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Studies on the Toxicity of crude Methanol Extract of *Phallusia nigra* Savigny, 1816

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ABSTRACT

The current study reports the results of the acute, sub chronic oral toxicity of the crude methanol extract of a simple ascidian *Phallusia nigra* administered to albino rat models for 24 hours and fourteen days respectively. Hematological parameters, liver and kidney function tests were performed to assess the safety of the extract. The results of acute toxicity indicated that the LD_{50} of the extract is greater than 1600 mg/kg body weight. Severe gross behavioral changes like irritability, tremor, labored breathing, staggering and convulsion were noted at a dose of 800 mg/kg body weight and above only. No mortality was observed during the acute and sub chronic toxicity studies on treatment with a dose of 200, 400, 800 and 1600 mg/kg body weight for 24 hours and fourteen days. Hematological, serum biochemical parameters of the liver and kidney were well within normal limits indicating non toxic nature of the extract.

Keywords: *Phallusia nigra,* Toxicity, Acute, Sub chronic, Liver function, Kidney function.

1. INTRODUCTION

Marine organisms are a rich source of structurally novel and biologically active metabolites. Primary and secondary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. Ascidians are marine sedentary organisms and they belong to biofouling community. They are found in piers, pilings, harbor installations, materials used for aquaculture operations etc. *Phallusia nigra* is a simple ascidian found in plenty in Tuticorin coast. Since the report of *Phallusia nigra* from Tuticorin coast of India [1], studies on the ecology, distribution, seasonal variation in the occurrence, taxonomy [2], breeding biology, recruitment and succession in the fouling community, role as bioindicators [3], association with coral reef [4], antimicrobial activity to human pathogens [5, 6, 7], food value [8], larvicidal potency [9], chemical investigation [10, 11, 12] and pharmacology [13, 14, 15] have been attempted. However, systematic toxicity studies of the crude extract of *Phallusia nigra* has not been carried out so far. The present study has been designed to assess the acute and sub chronic oral toxicity studies of the methanol extract of *Phallusia nigra*.

2. MATERIALS AND METHODS

2.1. Animal material

Samples of *Phallusia nigra* were collected from Tuticorin coast by SCUBA diving. They were identified using key to identification of Indian ascidians [16]. A Voucher specimen AS 2083 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-628 002.

2.1.1. Taxonomic status

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

2.2. Preparation of extract

Epibionts adhering to the test were carefully removed, washed repeatedly with sterile sea water, dried under shade, homogenized to get a coarse powder and stored in an airtight container. The powder was extracted with methanol 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.

2.3. Experimental Animals

Adult Wister Male rats weighing about 180-200 gm were used for the experiments. Healthy animals were purchased from Central Animal House, Annamalai University, Chidambaram, Tamil Nadu, India. The animals were acclimatized to the laboratory environment for a week during which they were allowed free access to clean water and pellet food. A constant 12 hours of darkness and 12 hours of light schedule, room temperature (24±2°C) and humidity (60-70 %) were maintained in a well ventilated animal house. The experiments were conducted according to the rules and regulations of the Animal Ethical Committee, Government of India.

2.4. Experimental protocol

2.4.1. Acute oral toxicity

Different doses of the methanol extract of *Phallusia nigra* was blended with gum acacia and 2 ml of 2 % vanillin as flavoring agent and were administered orally to five groups of six rats. The animals in group I served as control and received normal saline. Group II, III, IV and V were treated with 200, 400, 800 and 1600 mg/kg body weight of extract respectively through an oral canula attached to a graduated syringe. 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, and LD_{50} calculated by the modified method of Aliu and Nwude [17].

2.4.2. Sub chronic oral toxicity

The animals were divided into five groups of six each. Group I received normal saline and group II, III, IV and V were treated with 200, 400, 800 and 1600 mg/kg body weight of the extract respectively for fourteen days. During the course of the experiment, the animals were weighed every three days, food and water intake 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 carried out by standard procedure [18, 19, 20, 21, 22, 23, 24, 25].

2.5. 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.

3. RESULTS AND DISCUSSION

During the 24 h acute oral toxicity study no mortality was observed indicating that the LD_{50} is above 1600 mg/kg body weight. Severe gross behavioral changes like irritability, tremor, labored breathing, staggering and convulsion were noted at a dose of 800 mg/kg body weight and above only (Table 1 and 2). No other abnormal signs or mortality were noted during the 14 days sub chronic oral toxicity studies. During the experimental period, no significant change in the total count, RBC, platelets, percentage of hemoglobin and haematocrit was noted showing normal haemopoiesis and absence of anemia (Table 3).Bilirubin is formed from degeneration of haemoglobin by the action of reticuloendothelial system. Increased bilirubin level reflects the depth of jaundice [26]. Variation in the total protein, albumin, globulin can be assigned to the damage of hepatocellular inclusions leading to decrease in protein synthesis and accumulation of triglycerides resulting in fatty liver [27].

Table - 1: Acute oral toxicity of the methanol extract of *Phallusia nigra*

Group	Dose mg/kg bw	No. of Rats	Death	Dose difference	Mean death
Group I	saline	6	0	0	
Group II	200	6	0	200	-
Group III	400	6	0	400	-
Group IV	800	6	0	800	-
Group V	1600	6	0	1600	-

Table - 2: Gross behavioural changes observed with methanol extract of *Phallusia nigra*

Group	Dose mg/kg	Signs & symptoms (No of animals)	Score
Group I	saline	Irritability (0) Tremor (0) Laboured breathing (0) Staggering (0) Convulsion (0) Death (0)	Normal
Group II	200	Irritability (0) Tremor (0) Laboured breathing (0) Staggering (0) Convulsion (0) Death (0)	Good Normal activities
Group III	400	Irritability (1) Tremor (0) Laboured breathing (1) Staggering (2) Convulsion (1) Death (0)	Poor Normal activities not seen
Group IV	800	Irritability (4) Tremor (4) Laboured breathing (4) Staggering (4) Convulsion (3) Death (0)	Very poor Normal activities not seen
Group V	1600	Irritability (4) Tremor (4) Laboured breathing (4) Staggering (4) Convulsion (3) Death (0)	Very poor Normal activities not seen

Table - 3: Effect of methanol extract of *Phallusia nigra* on hematological parameters

Parameter	Units	Group **I**	Group II	Group III	Group IV	Group **V**
Total count	cells/cu mm	7860±40	6920±35	7240±20	7110±30	7900±20
Hemoglobin	%	12.36±1.56	11.67±1.05	12.98±1.69	13.04±2.11	12.67±1.34
RBC	10x6 ul	4.52±0.23	4.03±0.12	4.79±0.32	4.98±0.54	4.57±0.22
Haematocrit	%	37.21±0.56	36.43±0.94	38.66±1.01	39.59±1.77	37.11±0.78
Platelets	10x3 ul	197.55±12.35	186.28±10.25	193.33±8.34	194.22±11.51	198.55±10.50

Values are mean ± S.E.M. (n = 6)

Table - 4: Effect of methanol extract of *Phallusia nigra* on liver function

Parameter	Units	Group **I**	Group II	Group III	Group IV	Group **V**
Total bilirubin	Mg/dl	0.78±0.02	0.93±0.05	0.96±0.03	0.92±0.03	0.85±0.04
Total protein	Gm/dl	6.98±0.11	7.48±0.23	7.59±0.17	7.56±0.33	7.88±0.74
Albumin	Gm/dl	4.05±0.21	4.28±0.73	4.78±0.33	4.49±0.43	4.21±0.22
Globulin	Gm/dl	2.93±0.11	3.30±0.23	3.41±0.26	3.57±0.12	3.62±0.13
SGPT	(U/L)	16.57±0.01	15.58±0.03	16.26±0.05	16.56±0.06	16.99±0.06
SGOT	(U/L)	23.11±0.04	21.93±0.04	23.58±0.06	24.45±0.03	23.21±0.06
ALP	(U/L)	143.34±1.99	156.27±2.55	169.72±2.48	166.39±2.66	168.22±2.31

Values are mean ± S.E.M. (n = 6)

Table - 5: Effect of methanol extract of *Phallusia nigra* on renal function

Parameter	Units	Group I	Group II	Group III	Group IV	Group V
Sodium	mM/L	154.66±3.82	163.54±2.12	160.23±1.12	164.32±5.42	162.48±3.16
Potassium	mM/L	4.76±1.04	4.50±1.62	4.72±1.16	4.68±1.24	4.39±1.36
Bicarbonate	mM/L	20.46±2.88	24.12±2.68	23.80±2.55	21.74±2.96	22.54±2.43
Chloride	mM/L	100.08±2.26	102.14±3.63	101.46±2.85	99.78±3.49	99.54±3.88
Glucose	mg/dl	73.15±6.35	80.50±4.66	81.8± 4.10	72.90±5.80	74.56±3.77
Urea	mg/dl	16.4±1.78	13.5±1.67	12.40±0.98	13.33±1.20	14.66±1.55
Creatinine	mg/dl	0.89±0.18	0.81±0.06	0.86±0.21	0.85±0.08	0.84±0.26

Values are mean ± S.E.M. (n = 6)

Any alteration in the level of SGPT, SGOT, ALP reflects the structural and functional dysfunction of hepatocellular membrane or disintegration of cells recording liver damage [28,29]. In the present study hepatic biochemical parameters like 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 (Table 4). 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 5). This investigation thus provides evidence for the total safety profile of the methanol extract of *P. nigra,* 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.

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4. REFERENCES

1. Meenakshi VK. Occurrence of a new ascidian species – *Distaplia nathensis* sp. nov. and two species - *Eusynstyela tincta* (Van name, 1902), *Phallusia nigra* (Savigny,1816), new records for Indian waters, Indian Journal of Marine Sciences, 1998; 27: 477-479.
2. Meenakshi VK. Studies on a few aspects of ascidians-Taxonomy, Biofouling, Bio indicators and Economic importance, Final Technical report submitted to the University Grants commission, Hyderabad. 1996; 1-25.
3. Meenakshi VK. Biology of a few chosen ascidians, Ph. D., Thesis, Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India, 1997; 157-217.
4. Senthamarai S. Ascidians associated with coral reef in Tuticorin coast, M.Phil. Thesis, Manomanian Sundaranar University, Tirunelveli, Tamilnadu, India, 2004; 1-81.
5. Chandramathi V. Studies on the antibacterial activity of methanol extract of two chosen ascidians, M. Phil., Thesis, Manonmaniam Sundaranar University, Tamilnadu, India, 1997; 1-35.
6. Meenakshi VK. Screening of a few chosen ascidians of Tuticorin coast for antimicrobial activity, Final technical report submitted to University grants Commission, Hyderabad, 2006; 1-38.
7. Gopalakrishnan S, Shanmuga Priya D, and Meenakshi VK. Antimicrobial activity of the methanolic extract of *Phallusia nigra* Sav, Journal of Natural products and plant resourses, 2012; 2: 579-583.
8. Ajitha Kumary V. Food value of a few ascidians, M.Phil, Thesis, Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India, 1994; 1-39.
9. Paripooranaselvi M and Meenakshi VK. Screening of a few chosen ascidians of Tuticorin coast for larvicidal activity, Annals of Biological Research, 2012; 3: 3849-3852.
10. Gopalakrishnan S, Meenakshi VK and Shanmuga Priya D. Chemical investigation of

the simple ascidian *Phallusia nigra* Savigny, 1816 of Tuticorin coast by GC – MS, International Journal of Pharma and Biosciences, 2011; 2: 382 -387.

11. Gopalakrishnan S, Shanmuga Priya D and Meenakshi VK. Pharmacognostical and Preliminary Phytochemical Evaluation of *Phallusia nigra* Sav, Global Journal of Pharmacology, 2013; 7: 39-44.
12. Meenakshi, VK, Paripooranaselvi M, Gomathy S and Chamundeswari KP. GC-MS analysis of the ethanolic extract of *Phallusia nigra* Savigny, 1816, Proceedings of National Conference on Frontiers In Spectroscopy - 2012; 2012: 12-20.
13. Gopalakrishnan S, Meenakshi VK and Shanmuga Priya D. Antipyretic and Analgesic activity of *Phallusia nigra* Savigny, 1816, Annals of Biological Research, 2011; 2: 192-196.
14. Gopalakrishnan S, Shanmuga Priya D and Meenakshi VK. Wound healing activity of the methanolic extract of *Phallusia nigra* Sav, International Journal of Chemical and Pharmaceutical Sciences, 2012; 3: 45-51.
15. Gopalakrishnan S, Meenakshi VK and Shanmuga Priya D. Anaesthetic activity of *Phallusia nigra* Savigny, Annals of Biological Research, 2012; 3: 1863-1865.
16. Meenakshi VK. Marine Biodiversity - Taxonomy of Indian Ascidians, Final technical report submitted to Ministry of Environment and Forest, New Delhi, 2003; 1-19.
17. Aliu OY, Nwude N. Veterinary Pharmacology and Toxicology Experiments, A. B. U. Publishers Ltd, Zaria, Nigeria, 1982; 94-96.
18. Balistrei WR and Shaw LM. Liver function In: Fundamental of Clinical chemistry, (Ed) Tietz N.W.3rd edition. W.B. Saunders Company, Philadelphia, 1987; pp. 729-761.
19. Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ. Protein measurement with the folin's phenol reagent, Journal of Biological Chemistry, 1951, 265-275.
20. James S, Bilbiss L and Muhammad BY. The effect of *Catharanthus roseus* (L.) G.Don. 1838 aqueous leaf extraction on some liver enzymes, serum proteins and vital organs, Science World Journal, 2007; 2: 5-9.
21. Reitman S and Frankel SA. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases, American Journal of Clinical Pathology, 1957; 28: 56-63.
22. King EJ and Armstrong AR. Determination of serum and bile phosphatase activity, Canadian Medical Association Journal, 1934; 31: 56-63.
23. Sasaki T, Matsuy S and Sanae A. Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose determination, Ransho Kagajc, 1972; 346-350.
24. Varley H. Practical clinical chemistry, Arnold Heinemann Publication Private Limited, 1976; 452.
25. Owen JA, Iggo JB, Scangrett FJ and Steward IP. Determination of creatinine in plasma serum, a critical examination, Journal of Biochemistry, 1954; 58: 426-437.
26. Wooton IDP. In: Microanalysis in Medical Biochemistry, (4th edn). J & A Churchill Ltd., London, 1964; 138.
27. Takate SB, Pokharkar RD, Chopade VV and Gite VN. 2010. Hepato-protective activity of the ethyl acetate extract of *Launaea intybacea* (JACQ) Beauv in paracetamol induced hepato-toxicity in albino rats, International Journal of Pharmaceutical Sciences Review and Research, 2010; 1: 72-74.
28. Wasan KM, Najafi S, Wong J and Kwong M. Assessing plasma lipid levels, body weight, and hepatic and renal toxicity following chronic oral administration of a water soluble phytostanol compound FMVP4, to Gerbils, Journal of Pharmaceutical Science, 2001; 4, 228-234.
29. Wintrobe MM, Lee GR, Boggs DR, Bithel TC, Athens JW and Foerester J. Clinical Hematology, (5th edn), Philadelphia, Les and Febiger, PA, 1961; 326.