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Hepatoprotective effect of darhald (*Berberis aristata* DC.) against chemically induced hepatic damage

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ORIGINAL RESEARCH ARTICLE

ABSTRACT

Background: *Berberis aristata* is a medicinally important herb and used for centuries in Unani system of medicine for the treatment of various ailments. In the present paper, aqueous-ethanolic extract (50%, v/v) of the bark of *Berberis aristata* is shown to possess hepatoprotective activity.

Materials and Methods: Pretreatment of rats with the doses of 150 mg/kg and 200 mg/kg, p.o. of the plant extract for seven days significantly ameliorated the liver damage in rats exposed to the hepatotoxic compound galactosamine. The degree of protection was measured by using biochemical parameters like serum glutamate pyruvate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), alkaline phosphatase (ALP), serum bilirubin (TB) and metabolic parameters like total protein (TP), serum cholesterol, serum urea and wet liver weight. Since, hepatoprotective effect may be exerted, at least partly due to anti-oxidant activity; therefore, the test drug was also studied for TBARS test (Thiobarbituric Acid Reactive Substance).

Results: Only higher dose of Darhald (200 mg / kg; p.o.) exert significant protection, whereas lower dose (150mg/kg; p.o.) was ineffective in reducing SGPT and SGOT concentration significantly against acute damage produced by Galactosamine.

Conclusion: The aqueous-ethanolic extract (50%, v/v) showed a significant hepatoprotective activity comparable with that of Silymarin (100 mg/kg, p.o.). Histopathological studies further confirmed the hepatoprotective activity when compared with the galactosamine treated group.

Keywords: *Berberis aristata*, Liver, Chemical, SGOT, SGPT, ALP.

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INTRODUCTION

In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on rise, jaundice and hepatitis are the two major hepatic disorders that account for a high death rate (Gujrati *et al.*, 2007). No effective measures are available for the treatment of liver diseases in modern medicine. In such a situation, Traditional medicines particularly Unani medicine is approached. In Unani system of medicine, bark of *Berberis aristata* DC. is known as Darhald. It is frequently, prescribed in the treatment of hepatomegaly, hepatitis, splenomegaly etc (Ghani, 1921) and is used as Anti-inflammatory, cholagogue and resolvent (Khan, 1313H., Ghani, 1921).

Berberine, an alkaloid is the main constituent of *Berberis aristata* DC. and it showed anti-inflammatory (Akhter *et al.*, 1977), hepatoprotective (Janbaz and Gilani, 2000., Fang *et al.*, 2005) and hypolipidemic (Jean-Marie *et al.*, 2006) activities. The effect of Berberine on cardiovascular system in rats (Rastogi and Mehrotra, 1998., Zuiju *et al.*, 2000., chi-wai *et al.*, 2005), rabbit platelets and endothelial cells (Cai, G *et al.*, 2003). Alcoholic extract of *Berberis aristata* DC. showed significant anti-tumour activity in EAC (Ehrlich ascites carcinoma) model (Pai *et al.*, 2006). There was no scientific report available on the traditional claims of the effect of Darhald in liver disorders. Therefore, we investigated the anti-hepatotoxic effect of

hydroalcoholic extract of *Berberis aristata* against Galactosamine induced hepatic damage in rats.

MATERIALS AND METHODS

Plant material

The dried barks of *Berberis aristata* DC. were procured from Khari boali market of old Delhi. The identity of both the crude drugs samples were confirmed by Department of Raw Materials Herbarium & Museum, NISCAIR, Dr. K.S. Krishnan Marg, New Delhi.

Animals

Albino rats of wister strain weighing 150-200gm were obtained from Animal House, Department of IImul Advia. Experiment was conducted after getting approval from the departmental ethical committee for animal care and use.

Extracts

The dried barks of *Berberis aristata* DC. were crushed and powdered coarsely and then hydroalcoholic extract (50:50) was prepared using soxhlets apparatus. The solvent was removed under reduced pressure and the extract yield was 18 % (w/w) in terms of starting material.

Liver Function Test

The protective effect of the test and control drugs was studied by estimating the concentration of biochemical and metabolic markers of liver function in blood viz Serum Bilirubin (Malloy and Evelyn,1937), Serum Alanine Transaminase (ALT / SGPT) (Reitmen and Frankel,1957), Serum Aspartate Transaminase (AST / SGOT) (Reitmen and Frankel,1957), Serum Alkaline Phosphatase (ALP) (Kind and Kings,1954), Serum Total Protein (Biuret and Dumas,1971), Serum Total Cholesterol (Wybenga and Pileggi,1970), Serum Urea (Henry *et al.*, 1974) and Wet Liver Weight (Kuttan,2000).

Albino rats were divided into 5 groups (n=6 in each group). Group I served as vehicle control and received distilled water (1ml/kg; p.o) for seven days. Group II served as Galactosamine intoxicated control and received vehicle for seven days. Group III served as standard and received Silymarin (100 mg/kg; p.o) while Group IV and Group V were pretreated with 50% ethanolic extract of *Berberis aristata* (150 and 200 mg/kg, p.o. respectively) for 7 days. Liver damage was induced in all groups (except group I) with Galactosamine (200mg/kg, i.p.) on the seventh day, 1hr after drug administration.

After 24hr of Galactosamine treatment, the animals were sacrificed by cervical dislocation. After measuring the body weight, the blood was collected in plain test tubes for estimating the concentration of biochemical and metabolic markers of liver function.

Estimation of Lipid Peroxidation (TBARS Test)

The test was carried out according to the method of Okhawa *et al* 1979. 0.2 ml of sub cellular fraction of liver (as 10 % w/v homogenate with chilled 0.15M KCL) was mixed with 1.0 ml of 20 % acetic acid. Subsequently 0.2 ml of 8.0 % aqueous sodium dodecyl sulphate was mixed. After this 1.5 ml of 0.8 % Thiobarbituric acid and 1.1 ml of double distilled water added. The reaction mixture was incubated in a boiling water bath for an hour. After cooling to room temperature, 3.0 ml of n-butanol was mixed in each test tube. The reaction mixture was then centrifuged at 10,000 Xg for 15 minutes. A clear butanol supernatant was used for measuring the O.D. at 532 nm against the blank. The protein was also determined simultaneously by the method of Lowry *et al.*, 1951.

Liver Histological Studies

For histological study, the sections of liver were removed and fixed in 10 % formaline. The fixation was done immediately to check autolysis, to preserve as nearly as possible the natural state of the tissue cells. Care was taken to keep the volume of the fixative (Mukherjee, 1988). The tissue was processed and sections were cut. The slides were prepared and stained with haematoxyline and eosin stain and studied for histopathological change by light microscopy under various magnifications.

Statistical analysis

All values have expressed as mean +- S.E.M. Statistical significance was determined by using ANOVA followed by Pair-wise comparison by students't' Test.

RESULTS

Effects of lower and higher doses of Darhald on Liver function Test

In the animals in Group I, used as Plain control, SALT, SAST, S. Bilirubin, S. Alkaline. Phosphatase, S. Total Protein, S. Urea, S. Cholesterol and wet liver weight were seen to be 37.7 ± 5.87 units / ml, 33.0 ± 3.89 units / ml, 1.58 ± 0.43 mg / dl, 12.0 ± 1.57 KAU / dl, 6.25 ± 0.24 gm / 100 ml, 55.43 ± 3.80 mg / dl, 102.21 ± 4.09 mg / dl and 3.69 ± 0.21 gm / 100 gm b.w. respectively.

In Galactosamine treated animals (Group II), SALT, SAST, S. Bilirubin, S. Alkaline Phosphatase, S. Urea, S. Cholesterol were increased significantly while S. Total Protein was decreased significantly as compared to Group I, the values being 65.7 ± 6.35 units / ml ($P < 0.001$), 70.33 ± 2.39 units / ml ($P < 0.001$), 3.56 ± 0.23 mg / dl ($P < 0.01$), 22.20 ± 1.74 KAU / dl ($P < 0.001$), 80.80 ± 1.97 mg / dl ($P < 0.001$), 132.55 ± 4.71 mg / dl ($P < 0.01$), 6.25 ± 0.24 gm / 100 ml ($P < 0.01$), respectively. The increased produced in Wet liver weight was found to be 4.66 ± 0.83 , but it was statistically not significant.

In the animals treated with Silymarin and Galactosamine (Group III), SALT, SAST, S. Bilirubin, S. Alkaline Phosphatase, S. Urea, S. Cholesterol were decreased significantly while S. Total Protein was increased significantly as compared to Galactosamine treated Group, the values being 46 ± 2.53 units / ml ($P < 0.01$), 55.33 ± 3.49 units / ml ($P < 0.01$), 2.13 ± 0.26 mg / dl ($P < 0.05$), 14.83 ± 0.60 KAU / dl ($P < 0.001$), 67.06 ± 2.40 mg / dl ($P < 0.001$), 107.77 ± 6.97 mg / dl ($P < 0.01$), 7.42 ± 0.26 gm / 100 ml ($P < 0.01$), respectively. The decrease produced in Wet liver weight was seen to be 3.79 ± 0.21 respectively, but it was statistically not significant.

In the Group IV animals, treated with lower dose of Darhald (150 mg / kg) and Galactosamine, S. Bilirubin, S. Urea, S. Cholesterol, were decreased significantly, while S. Total Protein was increased significantly in comparison to the Galactosamine treated Group, the values were found to be 2.29 ± 0.33 mg / dl ($P < 0.05$), 73.86 ± 1.24 mg / dl ($P < 0.05$), 103.33 ± 4.47 mg /

dl ($P < 0.01$), 7.16 ± 0.27 gm / 100 ml ($P < 0.05$), respectively. The decrease observed in SALT, SAST, S. Alkaline Phosphatase and Wet liver weight was statistically not significant, values being 55.7 ± 4.42 units / ml, 66.7 ± 4.94 units / ml, 20.7 ± 1.67 KAU / dl and 4.57 ± 0.31 gm / 100 gm b.w. respectively.

In the Group V, treated with higher dose of Darhald (200 mg / kg) and Galactosamine, SALT, SAST, S. Bilirubin, S. Alkaline Phosphatase, S. Urea, S. Cholesterol were significantly decreased. while S. Total Protein was increased significantly as compared to the Galactosamine treated Group, the values were found to be 50.3 ± 2.03 units / ml ($P < 0.05$), 59.0 ± 2.62 units / ml ($P < 0.05$), 1.82 ± 0.37 mg / dl ($P < 0.01$), 17.5 ± 1.02 KAU / dl ($P < 0.01$), 69.14 ± 1.00 mg / dl ($P < 0.001$), 99.99 ± 7.69 mg / dl ($P < 0.001$), 7.55 ± 0.23 gm / 100 ml ($P < 0.01$), respectively. The decrease produced in Wet liver weight was statistically not significant, values being 4.02 ± 0.31 gm / 100 gm b.w.

Table 1. Effect of Test drugs and Silymarin (Standard) on biochemical parameters of liver functions in Galactosamine Induced Hepatic Damage.

Group	S. ALT / SGPT (Units / ml) (Mean \pm SE)	S. AST / SGOT (Units / ml) (Mean \pm SE)	Serum Bilirubin (mg / dl) (Mean \pm SE)	S. Alk. Phosp. (KAU / dl) (Mean \pm SE)
Plain Control	37.7 ± 5.87	33.0 ± 3.89	1.58 ± 0.43	12.00 ± 1.57
Galactosamine Control (200 mg / kg)	65.7 ± 6.35 $y^3 z^2 b^1$	70.33 ± 2.39 $y^3 z^2 b^1$	3.56 ± 0.23 y^2	22.20 ± 1.74 $y^3 z^3 b^2 c^2 d^3$
Standard (Silymarin) (100mg / kg)+ Galactosamine (200 mg/kg)	46 ± 2.53 x^2	55.3 ± 3.49 $y^3 x^2 a^1$	2.13 ± 0.26 x^1	14.8 ± 0.60 $x^3 a^2$
Darhald (150 mg / kg) + Galactosamine (200 mg/kg)	55.7 ± 4.42 y^2	66.7 ± 4.94 y^3	2.29 ± 0.33 x^1	20.7 ± 1.67 y^3
Darhald (200 mg / kg) + Galactosamine (200 mg/kg)	50.3 ± 2.03 x^1	59.0 ± 2.62 $y^3 x^1$	1.82 ± 0.37 x^2	17.5 ± 1.02 $y^2 x^2$

n = 6

x = Against Galactosamine (200 mg / kg) y = Against Plain Control
z = Against Standard (100 mg / kg) a = Against Darhald (150 mg / kg)
b = Against Darhald (200 mg / kg) 1 = P < 0.05, 2 = P < 0.01, 3 = < 0.0.

Table 2. Effect of test drugs and Silymarin (standard) on metabolic parameters of liver functions in galactosamine induced hepatic damage.

Group	Total Protein (gm / 100ml) (Mean \pm SE)	Serum Urea (mg / dl) (Mean \pm SE)	S. Cholesterol (mg / dl) (Mean \pm SE)	Wet liver weight (Liver wt / 100 gm body wt) (Mean \pm SE)
Plain Control	7.65 ± 0.27	55.43 ± 3.80	102.21 ± 4.09	3.69 ± 0.21
Galactosamine Control (200 mg / kg)	6.25 ± 0.24 $y^2 z^2 a^1 b^2$	80.80 ± 1.97 $y^3 z^3 a^1 b^3$	132.55 ± 4.71 $y^2 z^2$	4.66 ± 0.83 NS
Standard (Silymarin) (100mg / kg) + Galactosamine (200 mg/kg)	7.42 ± 0.26 x^2	67.06 ± 2.40 $y^3 x^3$	107.77 ± 6.97 x^2	3.79 ± 0.21 3.80 NS
Darhald (150 mg / kg) + Galactosamine (200 mg/kg)	7.16 ± 0.27 x^1	73.86 ± 1.24 $y^3 x^1$	103.33 ± 4.47 x^2	4.57 ± 0.31 NS
Darhald (200 mg / kg) + Galactosamine (200 mg/kg)	7.55 ± 0.23 x^2	69.14 ± 1.00 $y^3 x^3$	99.99 ± 7.69 x^3	4.02 ± 0.31 NS

n = 6, x = Against Galactosamine (200 mg / kg) y = Against Plain Control
z = Against Standard (100 mg / kg) a = Against Darhald (150 mg / kg)
b = Against Darhald (200 mg / kg) 1 = P < 0.05, 2 = P < 0.01, 3 = < 0.0

Effects of lower and higher doses of Darhald on Lipid Peroxidation

The Malondialdehyde (MDA) concentration was found to be 1.87 ± 0.26 in the Plain control Group, while it was increased to 3.40 ± 1.49 in Group II (200 mg / kg of Galactosamine). In the standard Group (100 mg / kg of Silymarin), the concentration of MDA was found to

be 1.21 ± 0.43 ($P < 0.05$), which was significantly lower than that in Galactosamine Group. In test drugs treated Group: Group IV (150 mg / kg of Darhald) and Group V (200 mg / kg of Darhald), the concentration of MDA was found to be 1.30 ± 0.17 ($P < 0.05$) and 1.23 ± 0.27 ($P < 0.05$), respectively, which was decreased significantly as compared to the Galactosamine Group.

Table-3. Effect of test drugs and Silymarin (standard) on lipid peroxidation in galactosamine induced hepatic damage.

Group	Lipid Peroxidation (n mole of MDA / mg of protein) (Mean \pm SE)
Plain Control	1.87 ± 0.26
Galactosamine Control (200 mg / kg)	$3.40 \pm 1.49z^1 a^1 b^1 d^1$
Standard(Silymarin)(100 mg / kg) + Galactosamine (200 mg / kg)	$1.21 \pm 0.43 x^1$
Darhald (150 mg / kg) + Galactosamine (200 mg / kg)	$1.30 \pm 0.17 x^1$
Darhald (200 mg / kg) + Galactosamine (200 mg / kg)	$1.23 \pm 0.27 x^1$

n = 6, x = Against Galactosamine (200 mg / kg)

y = Against Plain Control

z = Against Standard (100 mg / kg)

a = Against Darhald (150 mg / kg)

b = Against Darhald (200 mg / kg) 1 = $P < 0.05$,

2 = $P < 0.01$, 3 = $P < 0.001$, NS = Not Significant.

Histological Studies

In the histological study the Plain Control Group showed normal liver architecture with no evidence of fatty change, necrosis or inflammation.

The histological examination of Group II animals treated with Galactosamine, showed partially distorted liver architecture, no fatty degeneration, mild portal inflammation, marked focal cholestasis (Bile stasis) in parenchyma with encysted liver fluke, marked dilatation of portal veins, mild portal and periportal fibrosis.

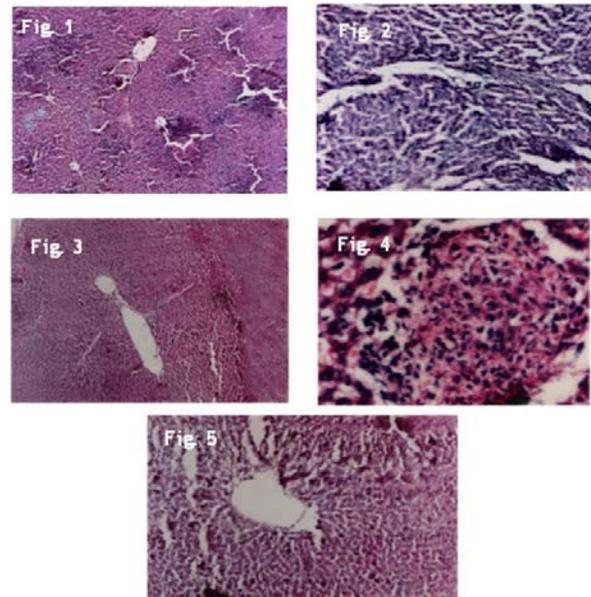
In the Silymarin treated Group, showed well maintained liver architecture, with no fatty change and necrosis, mild portal inflammation and increase in fibrous elements (mild fibrosis), and regenerating hepatocytes.

The animals in Group IV, treated with Darhald (150 mg / kg) showed well preserved liver architecture, portal and central vein dilatation with intense mononuclear infiltrate around portal tract, no fatty change are observed. Intrahepatic cholestasis with feathery degeneration is also noted in some hepatocytes. Regenerating hepatocytes, mild periportal inflammation and fibrosis were also observed.

The animals in Group V, treated with Darhald (200 mg / kg), showed well maintained liver architecture with no fatty change and necrosis, mild portal inflammation, mild increase in fibrous elements, mild inflammation around central vein (zone-3) and evidence of regeneration.

DISCUSSION

In our study Silymarin was found to be more effective than Darhald. Only higher dose of Darhald (200 mg / kg; p.o.) exert significant protection, whereas lower dose (150mg/kg; p.o) was ineffective in reducing SGPT and SGOT concentration significantly against acute damage produced by Galactosamine.



Figures: Fig. 1. Photomicrograph of plain control group; Fig. 2. Photomicrograph of galactosamine treated group; Fig. 3. Photomicrograph of Silymarin treated group in Galactosamine induced damage; Fig. 4. Photomicrograph of Darhald (150 mg / kg) treated group in Galactosamine induced damage; Fig. 5. Darhald (200 mg / kg) treated group in Galactosamine induced damage.

It is known that Galactosamine produces hepatic damage injury through disrupting the permeability of plasma membrane, which leads to elevation in the levels of Serum enzymes (Abul *et al.*, 2005). Silymarin and the test drug Darhald at higher dose exert hepatoprotective effect might be due to their effect against cellular leakage and loss of functional integrity of the cell membrane in hepatocytes i.e. it possesses membrane stabilizing property, indicated by significant decrease in SGPT, SGOT and S. ALK. Phosphatase. By seeing the effect of test drug on Lipid Peroxidation it can be suggested hepatoprotective effect of Darhald, may be lie at least, partially due to its anti-oxidant activity.

CONCLUSIONS

The aqueous-ethanolic extract (50%, v/v) showed a significant hepatoprotective activity comparable with that of Silymarin (100 mg/kg,p.o.). Histopathological studies further confirmed the hepatoprotective activity when compared with the galactosamine treated group.

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CONFLICT OF INTEREST

None declared.

REFERENCES

- Abul KN, Pillai KK, Pal SN, Aqil M. Free radical scavenging and Hepatoprotective activity of jigrine against Galactosamine induced hepatopathy in rats. *Journal of Ethanopharmacology*. 2005;97(3): 521-525.
- Akhter MH, Sabir M, Bhide, NK. Anti-inflammatory effect of Berberine in rats injected locally with Cholera toxin. *Indian Journal of Medical Research*. 1977; 65:133-141.
- Cai-G H, Zhong LC, Shan JW, Hua J, Bing HJ. Effect of Berberine on Arachidonic acid metabolism in rabbit platelets and endothelial cells. *Thrombosis Research*. 2003;106 (4-5): 223-227.
- Chi-Wai L, Xiao-Qiang Y, Zhen-Yu C, Wing-Hung K, Yu H. Cardiovascular action of Berberine. *Cardiovascular Drug Reviews*. 2005;19(3): 234-244.
- Ghani N. Khazeenat-ul-Advia. 1st Edi, Matba Munshi Nawal Kishore, Lucknow, Vol.2, pp. 530-531 (b.a), 1921.
- Jean-Marie B, Nicolos A, Pascal G, Raphaell G, Sandreine M. Inhibition of Lipid synthesis through activation of AMP kinase: an additional mechanism for the hypolipideamic effects of Berberine. *Journal of Lipid Research*. 2006;47(6):1281-1288.

Khan MA. Muheet-e-Azam. *Mataba Nizami, Kanpur*, Vol.3, pp. 5 (b.a), 1313 H.

Kuttan G. Immunomodulatory effect of some naturally occurring sulphur-containing compounds. *Journal of Ethnopharmacology*. 2000;72:93-99.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry*. 1951;193:265-275.

Mallay HT, Evelyn KA. Estimation of Serum Bilirubin Level. *The Journal of Biological Chemistry*. 1937;119:481-4.

Ohkawa H, Ohishi N, Yagi K. Assay for Lipid Peroxides in animal tissues by Thiobarbituric Acid Reaction. *Analytical Biochemistry*. 1979;95:351-358.

Reitman S, Frankel S. A colorimetric method for the determination of Serum Glutamic Oxaloacetic and Glutamic Pyruvic Transaminases. *American Journal of Clinical Pathology*. 1957;28:56-63,

Wybenga DR, Pileggi VJ, Dirstine PH, John Di Giorgio. Direct Manual Determination of Serum Total Cholesterol with a Single Stable Reagent. *Clinical Chemistry*. 1970;16:980.