administration significantly ($p < .05$) suppressed CAT activities in tissues (liver and kidney) relative to the control animals (CN). Subsequent administration of SE at 400 and 800 mg dosage significantly improved the enzyme activity in the liver (16.77 and 30.21%) compared with the toxicant group (CT). CAT activity in the kidney was only significantly enhanced (28.49%) by the extract at 800 mg dosage. This effect was comparable to those of BHT and L-AA.

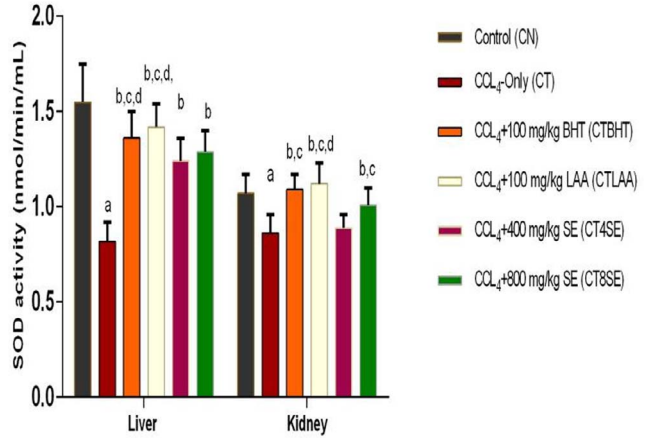

Fig. 2. . Effects of SE, BHT & L-AA on Super oxide dismutase (SOD) activity in liver and kidney of CCl4 – treated rats.

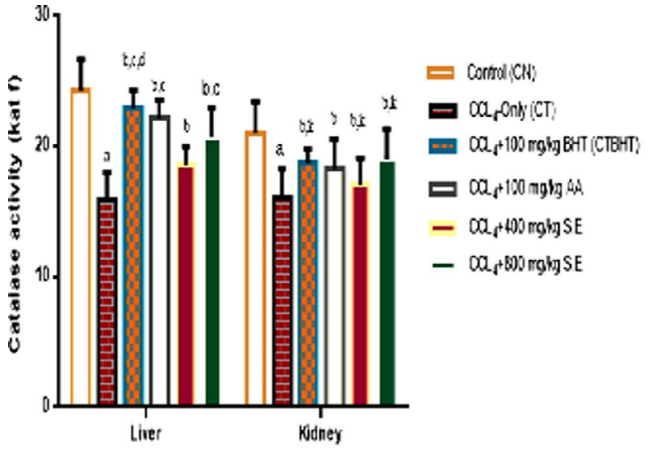

Fig. 3. . Effects of SE, BHT & L-AA on Catalase (CAT) activity in liver and kidney of CCl4 – treated rats.

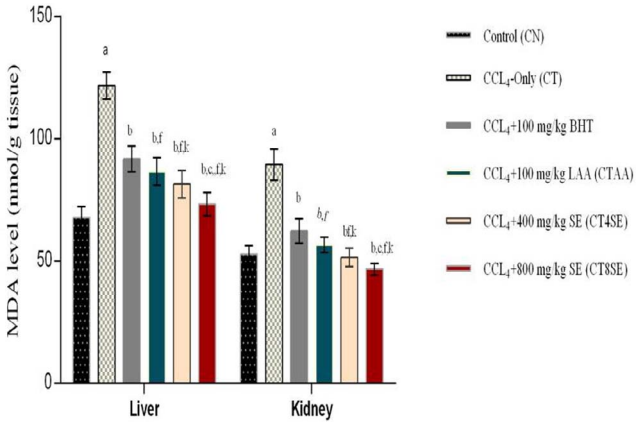

Fig. 4. . Effects of SE, BHT & L-AA on MDA concentration in liver and kidney of CCl_4 – treated rats.

3.4. Effects of SE extract on MDA concentration in the liver and kidney of Rats

Fig. 4 shows that CCl_4 caused significant ($p < .05$) increase in liver and kidney MDA concentration compared to the control animals. SE extract at 400 and 800 mg/kg BW, significantly reduced the level of MDA in both organs (respectively by 53.05 and 69.74% in the liver and 42.24 and 47.75% in the kidney). These values were significantly higher than those calculated for BHT (34.62 and 30.36%) and L-AA (28.81 and 36.64%) for liver and kidney respectively.

3.5. Effects of SE extract on GST activities in the liver and kidney of Rats

Fig. 5 summarizes the effects of SE extract on GST activity in CCl_4-treated rats in comparison with BHT and L-AA. CCl_4 significantly ($p < .05$) increased GST activity by 63.05 and 98.23% respectively in the liver and kidney of rats relative to the control group. In response, SE extract only significantly minimized the upsurge in hepatic GST activity by 8.6% at 800 mg dosage compared to the toxicant group Whereas BHT and L-AA significantly curtailed the CCl_4-induced elevation of GST activities in both liver and kidney by 34.81 and 38.76% and 39.25 and 42.47% respectively.

3.6. Effects of SE extract on GSH concentration in the liver and kidney of Rats

As depicted in Fig. 6, GSH concentration in the liver and kidney of

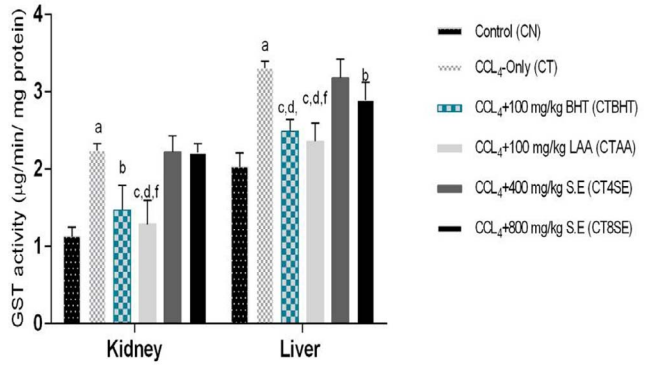

Fig. 5. . Effects of SE, BHT & L-AA on Glutathione-s-transferase (GST) activity in liver and kidney of CCl_4 – treated rats.

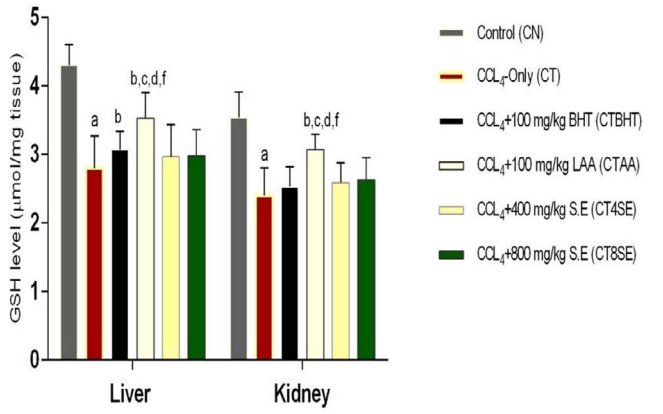

Fig. 6. . Effects of SE, BHT & L-AA on Glutathione (GSH) concentration in liver and kidney of CCl4 – treated rats.

rats treated with CCl_4 alone when compared to control group decreased significantly by 36.88 and 32.20% respectively. Co-treatments of rats with SE extract caused slight but non significant increase in kidney GSH level (9.58%) only at 800 mg/kg BW relatively to the untreated CCl_4-exposed animals. This observation was also noted with BHT, one of the standard antioxidants used as bench mark for SE. Conversely, L-AA significantly improved the concentration of GSH both in the liver and kidney by 26.07 and 27.91% respectively.

4. Discussion

The ability of carbon tetrachloride (CCl_4) to generate free radicals and causes cellular oxidative damage in *vivo* is well known [13,14,22]. Hence, the *in vivo* antioxidant potential of SE extract was assessed against CCl_4-radical generating system in the present study. Shaker et al. [23] in a previous study gave insight into the mechanism by which CCl_4 cause oxidative damage in body tissues. They hinted that the biotransformation of CCl_4 results in the generation of highly reactive and unstable free radicals (CCl_3^{-1}) that cause endoplasmic reticulum lipid peroxidation and cellular damage.

In line with the above, CCl_4 in this study generated reactive species which among other things lowered tissue protein, suppressed enzymatic antioxidant defenses (SOD and CAT activities), and caused peroxidation of membrane lipids. Conversely, SE extracts significantly replenished protein concentration and improved the activities of some antioxidant enzymes altered by CCl_4 in experimental rats. This observation supports previous reports on the ability of plant extracts to curtail free radical assaults in experimental animals [24–26].

SOD and CAT along with some minerals (Se, Mn, Cu and Zn) constitute the first line antioxidant defense system. Under normal physiological conditions, the superoxide radicals (O_2^*) generated in tissues through metabolism is catalytically converted to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) by superoxide dismutase (SOD) [27]. H_2O_2 when accumulated is toxic to body tissues or cells. Also, it is converted to deleterious hydroxyl radical (OH^*) in the presence of Fe^{2+} through Fenton reaction. In other to prevent this phenomenon, catalase (another antioxidant enzyme) breaks down H_2O_2 into water and oxygen, consequently curtailing free radical-induced damage [28]. The collective role of these enzymes is therefore critical in the entire defense strategy and mechanism against free radicals which are continually being generated in biological systems, either by accident of chemistry or on purpose.

However, when the activities of these enzymes are suppressed, the attendant effect is free radical-induced oxidative damage via a phenomenon known as oxidative stress. Oxidative stress is often characterized by formation of high level of Malondialdehyde (MDA) [29,30]. A number of compounds that generate reactive species elevate MDA level in biological systems, suppress antioxidant enzymes activity and lower the intracellular concentration of antioxidant molecules. This view is consistent with the findings in the present study in which CCl_4 administration caused significant MDA elevation and concomitant decrease in SOD and CAT activities as well as GSH concentration in the liver and kidney of rats. The decrease in SOD and CAT activities and simultaneous increase in MDA could be adduced to the accumulation of free radicals such as superoxide anion radical (O_2^*) and hydroxy radical (OH^*) generated from CCl_4 metabolism in the rats. This is consistent with the submissions of previous authors indicating that accumulation of lipid peroxides results after exposure to acute dose of CCl_4 [31,32].

On the contrary the ability of SE extract to significantly lowered lipid peroxidation and increased SOD and CAT activities suggest that the extract contains polyphenolic compounds with antioxidant characteristics or other phyto molecules with the ability to transfer electrons *in vivo*, and consequently able to mop up or scavenge free radicals [33,34]. In a previous study, through HPLC-MS analysis in dynamic MRM mode (using the optimized compound-specific parameters), we identified the presence of bioactive compounds such α-tocopherol, amentoflavone, lupeol and luteolin-7-O-glycosides) in SE [10]. The presence of these compounds which are well known for their antioxidant functions may have curtailed peroxidation of membranes by CCl_4-generated free radicals [33] and provided a synergistic support to SOD and CAT as noted in CCl_4-exposed rats treated with SE in the current study.

GSH is a thiol-containing tripeptide (γ-glutamyl-cysteinyl-glycine) which plays a significant role in both scavenging reactive oxygen species (ROS) and in the detoxification of xenobiotics [35,36]. In this investigation reduced GSH concentration in the liver and kidney of rats exposed to CCl_4 alone when compared to control group decreased significantly, an observation which is consistent with an earlier report by Brito et al. [37]. In contrast, co-treatments of rats with SE extract particularly at 800 mg/kg BW caused an increase in hepatic GSH relatively to CCl_4-animals left untreated.

The decrease in hepatic GSH content is obviously connected to CCl_4-induced toxicity and it may be imputable to exhaustive use of the endogenous antioxidant in a bid to curtail the free radicals generated from CCl_4 metabolism. This observation agrees with earlier findings which associated lowering of reduced GSH with the presence of reactive species or free radicals in cells [38,39]. On the other hand the increase in reduced GSH observed in rats following subsequent treatment with SE extract is likely due to the supportive antioxidant role offered by the extract. In essence, the combined protective effects of the extract and the endogenous GSH may have shielded the endogenous antioxidant against exhaustive usage. It may have also resulted from a moderate activation of glutathione reductase, an antioxidant enzyme which plays a crucial function in the conversion of oxidized glutathione to reduced glutathione at the expense of NADPH through GSH- GSSG cycle in the cell.

5. Conclusion

Overall, the outcome of this study suggests that SE extract appears to have the phyto-proficiency to protect against membrane peroxidation and improve the activities of some first line antioxidant enzymes such a SOD and CAT *in vivo* in the face of overwhelming reactive species.

Values are expressed as mean ± standard error of mean (SEM), n = 6. CN = Control rats treated vehicle for the extract (corn oil, 0.5 mL, p.o), CT = Rats treated with CCl_4 only (20% 2 mL/kg BW, single dose, i.p), CT4SE = Rats treated with 400 mg of SE extract per kg BW of rat, p.o after CCl_4 administration, CT8SE = Rats treated with 800 mg of SE extract per kg BW of rat, p.o after CCl_4 administration, CTBHT = Rats treated with 100 mg of BHT per kg BW of rat, p.o after CCl_4 administration, CTLAA = Rats treated with 100 mg of LAA extract per kg BW of rat, p.o after CCl_4 administration. a = significant when compared to NC, b = significant when compared to CT, c = significant when compared to CT4SE, d = significant when compared to CT8SE, f = significant when compared to CTBHT, k = significant when compared to CTLAA.

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Conflict of interest statement

The authors declare that there is no conflict of interest in respect to this article.

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