Int J Pharm Bio Sci 2013 Apr; 4(2): (P) 1050 - 1055

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ACUTE TOXICITY STUDIES OF METHANOLIC BARK EXTRACT OF *FICUS RACEMOSA*AND ROOT EXTRACT OF *CISSAMPELOS PAREIRA*

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ABSTRACT

The acute toxicity study of *Ficus racemosa* and *Cissampelos pareira*, was investigated. The acute toxicity test of the extracts was determined according to the OECD guidelines No. 420 (Organization for Economic Co-operation and Development). Female Wistar rats (150–180 g) were used for this study. A starting dose of 2000 mg/kg (P.O.) of the test samples were given to various extract groups containing 5 rats in each group. Rats were randomly selected for the study and tail marked to provide individual identifications. Rats were observed immediately after dosing during first 30 minutes, periodically during the first 24 hours, with special attention given during first 4 hours, and daily thereafter for 14 days (OECD guidelines, 2001). During the first four hours rats were tested for following various responses like behavioral response,neurological response and autonomic response and were accessed by their respective parameters.All the animals were found healthy and the extracts were found to be safe up to a dose of 2000 mg/kg.

KEYWORDS: Acute toxicity, *Cissampelos pareira, Ficus racemosa,* Methanolic extracts, Wistar Rats.

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INTRODUCTION

Acute toxicity refers to those adverse effects occurring following oral or dermal administration of a single dose of a substance or multiple doses given within 24 hours, or an inhalation exposure of 4 hours.Recent years have witnessed a renewed interest in plants as pharmaceuticals. It is presumed that herbal drugs have lesser side effects than allopathic medicines.*Ficus racemosa* Linn. (commonly known in all over India as Udumbara, Gular fig.) has been recommended for the treatment of diarrhoea, diabetes, hypertension, gastric ulcer, wound healing etc. *Ficus racemosa,* Linn.showed a wide range of pharmacological actions like hypoglycemic, hypolipidemic, renal, anti-carcinogenic, anti-diuretic, anti-tussive, hepatoprotective, radio protective, anti-ulcer, anti-inflammatory, anti-diarrhoea and anti-fungal[1].*Cissampelos pareira* Linn. possesses antibacterial, anti-inflammatory, antihistamine, antioxidant, antispasmodic, diuretic, hypertensive, muscle relaxant, uterine relaxant, antiseptic, aphrodisiac, analgesic, antihemorrhagic, cardio-tonic, diaphoretic, expectorant, febrifuge, hepatoprotective stimulant and tonic activities[1].This interest is channeled into the discovery of new biologically-active molecules by the pharmaceutical industry and into the adoption of crude extracts of plants for self-medication by the general public[2]. A number of studies have reported the toxic effects of herbal medicines[2-5].

Tomato*:Lycopersicumlycopersicon (=Solanumlycopersicum)*. Like many other nightshades, tomato leaves and stems contain solanine that is toxic if ingested, causing digestive upset and nervous excitement. Use of tomato leaves as a tea (tisane) has been responsible for at least one death[1]. Leaves, stems, and green unripe fruit of the tomato plant also contain small amounts of the poisonous alkaloid tomatine[2].

Indian pea*:Lathyrus sativus*,a legume grown in Asia and East Africa, used as an insurance crop during famines. Like other grain legumes, *L. sativus* produces a high-protein seed. The seeds contain variable amounts of β-N-Oxalyl-L-α, β-diaminopropionic acid or ODAP, a neurotoxic amino acid[3].

Nutmeg*: Myristicafragrans* contains myristicin, which is a naturally occurring insecticide and acaricide with possible neurotoxic effects on neuroblastoma cells[4]and cultivated Potato*(Solanum tuberosum)* varieties contain lower toxin levels[5].Studies of medicinal plants using scientific approaches showed that various biological components of medicinal plants exhibit a variety of properties and can be used to treat various ailments. *Ficus racemosa* and *Cissampelos pareira*, these two plants have been selected for the acute toxicity study to explore for further investigations. So much has been done in screening medicinal plants for efficacy based on traditional claims while less emphasis is placed on the issue of safety, as reports of efficacy far outnumber those of toxicity, probably as a result of the greater demands for resources and time. Hence pharmacological and toxicological evaluations of medicinal plants are essential for drug development[6,7]. In the present study, the acute toxicity study of the methanolic extract of the bark of *Ficus racemosa* and root of *Cissampelos pareira* were evaluated in Female Wistar rats to assess its safety or otherwise, since the findings are important considering the usage of the plants by human beings.

MATERIALS AND METHODS

(i) Experimental Plants (Herbals)

The fresh barks of *Ficus racemosa* and fresh roots of *Cissampelos pareira* were collected from the tribal areas of Tanginiguda, Malkangiri district of Odisha state, South India during the flowering stage in the month of March 2012. Both the herbal species were identified, confirmed and duly authenticated by Dr. S. K.

Dash, Professor and Head, P.G. Department of Biosciences, CPS. Mohuda, Berhampur (Odisha) and were preserved in the institutional museum of College of Pharmacy (Poly), Pandharpur of Solapur district, Maharashtra for future reference. Barks were washed and shadedried for 20 days, powdered to get homogeneously coarse powder allowing it to pass through sieve of mesh No.20. This powder was stored in air tight container and used for further successive extraction. In the same manner roots of *Cissampelos pareira* were powdered and preserved in air tight container.

(ii) Extraction

1.Air-dried, powdered barks of *Ficus racemosa* was Soxhlet extracted with methanol.
2.Powdered roots of *Cissampelos pareira* was air dried and Soxhlet extracted with methanol.
Moreover, the extract was subjected to preliminary phytochemical screening for the detection of various plant constituents.

(iii) Animals

Animal study was performed in the division of Pharmacology, B. R. Nahata College of Pharmacy, Mandsaur, with permission from the Institutional Animal Ethical Committee (CPCSEA No. 1019/C/06/CPCSEA and Regd. No.009/Ph.D./2012/IAEC/MIP/Mandsaur).
Female Wistar rats weighing (150-200g) were used for the study. They were kept in clean plastic cages in a12 h. light/dark cycle with litter changed every week. They were fed with mice cubes and water*ad libitum*. A standard protocol was observed in accordance with the Good Laboratory Practice(GLP) Regulations of the WHO (1998).

(iv) Phytochemical screening

The methanolic bark extracts of *Ficus racemosa* and root extracts of *Cissampelos pareira* was screened as described by standard methods[8,9,10].

(v) Acute toxicity studies

The acute toxicity test of the extracts was determined according to the OECD guidelines No. 420 (Organization for Economic Co-operation and development). Female Wistar rats (150–180 g) were used for this study. After the sighting study, starting doses of 2000 mg/kg (P.O.) of the test samples were given to various extracts groups containing 5 rats in each group. Rats were randomly selected for the study and marked to provide individual identifications. Rats were observed immediately after dosing during first 30 minutes, periodically during the first 24 hours, with special attention given during first 4 hours, and daily thereafter for 14 days (OECD guidelines, 2001). During the first four hours rats were tested for following various responses. Behavioral Response: Behavioral response was assessed by parameters like, alertness, stereo type, irritability, fearfulness, touch response, pain response, spontaneous activity, grooming and restlessness Neurological Response: Neurological response was assessed by parameters like, righting reflex, limb tone, grip strength, twitching, abdominal tone, pinna reflex, corneal reflex, straub tail, tremors and convulsions. Autonomic Response: Autonomic response was assessed by parameters like, defecation, writhing, urination, piloerection, heart rate, respiratory, pupil size and skin color.

RESULTS

Qualitative chemical evaluation was carried out by using test solutions. Test solutions were prepared by dissolving the extracts in specific menstrum and clear, transparent solutions were used for testing. The extracts were tested for the presence of carbohydrates, alkaloids, tannins, flavonoids, saponins, proteins, fats, steroids and triterpenoids.

Table 1
Phytochemical screening

Plant Constituents	*Ficus racemosa*	*Cissampelos pareira*
Carbohydrates	(--)	(--)
Alkaloids	(--)	(+)
Tannins	(+)	(+)
Flavonoids	(+)	(+)
Saponins	(+)	(--)
Proteins	(--)	(--)
Fats	(--)	(--)
Steroids	(+)	(+)
Triterpenoids	(--)	(+)

(+) - Presence, (--) – Absence.

Till the end of 14th day toxic symptoms were not observed in the treated groups. All the animals were found healthy and the extracts were found to be safe up to a dose of 2000 mg/kgas shown in Table-2.

Table 2
Acute toxicity study of extracts

Plant name(s)	Results after 14 days
	Methanol Extracts
Ficus racemosa	+ + + + +
Cissampelos pareira	+ + + + +

(+) - Alive, (-) – Dead

The above table follows OECD 2001, Guideline for testing of chemicals; acute oral toxicity, Environmental health and safety monograph series on testing and adjustment no.420.

DISCUSSION

Acute toxicity test give clues on the range of doses that could be toxic to the animal. It could also be used to estimate the therapeutic index (LD50/ED50) of drugs and Xenobiotics[11].Phytochemicals are thought to have a positive or negative effect on an animal. Acute toxicity study[20] of *Ficus* and *Cissampelos* species has many reports. However these two species were found to be safe at a higher dose of 2000mg/kg. When administered to wistar rats. As per preliminary phytochemical screening both the plants contain tannins, flavonoids,alkaloids, triterpenoids and phytosterols; which have negative/positive impacts beyond the actual dose. Hence the safety measures of phytochemicals are to be maintained through acute toxicity study.Tannins and Flavonoids are thought to have both proxidant and antioxidant effects on the body. While the antioxidant protects the tissues and organs, the proxidant damages the tissues and organs. The alkaloids are the secondary plant metabolites act on a diversity of metabolic systems in humans and other animals. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Most triterpenoid compounds in adaptogenic plants are found as saponin glycosides which refers to the attachment of various sugar molecules to the triterpene unit. These sugars can be easily cleaved off in the gut by bacteria, allowing the aglycone (triterpene) to be absorbed[15].This allows them insert into cell membranes [16] and modify the composition, influence membrane fluidity[17], and potentially affect signaling by many ligands and cofactors[18]. Phytosterols may inhibit lung, stomach, ovarian and breast cancers[19].The weight changes of the animals during the period of observation which was

more visible at higher doses, suggest the presence of tannins and other phenolic which are thought to interfere with absorption of nutrients making them unavailable and thereby reducing feed intake[12]. Even though the animals were fed with adequate diet, the methanolic extract at higher doses could have caused the interference since phytochemical studies showed the presence of tannins. The extracts should not affect the hematological parameters of the animals. This is very surprising because the extracts contained the presence of saponins which has been reported to have deleterious haemolysing effect on circulating erythrocytes[13]. The absence of gross and histopathological lesions in the organs could suggest the level of safety of the aqueous extract on the animals. However, further long-term toxicological studies (chronic toxicity), are needed inorder to establish it as medicine. Though the phytochemical screening revealed many chemical constituents, which could affect the animal positively or negatively as a result of prolong usage, it is recommended that a long-term study be conducted. The effect on hemoglobin concentration (Hb), white blood cell (WBC) count and packed cell volume (PCV) indicated the unlikelihood of the extract to induce anemia even after long use[1].

CONCLUSION

The use of plants and plant preparations has been in existent since prehistory. The World Health Organization (WHO) reported that about 80% of the world's population depend mainly on traditional medicine and the traditional treatment involve mainly the use of plant extracts (WHO, 1993). In the present study, the root extract of *Cissampelos pareira* and bark extracts of *Ficus racemosa* showed promising activity against acute toxicity. The result of this study confirmed that *Ficus racemosa bark* and *Cissampelos pareira* root could be safe and beneficial in the management of dosage regimen.

ACKNOWLEDGEMENT

Sincere thanks are due to Prof. Edwin Jarald, HOD, Pharmacognosy cum Assistant Coordinator, TIFACCORE in Green Pharmacy of B. R. Nahata College of Pharmacy for his valuable help.

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