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Research Paper

Preliminary Phytochemical Screening of Ethanolic Extract of Berberis Aristata and Its Acute Toxicity Testing

Zoology

KEYWORDS : Berberis aristata, acute toxicity, phytochemical screening.

Radhika Sharma Ph.D Research Scholar, Department of Zoology, Panjab University, Chandigarh India

Vijay Lakshmi Sharma Professor Department of Zoology, Panjab University, Chandigarh India

ABSTRACT

The main aim of our study is to investigate the phytochemical constituents of the ethanolic extact of Berberis aristata and to do its acute toxicity testing, as it is a potential herb that has been found to have many beneficial properties which can be utilized to treat many ailments. Plants are considered to have medicinal properties, it becomes very important to check whether they have any toxic effects associated with them which make them unsuitable for human consumption. For this purpose acute toxicity testing was done following OECD-423 guidlines by using limit test of Lorke. The results of phytochemical investigations revealed the presence of some very important secondary metabolites like alkaloids, glycosides, flavanoides and tannins and by acute toxicity testing it was found that the LD50 of B. aristata was found out to be greater than 5g per kg body weight which is the highest dose that can be given to the rodent. Safety usage of this extract in traditional medicine vis-a-vis its phytochemical consideration is discussed in this work.

INTRODUCTION-

Herbal plants are elemental foundation of traditional medicine which has been used by people for thousands of years. Traditional systems of medicine like Ayurveda have been in trend for centuries in all over the world. A lot of world's population still depends on herbs and their products for primary health care requirements. To face the challenge of toxic side effects modern medicines and the deficiency of medicines for a variety of chronic ailments have brought into light the field of herbal medicine, which provide treatments to many health issues. As a consequence, the usage of plant based preparations has been increasing all over the world (British Medical Association, 1993). Varieties of plants and their growing conditions according to geographical origin often play role in determining the quality and efficacy of herbals (Kamboj, 2000). Plants or any drugs must be ensured to be safely tested before they could be used as medicine. Very important step in fortifying that drugs are safe in use is to conduct different toxicity tests in appropriate animal models.

Berberis aristata also known as Indian barberry, daruharidra belongs to family Berberidaceae, is an erect spinous shrub found in the sub Himalayan regions at an altitude of about 800-2500 m and also Nilgiri hills of South India. It is 2-3m in height, has yellow coloured wood, bark is brown, roots are deep yellow in colour, spines are modified leaves, verticilliate phyllotaxy, lanceolate, simple, leathery, toothed, acuminate, sessile with reticulate pinnate venation. *Berberis aristata* is known to have hepatoprotective, antidiarrheal, anti PAF, cardiotonic, anti-microbial, ophthalmic properties and properties related to CNS (Potdar *et al*., 2012). *B.aristata* DC extract called Rasaut used as alternative and deobstruent and are used in skin disease, menorragia, diarrhea, jaundice and infection of eye (Chakravarti *et al*.,1950), anti-osteoporotic activity (Yogesh *et al*.,2011) and has hyperglycemic and antioxidant properties (Singh and Kakkar. 2009). Not much is known about its toxicological properties. Before use of any herbal plant as a medicine it is very important to know about the constituents it is made of. For this purpose preparation and testing of plant extracts before use becomes very important part. The aim of this work is to do the prelimnary phytochemical screening of ethanolic extract of *B.aristata* and to evaluate the toxic potential of this plant with the view to endorse or refute the safety usage of its ethanolic extract in traditional medicine.

MATERIAL AND METHODS-

COLLECTION, PREPARATION OF HERBARIUM AND IDENTIFICATION-

Plant materials were collected from Palampur (Kangra distt), Himachal Pradesh in April, 2014. Herbaria were made by following the method of Plain, 1968. Plant identification was done at the Botany Department, Panjab University, Chandigarh and voucher no. was obtained. The specimen of plant is kept in the department of Botany, Panjab University, Chandigarh for future refrence.

Plant Name	Voucher No.
Berberis aristata	5197



Figure 1: Berberis aristata herbarium



Figure 2: SOXHLET EXTRACTION

PREPARATION OF PLANT EXTRACT-

Plants of Berberis aristata were washed thoroughly to free them from debris and cut into small pieces, all plant parts were separated and shade dried for 15-20 days. 100g of powdered plant was subjected to soxhlet extraction with 250ml of ethanol as a

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solvent as it was most suitable solvent. Cycles of extraction were done at a temperature of 75 degree Celsius. Filteration of extract was done using whattman filter paper. After extraction filtered extract was evaporated to dryness by rota-evaporator. Then it was lyophilized for long shelf life. The weight of total powdered plant and total obtained extract was noted down and %yield was calculated.

PHYTOCHEMICAL SCREENING

To check the different phyto-constituents present in the extracts, phytochemical screening of the extracts was done. Different chemical tests were performed using protocol of Harbone, 1973 for establishing the profile of ethanolic extract of *B. aristata* to check their chemical composition. The extract was hydrolyzed with dilute HCl and the following tests were performed separately with each extract and results were noted carefully.

Wagner's test for detection of alkaloids

To a small amount of extract, 2 ml of Wagner's reagent (Iodine-Potassium iodide solution) was added by the side of the test tube. Formation of reddish brown precipitates confirmed the presence of alkaloids.

Barfoed's test for detection of carbohydrates

To 1 ml of extract, 1 ml of Barfoed's reagent (copper acetate dissolved in glacial acetic acid) was added and heated on a boiling water bath for 2 minutes. Appearance of red precipitates indicated the presence of carbohydrates.

Borntrager's test for detection of glycosides

50 mg of extract was hydrolysed with conc. HCl for 2 hours on water bath, filtered and the hydrolysate was obtained. To this hydrolysate, equal volume of chloroform or dichloromethane was added and shaken. Then lower chloroform layer was separated and shaken with half the volume of 10% ammonia solution. Rose pink colour in the ammonical layer indicated the presence of glycosides.

Alkaline reagent test for the detection of flavonoids

Aqueous solution of extract was treated with few drops of 10% ammonium hydroxide solution and observed for intense yellow coloration which disappeared on addition of dilute HCl.

Foam test for detection of saponins

The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A 2 cm layer of foam indicated the presence of saponins.

Millon's test for detection of proteins and amino acids

To 2 ml of extract, few drops of Millon's reagent were added. Appearance of white precipitate confirmed the presence of proteins.

Ferric chloride test for detection of phenolic compounds and tannins

About 0.5 mg of dried powdered samples were boiled in 20 ml water in test tubes and filtered. A few drops of 0.1% ferric chloride solution was added and observed for brownish green or blue-black colouration which confirmed the presence of tannins.

Salkowski test for detection of terpenoids

1 ml of test solution was taken in a clean and dried test tube and 2 ml chloroform and few drops of sulphuric acid were added into it. Shaken well and allowed to stand for some time. A reddish brown colour at the interface confirms the presence of terpenoids.

ANIMALS USED-

For the experiment 10 adult white albino females rats (wistar stock) weighing 150-200g were obtained from central animal house Panjab University. All rats were kept in the environmental condition of temperature- 22±2º C, humidity- 70±4. They were given ad libitum and tap water. The care of rats was done in accordance to internationally accepted standard guidelines for animal usage.

ETHICAL CLEARANCE

The ethical clearance for conducting the experiment was obtained from the Institutional Animal Ethics Committee, Panjab University, Chandigarh and the laboratory animals were used in accordance with the 'Guide for the Care and Use of Experimental Animals' approved by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

ACUTE TOXICITY TESTING

To check whether the plants extract has any toxic effects or not, acute toxicity testing was done. Acute oral toxicity study was performed as per OECD-423 guidelines (Acute Toxic Class Method). To calculate the LD_{50} of the plant, limit test of Lorke (1983) was followed. For the experiment, group of 10 rats were taken. The animals were subjected to overnight fasting before dosing, given only water after which the plant extract was administered orally at the dose level of 5g/kg body weight once. For first 24 hours, the animals were observed for any mortality and then they were observed for 14 days for any signs and symptoms of toxicity. Weights of rats were also recorded on 7th and 14th day and all other toxicity signs were also recorded. On 15th day, the rats were sacrificed and histology of major organs was done to notice any histopathological changes by method of Pearse (1968).

RESULTS AND DISCUSSION-

Different parts of *Berberis aristata* were dried and powdered, and then the extract was prepared by Soxhlet extraction method. The ethanolic extract was then evaporated to dryness in rota evaporator. The dried residues thus obtained were stored in screw capped vials at -4°C. Extract was evaluated for its Organoleptic properties and it was found that the colour of extract was yellowish-green, odor was sweet and consistency of extract was like a solid.

Table 1: PERCENTAGE YIELD AFTER EXTRACTION

PLANT NAME	PARTS USED	SOLVENT USED	WEIGHT OF DRIED PLANT	YIELD	PERCENTAGE YIELD
Berberis aristata	Leaves, roots, stem, seeds	Ethanol	2 Kg	300g	15%

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary screening of ethanolic extracts of plant was done to detect the presence of bioactive constituents present in the extract following the method of Harbone (1973).

Table 2: PHYTOCHEMICAL CONSTITUENTS PRESENT IN DIFFERENT PLANT EXTRACTS UNDER STUDY

S.No.	PHYTOCHEMICAL CONSTITUENTS	TEST PERFORMED	*B.ARISTATA* Extract
1	Alkaloids	Wagner's test	+
2	Carbohydrates	Barfoed's test	+
3	Glycosides	Borntrager's test	-
4	Saponins	Foam test	+
5	Proteins and Amino acids	Millon's test	+
6	Phenolic compounds and Tanins	Ferric chloride test	+
7	Terpenoids	Salkowski test	+
8	Flavonoids	Alkaline reagent test	+

Present (+), Absent (-)

ACUTE ORAL TOXICITY STUDIES

Acute lethal study of ethanolic extract of *B.aristata* showed that no animal died within first 24 hours after treatment of extract and there were no significant changes in the weights of rats on 7th and 14th day (Graph 1). There were no signs and symptoms of toxicity in the rats as shown in the table 3.

Graph 1: WEIGHTS OF RATS OF DIFFERENT GROUPS

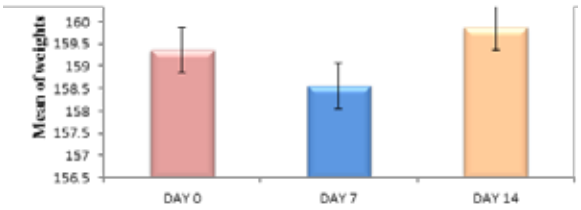

Table 3: Signs and symptoms observed during acute toxicity testing of ethanolic extract of *Berberis aristata*

Signs and Symptoms	Rat No.	Mortality	Feed intake	Water intake	Alertness	Skin colour	Eye colour	Urine output	Hyper activity
Group of 10 rats	1.	A	N	N	N	N	N	N	A
	2.	A	N	N	N	N	N	N	A
	3.	A	N	N	N	N	N	N	A
	4.	A	N	N	I	N	N	N	A
	5.	A	N	N	N	N	N	N	A
	6.	A	N	N	N	N	N	N	A
	7.	A	N	N	N	N	N	N	A
	8.	A	N	N	I	N	N	N	A
	9.	A	N	N	N	N	N	N	P
	10.	A	N	N	N	N	N	N	A

A – Absent, P – Present, N – Normal and I – Inactive

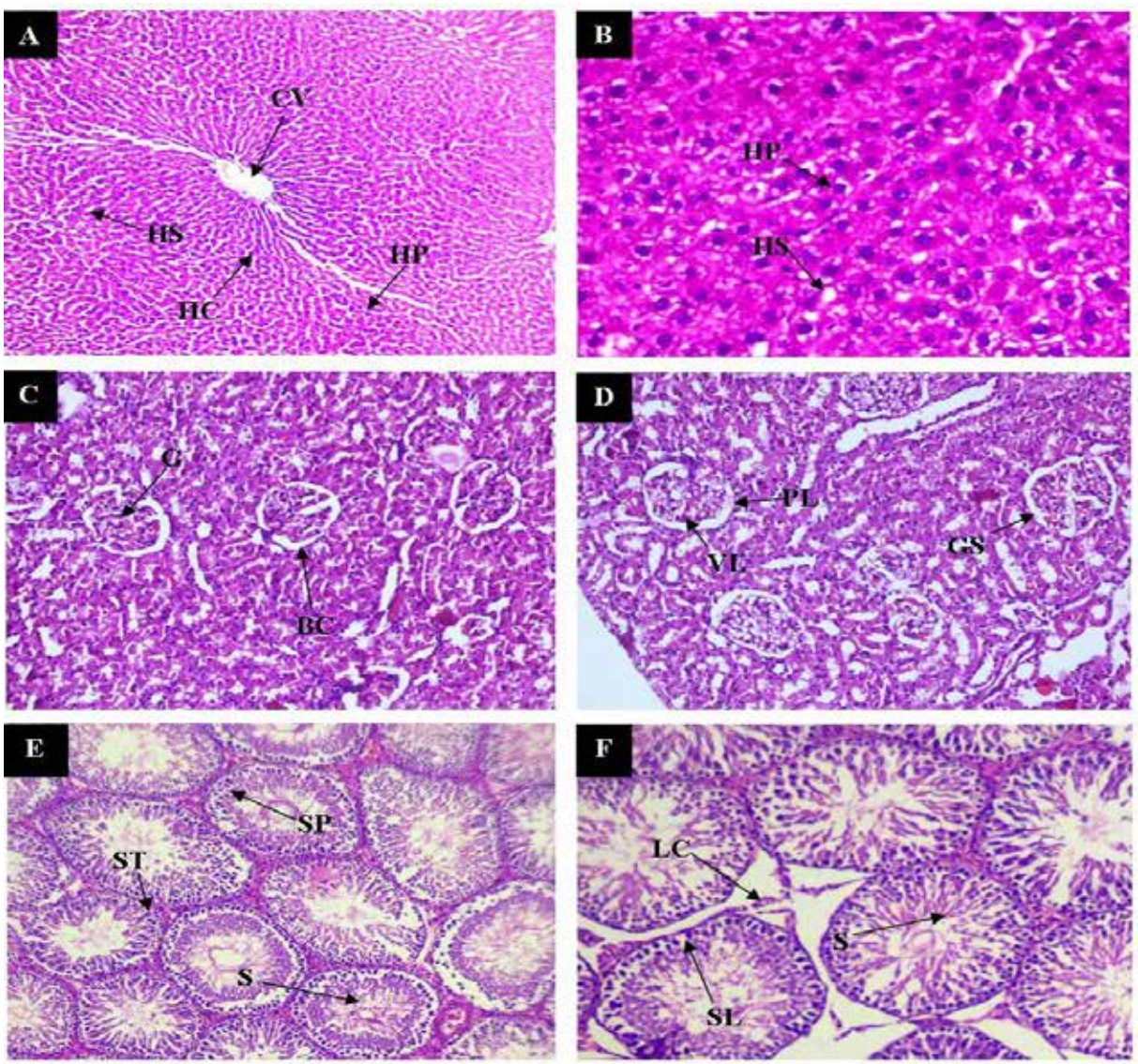

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Plate-1: Light micrographs showing histology of liver, kidney and testes during acute toxicity studies of *B.aristata*.

A, B: Normal histoarchitecture of liver showing hepatocytes arranged in cords around central vein and well defined sinusoids (10X, 40X);
C, D: Section of kidney showing showing normal histology with well extended glomerulus in bowman's capsule (40X, 40X);
E, F: Testes showing normal seminiferous tubules with concentric layers of spermatogonia, Leydig cells in the interstitial space and numerous sperms in the lumen (10X, 40X).

Abbreviations:
CV– central vein, HP– hepatocytes, HC– hepatic cord, HS– hepatic sinusoids, G– glomerulus, BC– Bowman's capsule, PL– parietal layer, VL– visceral layer, GS– glomerular space, S– sperms, ST– seminiferous tubules, SL– spermatogonial layers, SP– spermatogonia, LC– Leydig cells.

The rats groups administered with 5g/kg b.wt. of B. *aristata* ethanolic extracts did not show any histoarchitectural alterations (Plate-1). Histopathology of liver revealed normal structure of hepatocytes, central vein, hepatic cord and hepatic sinusoids. Kidney sections showed normal histology of Glomeruli, Bowman capsule and structure of testes also shoed normal seminiferous tubules with spermatocytes and spermatogonia, leydig cells and sperms. Hence it is concluded that LD_{50} of B. *aristata* must be > 5000 mg/Kg body weight. As the LD_{50} is > 5000 mg/Kg b.wt., So the plant extract is safe to be used at any dose which is less than 5000 mg/Kg b.wt.

In today's world where herbal medicine is in too much progression it becomes very important to study the properties of different plants which have some medicinal potential. The present study depicts the presence of different phytoconstituents in the ethanolic extract of *B.aristata.* In the *B.aristata* extract only glycosides were found absent all other phytoconstituents like alkaloids, carbohydrates, saponins, proteins, phenolic compounds, terpenoides and flavanoides were found present. All the protective properties of *B.aristata* are attributed to these phytoconstituents. Now among these which phytoconstituent is solely responsible for the activity of extract is still a debatable question and more studies involving higher teqniques are required in this field. As by acute toxicity study we came to know about the general idea that LD_{50} of *B. aristata* is greater than 5mg/kg body weight, and it is safe to be used in humans at any lower dose, detailed studies are needed in this direction however, it still serves a substantial cause as first pointer to the safety of toxic potential of a substance whose toxicity profile is not yet known (Kagbo and Ejebe, 2010).

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