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Hepatoprotective and antioxidant activity of *Odontonema Cuspidatum* (Nees) Kuntze against CCl_4-Induced Hepatic Injury in Rats

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Abstract
Objective: The present investigation deals with evaluation of hepatoprotective and antioxidant activity of total methanol extract and different fractions of *Odontonema cuspidatum* on the CCl_4 induced hepatotoxicity.
Material and methods: Albino Wister rats weighing (140±20 g) of either sex were divided into seven groups (n=8). Hepatotoxicity was induced by CCl_4 dissolved in olive oil (1:1, 2 ml/kg bw, S.c). Silymarin (100mg/kg) orally was used as standard drug. Test groups received total methanol extract and different fractions (aqueous, *n*-butanol and *n*-hexane fractions) of *Odontonema cuspidatum* aerial part in a dose of 400 mg/kg/day orally along with CCl_4. Treatment was given to all the tested groups daily for 5 days. The hepatoprotective effect of *Odontonema cuspidatum* was evaluated by assessment of biochemical parameters [(AST), (ALT), (ALP) and total bilirubin] and the antioxidant activity in the liver tissue was estimated by measuring the activities of antioxidant enzymes: reduced glutathione (GSH) as well as the level of lipid peroxidation by Malondialdehyde (MDA). Biochemical observations were also supported with histopathological examination of liver section.
Results: *Odontonema cuspidatum* methanol extract (OCME) (400mg/kg) exhibited a highly significant reduction ($p<0.05$) in AST, ALT, ALP and total bilirubin better than other fractions. *Odontonema cuspidatum* methanol extract in a dose of 400 mg/kg/d showed highly significant reduction ($p<0.05$) in MDA and rise in ($p<0.05$) in GSH better than other fractions. Histopathological examination of the liver denoted marked hepatoprotective effect of the total methanol extract by absence of histopathological lesions, decrease the extent of necrosis and fatty changes when compared to carbon tetrachloride group, while the other fractions showed above moderate effect on CCl_4 induced hepatotoxicity in rats.
Conclusion: *Odontonema cuspidatum* showed significant protection against carbon tetrachloride induced liver injury in rats by enhancing the antioxidant defense condition, lessening lipid peroxidation, and conserving against the pathological changes of the liver.

Keywords: Hepatoprotective; antioxidant; *Odontonema cuspidatum;* Carbon tetrachloride (CCl_4); silymarin.

1. Introduction
Liver is one of the important and vital organs in human body and the main site for metabolism and excretion. So it has an impressive role in the upkeep, performance and regulating homeostasis of the body. It participates in nearly all the biochemical pathways to growth, quarrel against disease, nutrient supply, energy supplying and reproduction[1].The major functions of the liver are metabolism of carbohydrate, protein and fat, secretion of bile, detoxification and storage of vitamins, iron and glucose or glycogen. Consequently, maintenance of a healthy liver is a decisive factor for overall health and well-being. But it is difficult to keep up the liver always in healthy form due to continuous exposure to environmental toxins, mistreating by poor drug habits, and prescribed & over-the-counter drug which can finally lead to various liver morbidness like hepatitis, cirrhosis etc. Also recent research in free radical biology propose the pathophysiological role of free radicals and oxidative stress in liver injury and damage[2]. Furthermore vitamin deficiency simultaneously with overproduction of free radicals and a reduced level of defensive enzymes is considered as the main offender for producing oxidative stress. But in contrast, the antioxidant defense system involving different enzymes such as catalase, glutathione peroxidase and superoxide dismutase, etc snare and devastate these free radicals [3]. So, liver diseases consider some of the fatal disease and one of the causes of high death rate in the world today[4]. Although of tremendous scientific advancement in the field of manufacturing of synthetic drugs used in the treatment of liver disorders, many of these synthetic agents are incompetent and with harmful side effects[5]. Recently, the attention of herbal medicines and their consuming

in treatment and curing various diseases and many dysfunctions is becoming increasingly popular and lionized wide acceptance[6]. Many plants have a vital role in management of liver diseases and there are many polyherbal formulations aimed to have hepatoprotective activities [7, 8]. Some herbals show promising activity through antifibrotic treatment [9-11], or in curing chronic hepatitis B [12, 13] and in treatment of chronic viral hepatitis C and hepatocellular carcinoma [14, 15]. *Odontonema cuspidatum* (Nees) Kuntze (syn.) *Odontonema strictum* commonly known as cardinal's guard or fire spike is perennial shrub belonging to family acanthaceae. It is native to Mexico but often cultivated in tropical areas and commonly planted as an ornamental for its attractive red tubular flowers. OC has been reported to have flavonoids, saponin, glycosides, tannins, steroids and terpenoids in leaves extracts [16]. OC is used in Burkina Faso for treatment of hypertension [17]. The literature scrutiny revealed the insufficient validated scientific reports on the chemical constituents and the pharmacological activities of OC, as well as there are no studies have been done on possible hepatoprotective activities of this plant, hence the aim of the present study was to evaluate the hepatoprotective effect of *O. cuspidatum* aerial part extracts against CCl_4-induced hepatotoxicity in rats. Hepatoprotection was determined by inspecting the activities of ALT, AST, ALP and total bilirubin in the serum of control and treated rats. The lipid peroxidation and antioxidant parameters MDA and GSH were evaluated in the liver homogenates to find the possible mechanisms of the hepatoprotective activity. A histopathological examination of liver sections was executed to confirm the hepatoprotective effect.

2. Material and methods

2.1. Chemicals

Carbon tetrachloride (CCl_4), ethylene diamine tetra acetic acid (EDTA), 5,5′-dithiobis-2 nitrobenzoic acid (DTNB, Ellman's reagent), potassium dihydrogen phosphate (KH2PO4), thiobarbituric acid (TBA), and silymarin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The assay kits were purchased from Spectrum, MDSS, GmbH, and Hannover, Germany.

2.2. Plant Material

The aerial parts of *O. cuspidatum* were collected on May 2013 from El-Orman Botanical Garden Giza, Egypt. The plant was botanically identified by Eng. Therese Labib, consultant of plant taxonomy at the Ministry of Agriculture and ex. director of El-Orman Botanical Garden, Giza, Egypt. A voucher specimen of *O. cuspidatum* has been deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assuit, Egypt under the number (OC02- 2013). The plant material was dried, powdered into fine particles and it becomes ready for use in the successive extraction.

2.3. Extract Preparation

The air-dried powdered aerial parts of *O. cuspidatum* (200 g) were extracted at room temperature by maceration with methanol (80% v/v) till exhaustion. The methanolic extract after filtration was concentrated under reduced pressure and collected in porcelain and allowed to dry. The dried methanolic extract (41 g) was digested with distilled water (300 mL) and the suspension was transferred to a separating funnel where the phytoconstituents were successively partitioned between the aqueous layer and *n*-hexane (300 mL × 3), methylene chloride (300 mL × 3), ethyl acetate (300 mL × 3) and *n*-butanol (300 mL × 3) till exhaustion. The acquisitioned fractions were dried under reduced pressure to give *n*-hexane fraction (4.50 g), methylene chloride (0.70 g), ethyl acetate (1.13 g), *n*-butanol (8.40 g) and aqueous (25.70 g) fractions, respectively.

2.4. Experimental Animals

Wister albino rats of either sex (140±20 g) obtained from the Animal House, Pharmacology Department, Faculty of Medicine, Assiut University were used. All animal rules were conducted in stratification with the internationally accepted principles for laboratory animals' use and care as found in the European Community Guidelines and Institutional Ethical Committee Approval was obtained. The study protocol was approved by the Animal Ethics Committee of Assiut University. Animals were housed under standardized environmental conditions in the Animal House in plastic cages at an ambient temperature (25±2 ºC) and relative humidity of 55-70%. A 12:12 hr light dark cycle was well-kept during the experiments. They were fed a standard diet, water was provided *ad libitum* and they were adapted for one week before entry into the later study. They were allowed free access to water and food throughout the period of the experiment. The animals were randomly divided into different groups (8 rats per treatment group). Each group was housed separately after recording its animal's body weight and had kept separate marks for identifying the dose level, group and individual number.

2.5. Acute Toxicity Study

The acute toxicity and lethality (LD_{50}) of the *O. cuspidatum* methanolic extract and fractions (aqueous, *n*-butanol and *n*-hexane fractions) were determined according to the method described by [18]. Substantially, the first phase of 9 rats for each extract or fraction were randomly divided into three groups (n=3), and were administered orally of 10, 100 and 1000 mg/kg of *O. cuspidatum* methanolic extract and fractions, respectively. In addition, a group of one rat was set up as a control group and was treated with 2% (v/v) Tween 80 in normal saline. They were observed for 24 h for general behavioral changes, physiological function and mortality. Since no death was registered, the second phase of doses 1600, 2900 and 5000 mg/kg of the methanolic extract and fractions were administered to a fresh batch of animals at one animal per dose. All animals were noted considerably on the day of treatment and surviving animals were monitored daily for 2 weeks for signs of acute toxicity. Retrieval and weight acquisition were seen as indications of having survived the acute toxicity.

2.6. Experimental Design

The animals were divided into seven groups, containing eight animals each. Group I served as (normal control) received distilled water (1 ml/kg, p.o.) daily for 5 days and olive oil (1 ml/kg, s.c.) on 2nd and 3rd day of the treatment. Group II served as (CCl_4 control) or (induced group) received distilled water (1 ml/kg, p. o.) daily for 5 days and CCl_4 : olive oil (1:1, 2 ml/kg, s. c.) on 2nd day and 3rd day of the treatment. Group III served as (positive control) or (silymarin group) received (100 mg/kg, p. o.) daily for 5 days and CCl_4 : olive oil (1:1, 2 ml/kg, s. c.) on 2nd day and 3rd day, 30 min after administration of standard drug. Groups IV, V, VI and VII

(tested groups) received *O. cuspidatum* methanolic extract (OCME), *n*-hexane, *n*-butanol and aqueous fractions respectively at a dose of 400 mg/ (kg p. o.) for 5 days and CCl_4 : olive oil (1:1, 2 ml/kg, s. c.) on 2nd day and 3rd day, 30 min after administration of methanolic extract and fractions of *O. cuspidatum* aerial parts. All the groups were given the above treatment daily for 5 days [19].

2.7. Estimation of Hepatoprotective Activity

The animals were sacrificed on 6th day under ether anesthesia and blood was collected by direct cardiac puncture into clean centrifuge tubes and left to stand for 30 min before centrifugation to avoid hemolysis. Samples were centrifuged at 3000 rpm for 15 min and the clear supernatant was collected into dry clean tube for biochemical tests as Alanine aminotransferase (ALT)[20], Aspartate aminotransferase (AST)[20], ALP[21] and total bilirubin[22]. Liver was dissected out into two parts. The first part was washed with 0.9% normal saline and a piece of 500 mg was homogenized with phosphate buffer (PH 7.4) then the homogenate was centrifuged. The supernatant was collected and stored at -70°C for estimation of lipid peroxidation (MDA) [23] and antioxidant enzyme (GSH) [24]. The second part was fixed in 10% formalin for histopathological examination.

2.8. Histopathological Examination

Liver tissue was fixed in 10% formalin, dehydrated in graded ethanol and embedded in paraffin wax and sliced into 5 μm thick sections in a rotary microtome (Leica, USA). Sections were prepared and stained with hematoxylin-eosin dye (Merck). The slides thus prepared were observed for histopathological features under the microscope (Zeiss, Germany) with ×400 magnification power [25].

2.9. Statistical Analysis

The mortality rate in group II was 25% (two rats), in group IV was 12.5% (one rat) and in group V was 12.5% (one rat), so the number of animals was taken for analysis were 6 animals. Data obtained were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ± S.E (n= 6 animals). The significant differences among values were analyzed using analysis of variance (one-way ANOVA) followed by Tukey's t test for comparison between different groups.($p< 0.05$ was considered as significant).

3. Result and Discussion

3.1. Acute toxicity study

The result of this investigation (Table 1&2) shows that there was no lethality or any symptoms of toxicity in the three groups of three rats that received 10, 100 and 1000 mg/kg body weight of each fraction and methanol extract of *O. cuspidatum* aerial parts at the end of the first phase of the study. At the end of the second phase of the study, there was neither death nor clear sign of toxicity in the groups of rats that received 1900, 2600 and 5000 mg/kg body weight of each fraction and methanol extract of *O. cuspidatum* aerial parts. There was no sign of toxicity or mortality recorded between all the dose groups throughout the two weeks experimental period. So, the LD_{50} of methanol extract and fractions of *O. cuspidatum* aerial part was greater than 5000 mg/kg b.w.

Table 1: Acute lethal effect of methanol extract and fractions of *Odontonema cuspidatum* aerial parts administered orally to Wistar albino rats*:*

Experiment / Treatment	Phase 1			Phase 2		
	Dose (mg/kg bw)	No Dead rats after 24 hrs	Treated rats after 24 hrs	Dose (mg/kg bw)	No Dead rats after 24 hrs	Treated rats after 24 hrs
control	0	0/3	0/3			
Total methanol ext.	10	0/3	0/3*	1600	0/1	0/1
	100	0/3	0/3	2900	0/1	0/1
	1000	0/3	0/3	5000	0/1	0/1
n-Hexane fr.	10	0/3	0/3	1600	0/1	0/1
	100	0/3	0/3	2900	0/1	0/1
	1000	0/3	0/3	5000	0/1	0/1
n-Butanol fr.	10	0/3	0/3	1600	0/1	0/1
	100	0/3	0/3	2900	0/1	0/1
	1000	0/3	0/3	5000	0/1	0/1
Aqueous fr.	10	0/3	0/3	1600	0/1	0/1
	100	0/3	0/3	2900	0/1	0/1
	1000	0/3	0/3	5000	0/1	0/1

(* Experiment was conducted into two phases; each dose group of phase-1 made up of 3 rats while those in phase 2 have 1 rat per group).

Table 2: Effect of oral administration of methanol extract and different fractions of Odo*ntonema cuspidatum* on the body weights of rats during acute toxicity experiment:

Experiment / Treatment		Phase 1		Phase 2
	Dose(mg/kg bw)	Weight gain (g) (x±SD)	Dose(mg/kg bw)	Weight gain (g)(x±SD)
control	0	33.00±4.582a		
Total methanol ext.	10	31.00±8.000[a]	1600	17
	100	25.00±6.245[a]	2900	6
	1000	12.33±6.110[a]	5000	5
n-Hexane fr.	10	19.66±8.020[a]	1600	12
	100	18.00±5.567[a]	2900	7
	1000	11.66±3.055[a]	5000	5
n-Butanol fr.	10	18.66±3.511[a]	1600	20
	100	10.66±3.214[a]	2900	12
	1000	13.00±3.605[a]	5000	8
Aqueous fr.	10	29.00±6.245[a]	1600	12
	100	22.33±6.027[a]	2900	9
	1000	12.33±2.516[a]	5000	8

Values are mean ± SD (standard deviation). Same superscripts indicate no significant difference ($p > 0.05$). Weight values in phase-2 (were n<3) were not compared due to absence of measure of variability.

3.2. Hepatoprotective Effect

The normal control group (received distilled water only) served as a baseline for all the biochemical parameters. A significant increase in the activity of the serum enzymes ALT, AST, ALP and Total bilirubin ($P< 0.05$) was observed in the CCl_4 group compared to the normal control group. Positive control (silymarin group) showed highly significant decrease ($p<0.05$) in AST, ALT, ALP and total bilirubin as compared to negative control group. The treatment of intoxicated rat with OCME and fractions at a dose of (400 mg/kg/day) produced a significant hepatoprotective effect and reduced the activity ($p<0.05$) of ALT, AST, ALP and total bilirubin compared to silymarin shown in (Table 3) and figures 1, 2, 3 and 4. OCME exhibited prognathous and noticeable hepatoprotective activity on intoxicated rats than other plant fractions.

Table 3: Effect of methanol extract and fractions of *Odontonema cuspidatum* aerial parts on biochemical parameters against CCl_4 induced liver injury:

Groups(n=6)	AST(IU/L)	ALT(IU/L)	ALP(IU/L/)	Total Bilirubin(g/dl)
Normal control	99.33±1.542	46.00±2.294	217.33±1.646	0.48±0.030
Negative control (CCl_4)	225.33±3.800 a*	165.83±6.508 a*	576.83±2.182 a*	3.54±0.030 a*
Positive control (silymarin)	139.33±2.275 a*	63.66±1.725 b*	278.83±1.701 a* b*	0.89±0.041 a* b*
Total methanol extract	164.00±2.280 a* b* c*	71.00±4.203 a* b*	316.00±1.673 a* b* c*	1.52±0.022 a* b* c*
n-Hexane fraction	191.00±3.803 a* b* c*	117.33±2.431 a* b* c*	484.00±1.879 a* b* c*	1.91±0.020 a* b* c*
n-Butanol fraction	176.16±1.851 a* b* c*	90.83±4.460 a* b* c*	367.66±1.453 a* b* c*	1.61±0.017 a* b* c*
Aqueous fraction	182.16±1.759 a* b* c*	94.50±3.603 a* b* c*	406.66±1.926 a* b* c*	1.74±0.018 a* b* c*

Values are expressed as mean ± S.E.M. of six animals; symbols represent statistical significance: *$p < 0.05$.
(a) Comparisons were made between group I vs. groups II–VII.
(b) Comparisons were made between group II vs. groups III–VII.
(c) Comparisons were made between group III vs. groups IV–VII.

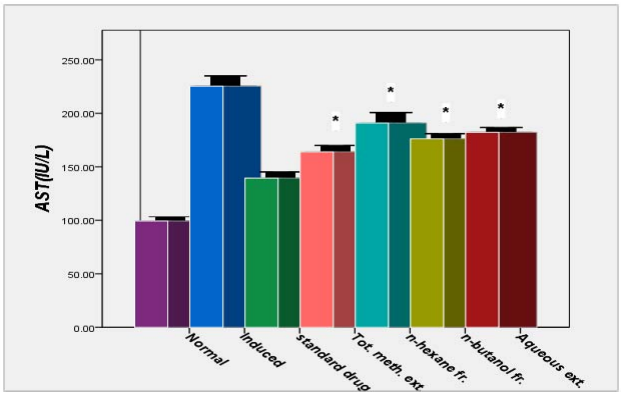

Fig 1: Effect of methanol extract and different fractions of *Odontonema cuspidatum* (Nees) Kuntze on serum level of AST enzyme

Values are expressed as mean ± S.E.M. of 6 animals. Statistical significant test for comparison was done by (one-way ANOVA followed by Tukey's multiple comparison test). *$p < 0.05$.

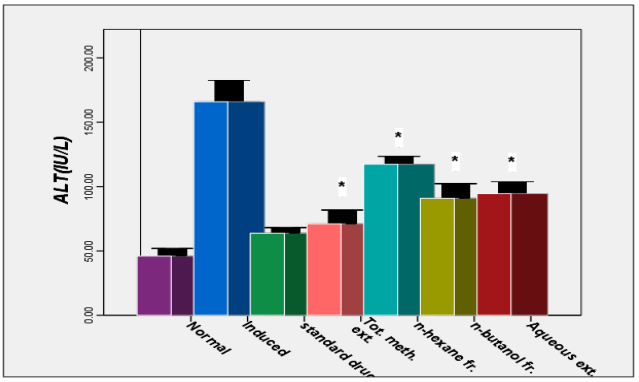

Fig 2: Effect of methanol extract and different fractions of *Odontonema cuspidatum* (Nees) Kuntze on serum level of ALT enzyme.

Values are expressed as mean ± S.E.M. of 6 animals. Statistical significant test for comparison was done by (one-way ANOVA followed by Tukey's multiple comparison test). *$p < 0.05$.

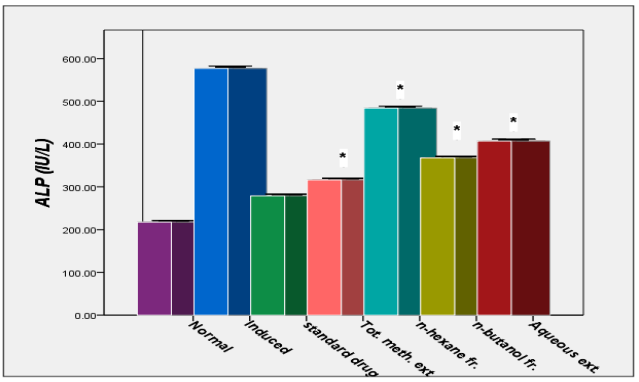

Fig 3: Effect of methanol extract and different fractions of *Odontonema cuspidatum* (Nees) Kuntze on serum level of ALP enzyme.

Values are expressed as mean ± S.E.M. of 6 animals. Statistical significant test for comparison was done by (one-way ANOVA followed by Tukey's multiple comparison test). *$p < 0.05$.

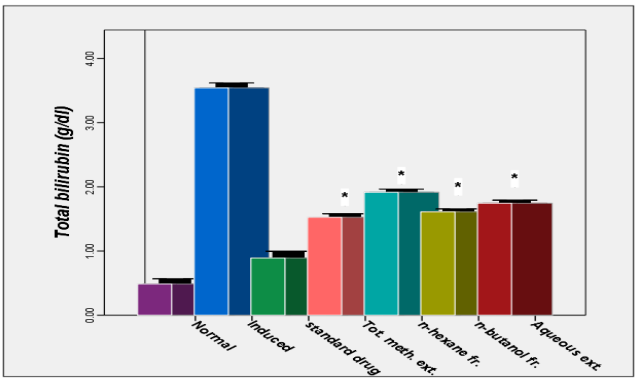

Fig 4: Effect of methanol extract and different fractions of *Odontonema cuspidatum* (Nees) Kuntze on serum level of Total Bilirubin

Values are expressed as mean ± S.E.M. of 6 animals. Statistical significant test for comparison was done by (one-way ANOVA followed by Tukey's multiple comparison test). *$p < 0.05$.

3.3. Antioxidant Activity

Levels of antioxidant and lipid peroxidation enzymes of negative control group were compared with normal control group. A marked reduction ($p<0.05$) in GSH and highly significant rise ($p<0.05$) in MDA values were observed in

CCl_4-intoxicated rat. Positive control (Silymarin group) showed highly significant reduction ($p<0.05$) in MDA, and rise ($p<0.05$) in GSH values when compared to negative control group. Test groups treated with OCME and fractions at a dose of 400 mg/kg/d showed significant reduction ($p<0.05$) in MDA, and highly significant rise ($p<0.05$) in GSH when compared with silymarin group. Shown in (Table 4) and figures 5 and 6. OCME exhibited noticeable antioxidant activity than other fractions. These results clearly elucidated the strong *in vivo* antioxidant activity provided by OCME.

Table 4: Effects of methanol extract and fractions of *Odontonema cuspidatum* aerial parts on lipid peroxidation and Antioxidant enzymes in CCl_4 induced liver injury

Groups (n=6)	MDA(nmol/g wet tissue)	GSH(µmol/g wet tissue)
Normal control	7.99±0.309	9.61±0.236
Negative control (CCl_4) (induced)	38.48±1.035 a*	0.90±0.215 a*
Positive control (silymarin)	11.94±0.354 a* b*	7.33±0.105 a* b*
Total methanol extract	14.85±0.222 a* b*	8.45±0.159 b*
n-Hexane fraction	20.46±0.880 a* b* c*	3.24±0.247 a* b* c*
n-Butanol fraction	15.48±0.259 a* b*	7.18±0.155 a* b*
Aqueous fraction	16.31±0.181 a* b* c*	6.03±0.104 a* b* c*

Values are mean ± S.E.M. (standard error mean) of six animals; symbols represent statistical significance:*$p < 0.05$.
(a) Comparisons were made between group I vs. groups II–VII.
(b) Comparisons were made between group II vs. groups III–VII.
(c) Comparisons were made between group III vs. groups IV–VII.

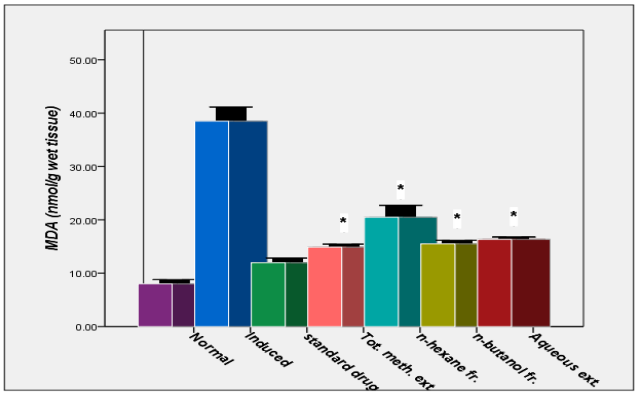

Fig 5: Effect of methanol extract and different fractions of *Odontonema cuspidatum* (Nees) Kuntze on tissue homogenate level of MDA.

Values are expressed as mean ± S.E.M. of 6 animals. Statistical significant test for comparison was done by (one-way ANOVA followed by Tukey's multiple comparison test). *$p < 0.05$.

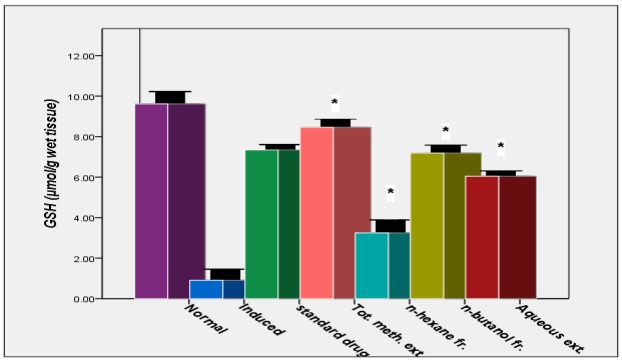

Fig 6: Effect of methanol extract and different fractions of *Odontonema cuspidatum* (Nees) Kuntze on tissue homogenate level of GSH.

Values are expressed as mean ± S.E.M. of 6 animals. Statistical significant test for comparison was done by (one-way ANOVA followed by Tukey's multiple comparison test). *$p < 0.05$.

3.4. Histopathological Observations

Histological study of liver sections of normal control group revealed the normal histological structure of hepatic lobule from central vein and concentrically arranged hepatic cords (Fig. 7a) Meanwhile, liver of rat from negative control (CCl_4) group showed chronic perihepatitis, ballooning degeneration of hepatocytes (Fig. 7b) and steatosis of hepatocytes (Fig.7c). reasonable improvement in the histopathological picture was noticed in liver of rat from positive control (silymarin) group as the examined sections revealed slight microvesicular steatosis of hepatocytes (Fig. 7d). However, liver of rat from total methanol extract group showed marked improvement. The examined sections from this group showed no histopathological lesions except hydropic degeneration of hepatocytes (Fig. 7e). Slight improvement in the histopathological picture was noticed in liver of rats from *n*-hexane fraction & *n*-butanol fraction groups. Liver sections of rat from *n*-hexane fraction group showed vacuolar degeneration of hepatocytes and inflammatory cells infiltration (Fig. 7f). Moreover, liver of rat from *n*-butanol fraction group revealed steatosis of hepatocytes (Fig. 7g). Steatosis of hepatocytes, inflammatory cells infiltration (Figs. 7h) were noticed in examined sections from aqueous fraction group.

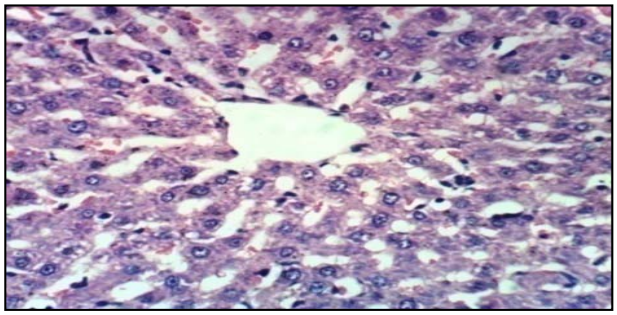

Fig. 7a: Normal control

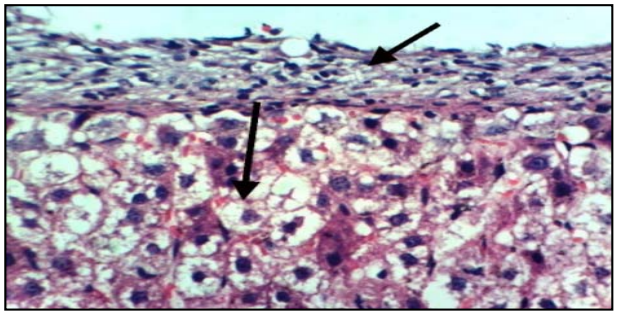

Fig. 7b: Negative control(CCL_4)

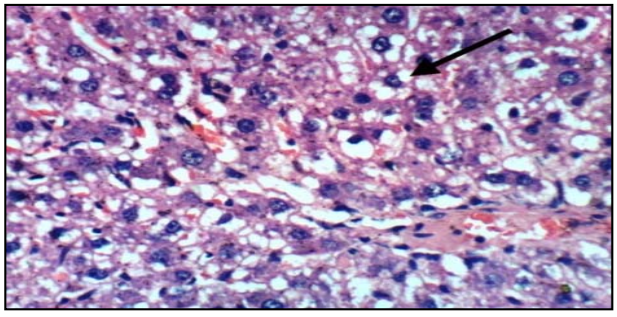

Fig. 7c: Negative control(CCL_4)

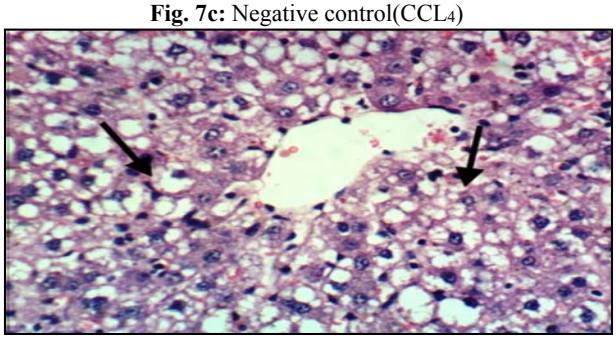

Fig. 7d: Positive control (silymarin)

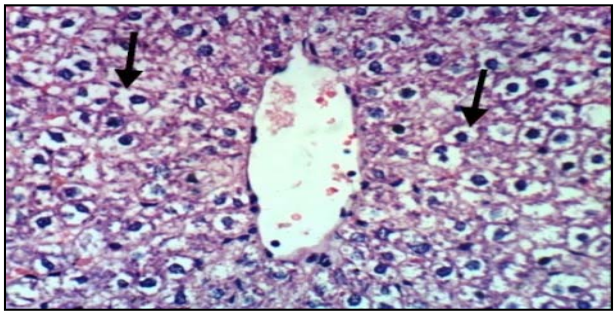

Fig. 7e: Total methanol extract 400

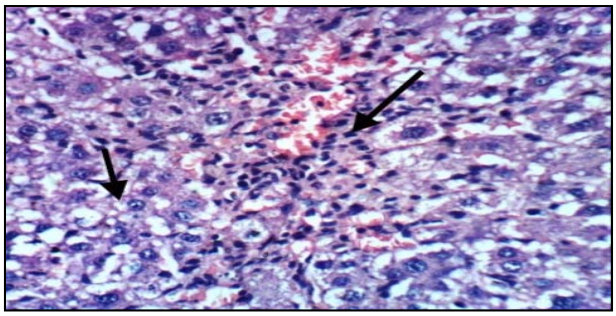

Fig. 7f: *n*-hexane fraction 400

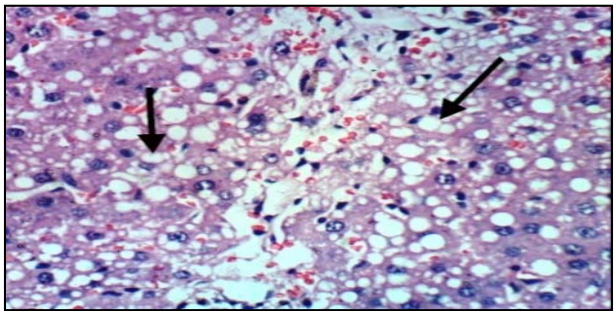

Fig 7g: *n*-butanol fraction 400

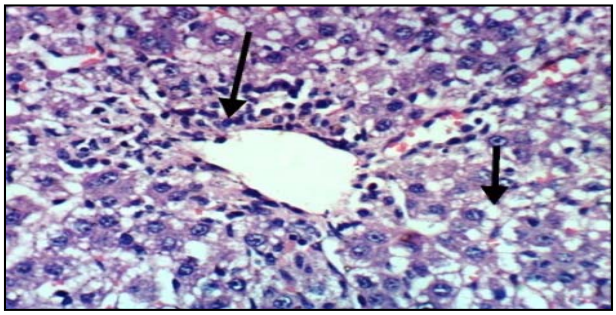

Fig. 7h: Aqueous fraction 400

Fig7: Hepatoprotective effect of OCME and fractions in CCl_4-intoxicated rat, (H and E staining, magnification x 400).

Group I (normal control) (Fig.7a): revealed the normal histological structure of hepatic lobule from central vein and concentrically arranged hepatic cords. Group II (CCl_4 group): showing chronic perihepatitis, ballooning degeneration of hepatocytes (Fig. 7b) and steatosis of hepatocytes (Fig.7c). Group III (CCl_4 + 100 mg/kg of silymarin): showing slight microvesicular steatosis of hepatocytes (Fig. 7d). Group IV (CCl_4 + 400mg/kg of OCME): showing no histopathological lesions except hydropic degeneration of hepatocytes (Fig. 7e). Group V (CCl4 + 400mg/kg *n*-hexane fr.) showing vacuolar degeneration of hepatocytes and inflammatory cells infiltration (Fig.7f). Group VI (CCl_4 + 400mg/kg *n*-butanol fr.) showing steatosis of hepatocytes (Fig. 7g). Group VII (CCl_4 + 400mg/kg aqueous fr.) showing Steatosis of hepatocytes, inflammatory cells infiltration (Figs. 7h).

4. Discussion

CCl_4 intoxication is a vastly used experimental model for liver injury in the screening of hepatoprotective activity of plant extracts or drugs. Administering CCl_4 to rats noticeably increases serum ALT, AST, ALP, and TB levels which reflects the severity of liver injury [26]. The exposure to this chemical is known to prompt oxidative stress by the formation of free radicals. Carbon tetrachloride induces hepatotoxicity through formation of reactive intermediate trichloromethyl free radical (CCl_3·) as a result from its bioactivation by cytochrome P450. Trichloromethyl free radical (CCl_3·) in presence of oxygen forms Trichloromethylperoxy radical ($CCl3O_2$·). As a result of formation of such these reactive intermediates, they covalently bind to cellular macromolecules producing lipid peroxidation which leads to injury of the membrane and leakage of cytosomal enzymes [27, 28]. Moreover, Trichloromethyl radicals also react with the sulfhydryl groups of GSH leading to its suppression [29]. In this study, significant increase in AST, ALT, ALP, and TB levels in the serum were noticed after administration of CCl_4, as reported earlier. The seepage of large quantities of enzymes into the blood stream was associated with centrilobular necrosis, ballooning degeneration of the liver and infiltration of the liver by lymphocytes. A marked increase of MDA level in the liver tissue in response to CCl_4 intoxication, articulating oxidative damage of the liver. Also, administration of CCl_4 reduced the levels of GSH in the liver tissue compared to the normal group. Pretreatment with *O. cuspidatum* methanolic extract, decrease the elevated levels of these enzymes and this can be inferred by the reduced amount of histopathological injuries. Lipid peroxidation has been embroiled in the pathogenesis of hepatic injury by the free radical reactive intermediates of CCl_4 and is accountable for cell membrane damage and consequent release of marker enzymes of hepatotoxicity [30]. In this study, significantly elevated levels of Thiobarbituric acid reactive substances (TBARS), products of membrane lipid peroxidation, noted in CCl_4 administered rats indicated hepatic damage. pretreatment with *O. cuspidatum* methanolic extract prevented lipid peroxidation and returned the increased MDA to its normal level which could be likely to the radical scavenging antioxidant constituents. GSH is the major non-enzymatic antioxidant and organizer of intracellular redox homeostasis, absolutely present in all cell types [31]. Administration of CCl_4 leads to a depletion in the glutathione level which can be an important factor in the CCl_4 toxicity. The mechanism of hepatoprotection by *O. cuspidatum* extract against CCl_4 toxicity might be due to reconquest of the GSH level. The

possible hepatoprotective activity of *Odontonema cuspidatum* against CCl_4-induced liver injury in rats may be due to its antioxidant activity as pointed out by protection against lipid peroxidation and reduced antioxidant levels, thereby minimizing free radical damage of hepatocytes.

5. Conclusion

Based on the results of this study, the hepatoprotective effect of *O. cuspidatum is* imputed to its ability to minify the rate of lipid peroxidation, to promote the antioxidant defense stature, and to conserve against the pathological changes of the liver induced by CCl_4 intoxication (fig.8). Further studies for long duration are required to find out the protective potential of *Odontonema cuspidatum* against chronic liver injury.

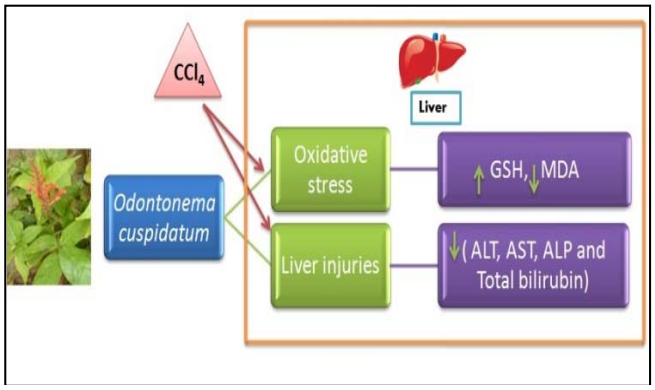

Fig 8:*Odontonema cuspidatum* preventsliver cell injuries perturbed by CCl_4 hepatotoxin.

6. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

7. Acknowledgment

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