Preliminary Phytochemical Screening And Acute Toxicity Testing Of Ethanolic Extracts Of *Acyranthes Aspera* And *Phyllanthus Niruri*

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Abstract- Herbs have always been the basis for medical treatments through much of human history and this traditional medicine is still very much practiced today. Many plants derived compounds are used in modern medicines that have proved to be very beneficial for man in getting rid of a lot of diseases. This study was carried out to determine the phytochemical constituents of the ethanolic extacts of *A.aspera* and *P.niruri* as these are potential herbs that have beneficial properties that can be utilized to treat many ailments. Also due to wide usage of herbs it becomes of utmost importance to check whether the plant is safe for human use. The results of phytochemical investigations revealed the presence of some very important secondary metabolites like alkaloids, glycosides, flavanoids and tannins. By such kind of analysis we can say that the healing properties of these herbs are attributed to the presence of these phytoconstituents also acute toxicity testing of these plants revealed they are safe to use in any dosage below 5g/kg body weight.

Keywords- *P.niruri*; *A.aspera*; phytochemical screening; acute toxicity testing

I. INTRODUCTION

Herbal plants are elemental foundation of traditional medicine which has been used by people for thousands of years. Traditional systems of medicine have been in vogue for centuries in all over the world. Roughly, 80% of world's population still depends on herbal products for their primary health care needs. The toxic side effects of drugs of modern medicine and the lack of medicines for many chronic ailments have led to the reemergence of herbal medicine, which provide possible treatments to many health problems. As a consequence, the use of plant based medicine has been increasing all over the world [1]. Varieties of plants and growing conditions according to geographical origin often play role in determining the quality and efficacy of herbals [2]. 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 toxicity testing of plant extracts before it comes to use becomes very important part.

Phyllanthus niruri belonging to family Euphorbiaceae, commonly known as Bhumyamalaki is approx. 50 to 70 centimeters tall and bears ascending herbaceous branches. The bark is smooth and lighter green in colour. It generally bears many pale green flowers along with some red ones. The fruits are of minimal size, smooth capsules which contains seeds. It is ethnobotanically known to have many therapeutic activities e.g. leaves as expectorant, diaphoretic and useful in strangury and sweats; seeds as carminative, laxative, astringent to the bowels, tonic to the liver, diuretic, diaphoretic, useful in bronchitis, ear ache, griping, ophthalmia and ascites [3]. An aqueous infusion of whole plant, which is employed as a stomachic, antiplasmodic, diuretic, against constipation and is also effective against fever including malaria, dysentery, gonorrhoea, syphilis, tuberculosis, cough, diarrhoea and vaginitis [4]. *Phyllanthus niruri* has antiviral, antiplasmodial, antidiabetic, analgesic, cardioprotective, anticarcinogenic, lipid lowering, anti-inflammatory, antibacterial and hepatoprotective activities.

Achyranthes aspera belongs to family Amaranthaceae, commonly recognized as Apamarga in ayurveda and is found as a weed that is growing upto 3 ft. in height. It has been traditionally used for a number of ailments. The plant is reported to have several medicinal properties and used as emmenogogue, purgative, diuretic, antimalarial, antihyperlidemic, estrogenic, antileprotic, antiplasmodic, cardiotonic, antibacterial and antiviral agent in traditional system of medicine. It is also used as antiasthmatic ajent and and in treatment of snake bite, urinary calculi, rabies, influenza, otorrhoea, piles, bronchitis, dirrhoea, renal dropsies, gonorrhea and abdominal pain [5]. The aim of this work is to find out the phytoconstituents present in these herbs and also to evaluate the toxic potential of these plants with the view to endorse or refute the safety usage of their ethanolic extracts in traditional medicine

II. MATERIAL AND METHODS

A. Collection of plant material-

Plant materials were collected from Palampur (Kangra distt), Himachal Pradesh during different times of the year. *P.niruri during* rainy season in month of July and *A. aspera* in month of October. Herbaria (fig 1) are made by following method of Plain [6]. Plant identification was done at the Botany Department, Panjab University, Chandigarh and voucher numbers were obtained: *Phyllanthus niruri*-20325 and *Achyranthes aspera*-20326.

B. Preparation of plant extracts-

Roots and leaves of *Acyranthes aspera* and *Phyllanthus niruri* were washed cut into small pieces and shade dried for

15-20 days. These plant parts were powdered thoroughly. The powdered plants were subjected to soxhlet extraction (fig 2) with ethanol as a solvent as it was most suitable on basis of solubility. After extraction filtered extract was evaporated to dryness by rota-evaporator. It was lyophilized to increase its shelf life. The weights of total powdered plants and total obtained extracts were noted down and percentage yield of both the plants was calculated.

C. 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 [7] for establishing the profile of ethanolic extract of *P.niruri* and *A. aspera* to check their chemical composition. The extracts were hydrolyzed with dilute HCl and the following tests were performed separately with each extract and results were noted carefully.

a) 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.

b) 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.

c) 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.

d) 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.

e) 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.

f) 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.

g) 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.

h) 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.

D. Experimental animal model

Adult male wistar albino rats with average weight of 150-200 g were used as test animals. They were obtained from the Central Animal House, Panjab University, Chandigarh. The animals were housed in spacious polypropylene cages under hygienic conditions and allowed to acclimatize for one week to their new environment. The animals were maintained in an environment of controlled temperature (25 ± 2°C) under a 12 hr. light-dark cycle. Standard commercial rat pellets (Ashirwad Industries, Punjab, Hindustan Lever, India) were given as food and tap water were provided *ad libitum*

E. Ethical clearance

The ethical clearance for conducting the experiment was obtained from the Institutional Animal Ethics Committee, Panjab University, Chandigarh under approval number (PU/IAEC/S/14/147) 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).

F. Experimental design for acute toxicity testing

To check whether these plant extracts have any toxic effects or not, acute toxicity testing of ethanolic extracts of *Phyllanthus niruri* and *Acyranthes aspera* 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 [8] was followed. For the experiment, 15 rats were taken for each plant, 5 rats per group. The animals were kept fasting for overnight before dosing, given only water after which A single oral low dose (LD) of 2000 mg/kg b.w. and a single oral high dose (HD) of 5000 mg/kg b.w. of *Phyllanthus niruri* and *Acyranthes aspera* were reconstituted as aqueous homogenous suspensions. The administration volume was set at 900 µl / kg b.w. Group 1, the control group (C), fed normal diet, and was gavaged 160 µl drinking water (once). Group 2, low dose group (LD) and group 3, high dose group (HD) were gavaged with the extract at a single administration with the doses indicated previously. 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 like (rising fur, draping, tremors, excitability, miosis, mydriasis, twitching, salivation, morbidity, etc. 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 (Liver, kidney and Testes) was done to notice any histopathological changes.

G. Histopathology studies

Histopathological studies were done by the method of Pearse (1968) to evaluate the changes in the basic structural pattern of the different tissues.

H. Statistical analysis

The statistical analyses were carried out using statistical package for social sciences SPSS rats' body weights were expressed as mean ± SD. Values in both groups were compared using paired-t-test. For analysis the level of statistical significance was fixed at $p<0.05$.

III. RESULTS

Fig 1: Showing herbaria of plants under study

Roots and leaves of *Phyllanthus niruri* and *Achyranthes aspera* were dried and powdered, then the extracts were prepared by Soxhlet extraction method. The ethanolic extracts were evaporated to dryness in rota evaporator. The dried residues thus obtained were stored in screw capped vials at -4°C.

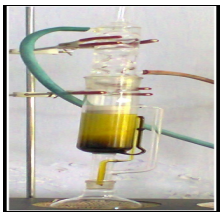

Fig 2: Soxhlet extraction

Table 1: Showing percentage yield after extraction

S. No.	Plant Name	Parts Used	Solvent Used	Weight Of Dried Plant	Yield	Percentage Yield
1.	*Phyllanthus niruri*	Leaves, roots	Ethanol	2.8Kg	350g	12.5%
2.	*Achyranthes aspera*	Leaves, roots	Ethanol	2.5Kg	380g	15.2%

Table 2: Phytochemical constituents present in different plant extracts under study

S. No.	PHYTOCHEMICAL CONSTITUENTS	TEST PERFORMED	STATUS	
			P.niruri	*A.aspera*
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

Limit test of Lorke was performed to study the acute toxicity effect of three different ethanolic extracts. Signs accompanying toxicity and possible death of animals were monitored for two weeks to ascertain the median lethal dose (LD_{50}) of the extract. Weights of rats were recorded on 7th and 14th day of the experiment.

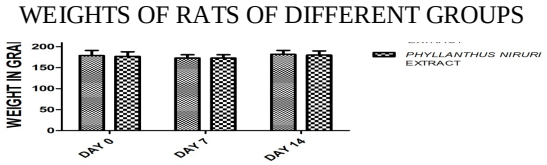

Fig 3: Showing weights of rats in different groups

Table 3: Showing different signs and symptoms of rats during acute toxicity testing in *P.niruri* and *A.aspera* group.

	SIGNS AND SYMPTOMS	*P.niruri* group	*A.aspera* group
1.	MORTALITY	ABSENT	ABSENT
2.	FEED INTAKE	NORMAL	NORMAL
3.	WATER INTAKE	NORMAL	NORMAL
4.	ALERTNESS	NORMAL	NORMAL-8 INACTIVE-2
5.	SKIN COLOUR	NORMAL	NORMAL
6.	EYE COLOUR	NORMAL	NORMAL
7.	URINE OUTPUT	NORMAL	NORMAL
8.	HYPERACTIVITY	ABSENT	ABSENT-7 PRESENT-3

The rats groups administered with 2g/kg b.wt. and 5g/kg b.wt. of *P.niruri and A.aspera* ethanolic extracts did not show any mortality and no symptoms of toxicity were observed in both lower and higher doses of extracts. The histoarchitecture of main organs i.e. liver, kidney and testes did not show any histoarchitectural alterations. Hence it is concluded that LD_{50} of *P.niruri and A.aspera* were found to be > 5000 mg/Kg body weight. As the LD_{50} is > 5000 mg/Kg b.wt., the plant extracts are safe to be used at any dose which is less than 5000 mg/Kg b.wt.

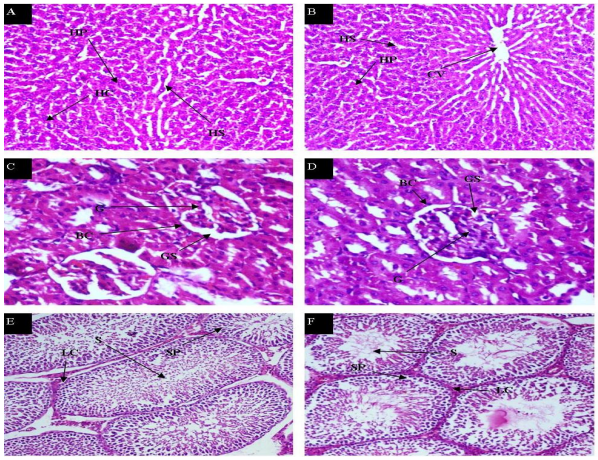

Plate 1: Light micrographs showing histology of liver, kidney and testes during acute toxicity studies of *Acyrathes aspera*

A, B: Normal histoarchitecture of liver showing hepatocytes arranged in cords around central vein and well defined sinusoids (10X, 10X); **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 (40X, 40X).

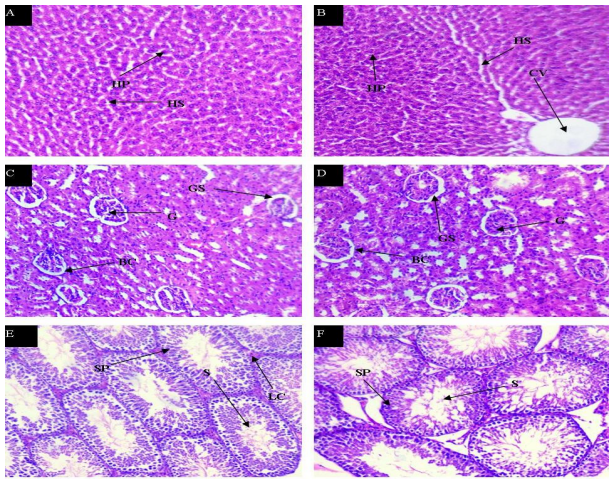

Plate 2 : Light micrographs showing histology of liver, kidney and testes during acute toxicity studies of *Phyllanthes niruri*.

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

IV. DISCUSSION

In todays 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 extracts of *P.niruri* and *A.aspera*. In the ethanolic extract of *P.niruri* proteins, phenolic compounds and tannins were found absent where as all the other phytoconstituents were found present. These constituents are the reason for such protective activities of *P.niruri*. In case of *A.aspera* it was found that glycosides, proteins, tannins, phenolic compounds and terpenoids were found absent the remaining phytoconstituents were responsible for its protective properties. These results were in accordance with results of Aarthi et al [9] and Tullanithi1 et al [10]. During acute toxicity testing of plant extracts no mortality was observed. In signs and symptoms of toxicity mild hyperactivity was found in *A.aspera* group which cannot be totally attributed to toxicity it may be due to hot weather conditions. All other symptoms were normal in both the treated groups even at higher dosage of 5g/kg bdy.wt. Weight difference on day 15 in rats was found to be not significant ($p>0.05$) when compared to day 0. Asare et al. [11] did acute toxicity studies of aqueous leaf extract of *Phyllanthus niruri* and have found the similar results. Reddy and Kamble [12] did toxicity study of *Achyranthus aspera* and found that the whole plant powder methanol extract of *Achyranthes aspera* were found to be nontoxic.

V. CONCLUSION

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the pharmacopoeia, these standards must be established. Phytochemical screening is first step in characterizing these crude extracts. *P.niruri* and *A.aspera* ethanolic extracts have shown presence of various phytoconstituents and all their protective properties are attributed to these phytochemicals only. Acute toxicity evaluation of the extracts classified them as non toxic to the experimental animals as LD50 > 5g/kg.bdy.wt. Chronic toxicity studies are suggested for exact LD50 determination.

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