ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 10, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

HEPATOPROTECTIVE ACTIVITY OF VARIOUS EXTRACTS OF *CLAUSENA DENTATA* (WILLD.) ROEM. (SYN. *CLAUSENA WILLDENOVII* WIGHT. AND ARN.) *RUTACEAE*

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Received: 20 May 2017, Revised and Accepted: 04 July 2017

ABSTRACT

Objectives: *Clausena* (Rutaceae) is a genus of about 23 species of unarmed trees and shrubs. The stem bark of *Clausena dentata* is used in veterinary medicine for the treatment of wounds and sprains. Even though *C. dentata* has a lot of potential medical uses, the study on pharmacological activities is in scarce. The present study was undertaken hepatoprotective activity of various extracts of *C. dentata.*

Methods: The plant *C. dentata* was collected from Kadagaman, near Tiruvannamalai, Tamil Nadu, India. The dry powder of stem bark (2.5 kg) was first soaked in hexane for 24 hrs. The extract was suction filtered. This was repeated for two more days, and similar extracts were pooled together and concentrated at 40°C using Buchi R - 153 Rotavapor. The residual plant material was extracted successively with chloroform and methanol same manner. Preliminary phytochemical test and hepatoprotective activity of various extracts of *C. dentata* against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats were carried out. Biochemical and histopathological changes were observed.

Results: The highly significant ($p<0.01$) reduction in the levels of serums glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, and bilirubin observed in the study in rats simultaneously treated with *C. dentata* extracts and CCl_4 as compared to CCl_4 alone treated.

Conclusion: CCl_4 induced hepatic damage was counteracted by the extracts of *C. dentata*. Changes were observed in enzymatic and histopathological level, when compared to CCl_4 alone treated group.

Keywords: *Clausena dentate,* Hepatotoxicity, Carbon tetrachloride, Biochemical changes.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i10.20127

INTRODUCTION

Clausena (Rutaceae) is a genus of about 23 species of unarmed trees and shrubs mainly grow in Indomalayan with a few in China, Africa, and Australia. 10 species are known to grow in India, of which five are of economic importance. The stem bark of *Clausena dentata* is used in veterinary medicine for the treatment of wounds and sprains [1]. The dried powdered rootstock is also used by the Kols, the tribes in Chotanagpur region, India, for decayed teeth. In Cambodia, the stem is considered bitter tonic and astringent [2]. The infusion is given for colic pain with diarrhea. *C. dentata* is used for digestion and as diuretic [3]. Hepatotoxicity has become one of the principle limitations of some important commonly used drugs. Because of its strategic placement in the body, toxins gain access first to the liver and make it one of the most vulnerable organs. Long-term exposure of the liver to various toxic insults may cause irreversible injury to the hepatic parenchyma and consequently chronic inflammatory changes [4]. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices as well as in traditional system of medicine in India. Most of the herbal drugs speed up the natural healing process of the liver. Therefore, the search for effective hepatoprotective drug continues [5]. Liver damage is a widespread pathology, which can influence these physiochemical functions and be caused by viral hepatitis, alcoholism, or liver-toxic chemicals, such as carbon tetrachloride (CCl_4) [6]. In this study, CCl_4 has been chosen, as its immense use in industry leads to severe exposure to mankind, resulting in acute liver disorder. CCl_4 induces successive hepatic changes consisting of hepatic steatosis, fibrosis, massive infiltration, and cirrhosis [7]. Considering the importance of the plant, the present study was undertaken to study the hepatoprotective effect of various extracts of *C. dentata* (Willd.) Roem. (Syn. *Clausena willdenovii* Wight. & Arn.) *Rutaceae.*

METHODS

Plant collection

The plant *C. dentata* (Willd.) Roem was collected in May 1999 from Kadagaman, near Tiruvannamalai, Tamil Nadu, India, and authenticated at Centre for Advanced Study in Botany, University of Madras, Chennai (Fig. 1). A voucher specimen of the plant has been deposited at the herbarium. The collected plant material was free from disease and free of contamination of other plants.

PHYTOCHEMICAL STUDIES

Preparation of extracts [8,9]

The dry powder of stem bark (2.5 kg) was first soaked, at room temperature, in hexane (1:4 w/v) for 24 hrs. The extract was suction filtered using Whatman filter paper. This was repeated for two more days, and similar extracts were pooled together and concentrated at 40°C under reduced pressure using Buchi R - 153 Rotavapor. The residual plant material was extracted successively with chloroform and methanol in the same manner as followed for hexane.

Preliminary phytochemical test

The different phytochemical chemical tests were performed for establishing profile of given extract for its chemical composition.

Hepatoprotective effect of various extracts of *C. dentata* against carbon tetra chloride induced hepatotoxicity in rats [10]

Requirements: Animals: Rats, toxic agent - CCl_4 (spectroscopic grade), solvent - Tween-80, 1%, anesthetic agent, anesthetic ether, standard enzyme kit, serum glutamate oxaloacetate transaminase (GOT), serum glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin (Dr. Reddy's Laboratories), and Instrument-Spectrophotometer Systronic UV-VIS 118.

Procedure

Adult male albino rats weighing about 200 g were maintained on a pellet diet (M/s.Hindustan Lever Limited, Mumbai). Institutional Animal Ethics Committee approved the study. The rats were divided into 11 groups of six each. Group 1: Control, Group 2: Toxicated with CCl_4 given intraperitoneally (0.7 mL/kg body weight), thrice a week for 2 weeks. Group 3, 4, and 5 were given CCl_4 I.P (0.7 mL/kg body weight) thrice a week for 2 weeks, and hexane extract (50, 100, 150 mg/kg body weight) orally. The same procedure was repeated with chloroform and methanol extract of 50, 100, and 150 mg/kg body weight orally to 6^{th}, 7^{th}, 8^{th}, 9^{th}, 10^{th}, and 11^{th} group of animals by feeding cannula once daily.

Biochemical studies

After completion of the experimental regimen, the rats were fasted overnight, and samples of blood 1.5 mL were collected from retro-orbital plexus of each rat and centrifuged immediately. The serum was separated and refrigerated until its use. This was assayed for ALP [11], GOT [12], GPT, and bilirubin [13] by standard spectrophotometric methods using standard enzyme estimation kits.

Histopathological studies

Two animals from each group were sacrificed under ether anesthesia. Whole livers were removed and preserved in formalin, by standard technique. Serial sections (5 μm) were cut and stained with hematoxylin and eosins were used for histopathological studies (Department of Pathology M.M.C at Chennai). The percentage of protection was calculated using the formula.

% Protection = 100/Carbon tetrachloride−Normal control×Drug and carbon tetrachloride/Normal control.

Statistical analyses

The data were subjected to analysis of variance and the significant differences among the mean compared with students t-test using SPSS PC+ software [14].

RESULTS

Qualitative chemical tests revealed the presence of various phytochemicals in hexane, chloroform, and methanol extracts of *C. dentata* (Table 1). Methanol extract showed a positive test for alkaloids. All the extracts contained carbohydrates, glycosides, amino acids, proteins, and volatile oils. Ferric chloride test showed the presence of phenolic compounds, in all the extracts. Saponins, phytosteroids, fixed oils, and fats were absent.

Biochemical changes

The biochemical changes due to the effect of extracts are presented in Table 2. The levels of the marker enzymes of serum GOT, GPT, ALP, and bilirubin were found to be significantly increased in CCl_4 treated Group II (Toxic) in comparison with the control. The treatment with extract + CCl_4 showed that the levels of serum GOT, GPT, ALP, and bilirubin reduced significantly when compared with the elevated level due to CCl_4 alone treated group.

Histopathological evaluation

Liver samples from the control and experimental animals treated at dose 150 mg/kg body weight were taken (two animals from each group) for histopathological studies on liver damage. Normal histology of rat liver showed the normal liver architecture, central vein, cards of hepatocytes, and portal triad having no sign of fatty degeneration (Fig. 1). The liver section of rat treated with CCl_4 showed diffuse and

Table 1: Qualitative phytochemical screening of various extracts of *Clausena dentata*

SI. No.	Phytochemical test	Hexane	Chloroform	Methanol
1.	Alkaloids			
	a. Mayer's reagent	-	-	+
	b. Wagner's reagent	-	-	+
	c. Hager's reagent	-	-	+
	d. Dragendorff's reagent	-	-	+
2.	Carbohydrates and glycosides			
	a. Molisch's test	+	+	+
	b. Fehling's test	+	+	+
	c. Barfoed's test	+	+	+
	d. Benedict's test	+	+	+
	e. Borntrager's test	+	+	+
	f. Legal's test	+	+	+
3.	Saponins			
	Foam test	-	-	-
4.	Proteins and amino acids			
	a. Millon's reagent	+	+	+
	b. Biuret reagent	+	+	+
	c. Ninhydrin reagent	+	+	+
5.	Phytosteroids			
	Liebermann – Burchard's test	-	-	-
6.	Fixed oils and fats			
	a. Spot test	-	-	-
	b. Saponification test	-	-	-
7.	Phenolic compounds and flavonoids			
	a. Ferric chloride test	+	+	+
	b. Gelatin test	+	-	-
	c. Lead acetate test	+	-	-
	d. Alkaline reagent	+	-	-
	e. Magnesium and hydrochloric acid reduction	+	-	-
8.	Gums and mucilages			
	Alcohol 95% test	+	-	-
9.	Volatile oils			
	Steam distillation	+	+	+

-: Negative, +: Positive

Table 2: Effect of various extracts of *Clausena dentata* on carbon tetrachloride induced hepatotoxicity

Sl. No.	Extract of *Clausena dentata*	Dose (mg/kg body wt.)	Mean value ± SD, (% protection)					ED_{50} (mg/kg body wt.)
			SGPT[b]	SGOT[b]	Alkaline[a] phosphatase unit/KA unit	Total[c] bilirubin	Direct bilirubin[c]	
1.	Control (Tween 80, 1%)		24.98±0.7705	86.21±0.2137	44.23±0.2160	0.2683±0.014	0.021±0.011	
2.	Carbon tetrachloride, (toxic)	0.7	60.91±0.9326	150.11±0.3189	87.4±0.3464	0.55±0.018	0.458±0.42	
3.	Hexane extract	50	51.58±0.098 (28%)	131.21±0.014 (28%)	70.86±0.4412 (28%)	0.472±0.001 (26%)	0.253±0.0012 (47%)	81.0
		100	40.90±0.014 (57%)	103.43±0.0187 (73%)	61.8±0.0753 (59%)	0.387±0.0079 (55%)	0.147±0.00014 (71%)	
		150	28.1±0.523 (88.8%)	93.08±0.1291 (89.5%)	48.15±0.1871 (90%)	0.31±0.016 (82.8%)	0.035±0.0051 (96%)	
4.	Chloroform extract	50	51.8±0.089 (25%)	132.01±1.569 (27%)	75.81±0.5384 (26%)	0.4893±0.0012 (21%)	0.2177±0.00081 (55%)	96.0
		100	42.18±0.034 (52%)	115.65±0.036 (54%)	63.58±0.1169 (55%)	0.4248±0.0011 (43%)	0.3412±0.0014 (27.7%)	
		150	33.71±0.3764 (75.8%)	96.50±0.3847 (83.9%)	49.8±0.2160 (85.2%)	0.37±0.014 (62.1%)	0.042±0.011 (95%)	
5.	Methanol extract	50	51.16±0.1211 (27%)	129.10±0.1414 (33%)	74.8±0.1414 (28%)	0.4735±0.0010 (26%)	0.3242±0.0014 (31%)	84.5
		100	37.18±0.077 (66%)	105.53±0.1633 (68%)	62.71±0.0758 (57%)	0.3658±0.0019 (53%)	0.1827±0.0016 (63%)	
		150	30.68±0.5565 (41.4%)	95.35±0.3450 (85.8%)	48.2±0.2160 (89.84%)	0.32±0.018 (79.32%)	0.035±0.0089 (96.8%)	

Values are mean±SD, (n=6); [a]KA (King-Armstrong), [b]Units/mL, [c]mg%. Unit=KA unit, 1 KA unit/dL=7.1 U/L, $p<0.01$ value are compared with control (unpaired t-test). SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamic pyruvic transaminase, SD: Standard deviation

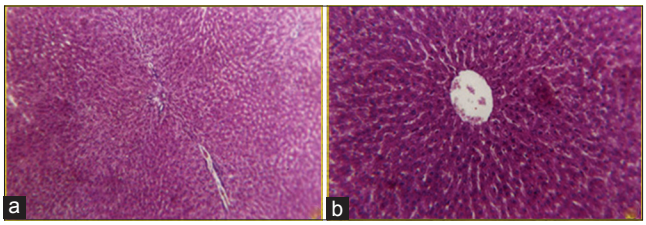

Fig. 1: Transverse section of normal rat liver showing central vein, cards of hepatocytes, portal triad, and no sign of fatty degeneration. (a) Under low power × 120. (b) Under high power ×1200

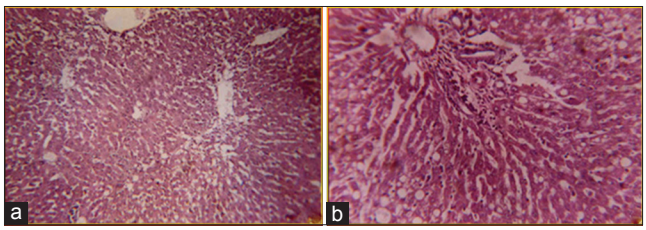

Fig. 2: Transverse section of carbon tetrachloride treatment liver showing severe fatty degeneration, sinusoidal dilation, and dysplastic nuclei in hepatocytes. (a) Under low power × 120. (b) Under high power × 10200

severe fatty change, dilated central vein, mononuclear cells in portal triad, dysplastic nuclei in hepatocytes, and sinusoidal dilation (Fig. 2). Liver section of CCl_4 + hexane extract (150 mg/kg body weight) treated group showed liver cells with mild dysplasia, central portion, and central veins filled with lymphocytes (Fig. 3). Liver section of CCl_4 + chloroform extract (150 mg/kg body weight) showed liver cells with central portion with mild dysplasia (Fig. 4). The liver section of CCl_4 + methanol extract (150 mg/kg body weight) treated group showed dilated central vein, cords of liver cells, sinusoidal dilation, mild dysplasia, and nuclear vacuolation (Fig. 5).

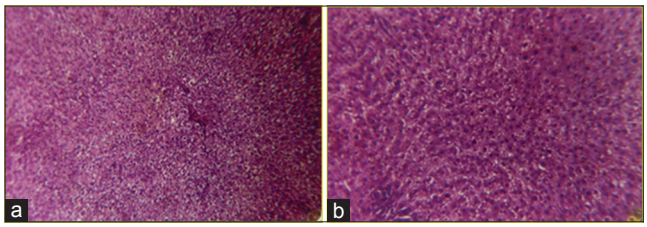

Fig. 3: Transverse section of rat liver after treatment with carbon tetrachloride + hexane extract of *Clausena dentata* showing mild dysplasia in liver cells, central portion, and central veins filled with lymphocytes. (a) Under low × 120. (b) Under high power × 1200

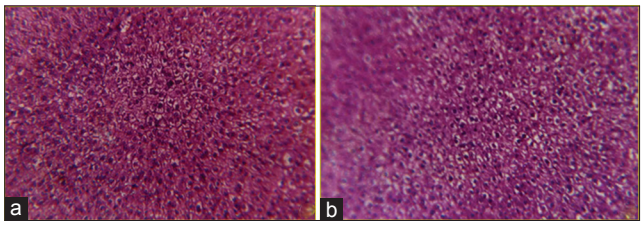

Fig. 4: Transverse section of rat liver after treatment with carbon tetrachloride + chloroform extract of *Clausena dentata* showing mild central portion with mild dysplasia in liver cells. (a) Under low × 120. (b) Under high power × 1200

The ED_{50} value was found by taking average value of all enzymatic percentage protection. The ED_{50} values of hexane, chloroform, and methanol extract were found to be 81, 96, and 84.5 mg/kg body weight, respectively.

DISCUSSION

The potentiation of carbon tetrachloride hepatotoxicity is supposed to be produced by enhanced production of active metabolites by the

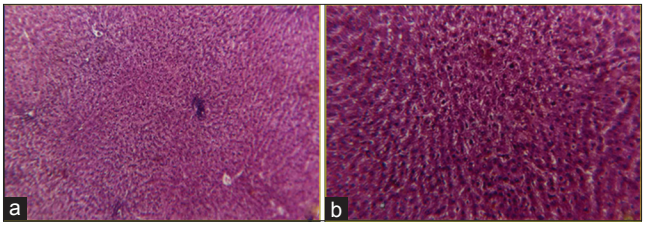

Fig. 5: Transverse section of rat liver after treatment with carbon tetrachloride + methanol extract of *Clausena dentata* showing mild dysplasia and nuclear vacuolation, dilated central vein, and cards of liver cells. (a) Under low × 120. (b) Under high power × 1200

ethanol activated mixed function oxidase system. This eventually led to the hepatocellular necrosis and was reflected in the experiment by marked changes in various enzymatic and nonenzymatic parameters of CCl_4 treated rats. The most serious delayed toxic effects of CCl_4 resulted from its hepatotoxic and nephrotoxic actions. Liver dysfunction begins soon after the ingestion of carbon tetrachloride.

The highly significant ($p<0.01$) reduction in the levels of serums GOT, GPT, ALP, and bilirubin observed in the study in rats simultaneously treated with *C. dentata* extracts and CCl_4 as compared to CCl_4 alone treated, also indicated that the extracts affected the important biochemical reactions which may be beneficial in reducing hepatic damage.

Extracts (hexane, chloroform, and methanol) of *C. dentata* were effective in counteracting the toxic effects of CCl_4 as shown by reversed levels of the altered biochemical, histopathological parameters, both in serum as well as liver in the order of hexane, methanol, and chloroform extract, and the ED_{50} value was found to be 81, 96, and 84.5 mg/kg body weight, respectively. This indicated that *C. dentata* contained the anti-inflammatory properties, may be contributing to the hepatoprotective effect.

As the phytochemical investigation revealed that *C. dentata* contained coumarins such as 3-{1,1 dimethylallyl) xanthyletin, dentatin, nordentatin, and carbazole alkaloid, these may be responsible for hepatoprotective activity. The coumarin content was more in hexane extract which may be the reason for more curing rate than the other two extracts as evidenced by HPTLC chromatogram.

CONCLUSION

CCl_4 induced hepatic damage was counteracted by the extracts of *C. dentata*. Changes were observed in enzymatic and histopathological level, when compared to CCl_4 alone treated group.

ACKNOWLEDGMENT

The authors acknowledge the Department of Pathology, Madras Medical College, Chennai, for continuous support throughout the project.

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