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# Antioxidant, hepatoprotective and hypolipidemic effects of methanolic root extract of *Cassia singueana* in rats following acute and chronic carbon tetrachloride intoxication

Ottu OJ, Atawodi SE*, Onyike E

*Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria*

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ABSTRACT

**Objective:** To evaluate *in vivo* antioxidant and hepatoprotective activities of the methanolic extract of the root of *Cassia singueana* in rats following acute and chronic carbon tetrachloride intoxication. **Methods:** Malondialdehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin as indices of liver damage and lipid peroxidation were detected in rats after intraperitoneal administration of extract (5 mg/kg). **Results:** The liver, kidney and heart showed significant reduction ($P<0.05$) in the levels of MDA from (0.18±0.04), (0.23 ±0.07) and (0.26±0.10) nmol/mg respectively in the $CCl_4$ control to (0.15±0.03), (0.17±0.04) and (0.17 ±0.07) nmol/mg protein in groups pre−treated with the extract for three days at 5 mg/kg). Similarly, compared to the $CCl_4$ control, significant reduction ($P<0.05$) in serum AST, ALT and bilirubin as well as in level of total cholesterol and MDA with concomitant increase in HDL cholesterol, superoxide dismutase and catalase levels when $CCl_4$−intoxicated rats were treated with *Cassia singueana* root extract for two weeks. **Conclusions:** These results suggest that methanolic extract of *Cassia singueana* contain potent antioxidant compounds that can offer significant protection against hepatic and oxidative injuries.

## 1. Introduction

The oxidation of lipids, proteins, carbohydrates, DNA and other biological molecules by toxic reactive oxygen species (ROS) can cause DNA mutation which serve to damage target cells and tissues, often resulting in cell senescence and death[1]. However, a potent scavenger of these (ROS) may serve as a possible preventive intervention for the free radicals generated by these species and the diseases they cause. This warrants the search and study of natural antioxidants, which have indeed generated great interest in the past few decades[2,3]. Hepatotoxicity has been reported as one of the damages caused by free radicals[4]. Therefore, carbon tetrachloride, a potent hepatotoxin metabolised by cytochrome P450 to yield toxic intermediate, trichloromethyl radicals which cause increase in hepatic lipid peroxidation and consequently liver damage and oxidative stress, and hence used widely as a model of hepatotoxicity to evaluate

hepatoprotective effects of natural products, was utilized was this study.

The antioxidant and hepatoprotective properties of some Nigerian and African medicinal plants and plant foods have been studied[5−18]. These reports suggested the antioxidant and hepatoprotective potential of *Sacoglottis gabonensis,* a plant used in Nigerian beverages, against 2,4− dinitrophenylhydrazine induced membrane peroxidation, and also demonstrated the *in vitro* and in some cases, *in vivo* antioxidants effect of *Hibiscus esculentus, Dacryodes edulis* (G.Don), *Khaya senegalensis, Moringa oleifera, Guiera Senegalensis,* J.F, *Canarium schweinfurthii, Syzygium aromaticum* (L.) Merr.& Perry, *Anogeissus leiocarpus, Gongronema latifolium, Crassocephalum crepidioides, Thonningia sanguinea* and *Trichilia roka* Chiov. (Meliaceae). However, nothing appears to be known about the antioxidant effect of *Cassia singueana*, an important savannah medicinal plant found in Nigeria and other West African countries.

*Cassia singueana* is a shrub of up to 10 m high with trunks of up to 35 cm in diameter. It inhabits the Sahel Sudan

Savannah vegetation belt on all soil types. It is widely distributed in Africa in countries such as Niger, northern Nigeria, Mali, Sudan, Eastern and Southern Africa. In northern Nigeria, *Cassia singueana* is as febrifuge known for its therapeutic effect in treating feverish conditions, acute malaria and conjunctivitis[19], just as the activities of the methanol extract of the root bark of this plant against rodent plasmodium infection, pyrexia and inflammation in mice and rats have been reported[20]. Our mini–survey at the beginning of this work also revealed that the root of the plant is used for treatment of liver related diseases in some parts of northern Nigeria. Therefore, since there appears to be no available report on the antioxidant and hepatoprotective effects of *Cassia singueana* roots, we undertook the present study to evaluate the hepatoprotective and antioxidant effects of the methanol extract of the root of the plant in rats intoxicated with carbon tetrachloride (CCl₄).

## 2. Materials and methods

### 2.1. Chemicals

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, total (HDL) cholesterol and high density lipoprotein cholesterol kits were obtained from Randox Laboratory Ltd. U.K. Methanol, ethanol, alpha–tocopherol, carbon tetrachloride, thiobarbituric acid, trichloroacetic acid and other chemicals were obtained from Sigma–Aldrich Germany.

### 2.2. Plant material

The root of *Cassia singueana* plant (Family: Caesalpinaceae) was obtained through a traditional herbal practitioner in Danga town, Katsina State, Nigeria and was identified in the Herbarium Section of the Biological Science Department of Ahmadu Bello University, Zaria, Nigeria, where a Voucher Number 6863 was assigned.

### 2.3. Experimental animals

Wistar strain of male Albino rats, body weight ranging from 160–200 g bred in the Animal House of the Department of Biochemistry, Ahmadu Bello University, Zaria were used. The rats were fed on pelleted commercial growers mash (Vital feed, Jos, Nigeria), They were kept at room temperature and were maintained *ad libitum* on tap water and growers mash (Vitafeeds, Jos, Plateau State Nigeria) except in the last 15 h before termination of the experiment. The animals were housed in plastic cages under conditions of 12 h light / 12 h dark cycle and at 25 ℃. The rats were weighed prior to commencement and termination of the experiment.

### 2.4. Extraction of plant materials

The roots of *Cassia singueana* were collected and rapidly washed under running tap water. They were air–dried at room temperature and made into fine powder using mortar and pestle. Pulverized material (35 g) was placed in the thimble of soxhlex extractor and extracted first, using petroleum ether (300 mL) for 8 h, and then methanol (300 mL), three times for 5 h each. The methanol extracts were combined and dried *in vacuo* at 45 ℃ using a rotary evaporator. The yield for the methanol extract was 32%.

### 2.5. Experimental grouping and treatment

The capacity of the extract to protect against hepatic injury and oxidative stress was investigated by randomly dividing the animals into the following groups with six rats each: solvent only (corn oil); vitamin E only (50 mg/kg); Vitamin E pre–treatment + CCl₄; *Cassia singueana* only (5mg/kg) and *Cassia singueana* pre–treatment + CCl₄ and CCl₄ only. However, to establish the ameliorative effect of *Cassia singueana* on pre–existing oxidative stress condition, the following groupings were used: solvent only (corn oil); *Cassia singueana* only; and CCl₄ pre–treatment + *Cassia singueana*; vitamin E only and CCl₄ only. All carbon tetrachloride treatments were performed at a dose of 0.6 mL/kg from a 33.3% solution in corn oil.

In the experiment designed to study the protective effects of the methanol extract of the root of *Cassia singueana* against oxidative stress, the animals were pre–treated with the extract (5 mg/kg) for three days before intoxication with carbon tetrachloride (0.6 mL/kg), which was administered one hour after the extract treatment on the third day, while for the ameliorative effect of *Cassia singueana* methanolic extract on existing oxidative stress and liver damage conditions, carbon tetrachloride (0.6 mL/kg) was administered 1 h before extract (5 mg/kg) or vitamin E (50 mg/kg) on the first day, and then extract and vitamin E administration were continued at a dose of 2.5 mg/kg and 10 mg/kg, respectively, for another 12 d before termination of the experiment.

### 2.6. Animal sacrifice

All animals were sacrificed 24 hours following last administration of drug or *Cassia singueana* extract. Animals were sacrificed under chloroform anaesthesia and whole blood was collected and allowed to stand for two hours for collection of serum. All sera samples were kept in Eppendorf tubes and stored at −20 ℃ until required for assay of biochemical parameters[13–15,21]. The organs were immediately harvested, rapidly rinsed in ice–cold normal saline and homogenized or stored at −20 ℃ for analysis of malondialdehyde as indicator of lipid peroxidation.

## 2.7. Tissue homogenization

The whole liver, kidneys and heart from each animal was removed after sacrificing the animal and were rinsed in normal saline and immediately stored in deep freezer. Tissues were homogenized in 10 parts in ice–cold potassium phosphate buffer (pH 7.4) using mortar and pestle. The homogenate was centrifuged at 3 000 ×g for 15 min and the supernatant collected. Protein concentration of the sample was determined by Biuret method, using bovine serum albumin as standard.

## 2.8. Assay for lipid peroxidation

Malondialdehyde level as indicator of Lipid peroxidation was determined as thiobarbituric acid reactive substances as described by earlier workers[14,15,22]. In this reaction, lipid peroxidation induced by the administered $CCl_4$ generates peroxide intermediates which upon cleavage release malondialdehyde, a product that reacts with thiobarbituric acid to form a coloured complex which is measured at 535 nm. In summary, the method is as follows; one millilitre of 14% trichloroacetic acid was measured into a test tube, 1ml thiobarbituric acid (0.6%) and 50 $\mu$ L of the tissue homogenate were then added. The mixture was incubated at 80 ℃ for 30 min in a water bath, allowed to cool rapidly in ice for 5 min followed by centrifugation at 3 000 ×g for 10 min. Malondialdehyde was measured colorimetrically at 535 nm and the level of the level of lipid peroxidation was calculated using the molar extinction coefficient of malondialdehyde ($1.56×10^5$ mol/L/cm) using the formula, A = $\Sigma$CL where A = absorbance, $\Sigma$ = molar coefficient, C = concentration and L = path length. All MDA concentrations were expressed in nmol/mg tissue protein.

## 2.9. Determination of the activity of endogenous antioxidant enzymes

The ability of the extract to boost the capacity of the endogenous antioxidant enzymes was evaluated by determining the activity of two enzymes, namely catalase (CAT) and superoxide dismutase (SOD) as follows:

### 2.9.1. CAT

CAT activity was measured using the method of Abei[23]. Briefly, the method is as follow: 10 $\mu$ L of serum was added to test tube containing 2.80 mL of 50 mM phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of freshly prepared 30 mM $H_2O_2$ and the decomposition rate of $H_2O_2$ was measured at 240 nm for 5 min. on a spectrophotometer (Jenway 640 UV/Vis). A molar extinction coefficient of 0.041 1 $mM^{-1}cm^{-1}$ was used to calculate the catalase activity.

### 2.9.2. SOD

SOD activity was evaluated according to the method described by Martin and co–workers[24]. In this assay, auto–oxidation of hematoxylin is inhibited by SOD at the assay pH; the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. The amount of SOD in the sample is determined in the "standard cytochrome C" SOD unit by measuring the ratio of auto–oxidation rates in the presence and absence of the sample. The method can be summarized thus; exactly 920 $\mu$ L of assay buffer was added into clean test tube containing 40 $\mu$ L of sample, mixed and incubated for 2 mins at 25 ℃, following which 40 $\mu$ L of hematoxylin solution was added. This was mixed quickly and the absorbance was measured immediately at 560 nm.

## 2.10. Determination of liver function parameters

Aspartate aminotransferase and alanine aminotransferase were determined colorimetrically at 546 nm using Randox assay kits based on the principle described by Reitman and Frankel[25]. Also, using the Randox kit, the colorimetric assay method for conjugated bilirubin involved reaction with diazotized sulphanilic acid in alkaline medium to form a blue complex, while total bilirubin was determined in the presence of caffeine, which releases albumin–bound bilirubin that then reacts with diazotized sulphanilic acid[26].

## 2.11. Determination of total and HDL–cholesterol

The method of Roeschlau and colleagues[27] was applied using assay kits (Randox Laboratories Ltd, UK) to determine the total serum cholesterol spectrophotometrically at 546 nm after enzymatic hydrolysis and oxidation. On the other hand, the HDL cholesterol was determined using assay kits (Randox Laboratories Ltd, UK) after low density lipoprotein (LDL) and chylomicron fractions were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction contained in the supernatant was assayed colorimetrically 540 nm.

## 2.12. Statistical analysis

The results on malondialdehyde (MDA), AST, ALT, bilirubin, catalase, total and HDL–cholesterol are presented as mean ± standard deviation and statistical evaluation was performed using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test to separate the means that are statistically different. The significance level was set at $P<0.05$.

**Table 1**

Levels of serum aspartate aminotransferase and alanine aminotransferase, direct bilirubin and total bilirubin of rats intoxicated with $CCl_4$ following 3 days pre–treatment with methanolic extract of *Cassis singueana* (5 mg/kg).

| Group | AST (U/L) | ALT (U/L) | Direct bilirubin ($\mu$ mol/L) | Total bilirubin ($\mu$ mol/L) |
|---|---|---|---|---|
| Solvent | 143.00 ± 15.15[b] | 66.00 ± 3.52[b] | 16.34 ± 1.14[b] | 18.58 ± 1.39[a] |
| $CCl_4$ only | 164.50 ± 16.99[b] | 81.40 ± 3.01[a] | 21.80 ±1.62[b] | 33.78 ± 2.06[c] |
| Vit. E only | 122.00 ± 11.23[a] | 42.00 ± 3.45[a] | 13.78 ± 1.40[a] | 17.65 ± 1.45[a] |
| Vit. E+$CCl_4$ | 140.00 ± 16.18[b] | 63.50 ± 4.07[b] | 17.37 ± 1.47[b] | 20.54 ± 2.30[b] |
| Extract only | 128.00 ± 10.40[a] | 45.20 ± 3.42[a] | 15.60 ± 1.66[b] | 18.06 ± 1.06[a] |
| Extract+$CCl_4$ | 149.00 ± 14.35[b] | 62.60 ± 2.82[b] | 18.65 ± 1.34c | 21.76 ± 1.84[b] |

Values with different superscripts down the column are significantly different at *P*<0.05.

**Table 2**

Serum aspartate aminotransferase, alanine aminotransferase, direct bilirubin and total bilirubin levels in rats administration methanolic extract of *Cassia singueana* root (2.5 mg/kg) for 14 days following initial $CCl_4$ intoxication (0.6 mL/kg).

| Group | AST (U/L) | ALT (U/L) | Direct bilirubin ($\mu$ mol/L) | Total bilirubin ($\mu$ mol/L) |
|---|---|---|---|---|
| Solvent | 201.00 ± 21.76[c] | 73.50 ± 4.45[b] | 16.78 ± 0.69[a] | 22.28 ± 1.04[a] |
| $CCl_4$ only | 358.20 ± 28.84[d] | 151.20 ± 9.17[d] | 23.27 ± 1.26[b] | 37.71 ± 3.95[c] |
| $CCl_4$+Vit. E | 160.70 ± 22.94[b] | 80.40 ± 6.26[c] | 17.02 ± 1.36[a] | 26.90 ± 4.98[b] |
| $CCl_4$+Extract | 172.90 ± 20.76[b] | 87.60 ± 5.41[c] | 19.38 ± 2.04[c] | 27.68 ± 2.66[b] |
| Extract only | 124.00 ± 14.32[a] | 36.90 ± 4.00[a] | 15.84 ± 2.16[a] | 21.50 ± 4.65[a] |
| Vitamin E only | 114.00 ± 10.66[a] | 38.40 ± 3.92[a] | 14.96 ± 2.07[a] | 19.83 ± 4.51[a] |

Values with different superscripts down the column are significantly different at *P*<0.05.

**Table 3**

Levels of MDA in organs of rats intoxicated with $CCl_4$ following 3 days pre–treatment with methanolic extract of *Cassis singueana* (5 mg/kg).

| Group | Liver(nmol/mg protein) | Kidney (nmol/mg protein) | Heart (nmol/mg protein) |
|---|---|---|---|
| Solvent | 0.17 ± 0.04[b] | 0.20 ± 0.05[c] | 0.19 ± 0.05[c] |
| $CCl_4$ only | 0.18 ± 0.04[b] | 0.23 ± 0.07[c] | 0.26 ± 0.10[d] |
| Vit. E only | 0.10 ± 0.02[a] | 0.13 ± 0.04[b] | 0.10 ± 0.02[a] |
| Vit. E+$CCl_4$ | 0.15 ± 0.03[b] | 0.14 ± 0.04[b] | 0.14 ± 0.03[b] |
| Extract only | 0.10 ± 0.03[a] | 0.14 ± 0.03[b] | 0.12 ± 0.06[a] |
| Extract+$CCl_4$ | 0.15 ± 0.03[b] | 0.17 ± 0.04[c] | 0.17 ± 0.07[c] |

Values with different superscripts down the column are significantly different at *P*<0.05.

## 3. Results

The results showed that whereas $CCl_4$ caused significant increase (*P*<0.05) in liver function parameters such as aspartate aminotransferase, alanine aminotransferase and bilirubin (direct and total), pre–treatment (Table 1) or treatment (Table 2) with methanolic extract of *Cassis singueana* root resulted in significantly lowered levels (*P*<0.05) of these parameters, and no significant difference (*P*>0.05) existed between the vitamin E treated and the extract treated group.

The concentration of MDA was measured as an index of the extent of lipid peroxidation in $CCl_4$ and extract treated groups. It was observed that pre–treatment (Table 3) or treatment (Table 4) with methanol extract of *Cassia singueana* root significantly reduced (*P*<0.05) the levels of MDA in all organs evaluated in rats intoxicated with $CCl_4$ compared to the $CCl_4$ control, especially in the liver and the kidney. Similarly, in the $CCl_4$ intoxicated rats treated for two weeks with methanol extract of *Cassia singueana* root or with vitamin E, the levels of two endogenous antioxidant enzymes, catalase and superoxide dismutase,

were significantly (*P*<0.05) boosted when compared to the $CCl_4$ control (Figures 1 and 2). *Cassia singueana* root extract also exhibited significant (*P*<0.05) lipid lowering capacity by reducing the levels of total cholesterol with a concomitant increase in the level of HDL cholesterol when compared to the $CCl_4$ control (Figures 3 and 4).
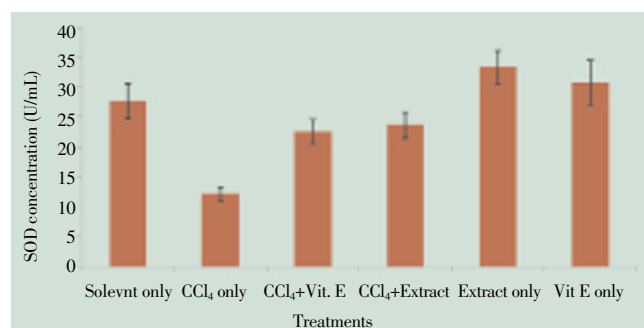


**Figure 1.** Levels of SOD activity of rats undergoing 72 hourly $CCl_4$ intoxication (0.3 mL/kg) with concomitant daily administration of methanolic extract of the root of *Cassia singuena* (2.5 mg/kg) for 12 days after initial $CCl_4$ dose of 0.6 mL/kg.

**Table 4**
Levels of MDA in organs of rats administration methanolic extract of *Cassis singueana* root (2.5 mg/kg) for 14 days following initial $CCl_4$ intoxication (0.6 mL/kg).

| Group | Liver(nmol/mg protein) | Kidney (nmol/mg protein) | Heart (nmol/mg protein) |
|---|---|---|---|
| Solvent | $0.17 \pm 0.05^b$ | $0.19 \pm 0.05^c$ | $0.16 \pm 0.04^c$ |
| $CCl_4$ only | $0.22 \pm 0.04^b$ | $0.24 \pm 0.06^c$ | $0.19 \pm 0.06^d$ |
| $CCl_4$+Vit. E | $0.18 \pm 0.03^c$ | $0.16 \pm 0.03^b$ | $0.14 \pm 0.03^b$ |
| $CCl_4$+Extract | $0.18 \pm 0.02^c$ | $0.17 \pm 0.04^b$ | $0.16 \pm 0.05^c$ |
| Extract only | $0.15 \pm 0.02^b$ | $0.13 \pm 0.03^a$ | $0.13 \pm 0.04^b$ |
| Vitamin E only | $0.11 \pm 0.02^a$ | $0.12 \pm 0.02^a$ | $0.10 \pm 0.01^a$ |

Values with different superscripts down the column are significantly different at $P<0.05$.
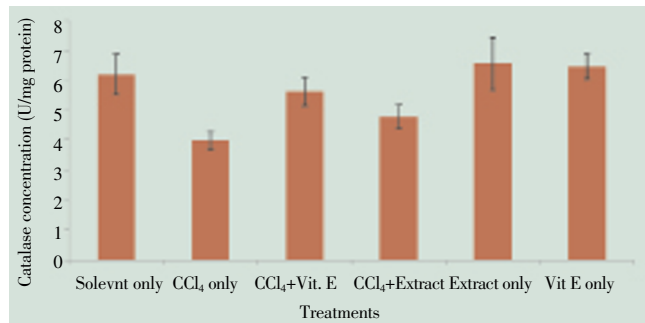


**Figure 2.** Serum catalase activity in rats administered methanolic extract of *Cassis singuena* root (2.5 mg/kg) for 14 d following initial $CCl_4$ intoxication (0.6 mL/kg).
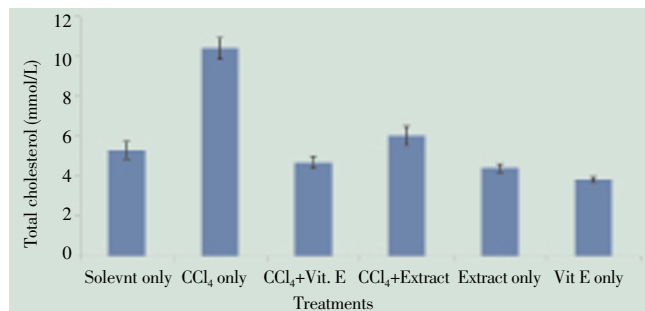


**Figure 3.** Serum total cholesterol in rats administered methanolic extract of *Cassis singuena* root (2.5 mg/kg) for 14 d following initial $CCl_4$ intoxication (0.6 mL/kg).
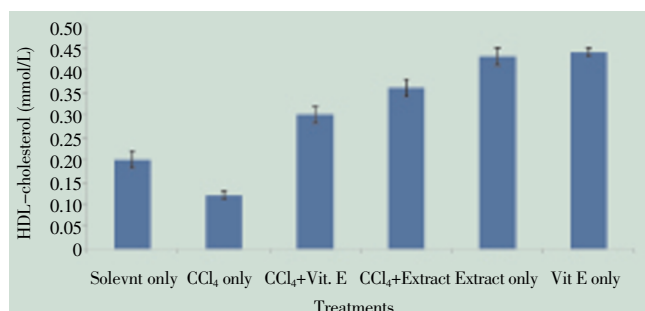


**Figure 4.** Serum total HDL–cholesterol in rats administered methanolic extract of *Cassis singuena* root (2.5 mg/kg) for 14 d following initial $CCl_4$ intoxication (0.6 mL/kg).

## 4. Discussion

The antioxidant and hepatoprotective effects of the methanolic extract of the root of *Cassia singueana* against $CCl_4$ induced injury in albino rats have been evaluated in this study. When the liver cell plasma membrane is damaged by a hepatoxin like $CCl_4$, a variety of enzymes normally located in the cytosol are released into the blood stream. Measurement of the activities of these serum marker enzymes is therefore a good assessment of liver function[28] in disease and experimental animal models. The elevated activities of AST and ALT observed in $CCl_4$ control rats in this study, are thus consistent with the extensive liver damage induced by the toxin.

The efficacy of any hepatoprotective drug is essentially dependent on its capacity to either reduce the harmful effects or maintaining the normal physiologic function which has been disturbed by the hepatotoxic agent. In line with this, administration of the methanol extract of *Cassia singuena* extract significantly ($P<0.05$) protected or ameliorated the changes associated with $CCl_4$–induced liver damage as indicated by a significant increase ($P<0.05$) in the activity of two liver enzymes, AST and ALT, as well as depressed the generation of free radicals that caused lipid peroxidation, which in turn could produce hepatocellular damage and enhanced production of fibrotic tissues.

The tendency of these enzymes to return towards a near normal level in extract–treated or pre–treated groups is a clear manifestation of the hepatoprotective and mitigating effects of *Cassia singueana*. This is further supported by the fact that the elevated level of direct and total bilirubin which can be deemed as a useful index of the severity of hepatocellular damage caused by $CCl_4$ treatment, were depressed by *Cassia singueana* treatment. Similarly, whereas the lowered levels of HDL–cholesterol and increased total cholesterol recorded in the serum of $CCl_4$–treated control group revealed the severity of hepatopathy[5], treatment or pre–treatment with the methanolic extract of the root of *Cassia singueana* significantly prevented or ameliorated this effect.

The increase in lipid peroxidation, a degradative process of membrane polyunsaturated fatty acid has been suggested to be caused by increase in malondialdehyde resulting from $CCl_4$ toxicity in the liver. The thiobarbituric acid reactive substances assay is the most popular method for estimation of malondialdehyde level, a reliable indicator of lipid peroxidation and free radical activity. Increased lipid peroxidation usually results in changes in cellular metabolism of the hepatic and extra–hepatic tissues, which ultimately leads to whole cell deformity and cell death[29,30].

However, administration of the methanolic extract of the root of *Cassia singueana* at daily intraperitoneal dose of 5mg/kg for three days significantly prevented the elevation of MDA in all tissues, especially the liver and the kidney. That the level of MDA is affected in the kidney by either $CCl_4$ or extract treatment to an appreciable level appears to suggest that the oxidative stress in the liver is quickly accompanied by similar effects in the kidney.

Increased synthesis of superoxide dismutase against superoxide anion radical ($^{\cdot}O_2^-$) production is usually an adaptive response of the cell to stimulation of gene transcription[31,32]. In the present study, a significant decrease ($P<0.05$) was observed in the activity of liver SOD in $CCl_4$ intoxicated rats, while treatment with methanol extract of *Cassia singueana* root promoted the hepato–protection by boosting the SOD level, and thus enhancing its free radical scavenging capacity. Similarly, level of catalase, another endogenous antioxidant defence enzyme was boosted in extract–treated animals compared to the $CCl_4$ control. Catalase traps the harmful hydrogen peroxide and converts it to water and oxygen. The depletion and or inhibition of catalase activity during $CCl_4$–induced toxicity may be due to the increased generation of reactive free radicals, which can create an oxidative stress in the cell[33]. That extract treatment boosted catalase activity, thus suggests that *Cassia singueana* root extract contained substances that protected liver tissues from free radicals responsible for the oxidative stress.

Furthermore, though increased formation of MDA in the $CCl_4$–treated groups of rats is an indication of lipid peroxidation, and depletion in CAT and SOD activities could be due to metabolic exhaustion arising from the increased production of ROS as evident from the increased lipid peroxidation levels. It has been suggested that superoxide radicals can even inhibit CAT activity and the increased hydrogen peroxide ($H_2O_2$) levels resulting from CAT inhibition could concomitantly inhibit SOD activity[34]. Thus, the increased formation of MDA observed for the $CCl_4$ control group could be due to both the increase in hepatotoxin–induced ROS formation and SOD inhibition[34–38]. Conversely, the decreased in MDA level caused by treatment with the *Cassia singueana* extract could be attributed to the decrease in lipid peroxidation level with concomitant increase in CAT and SOD activities, which should also accelerate the removal of the ROS.

It is well documented that most medicinal plants are enriched with phenolic compounds and flavonoids that confer potent antioxidant effect[21]. Previous studies have identified different classes of polyphenols, especially flavonoids as mostly responsible for many antioxidant and hepatoprotective effects that are observed in plant foods and medicinal plants[6–18]. Phytochemical studies have indeed revealed the presence of phenols, saponins, tannins and some traces of anthraquinones in different parts of the plant[39]. For example, from the roots of *Cassia singueana*, four tetrahydroanthracene derivatives, singueanol–Ⅰ and –Ⅱ, torosachrysone and germichrysone, were isolated in addition to pentacyclic triterpene lupeol and steroids (campesterol, β–sitosterol and stigmasterol). Also the stem bark and leaves have been reported to contain tannins[40], while, the presence of the flavonoids, leucopelargonidin, has been reported in the leaves[41]. The isolation of other phytochemicals from other extracts of different parts of the *Cassia singueana* that possess several therapeutic properties has been reported[42,43].

The ability of a substance or group of compounds to prevent hepatotoxicity and lipid peroxidation in vivo with concomitant decrease in total cholesterol while increasing level of HDL–cholesterol, as well as to boost the capacity of endogenous enzyme system to remove ROS has interesting implication in disease chemoprevention. This is because ROS has been identified as a major etiological factor in diseases like cancer, diabetes, cardiovascular dysfunction and neurodegerative disorders[34,35]. However, it remains to be known with certainty how *Cassia singueana* root extract is able to bring about the observed antioxidant and hepatoprotective effects. Nevertheless, put together, the results of this work suggest that methanol extract of *Cassia singueana* root possess strong hepatoprotective and nephroprotective potential, most probably because of its potent *in vivo* antioxidant and radical scavenging capacities.

## Conflict of interest statement

We declare that we have no conflict of interest.

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