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Anti-Hepatotoxic Effect and Phytochemical Analysis of *Berberis Aristata* Bark Against Carbon Tetrachloride-Induced Hepatic Damage in Rats

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Abstract

Aim: To evaluate anti-hepatotoxic activity and phytochemical analysis of *Berberis aristata* bark against carbon tetrachloride induced hepatic damage in rats. **Method:** The petroleum ether, ethyl acetate, chloroform, n-butanol, and ethanol extracts of *Berberis aristata* bark were evaluated for its anti-hepatotoxic activity using carbon tetrachloride induced hepatic damage in rats. Hepatoprotective activity was evaluated by measuring reduction in levels of serum marker enzymes namely serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and alkaline phosphatase after carbon tetrachloride treatment. **Result:** Carbon tetrachloride intoxication has resulted in increased serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and alkaline phosphatase levels. Among five different extracts treated groups, petroleum ether extract treated group has shown significant reduction in levels of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and alkaline phosphatase. **Conclusion:** The hepatoprotective activity of petroleum ether extract of the *Berberis aristata* bark may be due to presence of berberine or other alkaloids in combination.

Keywords
Berberis aristata, carbon tetrachloride, hepatoprotective activity, histopathological evaluation.

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INTRODUCTION

Plants constitute one of the major raw materials for drugs for treating various ailments of human being. Moreover, modern medicine does not have a suitable answer for many conditions such as liver

disorder and chronic conditions like asthma, arthritis etc. and this leads to increased interest in herbal drugs [1]. The herbal drugs used in Indian System of medicine are however, claimed to be effective and safe in liver ailments. Hepatoprotective, a class of therapeutic agents that includes many synthetic as well as natural products used to protect against hepatic damage induced by various toxins. Natural resources such as plants are always considered and used to explore new molecules for the therapeutic purposes. Hepatoprotective plants have an important place in the literature of Indian System of Medicine (ISM). Many plants mentioned in ISM are used either alone or in combination as a hepatoprotective.

Berberis aristata DC. (Berberidaceae) an erect, spine scent shrub, found in Himalayas from Garhwal to Bhutan, at altitude of 1800-3000m. The Plant is used as antiarthritic [2], antimicrobial [3-5], antiulcerogenic, antidiabetic, antidysentric, in ophthalmic, skin diseases [6]. The stem is used in amatisara (diarrhoea), medoroga (obesity), urustambha (stillness/loss of movement of legs), kapharoga (diseases due to excessive phlegm), karnaroga (diseases of the ear), mukharoga (diseases of oral cavity), netraroga (ophthalmic diseases), kandu (itching), vrana (wounds) and meha (diabetes) [7]. Decoction of root bark is used in malarial fever [8]. The root bark is employed in ophthalmic diseases, skin diseases and boils haemorrhoids, to wash ulcers and wounds in liver complaints [9], as antidiarrhoeal, in ear troubles backache and as a general tonic [10], as antihyperglycemic and antioxidant [11]. The *Berberis aristata* leaves are used to prevent acetaminophen-induced liver damage [12]. The alcoholic extract of the root inhibited the PAF-induced aggregation of rabbit platelets in a dose dependent manner [13].

The flowers were reported to yield five polyphenolic compounds, namely E-caffeic acid, quercetin, chlorogenic acid, meratin and rutin [14]. The bark afforded berberine chloride, palmatine chloride and a mixture of palamatine and bebrine chlorides [15]. The root showed presence of alkaloids, flavonoids, glycosides, saponins and sterol and absence of terpenoids [16]. Another study, however, reported the presence of alkaloids and tannins in the root but flavonoids and saponins were reported to be absent [17]. However, there is no report on hepatoprotective effect of the *Berberis aristata* bark. The present study deals with the levels of serum marker enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) for the prevention of carbon tetrachloride (CCl_4) induced hepatotoxicity by *Berberis aristata* bark extracts.

MATERIALS AND METHODS

Plant material

The powder of *Berberis aristata* bark was procured from local Herbal Supplier. The bark of plant was authenticated from Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. After authentication, barks of *B. aristata* deposited in the herbarium of the department of botany and were further subjected to observation of various parameters and extraction.

Chemicals

Carbon tetrachloride (S.D. Fine Chem Ltd., Mumbai), Silybon-140 (Micro Labs Ltd. Bangalore), Liv. 52 (Himalaya Drugs Company, India), Thiobarbituric acid (Loba Chemie Ltd., Mumbai), Trichloro acetic acid (NICE Laboratories Reagent), 5-5' Dithiobis-2-Nitro benzoic acid (Span Diagnostic, Surat), Glutathione standard (GSH) (Span Diagnostic, Surat), Disodium ethylene tetra acetic acid (EDTA) (S.D. Fine Chem Ltd., Mumbai), Meta phosphoric acid (Loba Chemie. Ltd, Mumbai), Sodium dihydrogen orthophosphate (Merck Ltd., Mumbai), Disodium hydrogen ortho phosphate (Merck Ltd., Mumbai), Tris-HCl (Qualigens, Excela R), Hydrogen Peroxide (H_2O_2) (S.D. Fine Chem Ltd., Mumbai), β-Mercapto Ethanol (E-Merck), Drabkin's reagent (Span Diagnostic, Surat), Cyammethemoglobin standard (Span Diagnostic, Surat), Estimation Kit (GOT & GPT) GOT Kit – Dr. Reddy's Laboratories, Hyderabad, GPT Kit – Span Diagnostic Ltd., Surat.

Preparation of extracts

After physical evaluation, powder of *Berberis aristata* bark weighing about 1480 gms was used for extraction with each solvent. This powder was divided into eight batches of approximately 180 gms in each batch. All the batches were exhaustively extracted with Pet. Ether (40^{o} – 60^{o}C) for defatting and then followed by ethyl acetate, chloroform, n-butanol, and ethanol using a Soxhlet apparatus successively. After each extraction the solvent was distilled off under reduced pressure by rotavapor vacuum flash evaporator to obtain respective extracts. The percent yields were noted for further studies.

Experimental animals

Albino rats (Wister strain) of either sex weighing 150-180 gms were used for the study. They were fed with a standard pellet diet and water ad libitum. Before their use in the experiment, the rats were kept in standard environmental conditions (25–28°C, 60–70% relative humidity and 12/12 h light/dark cycle).

Before conducting the experiment, ethical clearance was obtained from Institutional Animal Ethics Committee, Department of Chemcial Technology, Dr.Babasaheb Ambedkar Marathwada University, Aurangabad. (CPSCEA Reg.No.372/01/a/CPCSEA)

Acute Oral Toxicity – Acute Toxic Class Method

Toxicity testing was carried out according to OECD guidelines [18]. For acute toxicity studies, the Albino rats (Wister strain) of either sex weighing 150-180 gms totally two groups, each groups consists of three animals were used and they were treated with a single dose by gauage using a stomach tube to determine LD_{50} of various extracts. After treatment, the animals were observed for behavior changes and their mortality. From the study it was revealed that the extracts were found to be safe up to 2000 mg/kg body weight since there was no mortality. Hepatoprotective study was carried out by selecting doses as $1/10^{th}$ of the end point dose [19].

Hepatoprotective activity

Assessment of the hepatoprotective activity was done according to reported method [19]. Silymarin was taken as standard drug and Liv. 52 were used as reference drug. Animals were divided into nine groups each containing six animals with equal gender population as follows:

Group I: Served as Control and received single daily dose of liquid paraffin 1ml/kg b.w. I.P. for 10 days.

Group II: Served as positive control received 30% CCl_4 in liquid paraffin in dose of 1ml/kg b.w. I.P. for initial six days.

Group III: Was given 30% CCl_4 in liquid paraffin in dose of 1ml/kg b.w. I.P. for initial six days and Standard drug Liv. 52 in dose of 100mg/kg b.w. I.P. for the remaining days.

Group IV: Was given 30% CCl_4 in liquid paraffin in dose of 1ml/kg b.w. I.P. for initial six days and Standard drug Silymarin (Silybion) in dose of 100mg/kg b.w. I.P. for the remaining days of activity.

Group V: Treated with 30% CCl_4 in liquid paraffin in dose of 1ml/kg b.w. I.P. for initial six days and Petroleum Ether extract in dose of 125mg/kg b.w. I.P. once a day.

Group VI: Treated with 30% CCl_4 in liquid paraffin in dose of 1ml/kg b.w. I.P. for initial six days and Chloroform extract in dose of 150mg/kg b.w. I.P. once a day.

Group VII: Treated with 30% CCl_4 in liquid paraffin in dose of 1ml/kg b.w. I.P. for initial six days and Ethyl acetate extract in dose of 150mg/kg b.w. I.P. once a day.

Group VIII: Treated with 30% CCl_4 in liquid paraffin in dose of 1ml/kg b.w. I.P. for initial six days and Butanol extract in dose of 150mg/kg b.w. I.P. once a day.

Group IX: Treated with 30% CCl_4 in liquid paraffin in dose of 1ml/kg b.w. I.P. for initial six days and Ethanol extract in dose of 150mg/kg b.w. I.P. once a day.

Treatment duration was twelve days and the doses of CCl_4 were administered after every 24 hrs. The blood was collected on 11^{th} day of treatment from all animals by retro orbital method and subjected to different biochemical parameters.

Estimation of Biochemical Parameters

The rats were sacrificed 24 h after the administration of last dose under anesthesia using thiopentone sodium (35 g/kg b.w.i.p). The blood was collected and allowed to stand for 30 min at room temperature and then centrifuged to separate the serum. The separated serum was estimated for various biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT) [20], serum glutamate pyruvate transaminase (SGPT) [19], Serum Alkaline Phosphatase (ALP) [21, 22].

Statistical analysis

The mean value ± SEM was calculated for each parameter, each parameter was analyzed separately using ANOVA followed by Dunnets't' test. One-way ANOVA was applied to determine the significance of the difference between the control groups and rat treated with the test compounds.

Histopathological Studies

Liver was rapidly excised immediately after sacrifice. Liver was washed with normal saline (0.9%) and fixed in formalin (10%), serially sectioned and microscopically examined after staining with hematoxylin and eosin [23].

Qualitative Chemical Investigation

General qualitative chemical analysis was conducted to test the presence of various chemical components in the extract. The results obtained are tabulated in Table 1. The Hepatoprotective studies of different extracts obtained from *Berberis aristata* bark revealed that pet. Ether extract of *Berberis aristata* bark showed more significant activity; hence this extracts was taken for further phytochemical studies. The unsaponifiable matter of pet. Ether extracts was determined as per the method [24] and the %yield was recorded as 0.9gms.

High performance thin layer chromatography (HPTLC) study

High performance thin layer chromatography was performed on CAMAG TLC Sacnner-3 at 254nm and 580nm. The unsaponifiable matter of pet. ether extracts of *Berberis aristata* bark was taken for the study.

Five distinct spots were observed on the TLC Plate at a wavelength of 254 nm (Table No. 2) while seven distinct spots were observed at 580nm on TLC (Table No.3). The Rf values of these compounds are as listed in Table 2 and 3.

GC-MS Analysis

The unsaponifiable matter was separated from petroleum ether extracts of *Berberis aristata* it was utilized for further phytochemical analysis. GC-MS analysis of unsaponifiable matter of pet ether extract of *Berberis aristata* was performed on Shimadzu QP 5050 Machine (Figure 1). Library data of GC-MS confirmed presence of seven compounds (Table No. 4).

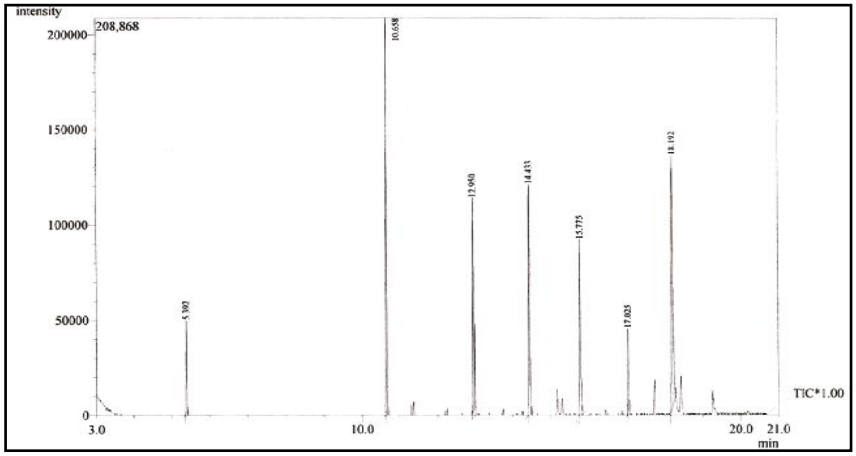


Figure 1: GC-MS profile of pet. Ether extract of *Berberis aristata* bark

HPLC analysis

The pet. ether extracts of *Berberis aristata* was taken for high performance thin layer chromatography. The sample was dissolved in 50% acetonitrile; TFA and 20µl sample is injected in C_{18} column of HPLC and numbers of peaks were observed.

The high performance liquid chromatography performed with unsaponified matter of petroleum ether extract showed 15 peaks having different retention time and their corresponding concentration (Table No. 5). The above results showed that these 15 peaks observed in the chromatogram are corresponding to 15 different compounds present in the unsaponified matter of petroleum ether extract of *Berberis aristata*.

Table No. 1: Qualitative chemical Investigation of *Berberis aristata* Bark.

Name of the Test	1	2	3	4	5
Test for Sterols					
01 Salkowski's Test	+	+	-	-	-
02 Libermann Buchardt's Test	+	+	-	-	-
03 Libermann's Test	+	+	-	-	-
Tests for Glycosides					
01 Baljets Test	-	-	-	+	+
02 Keller-Killani Test	-	-	-	+	+
03 Raymond's Test	-	-	-	+	+
04 Bromine Water Test	-	-	-	+	+
05 Legal's Test	-	-	-	+	+
Tests for Saponins					
01 Foam Test	+	+	+	+	+
02 Haemolysis Test	+	+	+	+	+
Tests for Carbohydrates					
01 Molisch's Test	-	-	-	-	-
02 Barfoed's Test	-	-	-	-	-

Name of the Test		1	2	3	4	5
03	Benedict's Test	-	-	-	-	-
04	Fehling's Test	-	-	-	-	-
Tests for Alkaloids						
01	Mayer's Test	-	+	-	-	+
02	Wagner's Test	-	+	-	-	+
03	Dragendorff's Test	-	+	-	-	+
04	Hager's Test	-	+	-	-	+
Tests for Flavonoids						
01	Ferric Chloride Test	-	-	+	-	-
02	Shinoda Test	-	-	+	-	-
03	Zn-HCl reduction Test	-	-	+	-	-
04	Alkaline reagent Test	-	-	+	-	-
05	Lead acetate Test	-	-	+	-	-

1: Pet. Ether extract, 2: Chloroform extract, 3: Ethyl acetate extract, 4: n-Butanol extract and 5: Ethanol extract.

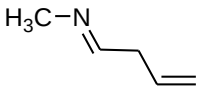
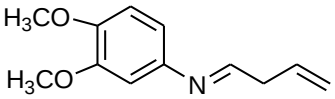
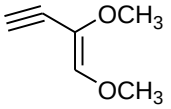
Table No. 2: - HPTLC analysis of unsaponified matter of Pet. ether extract of *Berberis aristata* at 254 nm

Peak. No.	Rf value	Percent Composition
01	0.13	2.09
02	0.19	2.04
03	0.31	4.72
04	0.50	19.23
05	0.76	9.49

Table No. 3: - HPTLC analysis of unsaponified matter of Pet. ether extract of *Berberis aristata* at 580nm

Peak. No.	Rf value	Percent Composition
01	0.13	0.63
02	0.16	1.53
03	0.30	3.29
04	0.43	5.31
05	0.57	16.24
06	0.66	18.35
07	0.79	20.46

Table No. 4: - GC-MS analysis of unsaponified matter of Pet. ether extract of *Berberis aristata*

Sr. No.	Name of compound	Structure
01.	N-(But-3-enylidene)-methanamine	
02.	N-(But-3-enylidene)-3,4-dimethoxybenzenamine	
03.	1,2-Dimethoxy but-1-en-3-yne	

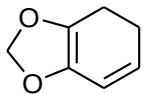
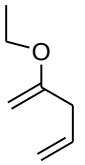
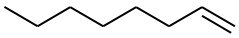
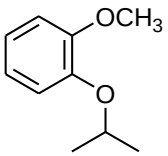
Sr. No.	Name of compound	Structure
04.	4,5-Dihydrobenzo-[1,3]-dioxole	
05.	2-Ethoxy penta-1,4-diene	
06.	n-Oct-1-ene	
07.	Isopropoxy-2-methoxybenzene	

Table No. 5: HPLC analysis of unsaponified matter of pet. Ether extract of *Berberis aristata*

Peak No.	Retention Time	% Composition
01	3.685	19.325
02	4.110	3.687
03	4.377	34.280
04	4.976	3.1932
05	6.301	2.062
06	6.806	6.629
07	7.926	1.192
08	9.199	2.432
09	9.438	3.373
10	9.893	0.745
11	10.650	1.520
12	10.976	0.908
13	11.312	12.371
14	11.936	1.422
15	17.290	6.855

Table No. 6: Effect of various solvent extracts of *Berberis aristata* bark on liver enzymatic levels in CCL_4 induced acute hepatic injury in rats.

Gr. No	Groups	Dose (mg/kg B.W)	SGOT (IU/L)	SGPT (IU/L)	ALP (KA/Units/100 ml)
I	Normal	-	150.66 ± 1.96	64.83 ± 2.04	46.83 ± 2.27
II	CCL_4 Control	-	284.16 ± 3.55	240.5 ± 1.94	89.33 ± 2.75
III	Liv.52 treated	100	251 ± 1.57	151.5 ± 1.64	59.5 ± 1.64
IV	Silymarin treated	100	236.83 ± 2.05	120.83 ± 1.44	51.83 ±1.42
V	Pet. Ether extract treated	125	159.83 ± 1.10	70.83 ± 1.014	58.5 ± 1.89
VI	Chloroform extract treated	150	167.5 ± 1.25	113.16 ±1.195	64.66 ± 1.02

Gr. No	Groups	Dose (mg/kg B.W)	SGOT (IU/L)	SGPT (IU/L)	ALP (KA/Units/100 ml)
VII	Ethyl acetate extract treated	150	198.16 ± 1.32	133.5 ± 2.045	70.5 ± 1.33
VIII	Butanol extract treated	150	214.66 ± 1.49	156.5 ± 1.408	75.16 ± 1.83
IX	Ethanol extract treated	150	181.5 ± 1.28	121.5 ± 1.78	65.83 ± 1.42

RESULTS AND DISCUSSION

To study the hepatoprotective effects we used the well-described CCl_4- model of rat liver fibrosis [25], in which the liver microsomal oxidizing systems connected with cytochrome P-450 produce reactive metabolites of CCL_4 such as trichloromethyl radical (CCL_3) or trichloroperoxyl radical (CCl_3O_3). These radicals cause lipid peroxidation, which produces hepatocellular damage and enhanced production of fibrotic tissue. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals [26].

Carbon tetrachloride intoxication in normal rats elevated the levels of SGPT, SGOT and ALP significantly indicating acute hepatocellular damage and biliary obstruction.

CCl_4 induced hepatotoxicity

Assessment of the hepatoprotective activity was done according to reported method [19]. Silymarin was taken as standard drug and Liv 52 was used as reference drug.

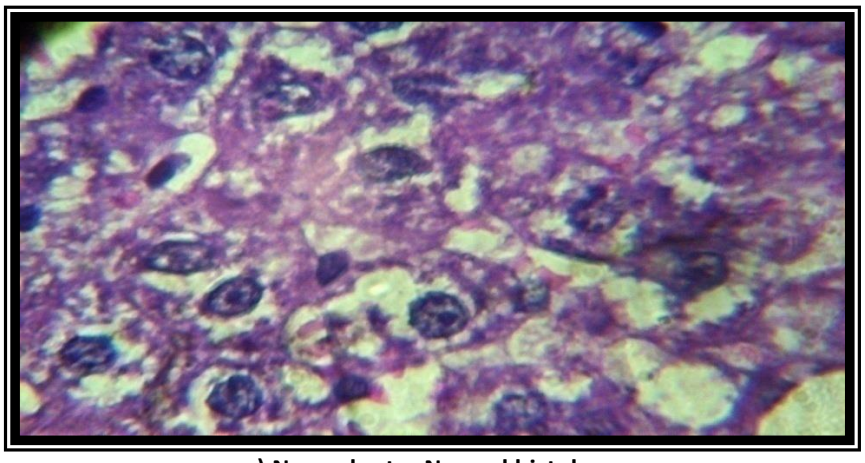
The results obtained from enzyme levels indicate that CCL_4 intoxication has resulted in increased SGOT and SGPT and ALP levels in treated group than the control group. The chloroform and Pet. Ether extract treated groups have shown reduced levels of SGOT and SGPT and ALP when compared to other extracts of the same plant. The enzymatic levels of SGOT, SGPT and ALT are tabulated in Table 6.

Mean values are ± SEM., n = 6 animals in each group. Comparison was made between: Group I vs. others. Statistical significance: $p < 0.001$

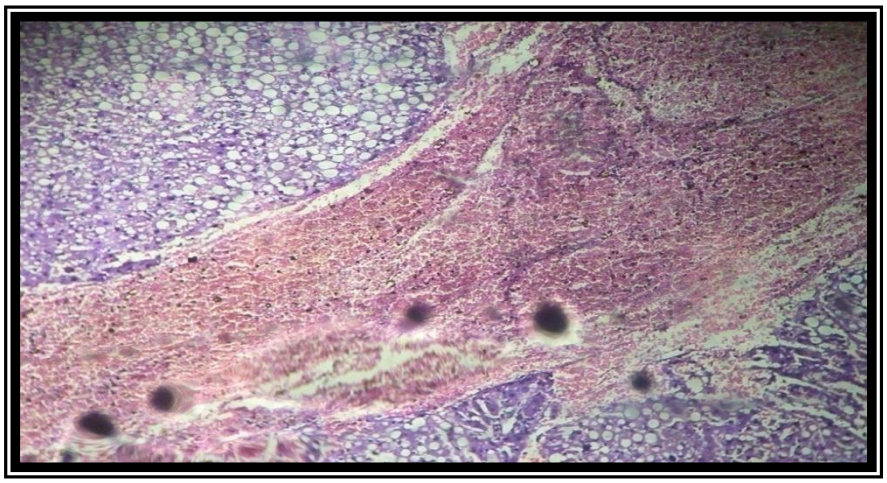
Histopathological evaluation

The microscopic histopathological evaluation of livers treated with various extracts and standards have revealed that the Pet. Ether extract of *Berberis aristata* bark were found to be of well preserved architecture and hepatocytes in normal pattern and did not show any degenerative changes. While the microscopic histopathological evaluation of marketed preparation like Liv.52 has shown normal hepatocytes in chords, normal sinusoids and normal kuffer cells.

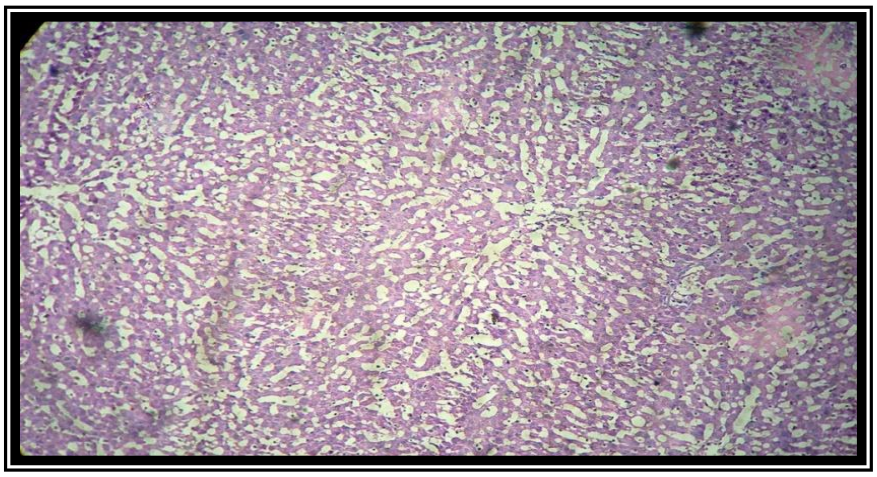
The details of histopathological observations of various extracts and standard drugs are indicated in Figure (a) to (i).



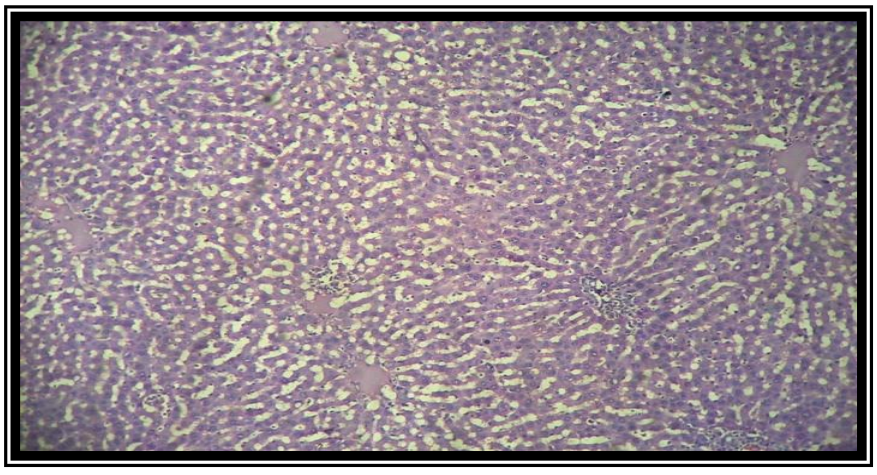
a) Normal rat: - Normal histology



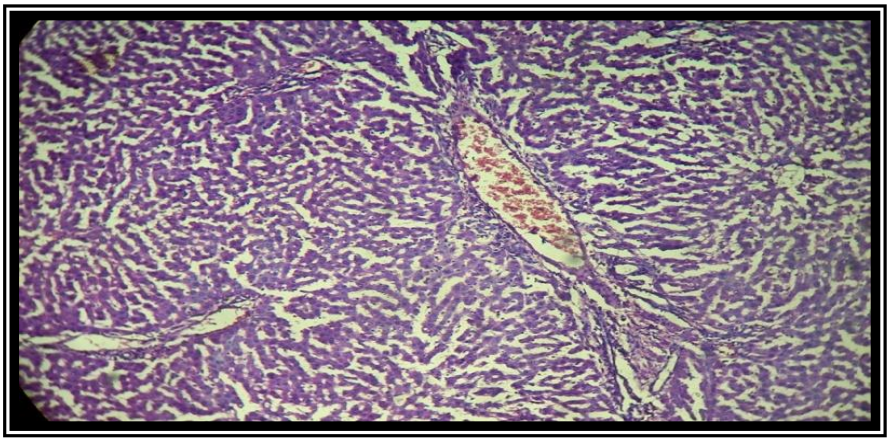
b) CCL_4 Control: - Marked congestion with diffused fatty infiltration of hepatocytes



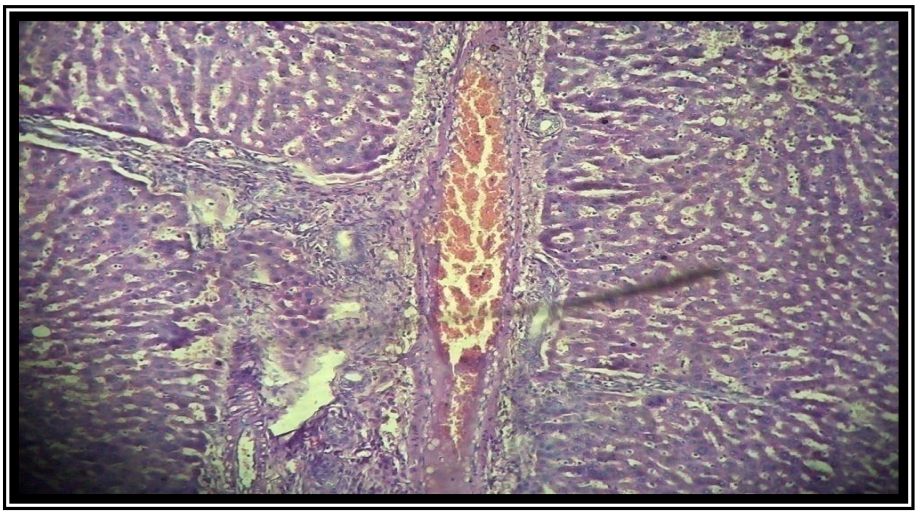
c) Liv.52 treated: - Centrilobular necrosis with defused mild fatty changes.



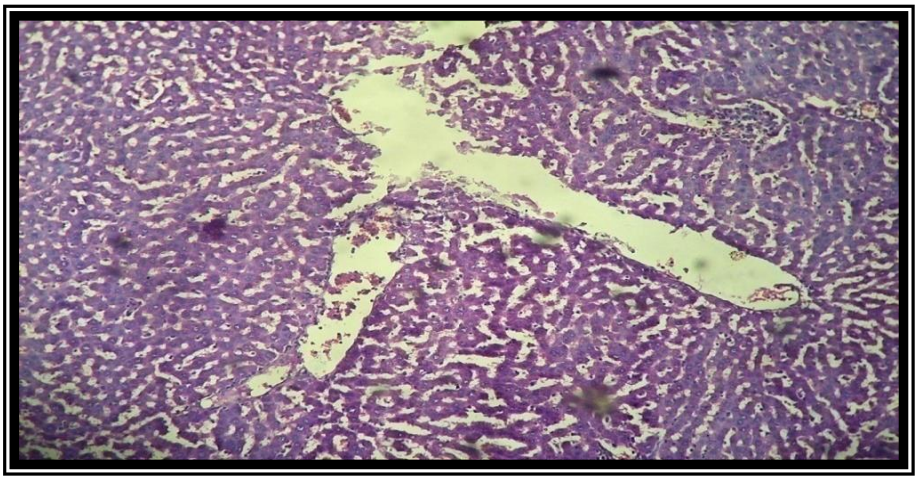
d) Silymarin treated: - Centrilobular necrosis with space round cells infiltration, sinusoids congested and mild fatty changes.



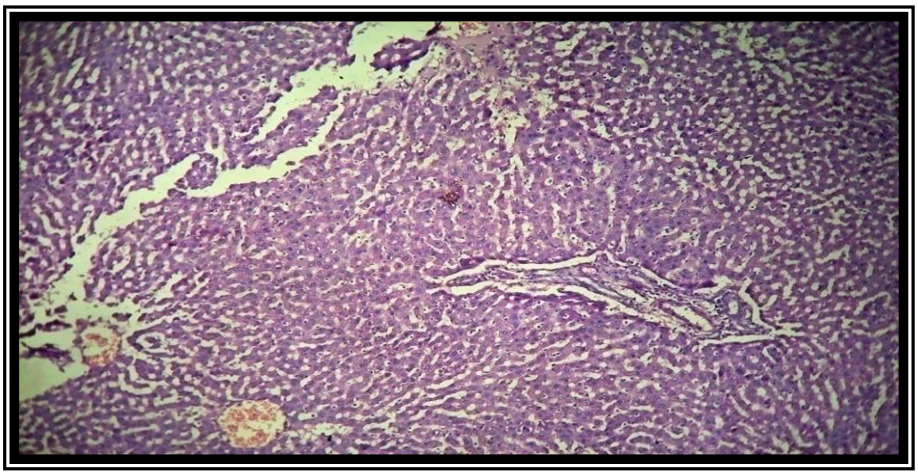
e) *Berberis aristata* Pet. Ether extract treated: - Congestion of hepatic artery with mild round cell infiltration in portal track.



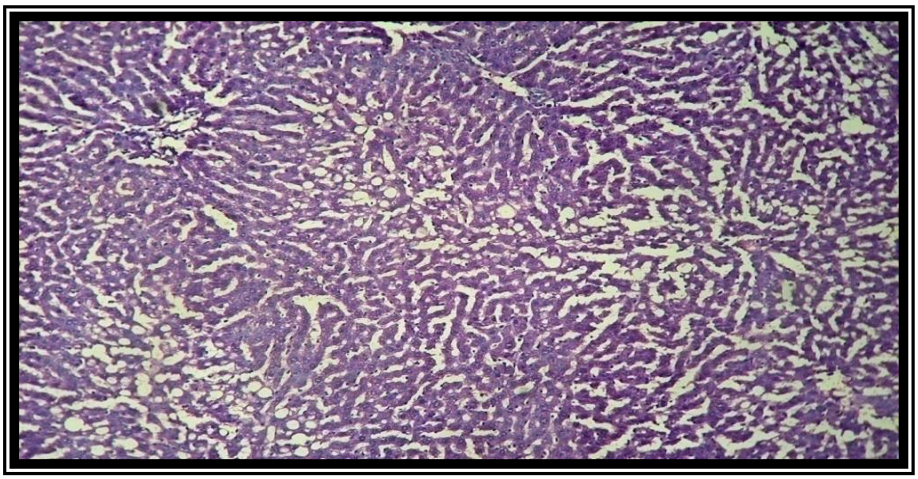
f) *Berberis aristata* Chloroform extract treated: - Congestion of hepatic artery, mild periportal fibrosis, and sinusoids congested.



g*) Berberis aristata* Ethyl acetate extract treated: - Mild congestion, mild portal triaditis.



h) *Berberis aristata* Butanol extract treated: - Marked congestion, tiny focus of hemorrhage and necrosis in parenchyma.



i) *Berberis aristata* Ethanol extract treated: - Marked centriolobular fatty change of fats.

CONCLUSION

The plant extract is reported to produce a long lasting, dose dependant decrease in blood pressure. The plant is also reported to be used as an intestinal antiseptic and bitter stomachic. It exhibits antineoplastic properties in addition to its hepatoprotective activity [24]. Since the traditional use of *Berberis aristata,* as hepatoprotective is well defined, this study was given a scientific base for the utility of *Berberis aristata* as a hepatoprotective.

A comparative histopathological study of liver from different experimental groups from these five extracts further corroborates the hepatoprotective efficacy of *Berberis aristata* bark. It is therefore suggested that petroleum ether extract of *Berberis aristata* showed significant results when compared with standard and reference groups. The extract showed rebuilding the hepatic damage, which was induced by CCl_4 and could prevent hepatobiliary damage in rats. The observation of a significant corrective effect of extracts from above plant part on biochemical parameters was supported by histopathological examination [25, 26, 27-29].

The phytochemical profile of the plant *Berberis aristata* of the family Berberidaceae shows the isoquinoline alkaloid berberine as the major bioactive constituent. Other alkaloids include berbamine, aromoline, karachine, palmitine, oxycanthine and oxyberberine [30]. The most bioactive constituent berberine [31] might be responsible for the protection of hepatic tissues. The hepatic protection of this plant may be due to berberine or combine effect of alkaloids present in chloroform extract of the stem.

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