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STUDIES ON ACUTE AND SUBCHRONIC ORAL TOXICITY OF *PHALLUSIA ARABICA* SAVIGNY, 1816

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ABSTRACT

Determination of toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. In the present study, acute and sub chronic oral toxicity of the ethanolic extract of *Phallusia arabica* on albino rats were investigated. The objective of safety evaluation for acute toxicity testing was done as per standard guidelines of OECD. Twenty four hours acute toxicity was performed with a single dose of 2000 mg/kg and subchronic studies with six sets of six albino rats receiving a dose ranging from 200-2000 mg/kg bw for 14 days. No mortality was observed even at a dose of 2000 mg/kg. Severe gross behavioral changes like irritability, tremor, labored breathing, staggering and convulsion were noted at a dose of 1600 mg/kg body weight and above only. Hematological, serum biochemical parameters of the liver and kidney were unaltered throughout the study indicating non-toxic nature of the extract. The result of the study suggests there is no obvious toxicity on treatment with ethanolic extract of *Phallusia arabica*.

Key Words:- *Phallusia arabica*, Toxicity, Acute, Subchronic, Hematology, Biochemical parameters.

INTRODUCTION

Ocean, a store house of living organisms has gained attention as a topic of research for evaluation of pharmacological properties and synthesis of novel new compounds. Natural products obtained from marine organisms consist of biologically active substances. The number of active compounds isolated from marine biota is increasing rapidly and now exceeds with hundreds of new compounds being discovered every year (Faulkner, 2002; Proksch and Muller, 2006). A large proportion of these biologically active metabolites have been extracted from marine organisms, especially sponges, ascidians, bryozoans, molluscs and some of them are currently in clinical trials (Proksh *et al.*, 2002). Although research on bioactive compounds from ascidians have been recently initiated, it is significant that the first marine natural product, Didemnin B entering into human clinical trial, is an ascidian metabolite. Ascidians are marine organisms with world-wide distribution and are utilized as food in various countries. *Phallusia arabica* is an important ascidian occurring as the major component of fouling community on the hull of ships, piers, pilings, harbour installations and materials used for aquaculture operations in the Tuticorin Port Area (Meenakshi *et al.*, 2004). A review of literature reveals that taxonomical, biofouling, toxicity, antimitotic, antibacterial, antimicrobial, HPLC, HPTLC, infrared, GC-MS studies and pharmacological aspects of a few species of ascidians are available (Meenakshi, 1996, 2003 and 2006; Paripooranaselvi *et al.*, 2012; Meenakshi *et al.*, 2012 and 2013; Ganeshan *et al.*, 2011; Bala Amutha *et al.*, 2010; Gopalakrishnan *et al.*, 2011, 2012 and 2013; Meenakshi *et al.*, 2012 and Kohila

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Subathra Christy *et al*., 2013). Studies on toxicity of ascidians from different parts of the world are rather few. Acute and subchronic toxicity of ethanolic extract of *Phallusia arabica* has not yet been studied. Hence, the present attempt has been made.

MATERIALS AND METHODS

Animal material

Samples of *Phallusia arabica* were collected from Tuticorin coast by SCUBA diving and identified using key to identification of Indian ascidians (Meenakshi and Senthamarai, 2004). A voucher specimen AS 2276 has been deposited in the National Collections of Ascidians in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin-628002.

Taxonomic status

Phallusia arabica is a simple ascidian belonging to the Phylum: Chordata, Subphylum: Urochordata, Class: Ascidiacea, Order: Enterogona, Suborder: Phlebobranchia, Family: Ascidiidae, Genus: *Phallusia*, Species: *arabica*.

Preparation of extract

100 gram powder was extracted with ethanol using Soxhlet apparatus, cooled to room temperature, evaporated in a rotary evaporator under reduced pressure to obtain a brown sticky residue which was used for toxicity studies.

Experimental animals

Adult male wistar albino rats weighing about 180-200 g were obtained from Central Animal House, Annamalai University, Chidambaram, Tamil Nadu, India. They were housed in standard environmental conditions of temperature at 24±1°C under a 12 h dark-light cycle, and allowed free access to drinking water and standard pellet diet. Rats were deprived of food except water 16-18 hour prior to the experiments. The rules and regulations of the Animal Ethical Committee, Government of India were followed.

Experimental protocol

Acute oral toxicity

Acute oral toxicity study was performed according to the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals 423 (OECD 2002). Six rats were administrated with a single oral dose of 2,000 mg/kg body weight ethanolic extract of *Phallusia arabica* while the control group received saline. They were placed under continuous observation for gross behavioral changes like irritability, tremor, labored breathing, staggering, convulsion and death for the first 2 h and then frequently during the next 24 h after which the number of dead rats if any were recorded.

Subchronic oral toxicity

A total of thirty six mature albino rats were used in this study. These were divided into six groups of six each. Group I received normal saline and group II, III, IV, V and VI were treated with 200, 400, 800, 1600 and 2000 mg/kg body weight of the extract respectively for fourteen days. Food and water intake were monitored daily. They were observed for toxic signs and symptoms like morbidity and mortality at an interval of 2, 4, 8 12, 16, 24 h and thereafter twice daily till the end of the experiment. 24 h after the last dose and 18 h of fasting, blood samples were collected through cardiac puncture, under chloroform anaesthesia into heparinised tube for haematological studies and non heparinised tube for liver and kidney function test. Total count, RBC, platelets were performed using Neubauer haemocytometer and estimation of haemoglobin by Sahli's haemoglobinometer. Biochemical profile of Liver and kidney were determined by standard procedure (Balistrei and Shaw, 1987; Lowry 1951; James *et al.,* 2007; Reitman and Frankel, 1957; King and Armstrong, 1934; Sasaki *et al.,* 1972; Varley, 1976; Owen *et al.,* 1954).

Statistical analysis

Values are presented as mean ± S.E.M and statistically evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's t - test.

RESULTS AND DISCUSSION

The results of acute oral toxicity and severe gross behavioural changes using the ethanolic extract of *Phallusia arabica* is given in Table 1. During the 24 h period no mortality was observed indicating nontoxic nature of the extract. Severe gross behavioural changes like irritability, tremor, labored breathing, staggering and convulsion were noted at a dose of 1600 mg/kg body weight and above only. No other abnormal signs or mortality were noted during the 14 days sub chronic oral toxicity studies.

Table 2 shows the results of hematological parameters. A marginal increase in total count, RBC, platelets and a minor variation in the percentage of hemoglobin and haematocrit was noted indicating normal haemopoiesis and absence of anemia.

The result of liver function is shown in Table 3. In the present study hepatic biochemical parameters like total bilirubin, protein, albumin, globulin, SGPT, SGOT and ALP were well within normal limits revealing the

safety profile of the extract on liver function even on its chronic use. Sodium, Potassium, Bicarbonate, Chloride, Glucose, Urea and Creatinine are considered as good indicators of renal function. The normal values of the renal biochemical parameters, including urea and creatinine suggest that the extract does not produce any sort of disturbance in the renal function (Table 4). Estimation of serum biochemical parameters after 14 days of subchronic oral toxicity studies indicated normal liver and kidney function. Similar observations have been reported from the ethanolic extract of *Microcosmus exasperatus* and methanolic extract of *Phallusia nigra* (Meenakshi *et al.*, 2012 and 2013). This is the first report on the acute and subchronic oral toxicity of ethanolic extract of *Phallusia arabica* indicating that it does not have any toxic effect on the liver and kidney. This investigation thus provides evidence for the total safety profile of the ethanol extract of *Phallusia arabica*, suggesting its safe use in single dose treatment and long term therapeutic application without producing any toxic effects. Hence further pharmacological studies can help to explore and establish the bioactive constituents of the extract which can be used safely for the treatment of various chronic diseases like diabetes and cancer in future.

Table 1. Subchronic oral toxicity with ethanol extract of *Phallusia arabica*

Group/ Dose mg/kg	Gross behavioural changes (No of animals)	Score
I- saline	Irritability (0) Tremor (0) Laboured breathing (0) Staggering(0) Convulsion (0) Death (0)	Normal
II-200	Irritability (0) Tremor (0) Laboured breathing (0) Staggering(0) Convulsion (0) Death (0)	Good Normal activities seen
III-400	Irritability (0) Tremor (0) Laboured breathing (0) Staggering(0) Convulsion (0) Death (0)	Good Normal activities seen
IV-800	Irritability (0) Tremor (1) Laboured breathing (0) Staggering(0) Convulsion (3) Death (0)	Fair Normal activities seen
V-1600	Irritability (2) Tremor (2) Laboured breathing (4) Staggering(4) Convulsion (3) Death (0)	Bad Normal activities not seen
VI-2000	Irritability (5) Tremor (5) Laboured breathing (6) Staggering(5) Convulsion (4) Death (0)	Bad Normal activities not seen

Table 2. Effect on hematological parameters

Group/ Dose mg/kg	Total count cells/mm^3	RBC million/mm^3	Platelets million/mm^3	Hemoglobin %	Haematocrit %
I- saline	7420±20	4.16±0.41	174.93±9.65	13.56±1.23	43.85±0.24
II-200	6120±24	4.68±0.25	197.45±10.65	11.23±1.95	33.27±0.26
III-400	7380±27	4.98±0.19	206.73±11.43	12.97±1.69	37.18±0.94
IV-800	7460±32	5.02±0.30	211.37±11.51	13.75±1.59	43.06±0.86
V-1600	7840±55	5.08±0.35	202.28±12.68	12.52±1.54	38.12±0.34
VI-2000	7901±22	5.88±0.27	198.33±12.13	12.72±1.50	37.25±0.45

Values are mean ± S.E.M. (n = 6)

Table 3. Effect on liver function

Group/ Dose mg/kg	Total bilirubin mg/dl	Total protein gm/dl	Albumin gm/dl	Globulin gm/dl	SGPT (U/L)	SGOT (U/L)	ALP (U/L)
I-Saline	0.72±0.02	7.84±0.13	4.26±0.46	3.58±0.27	12.93±1.24	18.54±0.98	113.65±4.48
II-200	0.76±0.04	7.43±0.17	4.23±0.18	3.00±0.28	14.63±1.07	16.75±0.34	129.54±3.95
III-400	0.93±0.05	7.89±0.47	4.35±0.48	3.54±0.72	13.93±1.04	18.34±1.13	118.54±4.07
IV-800	0.98±0.07	8.12±0.34	4.84±0.28	3.28±0.14	16.39±0.33	17.54±0.48	129.56±4.42
V-1600	0.91±0.02	8.24±0.16	4.73±0.42	3.51±0.25	16.64±0.43	16.64±0.93	113.65±5.13
VI-2000	0.87±0.03	8.34±0.35	4.56±0.32	3.55±0.26	16.95±0.56	16.70±0.65	115.45±5.15

Values are mean ± S.E.M. (n = 6)

Table 4. Effect on renal function

Group/ Dose mg/kg	Sodium m M/L	Potassium m M/L	Bicarbonate m M/L	Chloride m M/L	Glucose mg/dl	Urea mg/dl	Creatinine mg/dl
I-Saline	149.45±4.65	4.87±1.13	21.26±3.16	101.38±2.27	77.93±5.24	16.27±0.26	0.92±0.06
II-200	153.37±4.12	4.56±1.57	25.63±2.78	103.00±3.48	84.63±4.07	20.64±1.48	0.86±0.02
III-400	152.63±4.35	4.69±1.17	24.34±2.98	104.39±2.85	86.98±4.04	18.39±1.32	0.92±0.04
IV-800	157.49±5.67	4.72±1.24	23.84±2.28	102.28±2.74	74.39±5.33	19.22±0.96	0.81±0.03
V -1600	155.91±4.22	4.42±1.16	28.73±2.42	101.51±2.25	76.74±4.43	21.46±1.34	0.85±0.05
VI-2000	154.47±4.63	4.54±1.15	27.46±2.32	100.45±2.16	77.85±3.56	22.70±1.45	0.87±0.06

Values are mean ± S.E.M. (n = 6).

CONCLUSION

In conclusion, this study presents strong evidence of nontoxic effect of the ethanol extract of *Phallusia arabica*. The results show that the use of the ethanol extract of *Phallusia arabica* is safe and explains the extensive utilization of the marine organism in medicine.

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