



Research Article

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Effect of Roots of *Origanum majorana* in Methanolic and Ethyl Acetate Extracts on CCl₄-Induced Hepatotoxicity in Rat

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ABSTRACT

To evaluate the hepatoprotective activity of different solvent extracts of *Origanum majorana*. The root materials were shade dried and were extracted in a soxhlet apparatus successively with ethyl acetate and methanol. The solvent was removed by the process of distillation and the crude extract was dried under vacuum. The extracts were subjected to hepatoprotective activities, the extract producing significant activity was column chromatographed. The extract was preliminarily screened using thin layer chromatography (TLC) and nuclear magnetic resonance (NMR) to know the types of compounds present in the extracts. TLC was developed in n hexane: ethyl acetate solvent systems of different polarities and then plates were visualized under Iodine vapour exposure, UV short wave 254 nm and long wave 366 nm. Plates were sprayed with a solution of visualizing reagent 10% H₂SO₄ in methanol followed by heating in an oven at 110°C for up to five minutes. The methanolic extracts of *Origanum majorana* at different doses, Silymarin and Drug vehicle were administered *p.o* in sodium carboxy methyl cellulose suspension. The serum was used for the estimation of various biochemical parameters like SGOT, SGPT, ALKP, TBL, CHL, TPTN and ALB. The results clearly depicted that CCl₄ intoxication in normal rats elevated the serum levels of SGOT, SGPT, ALKP, TBL and CHL, where as decreased the levels of TPTN, ALB significantly when compared to control indicating acute hepatocellular damage and biliary obstruction leading to necrosis.

Keywords: CCl₄, Ethyl acetate, Hepatotoxicity, Methanol, *Origanum majorana*.

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INTRODUCTION

The liver disorders are one of the world problems. Despite its frequent occurrence, high morbidity and high mortality, its medical management is currently in

adequate, so far not yet any therapy has successfully prevented the progression of hepatic disease, even though newly developed drugs have been used to treat chronic liver disorders, these drugs have often side

effects. Therefore, that is an essential research about suitable herbal drugs that could replace the chemical ones. Liver injury due to chemicals (or) infectious agents may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure. However, no effective treatment that delays disease progression and complications has yet been found. Several recent studies suggest that traditional herbs and micronutrients such as carotenoids and selenium may be useful for this purpose. Carbon tetrachloride (CCl₄) is widely used for experimental induction of liver damage. The principle causes of carbon tetrachloride (CCl₄) are induced hepatic damage in lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals. Various medicinal plants have been used to treat for various diseases in all over the world. Nowadays, Indian medicinal plants are belonging to about 40 families were investigated as liver protective drugs.

In generally any hepatoprotective agent can act as antihepatotoxicity or hepatotropic agent but the vice versa is always not true. There are number of phytoconstituents from plants which have exhibited antihepatotoxic activity. Some of the reported constituents with pharmacologically/therapeutically proved claims may be enlisted as Silymarin, glycyrrhizin, (+) - catechin, saikosaponins, curcumin, picroside 1 and 2 and gomisin etc. [1] Acetylbergenin [2], Kolaviron a, flaovanone [3] was also reported for its hepatoprotective properties.

Origanum majorana: The herb belongs to the family Lamiaceae. The *O. majorana* is a bushy half-hardy perennial sub-shrub that is often grown as an annual. The *O. majorana* is 1-2 ft (0.3-0.6 m) tall with descending, multi-branched stems that spill over to create a mound. Since the stems take root where they touch the soil, the mound gradually increases in diameter. The leaves get up to a 1 in (2.5 cm) long and have a wonderful, very distinctive, perfumy fragrance when bruised. The flowers are tiny, less than 1/8 in (0.3 cm) long and arranged in burr like heads 1/2 in (1.3 cm) long. The herb is distributed North Africa, Turkey and SW Asia. It has naturalized in the Mediterranean region of southern Europe. It has been reported that Isorhamnetin has been isolated from the seeds of *O. majorana*). Hexacosanol, palmitic acid, β -sitosterol and its β -D-Glucoside, ursolic acid, astragal in, rutin, quercetin and two unidentified diterpenic acids have been isolated from *O. majorana*. It was reported that β -sitosterol, ursolic acid, rutin and quercetin were identified from the seeds of *O. majorana*. [4] 3-keto-olean-12-en-29-oic acid and Olean-12-en-3 β -ol-29-oic acid were reported from the seeds of *O. majorana*. [5] It was reported that leaves of *O. majorana* brewed in decoction were used as astringent in haemorrhoids. The plant is eaten as a vegetable and it was also used as a tonic and stimulant in Martinique. It was also reported that the roots of *O. majorana* were used in

decoction as an alternative. The leaves of *O. majorana* were reported to be used in headache and also found to possess significant anti-inflammatory activity in reducing paw oedema in rat model

The potential use of higher plants as a source of new drugs is still poorly explored. In most cases, only pharmacological screening or preliminary studies have been carried out and out of estimated only 5000 species have been studied for their medicinal use. [6] The hepatotoxicity of CCl₄ is due to the metabolic formation of the highly reactive trichloroethylene free radical which attacks the polyunsaturated fatty acids of the membrane of the endoplasmic reticulum and initiates a chain reaction. It is enhanced by induction of hepatic microsomal enzyme system and vice by antioxidants which move up the free radicals. The first cells to be debagged are those in the centrilobular region where microsomal enzyme activity is the greatest. The initial damage produced is highly localized in the endoplasmic reticulum which results in loss of Cytochrome P₄₅₀ leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty, a characteristic of CCl₄ poisoning. If the damage is severe, it leads to disturbances in the water and electrolyte balance of hepatocytes leading to an abnormal increase in liver enzymes in plasma, there by impairing mitochondrial functions, followed by hepatocellular necrosis. [7-8]

As natural product research continues to be an important part of the drug discovery, since ancient times, mankind has made use of plants in the treatment of various ailments because their toxicity factors. Recent interest in natural therapies and alternative medicines has made researchers pay attention to traditional herbal medicine. In the past decade, attention has been centred on scientific evaluation of traditional drugs with plant origin for the treatment of various diseases. Due to their effectiveness, with presumably minimal side effects in terms of treatment as well as relatively low costs, herbal drugs are widely prescribed; even when their biologically active constituents are not fully identified. We were developed interest in taking up the phytochemical investigation of selected plant species to explore hepatoprotective and the mechanism. There are a number of medicinal combinations in the traditional medicine which are commonly used as tonic for liver. In this study, we were focused on their hepatoprotective effects particularly against CCl₄.

MATERIALS AND METHOD

Plant Material

The roots of *C. epigaeus* were collected from Ananthagiri forest region, Visakhapatnam District, Andhra Pradesh, India in the months of March and May, 2006. The plant species were authenticated by Dr. M. Venkaiah, Taxonomist, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India. The Voucher specimens (TDR-BG- 24-07-2006)

were deposited in the institutional museum, College of Pharmaceutical Sciences, Andhra University, Visakhapatnam.

Extraction of the plant materials and sample preparation

The root materials were shade dried and were extracted in a Soxhlet apparatus successively with ethyl acetate and methanol. The solvent was removed by the process of distillation and the crude extract was dried under vacuum and stored in a dessicator prior to chromatographic separation. The extracts were subjected to anti-inflammatory, hepatoprotective and anti-microbial activities, the extract producing significant activity was Column chromatographed.

Preliminary extract screening

The extract was preliminarily screened using thin layer chromatography (TLC) and nuclear magnetic resonance (NMR) to know the types of compounds present in the extracts. TLC was developed in n hexane: ethyl acetate solvent systems of different polarities and then plates were visualized under Iodine vapour exposure, UV short wave 254 nm and long wave 366 nm. Plates were sprayed with a solution of visualizing reagent 10% H₂SO₄ in methanol followed by heating in an oven at 110°C for up to five minutes. The compounds develop various colours with this reagent.

Chromatographic techniques

The Column chromatography was done by standard procedure silica gel (400 g, finer than 200#, ACME) was used as adsorbent. The column was eluted with n-hexane, ethyl acetate and finally with methanol. Thin-layer chromatography was simultaneously used to identify and further separate compounds from the fraction using the same solvent system. The developing reagent is 10% H₂SO₄ in methanol.

Experimental Animals

Wistar albino rats of either sex weighing between 150-200 g were obtained from National Institute of Nutrition, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of 25 ± 2°C with an alternating 12 h light-dark cycle and relative humidity of 50 ± 15%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and by the Regulatory body of the government (Reg. no. 516/01/A/CPCSEA). They were fed with standard laboratory diet (supplied by Ratan Brothers, India) and water *ad libitum* during the experiment.

Experimental Design

The rats were given doses orally with extracts at different dose as mentioned below. Group I normal rats treated with Drug vehicle (1% Sodium CMC) and served as normal control, Group II rats were treated with hepatogens and Group III rats were treated with the standard drug Silymarin at 25 mg/kg body weight. All the doses were administered orally according to the body weight of the animals.

The extracts were administered orally in the following order Group-IV Received methanolic extract of *O. majorana* 200 mg/kg, Group-V Received methanolic extract of *O. majorana* 400 mg/kg, Group-VI Received methanolic extract of *O. majorana* 800 mg/kg. Group-VII Received ethyl acetate extract of *O. majorana* 200 mg/kg, Group-VIII Received ethyl acetate extract of *O. majorana* 400 mg/kg, Group-IX Received ethyl acetate extract of *O. majorana* 800 mg/kg.

CCl₄ induced hepatotoxicity: Carbon tetrachloride (CCl₄): 50% v/v solution of carbon tetrachloride was prepared in liquid paraffin. The solution was administered at the dose of 1.25 ml/kg b. wt. *i.p.* Methanolic and Ethyl acetate extracts obtained from roots of *O. majorana in vivo* on preliminary basis, against CCl₄ induced toxicity by assessing them through biochemical parameters. Each set of experiment was divided into groups consisting of control, toxicant, standard, and test. Groups consisted of 5 rats each unless otherwise mentioned. The protocol followed for CCl₄ induced hepatotoxicity on preliminary basis [9] was given below.

The protocol for CCl₄ induced hepatotoxicity

Group	0 h	24 h	48 h	72 h
Control	Vehicle	Vehicle	Vehicle	
CCl ₄	Vehicle	Vehicle + CCl ₄	Vehicle	With
Silymarin	Silymarin	Silymarin + CCl ₄	Silymarin	drawl of blood
Test	Extract	Extract + CCl ₄	extract	

Vehicle: 1% Sodium CMC, Test: Extracts prepared in 1% Sodium CMC.

The rats of control group received three doses of 1% Sodium CMC (1 ml/kg *p.o.*) at 24 h intervals (0 h, 24 h and 48 h). The animals in CCl₄ treated group received vehicle at 0 h vehicle followed by followed by CCl₄ diluted in liquid paraffin (1:1 *i.p.*) at a dose of 1.25 ml/kg, while at 48 h these animals received only vehicle. The test groups received the first dose of extracts at 0 h, second dose of extracts at 24 h, which was followed by a dose of CCl₄ and at 48 h the third dose of extracts. The positive control group received the first dose of silymarin (25 mg/kg) at 0 h, second dose of silymarin at 24 h followed by a dose of CCl₄ and at 48 h the third dose of silymarin. After 72 h blood was collected from all the groups, allowed to clot for the separation of serum. The serum was used for estimation of biochemical parameters. Serum Glutamic oxaloacetic transaminase (SGOT), serum Glutamic pyruvic transaminase (SGPT) was estimated by a UV - Kinetic method based on the reference method of international federation of clinical chemistry. [10] Alkaline phosphatase (ALKP) was estimated method by PNPP method [11], while total bilirubin (TBL) by jendrassik and grof method [12], total cholesterol (CHL) by CHOD - PAP method [13], total protein (TPTN) by color complexation with copper ions in an alkali solution. [14] Albumin was estimated by bromo cresol green method. [15] All the estimations were carried out

using standard kits on auto analyser of Merck make (300 TX, E. Merck-Micro Labs, Mumbai).

Statistical Analysis

The results are expressed as mean ± S.D from n=5 rats in each group. The significance of difference among the groups was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test.

RESULTS

Preliminary phytochemical analysis of the crude extracts was conducted according to the standard procedures and the results are tabulated.

The methanolic and ethyl acetate extracts were concentrated at low temperature (40-50°C) under reduced pressure. The ethyl acetate fractions of *O. majorana* were subjected to column chromatography, separately over silica gel column and the results are shown in Table. The fractions were monitored by using silica gel TLC and the fractions showing similar spots were mixed together.

Table 1: Percentage of the extractives obtained

S. No	Name of the plant Part	weight of the powdered material (kg)	% of Ethyl acetate Soluble extractives	% of methanol soluble extractives
1	<i>O. majorana</i> roots	2.0	8.50	6.50

Table 2: Preliminary phytochemical analysis of the crude extracts

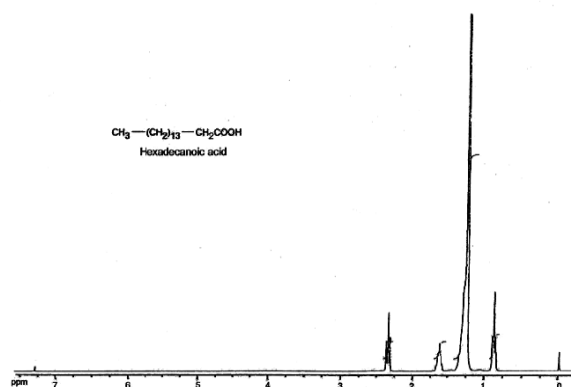
Extract	FeCl ₃	L. B reaction	Shinoda
<i>O. majorana</i> methanolic extract	+ve	+ve	-ve
<i>O. majorana</i> ethyl acetate extract	+ve	+ve	-ve

Table 3: Chromatography of the ethyl acetate fraction of *O. majorana* roots (OM)

Weight of extract taken	20 g
Weight of silica gel (100-200 mesh) used for adsorption	40 g
Column dimensions	60 mm × 210 cm
Weight of silica gel (100-200 mesh) used for packing the column	500 g
Volume of each fraction collected	500 ml

Fraction No.	Eluant Composition	Weight of residue (g)	Compound Isolated
1-10	Pure hexane	1.380	Waxy residue
11-28	5% ethyl acetate in hexane	0.891	OM-1
29-40	10% ethyl acetate in hexane	0.367	Yellow residue
41-56	15% ethyl acetate in hexane	0.103	-
57-64	20% ethyl acetate in hexane	0.364	White crystalline residue
65-81	25% ethyl acetate in hexane	0.354	--
82-89	30% ethyl acetate in hexane	0.720	Yellow residue
90-100	40% ethyl acetate in hexane	0.028	Mixture
101-110	50% ethyl acetate in hexane	0.921	Reddish brown residue
111-122	60% ethyl acetate in hexane	0.020	Mixture
123-132	70% ethyl acetate in hexane	1.120	Reddish brown

	hexane	residue	
133-142	90% ethyl acetate in hexane	0.974	Dark brownish residue
143-152	100% ethyl acetate	1.895	Intangible mass
153-164	5% methanol in ethyl acetate	3.52	Sticky matter
165-174	10% methanol in ethyl acetate	0.210	Mixture
175-183	30% methanol in ethyl acetate	0.982	Reddish residue
184-192	50% methanol in ethyl acetate	0.700	Reddish brown
193-201	70% methanol in ethyl acetate	2.152	Greenish colouring
202-210	100% methanol	3.163	Intangible mass



1H-NMR Spectrum of OM-1 (Hexadecanoic acid)

Structural Elucidation of Compound OM-1

The compound was obtained as colourless solid, m.p 60-62°C and its molecular formula C₁₆H₃₂O₂ was established by mass spectral data and elemental analysis. The IR spectrum (KBr) showed absorption bands for carbonyl (1712 cm⁻¹), hydroxyl (3350 - 3100 cm⁻¹) and long fatty alkyl chain methylene (2920 and 2840 cm⁻¹). The UV spectrum did not show any significant UV absorption above 200 nm. The ¹³C NMR spectrum showed resonances at 179.6 characteristic of an acid carbonyl and its adjacent methylene carbon at 34.1 followed by the second adjacent methylene carbon at 31.3, and the long chain methylene carbons appeared in the region 30.4-24.0 (several methylene carbons). The methylene carbon adjacent to the end methyl carbon appeared at 22.6 and the end methyl carbon appeared at 14.0. The ¹H NMR spectrum of the compound showed the signal at 2.49 (t, 2H, J=7.2 Hz) characteristic of methylene protons connected to an acid carbonyl function. The methylene protons of the fatty alkyl chain appeared at 1.74 (m, 2H) and 1.26 (br s, 24H) with an end methyl proton signal at 0.89 (t, 3H, J=7.1 Hz). The compound formed a monomethyl ester with an ethereal solution of diazomethane which indicated the presence of an acid function in the compound and the methoxy protons appeared at 3.66 (s, 3H) in ¹H NMR spectrum. The EIMS of the compound yielded the molecular ion peak at m/z 256 (M⁺) and the other fragment ions at m/z 228 (M⁺-CO) and 213 (MH⁺-CO₂), which indicated the presence of acid function in the molecule.

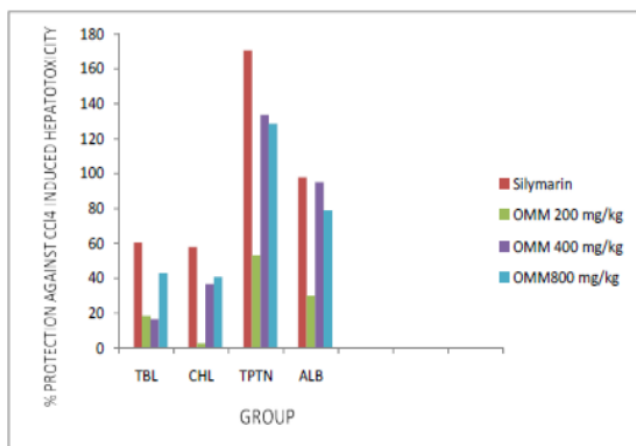


Fig. 1: Effect of the crude extract of OMM 200, 400 and 800 mg/kg along with silymarin 25 mg/kg body wt. on percentage protection of various biochemical parameters against CCl₄ induced hepatotoxicity.

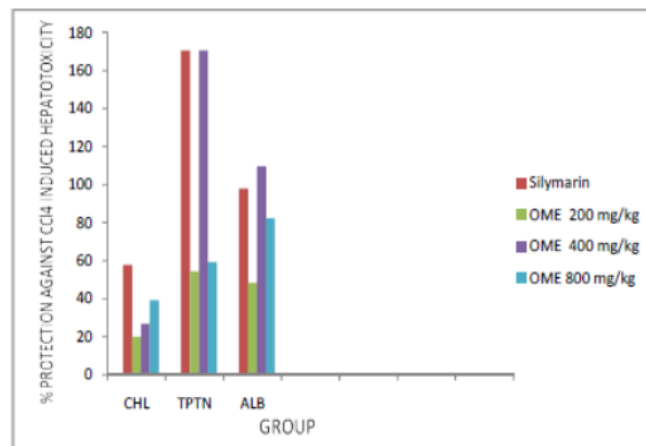


Fig. 2: Effect of the crude extract of OME 200, 400 and 800 mg/kg along with silymarin 25 mg/kg body wt. on percentage protection of various biochemical parameters against CCl₄ induced hepatotoxicity.

Table 4: Effect of Methanolic extracts of OM on CCl₄ induced hepatotoxicity in rats

Group	SGOT (IU/L)	SGPT (IU/L)	ALKP (IU/L)	TBL (mg/dl)	CHL (mg/dl)	TPTN (g/dl)	ALB (g/dl)
Control	108.4 ± 2.13	95.92 ± 3.23	218.60 ± 1.68	2.10 ± 0.18	110.6 ± 2.48	6.21 ± 0.12	3.90 ± 0.94
CCl ₄	315.4 ± 12.00	241.4 ± 4.58	428.1 ± 24.47	3.48 ± 0.68	270.7 ± 11.19	2.58 ± 0.82	1.98 ± 0.21
Silymarin	102.2 ± 1.71*	104.4 ± 0.8*	212.6 ± 1.68*	1.38 ± 11.19*	114.1 ± 0.42*	6.98 ± 0.17**	3.92 ± 0.18**
OMM 200 mg/kg	290.6 ± 5.31	251.7 ± 3.0	410.7 ± 2.99	2.84 ± 0.19	278.3 ± 4.94	3.95 ± 0.32	2.82 ± 0.48
OMM 400 mg/kg	132.6 ± 2.04*	132.0 ± 1.21*	271.6 ± 3.92*	2.9 ± 0.18*	171.4 ± 2.08*	6.03 ± 0.80***	3.86 ± 0.88**
OMM 800 mg/kg	112.7 ± 2.80*	110.2 ± 2.18*	252.6 ± 3.28*	1.99 ± 0.82*	160.2 ± 3.80*	5.90 ± 0.81***	3.54 ± 0.71**

Data expressed in mean ± SEM, n=5; Significant reduction compared to hepatotoxic group ***P<0.001; **P<0.01; * P<0.05

Table 5: Effect of Methanolic extracts of OM on Percentage protection against CCl₄ induced hepatotoxicity in rats

Group	SGOT	SGPT	ALKP	TBL	CHL	TPTN	ALB
Silymarin	67.59	56.62	50.33	60.34	57.85	170.54	97.98
OMM 200 mg/kg	7.86	4.26	4.06	18.39	2.80	53.10	29.78
OMM 400 mg/kg	57.95	45.31	36.55	16.66	36.68	133.72	94.94
OMM 800 mg/kg	64.26	54.35	40.99	42.81	40.79	128.68	78.78

Table 6: Effect of Ethyl acetate extracts of OM on CCl₄ induced hepatotoxicity in rats

Group	SGOT (IU/L)	SGPT (IU/L)	ALKP (IU/L)	TBL (mg/dl)	CHL (mg/dl)	TPTN (g/dl)	ALB (g/dl)
Control	108.4 ± 2.13	95.92 ± 3.23	218.60 ± 1.68	2.10 ± 0.18	110.6 ± 2.48	6.21 ± 0.12	3.90 ± 0.94
CCl ₄	315.4 ± 12.00	241.4 ± 4.58	428.1 ± 24.47	3.48 ± 0.68	270.7 ± 11.19	2.58 ± 0.82	1.98 ± 0.21
Silymarin	102.2 ± 1.71*	104.4 ± 0.8*	212.6 ± 1.68*	1.38 ± 11.19*	114.1 ± 0.42*	6.98 ± 0.17**	3.92 ± 0.18**
OME 200 mg/kg	279.3 ± 3.10	223.7 ± 2.42	361.3 ± 18.90	2.86 ± 0.10	216.7 ± 2.72	3.98 ± 0.32	2.94 ± 0.82
OME 400 mg/kg	122.2 ± 2.68*	116.9 ± 2.35*	249.1 ± 2.37*	1.92 ± 0.91*	198.6 ± 3.8*	6.98 ± 0.22**	4.15 ± 0.53**
OME 800 mg/kg	110.3 ± 2.61*	108.4 ± 2.10*	234.6 ± 3.01*	1.36 ± 0.61*	164.7 ± 5.42*	6.31 ± 0.10**	3.61 ± 0.19**

Data expressed in mean ± SEM, n=5; Significant reduction compared to hepatotoxic group ***P<0.001; **P<0.01; * P<0.05

Table 7: Effect of Ethyl acetate extracts of OM on Percentage protection against CCl₄ induced hepatotoxicity in rats

Group	SGOT	SGPT	ALKP	TBL	CHL	TPTN	ALB
Silymarin	67.59	56.62	50.33	60.34	57.85	170.54	97.98
OME 200 mg/kg	11.44	7.208	15.60	17.81	19.94	54.26	48.48
OME 400 mg/kg	61.25	51.45	41.81	44.82	26.63	170.54	109.59
OME 800 mg/kg	65.02	55.09	44.49	60.92	39.15	59.11	82.32

The length of the long fatty alkyl chain was also confirmed by the presence of homologous sequence ions at m/z 143, 157, 171, 185, 213. Based on the above spectral data, the compound BH-1 was characterized as hexadecanoic acid. The compound was reported for the first time from this species. The spectral data was in good agreement with that of hexadecanoic acid and the identity was confirmed by comparison with an authentic sample of hexadecanoic acid (BH-1), (mixed m.p and Co-TLC).

Result Shows Silymarin the standard drug at the dose of 25 mg/kg significantly reduced the increased levels of SGOT, SGPT, ALKP, TBL and CHL with the values 102.2 ± 1.71, 104.4 ± 0.8, 212.6 ± 1.68, 1.38 ± 0.05, and 114.1 ± 0.42 respectively and increased the levels of TPTN and ALB 6.98 ± 0.17 and 3.92 ± 0.18 respectively Methanolic extract of *O. majorana* at 400 mg/kg produced 132.6 ± 2.04, 132.0 ± 1.21, 271.6 ± 3.92, 2.9 ± 0.18, 171.4 ± 2.08, 6.03 ± 0.80 and 3.86 ± 0.88, where as methanolic extract of *O. majorana* at 800 mg/kg

produced 112.7 ± 2.80 , 110.2 ± 2.18 , 252.6 ± 3.28 , 1.99 ± 0.82 , 160.2 ± 3.80 , 5.90 ± 0.81 and 3.54 ± 0.71 .

Result shows Silymarin the standard drug at the dose of 25 mg/kg significantly reduced the increased levels of SGOT, SGPT, ALKP, TBL and CHL with the values 102.2 ± 1.71 , 104.4 ± 0.8 , 212.6 ± 1.68 , 1.38 ± 0.05 , and 114.1 ± 0.42 respectively and increased the levels of TPTN and ALB 6.98 ± 0.17 and 3.92 ± 0.18 respectively. Ethyl acetate extract of *O. majorana* at 400 mg/kg produced 122.2 ± 2.68 , 116.9 ± 2.35 , 249.1 ± 2.37 , 1.92 ± 0.91 , 198.6 ± 3.8 , 6.98 ± 0.22 and 4.15 ± 0.53 , whereas ethyl acetate extract of *O. majorana* at 800 mg/kg produced 110.3 ± 2.61 , 108.4 ± 2.10 , 234.6 ± 3.01 , 1.36 ± 0.61 , 164.7 ± 5.42 , 6.31 ± 0.10 and 3.61 ± 0.19 .

DISCUSSION

Liver, the largest gland functioning as an organ of storage, manufacturing and biotransformation is a vulnerable target for injury. Chronic alcohol consumption, exposure to toxic chemicals and certain drugs like paracetamol, tetracycline, antitubercular drugs, chemotherapeutic agents, NSAIDS, damage the liver cells (hepatocytes) in long run. Drug induced liver injury is a major health problem, the manifestations of which are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant liver failure. Modern medicine has provided us many drugs that alleviate liver diseases but compared to it herbal medicine is preferred because the latter is cost effective and considered to be a safe approach for treatment with minimal side effects. [16] In present study results clearly depicted that CCl₄ intoxication in normal rats elevated the serum levels of SGOT, SGPT, ALKP, TBL and CHL, where as decreased the levels of TPTN, ALB significantly when compared to control indicating acute hepatocellular damage and biliary obstruction leading to necrosis. The rats treated with the methanolic extracts of *O. majorana* and silymarin showed a significant ($P < 0.05$) decrease in all the elevated SGOT, SGPT, ALKP, TBL, CHL and significant increase ($P < 0.05$) in TPTN and ALB levels at 400 and 800 mg/kg. The rats treated with the ethyl acetate extract of *O. majorana* and silymarin showed a significant ($P < 0.05$) decrease in all the elevated SGOT, SGPT, ALKP, TBL, CHL and significant increase ($P < 0.05$) in TPTN and ALB levels at 400 and 800 mg/kg. The percentage protection was greater in ethyl acetate treated groups when compared to the methanolic extract treated rats.

The crude extract from the selected plant produced significant hepatoprotective activity and ethyl acetate extract has produced significant percentage protection

when compared to the methanolic extracts. Ethyl acetate extract of *O. majorana* and silymarin showed a significant ($P < 0.05$) decrease in all the elevated SGOT, SGPT, ALKP, TBL, CHL and significant increase ($P < 0.05$) in TPTN and ALB levels at 400 and 800 mg/kg. The extract must be studied further for dose dependency, toxicity studies and mechanism of action should be established.

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