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Acute Toxicity Study of Rhizome of *Drynaria Quercifolia* (L.) J. SM.

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ABSTRACT
Toxicology studies helps to determine short term and long term adverse effects of a drug. The present study was done to evaluate the acute toxicity of powder (*choorna*) of rhizome of *Drynaria quercifolia* (L.) J. Sm. 15 male and 15 female Wistar Albino rats were used for the purpose. Single time oral administration of suspension of powdered rhizome was given in four doses viz 0.2 g (Half the calculated effective dose), 0.4 g (calculated effective dose), 0.8 gm (Two times the calculated effective dose) and 1.6 gm (Four times the calculated effective dose) per 200g body weight of rat. Changes in body weight, food and water intake and cage side observations were done for 14 days. On 15th day gross necropsy were done. There was no abnormal cage side observations, mortality and pathological changes of internal organs on necropsy in any of the treated groups even at highest dose of 1.6 gm/ 200 gm body weight.

KEYWORDS: *Drynaria quercifolia* (L.) J. Sm., acute toxicity, rhizome, *choorna*.

INTRODUCTION
Drynaria quercifolia (L.) J. Sm. is a perennial epiphytic, epipetric or terrestrial fern commonly found in tropical climatic conditions. It has thick, stout, nearly flat rhizome with dense covering of reddish brown soft scales. Fertile foliage fronds and sterile nest fronds grown from this rhizome. Round circular shaped sori found on the bottom surface of foliage fronds.[1, 2] The drug is known as *Aswakatri* in Sanskrit and *Thudinthappala* in Malayalam. Its rhizome is used as ingredient of some ayurvedic formulations of external use.[3] Internal use of its rhizome among tribal peoples also reported.[1]

In vivo toxicology studies helps to determine short term and long term adverse effects of a drug. It can be conducted as acute, subacute, subchronic and chronic toxicity studies. Among these acute toxicity study are done to determine the short term adverse effect. It provides information on acute toxicity in humans and also give estimate of safe acute dose and potential target organ of toxicity. [4]

In a previous research work on acute and sub-acute toxicity of methanolic extract of rhizome of *Drynaria quercifolia* (L.) J. Sm. there was no toxic signs in 2000mg/kg body weight of mice and 1g/kg body weight of rat respectively.[5] In another study on acute toxicity in Swiss Albino mice using ethanol extract, hexane extract and ethyl acetate extract no lethal signs was found in 5g/kg, 1000mg/kg and 1000mg/kg respectively.[6] The toxic effect of the rhizome in its crude form is not yet evaluated. So that, in present study evaluation on acute toxic effect of powder (*choorna*) of dried rhizome of *Drynaria quercifolia* (L.) J. Sm. were done.

EXPERIMENTAL WORK

Materials

15 Male and 15 female Wistar Albino rats weighing 150 to 200 gm, rat cages, suspension of the drug, feeding bottles, weighing machine, permanent marker, beaker, feeding cannula, gloves, surgical scissors, scalpel.

Collection and preparation of drug

The plant was collected from Vadakara village, Ernakulam district, Kerala during the month of July 2019. The pharmacognostical identification was done in the Pharmacognosy unit, Department of Dravyaguna vijnanam, Govt. Ayurveda College, Tripunithura. The debris, decayed materials and fronds of the plant were removed. Then the rhizome was washed well to remove the physical impurities. Hairs on its outer surface were removed using a knife. It was then cut into small pieces and dried well under shade. The properly dried rhizome free from moisture was powdered and sieved through a mesh of 120 sieve size to get fine powder. It was then stored in an airtight container.

Animal procurement

A total of 30 Wistar Albino rats (15 female and 15 male) were procured from the proposed source, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. (Reg. No. 328/GO/Re/S//01/CPSEA). The animals were kept in animal house of Department of Dravyagunavijnanam, Government Ayurveda College, Tripunithura. They were acclimatized for standard laboratory conditions for 7 days before study and were fed with standard rat pellet and purified water for drinking. The study started when the animals attained a body weight of 150 to 200 gm.

Dose fixation

The classical reference on dose of *choorna* (powder) of *Drynaria quercifolia* (L.) J. Sm. is not available. *Sarangadhara Samhita*, a book that details various dosage forms in Ayurveda mentions that 12 gm is the dose of *choorna* (powder) for adult.[7] From that reference the effective dose of the powdered drug for rats was calculated by multiplying with conversion factor 0.018 of Paget and Barnes table.[8] 0.216 gm was found as the calculated effective dose for rat of 200 gm body weight. Dose of Formalin used was 0.05 ml.

Preparation of *choorna* (powder) for the study

By mixing 12 gm powder in 100 ml of distilled water the suspension of the powdered drug prepared. 1 ml of suspension contains 0.12 gm of the powdered drug. The dose of suspension given to various dosage forms were then calculated using this. The volume of suspension administered to a rat of 200 gm body weight in calculated effective dose was 1.8 ml. The volume of suspension given to each animal were then calculated according to the dose as well as the weight of animal.

Mode of administration

Through oral route suspension of the powdered drug was administered to the rats.

Dosing schedule

Single time administration was done for all the groups through oral route.

Grouping of animals

The animals were divided into 5 groups of 6 rats (3 males, 3 females) each. Group A (Control) received distilled water. Group B, C, D and E were treated groups received the powdered the drug in various doses as given in Table 1.

Table 1: Grouping of animals

Sl no	Groups	Doses given
1.	Group A – control (No treatment)	Distilled water (2 ml/200g body weight)
2.	Group B (Half the calculated effective dose)	0.2 g/200g body weight

3.	Group C (calculated effective dose)	0.4 g/200g body weight
4.	Group D (Two times the calculated effective dose)	0.8 g/200g body weight
5.	Group E (Four times the calculated effective dose)	1.6 g/200g body weight

Mode of administration of drug

Oral route with help of stomach tube.

Dosing schedule

Single time administration

Method

Grouped animals were kept in separate cages for easier observation. They were weighed shortly before the drug administration. Animals were given with standard diet and potable water and the daily intake of water and food by them were calculated from the amount left in the cage. After the administration of respective doses powdered drug, animals were observed individually during the first 30 minutes, periodically during the first 24 hours, with special attention during the first 4 hours, and daily thereafter, for a total of 14 days. Weight of animals were again taken one week after the start of experiment and on 14th day and changes in weight were calculated and recorded. Other observations include changes in fur, skin, eyes, and faeces breathing abnormalities, gait, tremors, convulsions, salivation, lethargy, sleep and coma. At the end of the test on 15th day animals were humanely killed by cervical dislocation and subjected to gross necropsy.

Ethics

Approval from the institutional animal ethics committee was obtained and the number was No B4/2601/2017/AVC.

RESULTS & DISCUSSION

Cage side observations of all the animals were tabulated below in table 2.

Table 1: Cage side observation of all animals

Sl no	Parameter	Cage side observations
1.	Condition of the fur	Normal
2.	Condition of the skin	Normal
3.	Subcutaneous swelling	Nil
4.	Eye changes- dullness/ opacities	Nil
5.	Ptosis	Nil
6.	Colour and consistency of faeces	Normal
7.	Breathing abnormalities	Nil
8.	Gait	Normal
9.	Tremor	Nil
10.	Convulsions	Nil
11.	Salivation	Normal
12.	Lethargy	Nil
13.	Sleep	Normal
14.	Coma	Nil

Reduction in body weight of animals indicates adverse effect of drug. If a particular dose of drug produce 10 % or more reduction in body weight it is considered as toxic, irrespective of whether or not it is

accompanied by any other changes.[9] In present study there was no significant reduction in body weight in any of the treated animals for all the 14 days as compared with the day before treatment. Changes in food and water intake by the treated animals was also negligible for all days.

In acute toxicity study mortality is a main criteria of evaluation. There was no mortality even at the highest dose level i.e. 1.6 gm/ 200 gm body weight. So all the animals were humanely killed by cervical dislocation method on the 15th day and subjected to gross necropsy. There was no gross pathological changes in internal organs of any of the treated animals.

In present study orally administered rhizome of *Drynaria quercifolia* (L.) J. Sm. did not shown any significant changes in body weight, food and water intake as well as in cage side observations by all the treated animals. There was no mortality and pathological changes in internal organs on necropsy even at highest dose of 1.6 gm/ 200 gm body weight.

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