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Methanolic Extract of Cassia Sieberiana Leaves: Antioxidant and Hepatoprotective Properties in Carbon Tetrachloride-Induced Hepatotoxicity in Rats

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ABS TRAC T

The study's objectives were to assess the methanolic extract of C. sieberiana leaves' hepatoprotective and antioxidant properties. Hepatoprotective, in vitro, and in vivo antioxidant activity is the study design. Location and Study Period: June 2023, Department of Veterinary Physiology, Pharmacology, Biochemistry, and Animal Health and Production, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State. ***Methodology:*** Using the cold maceration technique, Cassia sieberiana leaves were extracted for 48 hours using 80% methanol. Carbon tetrachloride (CCl_4)-induced hepatotoxicity in albino rats was used to assess the hepatoprotective efficacy of C. sieberiana extract (100, 200, and 400 mg/kg) and silymarin (100 mg/kg). Both in vitro (2, 2-diphenyl-1-picrylhydrazyl photometric test) and in vivo (malondialdehyde and catalase level assay) models were used to measure the antioxidant activity. Results: 9.5% w/w of dark green, pasty extract was produced after 48 hours of cold maceration extraction of C. sieberiana leaves. Saponins, alkaloids, flavonoids, terpenes/sterols, glycosides, and tannins were detected using the CSE's phytochemical spot test. When compared to the negative control group, the extract (100, 200, and 400 mg/kg) and silymarin (100 mg/kg) significantly ($p < 0.05$) increased the levels of alkaline phosphate, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in the serum of the treated rats. In the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) photometric experiment, the extract (25–400 µg/ml concentration) increased antioxidant activity in a concentration-dependent manner. In the DPPH photometric experiment, the extract's IC50 was 50 µg/ml. When compared to the negative control group, the extract and silymarin significantly ($p<0.05$) increased the amount of catalase in the treated rats. Additionally, as compared to the negative control group, the extract (400 mg/kg) and silymarin (100 mg/kg) significantly ($p<0.05$) reduced the amount of malondialdehyde in the treated rats. ***Conclusion*:** This research shows that C. sieberiana leaves have strong antioxidant and hepatoprotective properties. It supported the use of C. sieberiana leaves in traditional medicine to treat illnesses linked to liver damage.

Keywords: *Hepatoprotective Properties, Silymarin, Malondialdehyde, Carbon Tetrachloride, Catalase, and Cassia Sieberiana.*

1. INTRODUCTION

The liver is vital to the body's metabolism, detoxification, and secretion processes. Distortion of these metabolic processes is linked to liver disease [1]. As a result, liver illnesses continue to be one of the major public health issues, and there are many different liver conditions for which there is no effective therapy. Around 20,000 fatalities globally are attributed to liver diseases each year [2]. Even while oral hepatoprotective medicines have made significant strides in the treatment of liver illnesses, the hunt for novel medications is still ongoing since the current synthetic medications have a number of drawbacks [3-5]. Because the liver performs several essential processes in the human body and liver disease results in intolerable issues, the hunt for innovative plant-based treatments is currently underway [6, 7].

Cassia sieberiana DC is a member of the Fabaceae family. The English term for it is "drumstick," but its native names in Nigeria are "marga" in Hausa, "margaje" in Fulani, "kiskatigrai"

in Kanuri, and "ifo" or "aridantooro" in Yoruba. With hanging branches and vivid yellow blooms, it may reach heights of 10 to 20 meters [8]. The fruit (pods) are smooth, cylindrical, indehiscent, dark brown, 40–60 cm long, and around 1.5 cm in diameter. The plant is an open savannah tree that grows in the drier parts of thickets and forests. From Senegal to Cameroun, as well as Sudan and the Republic of Congo, the plant is extensively dispersed across the southern Sahel and Sudan savanna [9, 10]. Additionally, the majority of Nigeria is home to it [8]. In traditional medicine, different portions of the plants are used to cure ulcers, piles, gonorrhoea, fever, jaundice, and stomachaches [8]. Pharmacological actions such as antioxidant, anti-ulcer, antidiabetic, laxative, wound healing, and anti-malarial have been shown in the extract from different portions of the plant [11–13]. The goal of the current investigation was to determine if methanolic extract of Cassia sieberiana (CSE) leaves might protect rats' livers from carbon tetrachloride-induced hepatotoxicity.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Mr. A. O. Ozioko, a taxonomist at the Bioresource Development and Conservation Programme (BDCP), Enugu state, Nigeria, recognised fresh Cassia sieberiana leaves that were gathered in March 2013 from Nsukka. For reference, a voucher specimen with the catalogue number MOUAU/VPP/2013/11 was placed in the departmental herbarium.

2.2 Preparation of the Plant Extract

Using 80% methanol in a Winchester bottle, 200 g of dried and ground Cassia sieberiana leaves were extracted using the cold maceration technique for 48 hours at room temperature. Whatmann No. 1 filter sheets were used for filtering, and the filtrate was concentrated at 40ºC in a hot air oven to produce a 19 g residue. For the duration of this investigation, the extract was kept in a refrigerator at 4°C.

2.3 Experimental Animals

For this investigation, 35 male Albino Wistar rats weighing between 120 and 140 grams were acquired from the laboratory animal unit of the Department of Veterinary Physiology, Pharmacology, Biochemistry, Animal Health and Production at Michael Okpara University Agriculture, Umudike. They were kept in aluminium cages with five rats each, and they had unlimited access to clean drinking water and regular commercial pelleted meal (Vital feed®, Nigeria). They were maintained in a daily cycle of natural light and dark and at a typical ambient temperature. They were kept in compliance with the Guidelines for the Use and Care of Laboratory Animals [14]. Before the trial started, they were given two weeks to acclimatise. The animal ethics committee at Michael Okpara University of Agriculture authorised the protocol for the animal experiment.

2.4 Experimental Procedures

2.4.1 Phytochemical spot test

Using conventional methods, the CSE was examined for the content of alkaloids, flavonoids, tannins, glycosides, saponins, terpenes/sterols, carbs, and starch [15].

2.4.2 Oral acute toxicity study

The OECD guideline no. 425 (acute oral toxicity – Up-and-Down-procedure) was followed in the oral acute toxicity test of Cassia sieberiana methanolic extract. In summary, two groups of five rats each received oral doses of 1000 and 2000 mg/kg of CSE, respectively, and were monitored for toxicity and mortality for 48 hours [16].

2.4.3 Effects of CSE on carbon tetrachloride (CCl4)-induced hepatotoxicity rats

Five groups (I–V) of five male albino rats each were randomly selected. 10 ml/kg of distilled water was given to group I (negative control), 100 mg/kg of silymarin was given to group II (positive control), and 100, 200, and 400 mg/kg of CSE were given to groups III through V. For five days in a row, each animal received an oral dosage once per day. Each rat received an intraperitoneal injection of 20% CCl4 in liquid paraffin at a dosage of 2 ml/kg 24 hours following the last treatment (day 6). Following a 24-hour CCl4 therapy, rats were given chloroform anaesthesia, and their hearts were directly punctured to draw blood. Centrifugation at 2000 rpm for 10 minutes was used to separate the serum, which was then aspirated into sterile plain sample vials. Later, the rats were put to death by cervical dislocation, and their livers were removed right away to make liver homogenate.

2.4.4 Serum biochemical analysis

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) assays were performed using the Reitman and Frankel [17] technique, and the Klein *et al*., [18] method was used to quantify the blood level of alkaline phosphatase using Randox test kits. A standard diagnostic kit (Randox kit, Randox Laboratories, U.K.) was used to measure the serum total protein and albumin levels.

2.4.5 Determination of the lipid peroxidation (LPO) using liver homogenate

Using a modified technique as outlined by Draper and Hadley [19], the amount of thiobarbituric acid reactive substance (TBARS), a frequently used indicator of lipid peroxidation and malondialdehyde (MDA) generation, was quantified in liver homogenate. One millilitre of 14% trichloroacetic acid and one millilitre of 0.6% thiobarbituric acid

were added to the 50 millilitres of liver homogenate to deproteinise it. The liquid was chilled on ice for five minutes after being heated in a water bath at 100ºC for thirty minutes to finish the reaction. A UV spectrophotometer was used to measure the absorbance of the coloured product (TBARS) at 535 nm after centrifugation at 2000 rpm for 10 minutes. Using the formula A = ΣCL, where A = absorbance, Σ = molar coefficient, C = concentration, and L = path length, the concentration of TBARS was determined using the molar extinction coefficient of malondialdehyde (1.56 × 105 mol/L/cm). The findings were given in nmol/mg of protein.

2.4.6 Estimation of the effects of CSE on catalase activity in serum

The modified approach outlined by Aebi [20] and Atawodi [21] was used to measure the catalase activity in serum. In short, the procedure is as follows: 2.80 mL of 50 mM potassium phosphate buffer (pH 7.0) was put to a test tube along with 10 μL of serum. A spectrophotometer was used to monitor the rate of hydrogen peroxide breakdown at 240 nm for five minutes after 0.1 mL of fresh 30 mM hydrogen peroxide was added to start the reaction. Catalase activity was calculated using a molar extinction value of 0.041 mM−1cm−1. The findings were reported as µmol/mg of protein.

2.4.7 Determination of in vitro antioxidant activities of CSE using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) photometric assay

Using a spectrophotometer and the DPPH Assay [22], the extract's capacity to scavenge free radicals was evaluated. In a cuvette, 0.5 mM DPPH (in 1 ml of methanol) was combined with the test extract (2 ml) at various concentrations (25, 50, 100, 200, and 400 µg/ml). After 30 minutes of room temperature, dark incubation, the absorbance at 517 nm was measured. The antioxidant activity percentage was computed as follows after the concentrations were made in triplicate. Antioxidant activity (AA) = 100-[(absorbance of sample – absorbance of blank) ×100}/absorbance of control]. The negative control was 1.0 ml of the 0.5 mM DPPH solution plus 2.0 ml of methanol, whereas the blank was one millilitre of methanol plus 2.0 ml of the extract. The reference standard was ascorbic acid, or vitamin C [23].

2.4.8 Statistical Analysis

One-way analysis of variance (ANOVA) was used to analyse the data, which were shown as mean ± SEM. The least significant difference (LSD) between the several groups was used to distinguish the variant means. At the $p<0.05$ level, significance was acknowledged.

3. RESULTS

3.1 Preparation of the Extract

19 g of CSE were recovered, with a yield rate of 9.5% w/w dry matter. The extract had a pasty consistency and a dark green hue.

3.2 Phytochemical Spot Test

Saponins, tannins, terpenes/sterols, glycosides, alkaloids, and flavonoids were found in CSE, according to the phytochemical spot test.

3.4 Oral Acute Toxicity Test

All dosages (1000–2000 mg/kg) of the CSE administered orally were well tolerated, as no fatalities or clinical symptoms of toxicity were noted throughout the observation period.

3.5 Effects of *CSE* on the Liver Function Markers of Carbon Tetrachloride Treated Rats

Table 1 shows how carbon tetrachloride-treated rats' serum ALT, AST, and ALP activity were affected by CSE. When compared to the negative control rats, the extract (100, 200, and 400 mg/kg) and silymarin (100 mg/kg) substantially ($p<0.05$) decreased the activities of ALT, AST, and ALP in the serum of treated rats in a dose-dependent manner. Additionally, when compared to the negative control rats, the extract (100, 200, and 400 mg/kg) and silymarin (100 mg/kg) did not significantly ($p>0.05$) alter the serum albumin and total protein levels in the treated rats.

3.6 *In vivo* Antioxidant Effect of CSE on Carbon Tetrachloride Treated Rats

Table 2 shows the in vivo antioxidant effect of CSE on rats treated with carbon tetrachloride. The amount of malondialdehyde in the liver homogenate of the treated rats decreased in a dose-dependent manner at all extract (100, 200, and 400 mg/kg) and silymarin (100 mg/kg) dosages. When compared to the negative control group, the groups treated with CSE (400 mg/kg) and Silymarin (100 mg/kg) had considerably ($p<0.05$) reduced levels of malondialdehyde. Additionally, silymarin (100 mg/kg) and the extract (100, 200, and 400 mg/kg) produced a substantial ($p<0.05$) dose-dependent rise in the difference between the treated rats' serum catalase levels and those of the negative control animals.

Table 1: The effects of CSE on the liver function markers of carbon tetrachloride treated rats

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total protein g/dl	Albumin g/dl
Distilled water 10 ml/kg	415.00±5.00	250.50±3.50	129.51±1.7	6.90±0.02	3.41±0.05
Silymarin 100 mg/kg	224.50±10.50*	135.00±8.00*	64.01±9.99*	4.47±1.59	2.17±0.79
CSE 100 mg/kg	347.67±4.97*	203.33±6.01*	64.53±6.02*	4.85±0.08	2.38±0.84
CSE 200 mg/kg	316.67±2.73*	175.00±2.89*	62.60±7.06*	4.51±0.22	3.05±0.09

CSE 400 mg/kg	278.00±3.79*	159.67±1.76*	60.17±5.46*	4.76±0.18	2.34±0.28

**P<0.05 when compared to the negative control*

Table 2: In vivo antioxidant effect of CSE on carbon tetrachloride treated rats

Group	MDA (nmol/mg protein)	CATALASE (µmol/mg protein)
Distilled water 10 ml/kg	0.60±0.14	1.07±0.29
Silymarin 100 mg/kg	0.25±0.02*	3.49±0.19*
CSE 100 mg/kg	0.47±0.08	1.23±0.18
CSE 200 mg/kg	0.39±0.02	2.20±0.54*
CSE 400 mg/kg	0.27±0.10*	3.38±0.10*

**P < 0.05 when compared to the negative control*

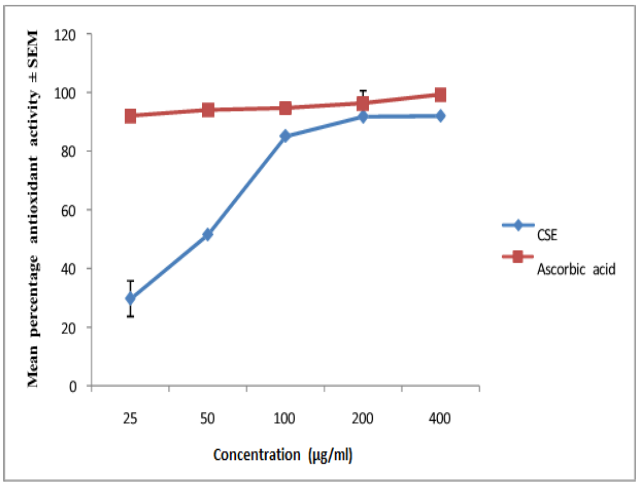

Figure 1: The *in vitro* antioxidant activity of CSE using DPPH photometric assay

4. DISCUSSION

Carbon tetrachloride-induced hepatoxicity was used to investigate the hepatoprotective activity of the methanolic extract of C. sieberiana. Both in vitro (DPPH radical scavenging) and in vivo (MDA and catalase) methods were used to investigate the antioxidant activity. 9.5% w/w of dark green, pasty extract was obtained using the 48-hour cold maceration technique of extracting C. sieberiana leaves. Saponins, alkaloids, flavonoids, terpenes/sterols, glycosides, and tannins were detected using the CSE's phytochemical spot test. This is consistent with Toma *et al.*,'s findings [8]. One or more of the phytochemical components may be responsible for the extract's hepatoprotective effects [24].

Even at the maximum dosage of 2000 mg/kg employed in this trial, no mortality or clinical symptoms were seen, indicating that the extract was well tolerated, according to an oral acute toxicity test. Other researchers have reached similar conclusions [16, 25].

A single intraperitoneal injection of 20% CCl4 in liquid paraffin at a dosage of 2 ml/kg caused hepatotoxicity. Hepatoprotective medications are often screened using the carbon tetrachloride-induced liver damage model. Trichloromethyl radical, an active metabolite of CCl4, mediates its hepatotoxic action. The endoplasmic reticulum's membrane lipids, which are rich in polyunsaturated fatty acids, undergo peroxidative destruction when the trichloromethyl radical covalently binds to macromolecules. Malondialdehyde (MDA), which is produced as a result of lipid peroxidation, damages biomembranes. Serum levels of cytosolic enzymes rise as a result of membrane damage. In this study, elevated blood levels of AST, ALT, and ALP as well as elevated levels of MDA in the liver homogenate of the rats in the negative control group demonstrated the induction of hepatotoxicity.

When compared to the negative control group, the pretreatment of rats with CSE (100, 200, and 400 mg/kg) and silymarin (100 mg/kg) prior to the induction of hepatoxicity resulted in a substantial ($p<0.05$) decrease in the blood levels of AST, ALT, and ALP. This suggests that CSE may have stabilised the plasma membrane and repaired the hepatic tissue damage brought on by CCl4. This is located in accord with the findings of other earlier researchers that the mending of the hepatic parenchyma and the regeneration of hepatocytes cause the blood levels of transferase and phosphatase to revert to normal.

The length of the trial may have contributed to the lack of a significant difference ($P>0.05$) in the blood levels of albumin and total protein between the treatment and control groups. When assessing the severity and chronicity of liver disease, serum albumin levels are crucial. Serum albumin levels are often normal or within the reference range in cases with acute liver illness. The DPPH photometric test was used to measure CSE's in vitro free radical scavenging activity. It is widely known that the DPPH photometric assay model may be used to screen compounds for their ability to scavenge free radicals. The free radical scavenging activity was increased by the CSE in a concentration-dependent manner. Lipid peroxidation inhibition is linked to the scavenging of DPPH radicals.

The amount of MDA in the liver homogenate was measured in order to assess the lipid peroxidation. This research found that the rats treated with CCl4 had higher levels of MDA in their liver

homogenate. The extract may have prevented the reductive dehalogenation of CCl4 to trichoromethyl radical, which is catalysed by cytochrome P450 in the liver cell endoplasmic reticulum, as the pretreatment with silymarin and CSE resulted in a decrease in the MDA level. The primary cause of CCl4 hepatotoxicity is the fast reaction of the trichloromethyl radical, which attacks microsomal lipids and causes their peroxidation. It also covalently bonds to proteins and microsomal lipids, starting a series of secondary biochemical events.

Increased reactive oxygen species (ROS) brought on by the CCl4 treatment are the cause of the lower catalase level seen in the negative control. When compared to the negative control group, the rats pretreated with silymarin (100 mg/kg) and CSE (100, 200, and 400 mg/kg) had higher levels of catalase. This might be because the metabolic activities that would have produced trichloromethyl radicals and other ROS from CCl4 were inhibited, which would have exhausted the catalase.

The antioxidant evaluation's findings imply that C. sieberiana's hepatoprotective action may be mediated by antioxidant activity. The inclusion of terpenoids, flavonoids, saponins, and tannins—all of which have been shown to have hepatoprotective and antioxidant properties—supports this.

5. CONCLUSION

The findings of this study show that C. sieberiana extract has a strong hepatoprotective effect in rats with liver damage caused by carbon tetrachloride. This might be because it contains certain phytochemicals or because it has antioxidant and free radical scavenging qualities. To identify the active hepatoprotective principle and ascertain its mode of action, further research is advised.

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