Haya: The Saudi Journal of Life Sciences **ISSN 2415-623X (Print)**
Scholars Middle East Publishers **ISSN 2415-6221 (Online)**
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

Toxicity evaluation of methanolic rhizome extracts obtained from Drynaria *quercifolia* (Linn.) J. Smith in experimental animals

Ranjan Padhy[1*], Sanjeeb Kumar Patro[2], Mrutyunjay Jena[3], Santosh Kumar Dash[1]
[1]P. G. Department of Biosciences, College of Pharmaceutical Sciences, Berhampur-760002, Odisha, India
[2]Department of Pharmacy, College of Pharmaceutical Sciences, Berhampur, -760002, Odisha, India
[3]P. G. Department of Botany,Berhampur University, Bhanja Bihar, Berhampur-760007, Odisha, India

***Corresponding author**
Dr. Ranjan Padhy

Article History
Received: 22.10.2017
Accepted: 25.10.2017
Published: 30.10.2017

DOI:
10.21276/haya.2017.2.7.4

Abstract: *Drynaria quercifolia* (Linn.) J. Smith has been used in many herbal folklore medicines in Ganjam - Gajapati districts of Odisha state, India. Study pertains to its toxicity profile was undertaken to establish its use as a safe drug for different ailments. Swiss albino mice and wistar albino rats were used for acute and sub-acute toxicity studies respectively. In acute toxicity model, the mice received a limit dose (2000mg/kg) of methanolic extract and kept under observation for 7 days. In sub acute toxicity studies, rats were treated with daily doses (1 g/kg body weight) of methanolic extract for 28 days. In acute toxicity study, samples did not show any mortality at the dose of 2000mg/kg body weight. Further, there were not any significant marked changes observed in hematological parameters too during sub acute toxicity tests. Methanolic extract (ME) of rhizomes of *Drynaria quercifolia* was found to be safe as a drug. It was without any toxicity at 2000mg/kg body weight and 1g/kg body weight during acute and sub acute toxicity evaluations respectively.
Keywords: *Drynaria quercifolia* (Linn.) J. Smith, Methanolic extract, rhizome, toxicity profile

INTRODUCTION

According to World Health Organization (WHO) reports, 80% of world's population depends on herbal medicines for their primary healthcare [1]. Hence WHO has shown great interest in documenting the use of medicinal plants used by tribal from different parts of the world [2]. Seeing its larger proportionate use, proper dosage monitoring and toxicological characteristics are required to be explored to keep the herbal practitioners abreast of the pros and cons of the medicaments in routine dispensing and their safety aspects as well. Preliminary phytochemical analysis of the methanolic and chloroform extracts of the plant rhizome showed the presence of flavanoids, saponins, phenolic compounds, tannins, steroids carbohydrates [3 & 4].

Toxicity study determines the adverse and lethal effect of a constituting chemical of drug value when administered into a biological system. This test is applied to animals, plants and microbial systems, while assessing and evaluating the toxic characteristics of a substance. Depending on the duration of drug exposure to animals, toxicological studies may be categorized into three types such as acute, sub-acute and chronic type [5 & 6]. Acute toxicity study determines the immediate toxic effect of the drug where a single dose in large quantities is given which involves estimation of LD_{50} value of a drug or chemical. Sub-acute toxicity study determines the effect of drug on biochemical and hematological parameters of blood and the histopathological changes occurring there in. in this study, repeated doses of drug were given in sub lethal quantity for a period of 14 to 21 days. On the other hand, chronic toxicity study involves determination of carcinogenic and mutagenic potential of a drug where the drug is given in different doses for three months to over a year period of time. Acute and sub acute toxicity evaluations are of much importance from the point of establishment of potential adverse effects of the herbal preparation and determination of the dose regimen for further studies. Recently, study of *in vitro* cytotoxicity using animal cell line culture is also carried out as one of the promising test criteria, the results of which seem to be at par in human system too.

Acute toxicity study defines the intrinsic toxicity of the chemical, Prediction of hazard to non-target species or toxicity to target species, determine the most susceptible species, provide information for the design and selection of dose level for prolonged studies etc. [7]. However, many toxicity studies are conducted

solely for the purpose of determining the LD_{50} of a chemical.

In the light of the above, the toxicity determination of an extract with ethno medicinal value was undertaken with the solvent extract of *Drynaria quercifolia* claimed as a medicinal plant for various ailments evident from the folklore literatures of Odisha state. Acute toxicity was studied using animal models like mice (*Mus musculus*). Morbidity was taken as the parameter of toxic property of the extract measured to determine the lethal dose (LD_{50}) and effective dose (ED_{50}). Both LD_{50} and ED_{50} values were used to calculate the therapeutic index of the crude drugs. Oral route of drug administration was followed for the purpose.

MATERIALS AND METHODS

The test substance

The various concentrations of ME (Methanolic extract) of rhizomes of *Drynaria quercifolia* were prepared in distilled water and were administered orally. Fixed dose (OECD Guideline no. 423, Annexure 2d) method of CPCSEA was adopted for toxicity studies.

Animals used

Swiss albino mice and Wistar albino rats were used for acute and sub-acute toxicity studies respectively. Animals were procured at least 2 weeks prior to study for acclimatization. Animal house was well maintained under standard environmental conditions of temperature 22 ± 2^0 C and room humidity 60±10% with 12 hours day and night cycle. Mice and rats were kept in groups of 6 per cage. They all were provided with standard commercial food pellets and water *ad libitum.* Proper Cleaning and sanitation was done every day. All animal experiments were carried out after obtaining the approval of institutional animal ethical committee *vide* registration number 1170/AC/08/CPCSEA and 1479/GO/c/11/CPCSEA.

Experimental procedure

Acute Toxicity Test

The acclimatized animals (mice weighing 20-30gm of either sex) were allowed to fast by withdrawing the food and water for 18 hours prior to the experiment. The prepared extracts were administered intraperitonially (i.p) following fixed dose (OECD Guideline no. 423, Annexure 2d) method of CPCSEA. Subsequent to administration, animals were observed closely for first 2hrs for any toxic manifestations. Common side effects such as mild diarrhea lose of weight and depression of treated animal groups was recorded within 7 days of observation. The toxicological effects were observed in terms of mortality at 2000 mg/kg body weight in all cases and expressed as the acute lethal dose (ALD_{50}).

Sub Acute Toxicity Test

The experimental animals (Wistar albino rats weighing 160±10gm) were divided into two groups of four animals each. One group was considered as experimental group while the other was used as control group. The methanolic extract was dissolved in 2% Tween 80 to get the concentration of 1mg/ml. Each albino rat of the experimental group was administered with 0.3 ml of sample solution containing 300µg of the herbal drug daily for 28 days. Similarly, the control group was administered with the same amounts of the solvent (vehicle) daily for 28 days. Oral route of administration was used for the purpose. The body weight of each animal was noted before and after the treatment of the animal with the herbal drug. Once the treatment was completed on the 28^{th} day biochemical studies were undertaken for which blood was collected from the jugular veins of each animal. For hematological study such as total and differential blood cell count, ESR and percentage of hemoglobin blood was drawn from tail vein of both groups before administration of the drug on 1^{st} day, 7^{th} day and after completion of the treatment. Along with hematological investigations gross general observation such as the behavior, CNS (central nervous system) excitation and depression, muscular weakness, salivation, diarrhea and the food intake of the rats were undertaken. Body weight was measured before and after drug treatment. Determination of haemoglobin percentage was done by Sahil's acid hematin method using Sahil's hemometer [8]. Differential count was estimated by microscopic method using Leishman's stain. Total count of RBC, WBC and platelet was carried out by haemocytometer and ESR was determined by Westergrens method using Westergren tube and stand [8].

Statistical analysis

All the values of the data were expressed as mean ± SEM. Significant differences were determined using a student t- test and the differences were considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

Use of herbal medicine is increasing day by day and it is now stand as one of the alternative to clinical therapy. But, it is often seen that these drugs are dispensed unscientifically by the traditional herbalists without knowing its proper dosage form. Ascertaining their safety and efficacy would be a ready reckon for the herbalists and to the persons in the profession as well. Thus, the sub acute and acute toxicity studies were undertaken to know about the safety aspects and select proper dosage form of the prepared herbal extract for further experiments.

Analysis of blood parameters are usually undertaken to evaluate toxic effects of a drug, as blood vascular system is the main medium and initially exposed system of the body. Any alteration in its components leads to change the normal functioning of

the body [9]. The change in body weight is also an indicator of adverse side effects resulted due to intake of toxic materials [10]. Effect on different organs is also an important criterion required to be evaluated.

In acute toxicity study, samples did not show mortality at the dose of 2000mg/kg body weight. Therefore, 2000mg/kg dose was considered as ALD_{50} cut off the dose under globally harmonized classification system (GHS) category 5 safe dose, as per OECD guideline 423 (Annexure 2d). Common side effects such as mild diarrhea loss of weight and depression in treated group of animals were not recorded with in the 7 days of observation. But, in present and further studies only 150mg/kg body weight dose was selected for *in vivo* studies. Since, published report suggested that >200mg/kg body weight of extracts for *in vivo* studies and >200µg/ml of extract concentrations *in vitro* are likely to be artificial despite of yielding reproducible effects. Even worse, such high concentrations may trigger non physiological effects resulting in artifacts [11].

During the sub acute toxicity tests, body weight was taken before and after treatment. These values were presented in table 1. Here it was found that the weight of control group increased by 1.79% and the experimental group by 1.6% respectively which were not significant. Neither change in the colour nor in the weight of the organs happened on application of the extract compared to the control group. The effect of the extract on the biochemical parameters showed little change which was not significant and hence there were not any adverse effect of the extract on the functions of the organs. The hematological parameters such as Hb, RBC, WBC, Platelets and differential count were compared with the control group and the change was also found to be insignificant (Table 2).

Table 1: The effects of ME (Methanolic extract) on average mean body weight (gm) in control and treated rats of sub acute toxicity studies

Treatment	Day 1	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28
Control group	159.5	161.66	163.33	165.0	165.83	166.66	169.16	171.66
MEDQ Treated (150mg/kg body wt.)	159.16	163.33	168.33	168.33	169.16	172.5	175.83	180.83

Table 2: Effect of ME on Hematological parameters of the control and treated group of rats after 28 days treatment

Group	RBC (million cells/cu.mm)	WBC (10^3cells/cu.mm	Haemoglobin (gm %)	Platelets (10^3/cu.mm)
Control	4.333±0.42	5.500±0.56	13.17±0.4	273.3±3.57
MEDQ treated (150mg/kg wt.)	4.833±0.70	5.833±0.47	14.33±0.3	289.2±5.54

Mean ± SEM (n = 4) $^{*}p < 0.05$. $^{**}p < 0.01$ vs control group. Control group received 0.05 ml 2% Tween 80 solution

CONCLUSION

In acute toxicity study, samples did not show mortality at the dose of 2000mg/kg body weight. Therefore, 2000mg/kg dose was considered as ALD_{50} cut off the dose under globally harmonized classification system (GHS) category 5 safe dose, as per OECD guideline 423 (Annexure 2d). Further, there were not any marked changes observed in hematological parameters including effect on other factors during sub acute toxicity tests concluded that it was safe to be used as a drug.

ACKNOWLEDGMENTS

Thanks are also due to the authorities of P. G. Department of Biosciences, CPS, Berhampur and P.G. Dept. Of Botany, Berhampur University, Berhampur for laboratory facilities to carry out the study. The authors also would like to acknowledge Botanical Survey of India, Kolkata for authentication of the plant specimen.

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