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Research Article

EVALUATION OF THE HEPATOPROTECTIVE AND ANTIOXIDANT EFFECT OF *BERBERIS ASIATICA* AGAINST EXPERIMENTALLY INDUCED LIVER INJURY IN RATS

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ABSTRACT

Berberis asiatica roots have been used for the treatment of affection of eyes, skin, rheumatism and jaundice could be a substitute for endangered species *Berberis aristata* exhaustively used by industries and in ayurvedic system of medicine. The present investigation describes hepatoprotective and antioxidant activities of dried aerial parts of *Berberis asiatica*, aqueous (AqBA) and methanol (MeBA) extracts, against CCl_4 –induced hepatic injury. Hepatic injury was achieved by injecting 2ml/kg s.c of CCl_4 in equal proportion with olive oil. AqBA and MeBA at dose levels of 200 and 300 mg/kg offered significant (*P<0.001*) hepatoprotective action by reducing the serum marker enzymes like serum glutamate oxaloacetate (SGOT), serum glutamate transaminase (SGPT). They also reduced the elevated level of serum alkaline phosphatase (ALP) serum acid phosphatase (ACP) and serum bilirubin. Reduced enzymic and non nonenzymic antioxidant levels and elevated lipid peroxide level were restored to normal by administration of MeBA and AqBA. Histopathological studies further confirmed the hepatoprotective activity of these extracts when compared with CCl_4 treated control groups. The result obtained were compared with silymarin (100mg/kg; p.o), the standard drug. In conclusion MeBA extract at (200mg/kg, p.o) showed significant *p<0.001* hepatoprotective activity similar to that standard drug, silymarin.

Keywords: *Berberis asiatica*; antioxidant activity; antihepatotoxic activity, free radicals

INTRODUCTION

Free radicals are reactive oxygen species (ROS) are inevitably generated due to incomplete reduction of O_2 in electron transfer reaction as a byproduct of biological reactions. The reactive oxygen species (ROS) such as superoxide anion radical (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$) have been implicated in the pathophysiology of various clinical disorders, including ischemia, reperfusion injury, atherosclerosis, acute hypertension, haemorrhagic shock, diabetes mellitus and cancer.[1] They play an important role in the inflammation process after intoxication with by ethanol, carbon tetrachloride or carrageenan.[2,3,4] It is well known that, free radicals are the reactive species derived from them cause damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury.[5] Scavenging of free radicals by antioxidants could reduce the fibrosis in the tissue. [6] Thus Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals.[7] numerous medicinal plants and their formulations are used for liver disorders in ethno medical practices as well as in traditional systems of medicine in India.[8]

Berberis asiatica Roxb. (Berberidaceae) is a very common substitute to "*Daruharidra,"* that is *Berberis aristata* DC which is used in ayurvedic system of medicine. Being an important medicinal plant, it is used extensively for treating variety of ailments. That is affection of eyes, skin diseases, jaundice and rheumatism.[9-11]

The major alkaloid of this plant is reported to be berberin.[12-13] Ethnomedicinal investigations revealed that the tribal kumaun region use the decoction of the root for treating the eye troubles and boils.[14] The extensive use of *Berberis aristata* by different pharmaceutical industries coupled with recent revival of interest in herbal medicines have led to ever increasing demand of this species. It as therefore becomes essential to search for a possible substitute for this species. The present investigations of methanol extract of *Bereberis asiatica* aerial parts studied for its possible anti hepatotoxic and antioxidant action in rats and aimed to evaluate the relationship between liver protective effects and antioxidant activity.

MATERIAL AND METHODS

Plant material

The aerial parts of *Berberis asiatica* Roxb. (Berberidaceae) were collected from Pithoragarh district of Uttarakhand during January 2007. The plants were identified by Dr. H.B Singh of National

Institute of Science communication and Information recourses (NISCAIR), New Delhi, India.

Preparation of extract

The dried ground aerial parts of *Berberis asiatica* were extracted by methanol and water separately using Soxhlet apparatus. The extracts obtained were dried under reduced pressure using rotary evaporator to get the crude aqueous extract (21%) and methanol extract (24 %) respectively.

Phytochemical screening

The known quantity of dried powdered drug was extracted in soxhlet with pet ether, methanol and water successively (Table.1) was tested for different constituents (Peach and Tracy, 1955) *viz.* steroids and triterpenoids (Liebermann-Burchard reaction), flavonoids (Shinoda test), alkaloids (Mayer's reagent),tannins(Ferric chloride test) and sugar (fehling solution test).The phytochemical investigations revealed that triterpenoids and alkaloids were present in methanol and water soluble parts, resin and sugar in pet ether fractions and tannins were present in water soluble parts.

Animals

Wistar albino rats (150-200g) of either sex roughly of the same age (8-10 weeks) were used for the present studies. They were housed in clean polypropylene cages (3 in each) and maintained in standard laboratory condition at ambient temperature (25±2^{0}C) with relative humidity (55-64%) and light and dark conditions (12/12h). They were provided with rat chow diet (Hindustan Liver limited Kolkata) and free asses of drinking water *ad libitum*. The experiments and procedure used in the study were approved by Institutional animal ethical committee (IAEC).

Chemicals

Silymarin, (Sigma chemicals, USA), C_2H_5OH, disodium hydrogen phosphate (Na_2HPO_4), Hydrogen peroxide (H_2O_2), dihydrogen potassium phosphate anhydrous (KH_2PO_4) and thiobarbituric acid were purchased from Merck India Ltd, Mumbai India.

Assessment of hepatoprotective activity

This was described recently (Tiwari et al., 2008). Briefly the animals were divided into six group's six animal each. Group I served as normal control were administered p.o., a single daily dose of 0.5% Tween 80(1ml) on all 5days and olive oil (1ml/kg) on days 2 and 3. Group II served as induction control, animal were administered a single daily dose of 0.5% Tween 80 (1ml) p.o., on all 5 days and on 2 and 3 day they were administered CCl_4, 2mL/Kg s.c., (1:1) dilution with olive oil (Lin et al., 1998) on 5 day. Group III-VI, served as *Berberis asiatica* treated groups were administered AqBA and MeBA extracts (200 and 300mg/kg, p.o.) all 5 days and a single dose of CCl_4 (2mL/Kg) s.c., on days 2 and 3, 30 min after extracts administrations and group VII Silymarin, the known hepatoprotective compound (100mg/kg.p.o.) for 5 days and a single dose of CCl_4 (2mL/Kg) s.c., on days 2 and 3, 30 min after silymarin administration. The food was withdrawn on preceding night of the experiment. On 5th day all the animals were sacrificed by mild ether anesthesia. Blood sample were collected from heart of each animal. Serum was separated for the estimation of the biochemical markers and liver was dissected out for the determination of antioxidant activity for histology studies liver tissue was collected.

Table 1: Phytochemical screening of extracts.

Phytoconstituents	Qualitative abundance		
	Pet. ether	Methanol	Water
Anthraquinones	_	-	-
Soluble tannins	+	++	++
Condensed tannins	+	++	+
Flavonoids	_	++	-
Alkaloids	+	+++	++
Indole alkaloids	-	++	+
Steroidal alkaloids	-	++	+
Saponins	+	+	-
Glycosides	-	-	-
Resins	+	-	-
Terpenoids	-	++	+

Levels of phytoconstituents were qualitatively determined based on chemical groups and thin layer chromatography on the following scale, - absent +present at low level, ++ present at moderate level, +++ present at high level.

Determination of enzyme level

The activities of serum glutamate pyruvate transaminase (SGPT), and serum glutamate oxaloacetate transaminase (SGOT) estimation of serum ALP, serum Bilirubin [Total and direct] were assayed by the reported methods[18, 19, 20] and carried out to asses the acute hepatic damage caused by CCl_4.

Assessment of antioxidant activity

Liver was excised washed thoroughly in 0.86% ice cold saline to remove the red blood cell. They were then gently blotted between the folds of filter paper and weighed in an analytical balance. Then it was suspended in 10 %(w/v) in ice cold buffer 0.05M then phosphate buffer cut into small pieces and homogenized using a polytron homogenizer at 20^{0}C. The homogenate was centrifuged at 3000 rpm for 20 min to remove the cell debris, unbroken cells, nuclei erythrocytes and mitochondria. The supernatant was used for the estimation of enzymic and non enzymic anti oxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), activities and the level of lipid peroxidation as described by the methods [21-24]

Histopathological Examination

The liver tissue was collected and immediately fixed in 10% formalin solution, dehydrated in gradual ethanol

(50-100%), cleared in xylene and embedded in paraffin. sections (4-5µm) were prepared and then stained with hematoxylin and eosin dye for photo microscopic observations.

Statistical analysis

The data represents mean ± SD. Results were analyzed statistically by one way ANOVA followed by Bonferroni's multiple comparison test between the data of control and treated groups using SPSS software (students' version), the minimum level of significance was set up at $p<0.05$ or less.

RESULTS

On preliminary phytochemical analysis various fraction of *Berberis asiatica* have shown the presence of steroids and triterpenoids, flavonoids, alkaloids tannins and sugar as represented in table.1

As shown in table 2 activities of serum GPT, GOT, acid and alkaline phosphatase were markedly elevated in CCl_4 treated animals compared to normal, indicating liver damage. Administration of methanol extract at 200mg/kg remarkedly prevented CCl_4 elevation of serum enzymes. The AqBA have shown nearly same effect at higher dose (300mg/kg).(fig 1(a) and fig(1b)

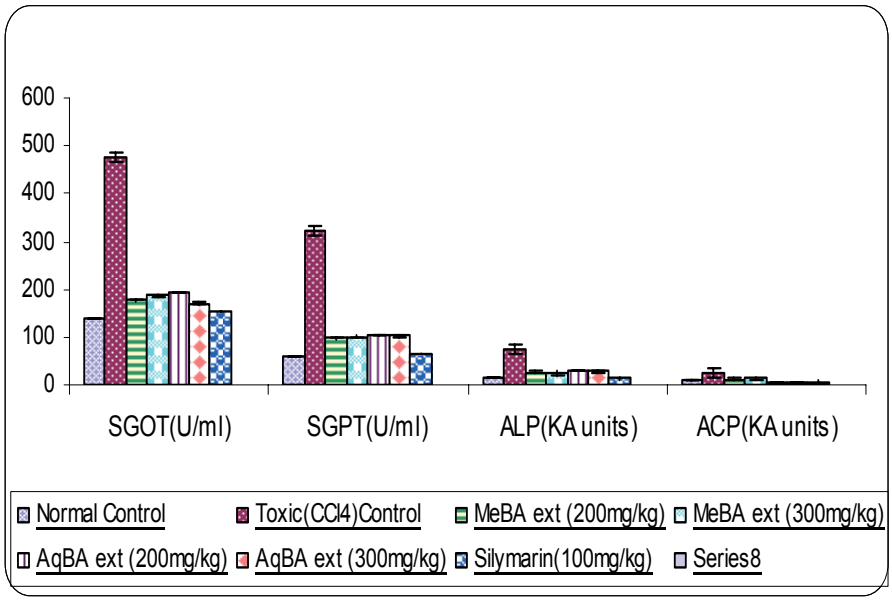

Fig. 1(a): Serum enzyme levels methanol extract treated animals

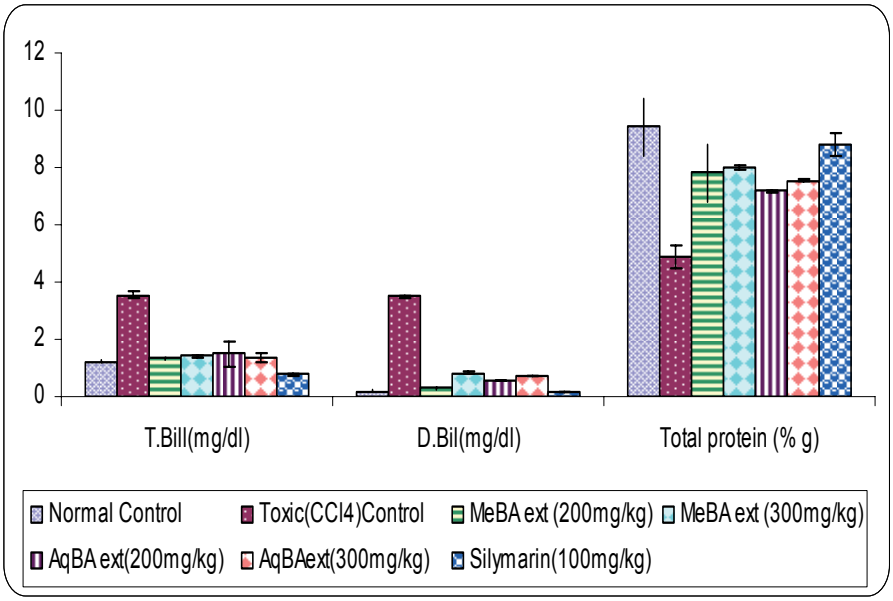

Fig. 1(b): Serum enzyme levels methanol extract treated animals

Table 2: Effects of methanolic extract of ariel parts *Berberis asiatica* on CCl_4 induced hepatotoxicity

Group	SGOT (U/ml)	SGPT (U/ml)	ALP (KA units)	ACP (KA units)	S.bilirubin(SB)(mg/dl)		Total protein g/dl
					T-Bill	D-Bill	
Normal Control	137.53 ± 4.22	58.98±3.12	14.57±1.40	9.41± 0.44	1.23±0.03	0.20±0.01	9.42±1.02
Toxic(CCl4)Control	475.77 ± 10.08**	320.52± 12.03**	74.34 ± 9.98**	26.41±0.83**	3.55±0.11**	1.50±0.03**	4.91±0.41**
CCl4+MeBA ext (200mg/kg)	177.01± 3.11**	97.14 ± 1.74**	26.42 ± 1.64**	11.98±0.22**	1.26±0.02**	0.19 ±0.02**	8.01±1.02**
(%protection)	(88.32)	(85.40)	(80.13)	(84.68)	(98.70)	(96.90)	(106..30)
CCl4+MeBA ext (300mg/kg	187.31 ± 2.78*	101.14±2.64*	25.44 ± 3.40*	12.41±1.42*	1.45± 0.01*	0.32 ± 0.04*	7.81±0.11*
(%protection)	(85.28)	(83.88)	(81.77)	(82.35)	(90.31)	(90.76)	(64.30)
CCl4+ AqBa ext (200mg/kg)	184.11± 2.81*	104.14± 1.32**	32.15 ± 1.43**	15.07±1.55**	1.51± 0.44**	0.67± 0.02**	7.19± 0.04**
(%Protection)	(86.34)	(82.73)	(70.55)	(66.70)	(87.93)	(63.00)	(50.55)
CCl4+AqBa ext (300mg/kg)	170.20 ± 1.71**	101.14±1.67**	29.42 ± 2.00**	14.51± 1.10**	1.61± 0.19**	0.75± 0.01**	7.55± 0.06**
(%Protection)	(90.34)	(83.88)	(75.11)	(70.0)	(83.62)	(57.0)	(58.53)
CCl4+Silymarin (100mg/kg)	151.94 ± 2.47**	65.45± 1.13**	12.41± 1.23**	7.34± 0.41**	0.79±0.04**	0.24 ± 0.01**	8.81±0.41**
(%protection)	(95.73)	(97.52)	(103.5)	(112.17)	(118.96)	(100.7)	(134.0)

Value are mean ± SD (n=6),*Statistically different at p<0.05, ** Statistically different at p<0.001

Table 3:Effects of Methanol extracts of *Berberis asiatica* aerial on Liver antioxidant enzymes and Lipid peroxidation on CCl_4 induced hepatotoxiciy

Group	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protiens)	MDA (nmol/mg protien)
Normal Control	27.20 ± 1.26	63.32 ± 3.10	33.32 ± 0.48	3.95± 0.11
Toxic(CCl4)Control	12.33 ± 1.04*	23.55 ± 5.45*	18.10 ± 2.36*	9.88± 0.03*
CCl4+MeSI ext (200mg/kg)	24.90 ± 1.56**	52.30 ± 4.03**	28.89 ± 3.61**	4.51. ± 0.02**
CCl4+MeSI ext (300mg/kg)	21.48 ± 3.83*	51.18 ± 2.01*	28.15 ± 1.45*	4.36 ± 0.02*
AQBA ext (200mg/kg)	22.68 ± 2.13**	48.31 ± 2.02**	29.51 ± 3.30**	4.65. ± 0.07**
AQBA ext (300mg/kg)	23.48 ± 1.02*	47.61 ± 2.01*	30.05 ± 1.04*	4.26 ± 0.03*
CCl4+Silymarin(100mg/kg)	19.40 ± 1.01**	61.14 ± 2.47**	36.14 ± 4.24**	1.01± 0.05**

Value are mean ± SD (n=6), *Statistically different at *p<0.05*** ,Statistically different at *p<0.001*

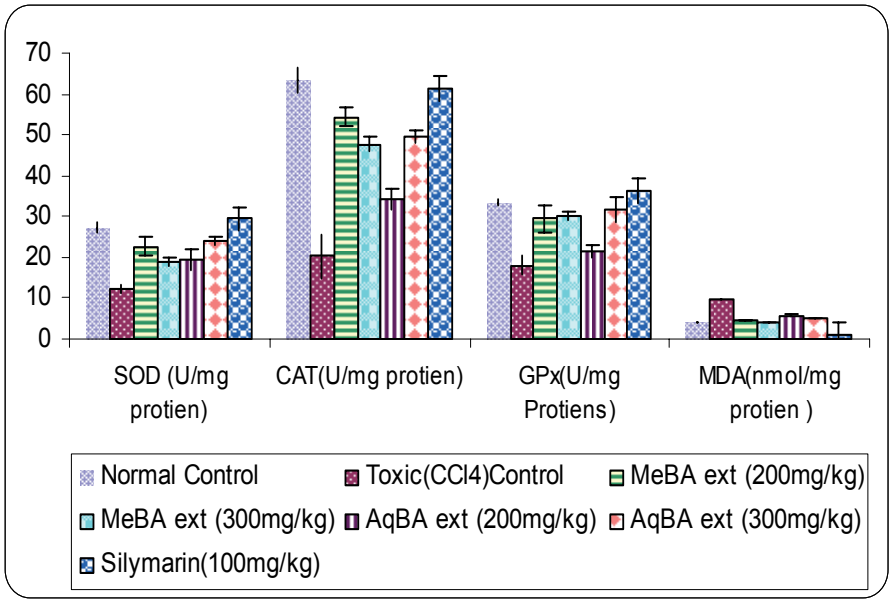

Fig. 2: Antioxidant effect of methanol extract

As given in table (3) reduced activities of enzymic and non-enzymic antioxidants and enhanced activity of lipid peroxidation were seen in the CCl_4 –treated group, where as standard Silymarin and the drug treated groups showed significant (p< 0.001) rise in antioxidant levels with reduction in lipid peroxidation level when compared with the CCl_4 –treated control group(fig 2)

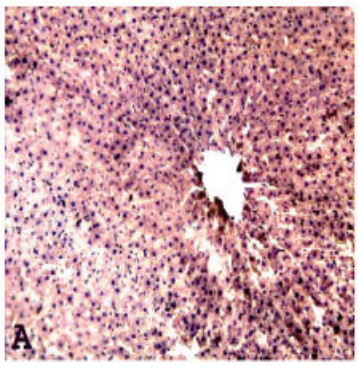

Fig. A: Liver cells of normal rats

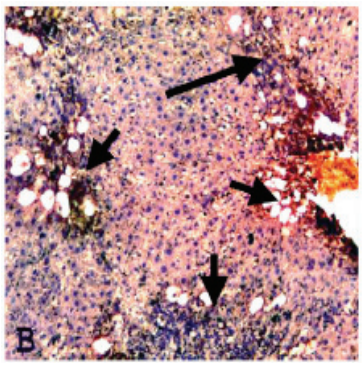

Fig. B: Liver cells of rats intoxicated with CCl_4

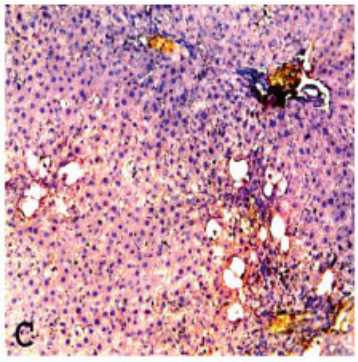

Fig. C: MeBA (200mg/kg) treated Liver cells

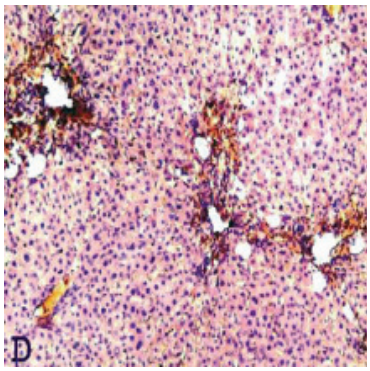

Fig. D: AqBA (300mg/kg) treated Liver cells and intoxicated with CCl_4 and intoxicated with CCl_4

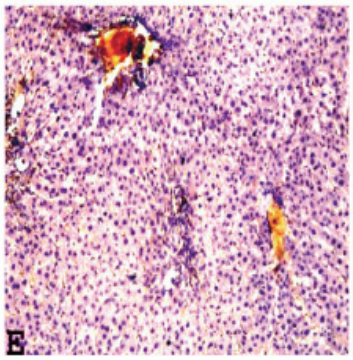

Fig. E: Silymarin treated (100mg/kg) treated Liver cells and intoxicated with CCl_4

From the tables and figures it is clear that the both MeBA and AqBA showed dose dependent hepatoprotective activity. However MeBA (200mg/kg) exhibited relatively higher protective action which is comparable with standard drug, silymarin (100mg/kg).

The Histopathological studies of liver showed fatty changes, swelling and necrosis with the loss of hepatocytes in CCl_4-treated group, whereas the drug treated groups showed absence of cell necrosis, but with minimal inflammatory conditions around the central vein. The MeBA (200mg/kg, p.o.) treated group showed minimal inflammatory condition with near normal liver architecture. The silymarin treated group showed almost normalization of fatty accumulation and necrosis. (Fig, A-E).

DISCUSSION

CCl_4- is biotransformed by cytochrome P-450 in the liver endoplasmic reticulum to the highly reactive trichloromethyl free radical. This free radical in turn reacts with oxygen to form trichloromethylperoxy radical, which may attack lipids on the membrane of endoplasmic reticulum more readily than the trichloromethyl free radical. The trichloromethyl peroxy radical leads to elicit lipid peroxidation, the distribution of Ca^+ homeostasis, elevation of hepatic enzymes and finally results in cell death[25].

It can be concluded from this investigation that, among the aqueous and methanolic extracts tested, the methanolic extract(200mg/kg) of the roots of *Berberis asiatica* possess more effective hepatoprotective activity against CCl_4 intoxication in rats because of its antioxidant bearing capacity. Our further detailed studies may, however, confirm the utility profile of this drug.

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