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Hepatoprotective and antioxidant effects of "Couroupita guianensis" on carbon tetra chloride - induced liver damage

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Abstract

To investigate the hepatoprotective and antioxidant activities of methanol leaves extract of *Couroupita guianensis* against carbon tetrachloride+ Olive oil -induced hepatotoxicity in rats.

The hepatoprotective activity of methanol extract of leaves of *Couroupita guianensis* was evaluated against carbon tetrachloride + Olive oil -induced hepatic damage in rats. *In-vivo* antioxidant activity of methanol extract of leaves of *Couroupita guianensis* was evaluated by various assays including lipid peroxidation, super oxide dismutase, reduced glutathione, catalase, glutathione peroxidase, and glutathione reductase. The methanol extract of leaves of *Couroupita guianensis* at dose of 250 and 500 mg/kg were administered orally once daily for seven days. Serum enzymatic levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), bilirubin and Lactate dehyrogenase were estimated along with estimation of superoxide dismutase (SOD), reduced glutathione, catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRD) and malondialdehyde (MDA) levels in liver tissues. Further histopathological examination of the liver sections was carried out to support the induction of hepatotoxicity and hepatoprotective efficacy. The substantially elevated serum enzymatic levels of SGOT, SGPT, ALP total bilirubin and total protein were found to be restored towards normalization significantly by the methanol extract of leaves of *Couroupita guianensis* in a dose dependent manner with maximum hepatoprotection at 500mg/kg dose level. The extract showed potent activities on reduced glutathione, SOD, CAT, GRD, GPx and MDA levels. The histopathological observations supported the biochemical evidences of hepatoprotection. The results of the present study strongly reveal that methanol extract of leaves of *Couroupita guianensis* has hepatoprotective and antioxidant activities against carbon tetrachloride -induced hepatic damage in experimental animals.

Keywords: *Couroupita guianensis*; serum transaminases; carbon tetrachloride; hepatoprotective; histopathology..

Introduction

The liver is the major site of xenobiotic metabolism and its injury can be caused by toxic chemicals, drugs, and virus infiltration from ingestion or infection [1,2]. In the absence of a reliable liver protective drug in the modern system of medicine, natural extracts from medicinal plants considered to be effective and safe are recommended for the treatment of liver disorders [3]. Silymarin, one of these compounds, is used as a standard reference and exhibits hepatoprotective effects and antioxidant activity by altering cytoplasmic membrane architecture and preventing the penetration of hepa-totoxic substances, such as carbon tetrachloride (CCl_4), thioacetamide and D-galactosamine [4,5]. *Couroupita guianensis* typically thought of to be an example of a medicative plant with a use in fashionable Chinese drugs. *Couroupita guianensis* (Aubl.) belongs to family called Lecythidaceae, could be a massive deciduous tropical tree 90' tall and autochthonous to the Amazon timberland. It's full-grown in Indian gardens as a decorative tree for its enticing flowers. In Tamil Naidu, it's referred to as Naglingam flower owing to Sivalingam form is visible at the center of the flower and snake designed spore is that the specialty of this flower and it's excellent essential oil. Juice made up of the leaves is employed to cure skin ailments, and shamans of South America have even used tree components for treating protozoal infection. Leaves and flowers of *Couroupita guianensis* used in the treatment of like upset, tumors, pain and inflammatory processes [6], cold, enteric gas formation and abdomen ache [7]. Various pharmacological activities of *Couroupita guianensis* were reported in literature such as analgesic & anti-inflammatory [8], antibacterial [9], antiulcer [10], anticancer [11], antidepressant [12], anti-fertility [13], antimicrobial [14,15], antipyretic [16], anxiolytic [17], immunomodulatory [18], neuropharmacological [19], wound healing [20], anti-arthritic, anti-stress, anti-diarrheal [21], ovicidal [22], anti-nociceptive [23], antifeedent & larvicidal [24]. Different secondary metabolites such as -amirin, β-amirin, β-sitosterol, tannins [25], ketosteroids [26]

and terpenoids, alkaloids, carbohydrates, proteins [27] were present in the *Couroupita guianensis*. Since various antioxidant compounds were isolated from this plant, *Couroupita guianensis* has good potential for development as an antioxidant and hepatoprotective agent.

Materials And Methods

Plant material

The plant material was collected from Tirupati (Andhra Pradesh), India and further identified, confirmed & authenticated by Dr. Madavchetty, Professor, Botany department, Sri Venkateswara University, Tirupati. Voucher specimen No (GIP-Plant No-002) has retained in GITAM Institute of Pharmacy, GITAM University.

Chemicals

All chemicals used in the study were of analytical grade. CCl_4 was procured from Krishna Chemicals Pvt. Ltd., Gujarat, and Silymarin from GVK Bio sci, Hyderabad.

Preparation of plant material

The collected leaves of *Couroupita guianensis were* washed with tap water. The leaves were cut in to small pieces and air-dried thoroughly under shade (at room temperature) for 2 months to avoid direct loss of phytoconstituents from sunlight. The shade dried materials were powdered using the pulverizer and sieved up to 80 meshes. It was then homogenized to fine powder and stored in air-tight container for furthers analysis.

Preparation of *Couroupita guianensis* extract

The leaves of *Couroupita guianensis were* refluxed with methanol in a soxhlet extractor for 72 hrs. The excess solvent was removed from the extract by vacuum rotary flash evaporator and concentrated over the hot water bath. Finally dried extract was stored in desiccators for hepatoprotective and antioxidant studies.

Preliminary phytochemical screening

The methanolic extract was subjected to various phytochemical studies to identify the presence of various phytoconstituents like alkaloids, steroids, flavonoids, tannins, glycosides, terpenes [28].

Toxicity study

The toxicity study was carried out using OECD guide lines No. 423. Three female mice of the same age group and weight were taken in a single dose up to the highest dose of 2000 mg/kg B/W orally. The animals were observed for 1 hr continuously and then hourly for 4 hr, and finally after every 24 hr up to 15 days for any mortality or gross behavioral changes [29]

In-vivo hepatoprotective activity

Experimental animals

Albino rats of either sex, weighing 200-250gm were procured and maintained in standard laboratory conditions. The animals were fed with standard pellet diet and water ad libitum. The study was approved by the Institutional Animal Ethical Committee (IAEC) and experiments were conducted as per the guidelines of CPCSEA.

Experimental design

A total of 30 rats were divided into5 groups of 6 rats each.
Group I served as normal control and received only the Vehicle (1mL/kg/day orally).
Group II received CCl_4 1mL/kg (1:1 of CCl_4 in olive oil) i.p. once daily for 7 days.
Group III received CCl_4 1mL/kg (1:1 of CCl_4 in olive oil) i.p. and silymarin 100 mg/kg orally (p.o.) for 7 days.
Groups IV, V were administered methanol extract of leaves of *Couroupita guianensis* at 250, and 500 mg/kg body weight p.o., respectively and dose of 1mL/kg i.p. of CCl_4 (1:1 of CCl_4 in olive oil) for 7 days. All rats were sacrificed by cervical dislocation 24hrs after the last treatment.

Biochemical parameters

The blood samples were allowed to clot for 45 minutes at room temperature. Serum was separated by centrifugation at 3500 rpm at 37^0C for 15 min and analysed for various biochemical parameters such as serum glutamic pyruvate transaminases (SGPT), serum glutamic oxalo acetate transaminases (SGOT), alkaline phosphatase (ALP), bilirubin, and Lactate dehydrogenase (LDH).

Antioxidant parameters

For estimating antioxidant activity, animals were sacrificed and liver was excised, rinsed in ice –cold normal saline followed by 0.15M Tris-HCl (pH-7.4) blotted dry and weighed. A10% w/v of homogenate was prepared in 0.15M Tris-HCl buffer and processed for the estimation of lipid peroxidation (TBARS). A part of homogenate after precipitating proteins with trichloroacetic acid (TCA) was used for estimation of glutathione. The remaining homogenate was centrifuged at 1500 rpm for 15 min at 4^0C. The supernatant thus obtained was used for the estimation of superoxide dismutase, GPx, GSH and catalase.

SGOT & SGPT

Serum transaminases (GOT and GPT) were determined by the method of [30]. Each substrate (0.5mL) [either -L-alanine (200mM) or L-aspartate (200mM) with 2mM - ketoglutarate] was incubated for 5 min at 37 C. A 0.1mL of serum was added and the volume shall be adjusted to 1.0mL with sodium phosphate

buffer (pH 7.4; 0.1M). The reaction mixture was incubated for 30 and 60 min for GPT and GOT, respectively. A 0.5mL of 2, 4-dinitrophenyl hydrazine (1mM) was added to the reaction mixture and left for 30 min at room temperature. Finally, the color was developed by the addition of 5mL NaOH (0.4 N) and the product formed was read at 505nm. Data were expressed as IUL^{-1}.

ALP

Alkaline phosphatase (ALP) was assayed by the method of [31]. The reaction mixture of 3.0 ml containing 1.5 ml of buffer (carbonate-bicarbonate buffer, 0.1M, pH 10.0), 1 ml of substrate and requisite amount of the enzyme sources was incubated at $37^{o}C$ for 15minutes. The reaction was arrested by the addition of 1.0 ml of Folins phenol reagent. The control tubes were received the enzyme after arresting the reaction. The contents was centrifuged and to the supernatant, 1.0 ml of 15% sodium carbonate solution, 1.0ml of substrate and 0.1ml of magnesium chloride (0.1M), was added and mixture shall be incubated for 10 minutes at $37^{0}C$. The colour was read out 640 nm against the blank.

Bilirubin

Bilirubin content was estimated by method of [32]. The two test tubes were taken and each into was added 0.2ml of serum sample and 1.8 ml of distilled water. To the unknown, 0.5 ml of diazo reagent and to the blank, 0.5 ml of 1.5% hydrochoric acid was added. Finally, to each tube, 2.5 ml of methanol was added and then allowed to stand for 30 minutes in ice and absorbance was read at 540nm. For a standard curve, the above standard was diluted 1in 5ml methanol. The amount of direct reacting bilirubin was determined similarly by substituting 2.5ml of water for 2.5ml of methanol. The values were expressed as mg/dl.

LDHactivity

Lactate dehydrogenase (LDH) activity was estimated in serum by the standard method [33,34]. The reaction mixture consisted of 0.1mL of nicotinamide adenine dinucleotide (NADH)-reduced disodium salt (0.02 M), 0.1mL of sodium pyruvate (0.01 M), 0.1mL of serum, and made up to 3mL with sodium phosphate buffer (0.1M; pH 7.4). The changes in the absorbance was recorded at 340nm at 30s interval each for 3 min and the enzyme activity was calculated using a molar extinction coefficient of 6.220M-1 cm-1 and it was expressed as nanomoles NADH oxidized min-1 mg-1 protein.

Estimation of antioxidant parameters

Lipid peroxidation assay

Lipid peroxidation (LPO) was measured by the method of [35]. Acetic acid 1.5mL (20%; pH 3.5), 1.5 of TBA (0.8%), and 0.2mL of sodium dodecyl sulfate (8.1%) was added to 0.1ml of supernatant and heated at $100^{o}C$ for cooled and 60 min. Mixture was cooled, and 5mL of n-butanol : pyridine (15 : 1) mixture and 1mL of distilled water was added and vortexed vigorously. After centrifugation at 1200g for 10min, the organic layer was separated and the absorbance was measured at 532nm using a spectrophotometer. Malonyldialdehyde (MDA) is an end product of LPO, which reacts with TBA to form pink chromogen–TBA reactive substance. It was calculated using a molar extinction coefficient of 1.56 X 105M-1 cm-1 and shall be expressed as nanomoles of TBARS mg-1 of protein.

Superoxide dismutase assay

Superoxide dismutase (SOD) activity was analyzed by the method described by [36]. Assay mixture contain 0.1mL of supernatant, 1.2mL of sodium pyrophosphate buffer (pH 8.3; 0.052M), 0.1mL of phenazine methosulfate (186 mM), 0.3mL of nitroblue tetrazolium (300 mM), and 0.2mL of NADH (750 mM). Reaction was started by the addition of NADH. After Incubation at $30^{o}C$ for 90s, the reaction was stopped by the addition of 0.1mL of glacialacetic acid. Reaction mixture was stirred vigorously with 4.0mL of n-butanol. Color intensity of the chromogen in the butanol was measured spectrophotometrically at 560nm and the concentration of SOD was expressed as Umg^{-1} of protein.

Reduced glutathione assay

Reduced glutathione (GSH) was measured according to the method of [37]. The equal quantity of homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.01 ml of this supernatant, 2ml of phosphate buffer (pH 8.4), 0.5 ml of 5'5-dithio, bis (2-nitrobenzoic acid) and 0.4ml double distilled water was added. Mixture was vortexed and the absorbance read at 412nm within 15 min. The concentration of glutathione was expressed as µg/mg of protein.

Catalase assay

Catalase activity (CAT) was measured by the method of [38]. A 0.1mL of supernatant was added to cuvette containing 1.9mL of 50mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0mL of freshly prepared 30mM H2O2. The rate of the decomposition of H2O2 was measured spectrophotometrically at 240 nm. Activity of CAT was expressed as Umg-1 of protein.

Glutathione peroxidase assay

Glutathione peroxidase (GPx) activity was determined by the method described by Wendel (1981). The reaction mixture consist of 400µL of 0.25M potassium phosphate buffer (pH- 7.0), 200 mL supernatant, 100 µL GSH (10 mM), 100 µL NADPH (2.5mM), and 100µL GRD (6UmL-1). Reaction was started by adding 100µL hydrogen peroxide (12mM) and absorbance was measured at 366nm at 1min intervals for 5 min using a molar extinction

coefficient of 6.22X 103M-1cm-1. Data was expressed as mU mg-1 of protein.

Glutathione reductase assay

Glutathione reductase (GRD) activity was assayed by the method of [39]. The assay system consist of 1.65mL sodium phosphate buffer (0.1M; pH 7.4), 0.1mL EDTA (0.5 mM), 0.05mL oxidized glutathione (1mM), 0.1mL NADPH (0.1 mM), and 0.05mL supernatant in a total mixture of 2mL. The enzyme activity was quantified by measuring the disappearance of NADPH at 340nm at 30s intervals for 3min. The activity was calculated using a molar extinction coefficient of 6.22 X 103M-1cm-1and was expressed as nanomoles of NADPH oxidized min-1 mg-1 protein. Protein content in the tissue was determined by earlier method reported [40], using bovine serum albumin (BSA) as the standard.

Histopathological studies

Paraffin sections of buffered formalin-fixed liver samples were stained with hematoxyline-eosin to study the histological structure of control and treated (toxicant, *Couroupita guianensis* leaf extract, silymarin) rats liver.

Statistical analysis

All values are expressed as mean S.E.M. Total variations present in a set of data were estimated by one-way analysis of variance (ANOVA) followed by T test. P values<0.05 were considered statistically significant.

Results

Percentage yield

The percentage yield of the methanol extract of *Couroupita guianensis* was found to be 24.6% given. The percentage yield of the methanol extract of *Couroupita guianensis* was mentioned in the Table No.1

Table No 1. Percentage yield of methanol extract of *Couroupita guianensis*

Extract Name	% Yield (w/w)
Methanol extract of *Couroupita guianensis*	24.6

Preliminary phytochemical screening of Couroupita guianensis

It was observed that the preliminary phytochemical screening of *Couroupita guianensis* showed the presence of carbohydrates, proteins, amino acids, steroids, alkaloids, glycosides reducing sugars, tannins and saponins in methanol extract, where as absence gums and oils. The preliminary phytochemical screening for various functional groups is tabulated as Table No. 2.

Table No. 2. Qualitative analysis (group tests) of methanol extract of *Couroupita guianensis*

Name of Phytochemical test	Observation report of methanol extract of *Couroupita guianensis*
Carbohydrates	+
Steroids	+
Flavonoids	+
Alkaloids	+
Amino Acids	-
Reducing Sugars	+
Tannins	+
Saponins	+
Gums	-
Oils	-

"+" indicates positive; "-" indicates negative

Toxicity study

Rat when fed with methanol extract of *Couroupita guianensis* up to 2000 mg kg^{-1},p.o. exhibited no mortality or any sign of gross behavioral changes when observed initially for 24 h, and finally up to 15 days.

Carbon tetrachloride induced hepatotoxicity

The impact of CCl_4 on the levels of SGOT, SGPT, ALP, LDH, bilirubin, and total protein in the serum were summarized in Table 3. The serum levels of above biochemical parameters were significantly ($P < 0.05$) increased; however, a significant decrease in the levels of total protein was observed in CCl_4 + Olive oil treated control compared to normal control. Treatment with methanol leaf extract of *Couroupita guianensis* and silymarin prior to CCl_4 + Olive oil intoxication, afforded protection by lowering of the above serum markers as well as by increasing the total protein content. Better protection was observed with the higher dose (500 mg/kg) of the extract.

Table: 3. Hepatoprotective parameters of methanol extract of *Couroupita guianensis* against CCl_4 + Olive oil induced damaged.

Biochemical Parameters	Control group	CCl_4 + Olive oil treated group	*Couroupita guianensis* methanol extract(mg/kg)		Silymarin (25mg/kg)
			250	500	
SGOT (IUL^{-1})	106±9.5	267±15.4	204±11.6	157±8.5	112±9.5
SGPT (IUL^{-1})	46.9±74	197.8±11.4	111.4±8.6	66.8±11.6	58.7±86
ALP (KA Units)	42.8±1.18	84.6±0.99	54.7±0.55	40.4±1.39	46.2±1.25
LDH (nanomoles NADH oxidized min^{-1} mg^{-1} protein)	375.6±11.4	544.8±14.5	486.8±8.5	446.2±0.05	414.7±0.13
Total bilirubin (mg/dl)	2.7±0.04	5.24±0.14	3.0±0.07	3.1±0.04	2.2±0.05
Total protein (mg/dl)	9.7±0.15	4.48±0.09	7.5±0.04	8.7±0.37	9.2±0.13

Data are given as mean SD of six animals. * Significant difference ($p < 0.05$) from control or CCl_4 + Olive oil -treated rats. ALP = alkaline phosphatase; BIL=bilurubin, LDH= Lactate dehydrogenase; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase.

In vivo antioxidant assays

As shown in the table 4, CCl_4 + Olive oil intoxication produced significant ($P < 0.05$) reduction in GSH,SOD,CAT, GPx, and GRD activities along with significantly increased lipid peroxidation level (expressed as MDA) when compared to normal control. Treatment with methanol leaf extract of *Couroupita guianensis* at doses 250 and 500 mg/kg b.w for 7 days showed significant higher levels of GSH, SOD,CAT, GPx, and GRD in addition to significant lower levels of hepatic MDA as compared to CCl_4 + Olive oil intoxicated rats.

Table.4. Effect of methanolic extract of *Couroupita guianensis* on biochemical parameters

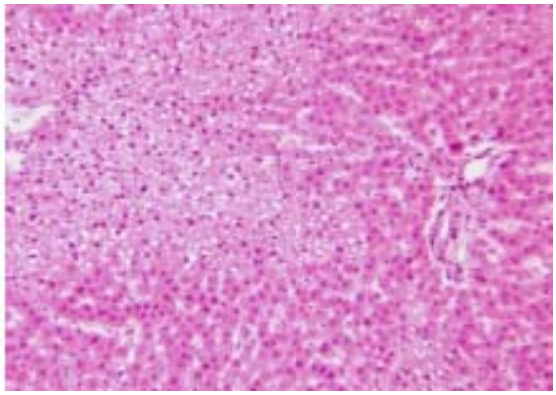
S.NO	Treatment	GSH (µmol/mg of protein)	SOD (u/mg of protein)	Catalase (u/mg of protein)	GPx (mU mg^{-1} of protein)	GRD (nanomoles of NADPH oxidized min^{-1} mg^{-1} protein.)	MDA (nanomoles of TBARS mg^{-1} cm of protein)
1	Normal control	10.2±07	61.4±8.5	198.6±0.06	286.6±0.99	18.6±1.39	4.7±03
2	Hepatotoxic Control	2.4±09	22.6±0.14	48.7±08	187.4±0.85	6.4±1.16	14.6±06
3	Standard	9.8±08	59.8±0.55	189.4±15	279.2±08	16.2±0.04	5.8±04
4	MECG 250 mg/kg	6.8±1.3	43.8± 03	134.6±1.5	201.6±0.07	10.4±0.37	9.2±0.5
5	MECG 500 mg/kg	8.4±1.2	54.6±015	169.8±13	244.8±0.08	14.8±0.13	7.4±04

Data are given as mean SD of six animals. * Significant difference ($p < 0.05$) from control or CCl_4 + Olive oil -treated rats. SOD= Super oxide dismutase; CAT= Catalase, GPx= Glutathione peroxidase; GRD = glutathione reductase; MDA = Malonyldialdehyde; RD = Reduced glutathione

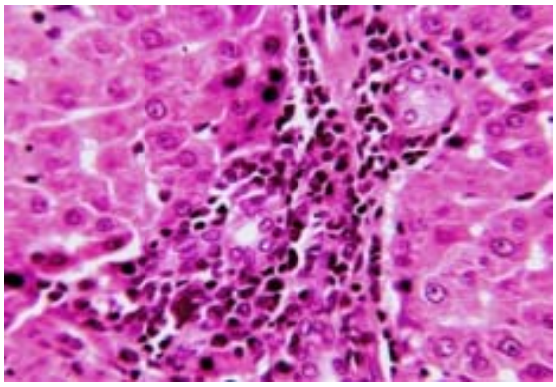
Histopathological observations

Histology of liver (Figure 1) of normal saline treated group showed normal architecture. The hepatic cords and the sinusoids were well visible (I). The histopathological studies of the liver showed fatty changes, swelling, necrosis, cell vacuolization, degenerated nuclei and inflammatory, infiltration with loss of hepatocytes in CCl4: olive oil (1:1 ratio) intoxicated rats in comparison with normal rats (II). Silymarin treated group showed considerable reduction in necrosis and damage of liver cells (III). The liver sections of rats

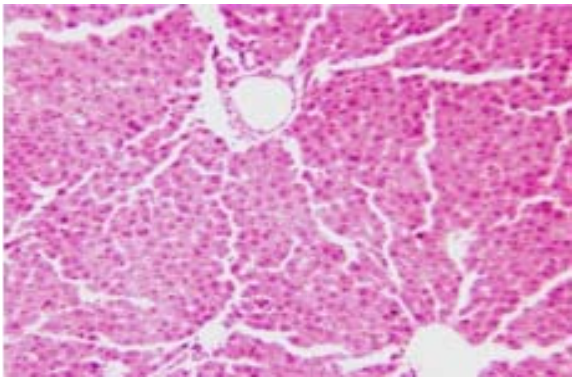
treated with the lower (250mg/kg) and higher (500mg/kg) doses of the extract showed reduced degeneration of hepatocytes, normalization of fatty changes, decrease in vacuolization and necrosis of the liver (IV & V).



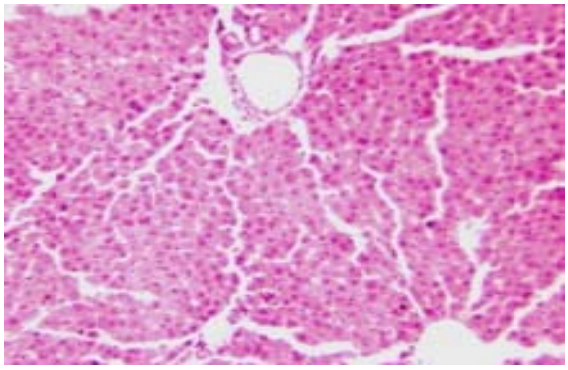
I-Normal



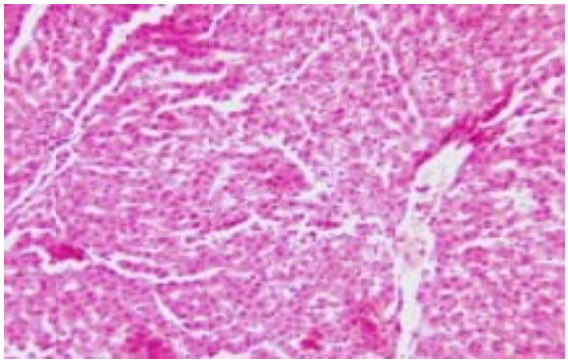
II- CCl4- Olive oil (1:1, 1 ml/kg)



III-Standard (Silymarin 25mg/kg)



IV-Low dose (250 mg/kg)



V-High dose (500 mg/kg)

Discussion

Acute toxicity studies revealed the non-toxic nature of the methanolic extract of *Couroupita guianensis* up to a dose level of 2000 mg/kg body weight in rats. There was no lethality or toxic reaction found at any of the doses selected during the study. In the present investigation, *Couroupita guianensis* methanol extract was evaluated for the induced hepatotoxicity in rat. The hepatotoxicity induced by CCl_4 + Olive oil hepatoprotective is due to its metabolite $CCl_3^{\bullet}$, a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage. Hepatocellular necrosis leads to elevation of the serum marker enzymes such as SGOT, SGPT, ALP, and bilirubin and which are released from the liver into blood [40]. The present study revealed a significant increase in the activities of SGOT, SGPT, ALP and serum bilirubin levels on [41,42], indicating considerable hepatocellular injury. Administration of *Couroupita guianensis* methanol extract at different doses level (250 and 500 mg/kg) attenuated the increased levels of the serum enzymes, produced by CCl_4 + Olive oil and caused a subsequent recovery towards normalization comparable to the control groups animals. The hepatoprotective effect of the *Couroupita guianensis* methanol extract was further accomplished by the histopathological examinations. *Couroupita guianensis* methanol extract at different dose levels offers hepatoprotection, but 500mg/kg is more effective

than the lower dose. In CCl_4 + Olive oil induced hepatotoxicity, the balance between ROS production and these antioxidant defenses may be lost, 'oxidative stress' results, which through a series of events deregulates the cellular functions leading to hepatic necrosis. The reduced activities of SOD, GRD, GPx and catalase observed point out the hepatic damage in the rats administered with CCl_4 + Olive oil but the treated with, 250 and 500mg/kg of *Couroupita guianensis* methanol extract groups showed significant increase in the level of these enzymes, which indicates the antioxidant activity of the *Couroupita guianensis*. Regarding non enzymic antioxidants, GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals, including CCl_4 + Olive oil. Furthermore, a decrease in hepatic tissue GSH level was observed in the CCl_4 + Olive oil [43] treated groups. The increase in hepatic GSH level in the rats treated with, 250 and 500 mg/kg of *Couroupita guianensis* methanol extract may be due to de novo GSH synthesis or GSH regeneration. The level of lipid peroxide is a measure of membrane damage and alterations in structure and function of cellular membranes. In the present study, elevation of lipid peroxidation in the liver of rats treated with CCl_4 + Olive oil was observed. The increase in LPO levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent the formation of excessive free radicals. Treatment with *Couroupita guianensis* methanol extract significantly reversed all the changes. Hence, it is possible that the mechanism of hepatoprotection of *Couroupita guianensis* may be due to its antioxidant activity.

Conclusion

In conclusion, this study showed that the methanol leaf extract of *Couroupita guianensis* has hepatoprotective effects that were proven by biochemical, antioxidant and histopathological analysis. The *Couroupita guianensis* methanol extract has shown dose dependent activity among which at the dose level of 500 mg/kg, p. o. shows greater activity which is comparable with the control and standard groups.

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