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IN VIVO ACUTE AND SUB ACUTE ORAL TOXICITY OF DIDEMNUM PSAMMATHODES

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ABSTRACT

Oral toxicological testing in animal models helps to identify the safety profile of drug leads. These are essential preliminary steps in the invention of new drugs. Acute and sub acute oral toxicity of the ethanol extract of colonial ascidian *Didemnum psammathodes* supplemented with and without Vitamin E was evaluated after single and 28-day repeated oral dosing in Swiss albino mice. The biochemical profile of liver, kidney and haematological parameters were also assessed adopting standard procedure. Single day oral dosing of 2000 mg/kg bw did not indicate any mortality. Administration of the extract at 100, 500, 1000 and 2000 mg/kg bw for 28 days indicated changes in behaviour above a dose of 500 mg/kg, but no mortality. Results of the hepatic, renal function tests and blood profile were within normal limits below 500 mg/k confirming the oral safety of the extract.

KEYWORDS: Acute, sub acute, oral, *Didemnum psammathodes.*

INTRODUCTION

Toxicity is the degree to which a substance can harm human or animals.[1] Toxic manifestations of drug leads can be identified by toxicological testing in animal models. In the development of new drugs, these tests form one of the preliminary and essential steps.[2] Acute toxicity test identifies the dose causing major adverse effects and sub acute toxicity assesses the side effects of repeated exposure to lower doses of the extract and to fix the safe dosage during pharmacological evaluations. It is also intended to monitor the systemic toxicity, gross behavioural changes, hepatic, renal function and haematology. Acute and sub acute oral toxicity studies are usually carried out to establish the safety profile before

performing *in vivo* mouse model experiments with the crude extracts. Reports on the acute and sub chronic oral toxicity of the ethanolic extract of simple ascidians *Microcosmus exasperatus*,[3] *Phallusia nigra*,[4] *Phallusia arabica*,[5] *Ascidia sydneiensis*[6] and the colonial ascidian *Ecteinascidia venui*[7] to Wistar rats are available. From India, there is lack of proven scientific data on toxicity, adverse effects and safety of crude extracts of marine organisms and comprehensive *in vivo* mouse model study to categorise the toxic effects of the extract of *Didemnum psammathodes* supplemented with and without Vitamin E.

OBJECTIVES

The objective of the present study is to evaluate the safety of ethanol extract of colonial ascidian - *Didemnum psammathodes* supplemented with (+) and without (-) vitamin E by acute and sub acute toxicity after single and 28-day repeated oral dosing in Swiss albino mice, estimate serum biochemical profile of liver, kidney and hematological parameters.

MATERIALS AND METHODS

Animal material

The specimens of colonial ascidian, *Didemnum psammathodes* were collected from the intertidal rocky shore area of Thoothukudi north break water in the month of July to November and identified. A voucher specimen AS 2273 and has been submitted in the National collections of the ascidians of the museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin.

Preparation of powder and extract

Epibionts adhering to the surface of the test were carefully removed. The specimen was washed several times with sterile sea water. It was dried under shade, homogenized to get a coarse powder and stored in an air-tight container. 100 g powder was extracted with ethanol using Soxhlet apparatus, cooled to room temperature and evaporated in a rotary evaporator to get a residue. This residue was used for further studies.

Experimental animal

Healthy adult Swiss albino mice weighing 20-25 g were procured from Central Animal House, Annamalai University, Chidambaram, Tamil Nadu, India. They were kept in clean proper ventilated cages and acclimatized to standard environmental conditions for one week. The temperature of the room was maintained at 24±2ºC, a relative humidity between 60-70% and 12 hours of dark and 12 hours light schedule. The animals were fed with standard pellet

diet and safe drinking water *ad libitum*. Prior to the experiments, animals were fastened by withholding food for 3-4 hours. Water was given without any restriction. After administration of the doses, food was withheld for 1-2 hours. The rules and regulations of the Animal Ethical Committee, Government of India were followed.

Experimental protocol

Acute oral toxicity studies

Acute oral toxicity of the ethanolic extract of *Didemnum psammathodes* supplemented with (+) and without (-) vitamin E were performed as per OECD guideline for testing of chemicals 423.[8] Principle of this method involves administering the substance to be tested to a group of experimental animals at one defined dose. Based on the results - presence or absence of mortality at the selected dose, further testing with the same dose, next higher or lower dose is decided. Swiss albino mice weighing 20-25 g were randomly distributed into three groups of six animals each. Group I was treated as control and received saline whereas Group II and III were considered as experimental. A single oral dose of 2000 mg/kg body weight of ethanolic extract of *Didemnum psammathodes* supplemented with vitamin E (+) was administered to group II while group III received the extract without (-) the vitamin supplement. The treatments were given through an intra gastric catheter in the morning at 9 am. One to two hours after oral treatment with the extract, the experimental animals were given free access to pellet food and water. They were kept under continuous observation for any toxic signs or symptoms including mortality once during the first 30 minutes, at an interval of one hour and then periodically during the first 24 hours and daily for the next 14 days. The percentage of mortality in each group was noted and recorded.

Sub acute oral toxicity studies

Sub acute oral toxicity of the ethanolic extract of *Didemnum psammathodes* to OECD guideline 407.[9] Mature Swiss albino mice numbering thirty were divided into five groups of six each. Group I which received normal saline was treated as control. Group II, III, IV and V were administered with 100, 500, 1000 and 2000 mg/kg body weight of the extract of *Didemnum psammathodes* supplemented with vitamin E (+) respectively through intra gastric catheter daily for a period of 28 days. Another set of four groups were treated in the same way with similar concentrations of the extract without vitamin supplement (-). Animals were supervised daily for food and water intake. They were carefully observed for clinical signs, gross behavioural changes like irritability, tremor, laboured breathing, staggering and

convulsion at different time intervals of 2, 4, 8, 12, 16, 24 hours, twice daily for a period of 28 days. At the end of the experimental period of 28 days, animals were deprived of food and water for 8 hours and then sacrificed. The blood sample was collected and serum biochemical profile of liver, kidney and haematological parameters were determined.[10-22] The percentage of haemoglobin was estimated by Sahli's haemoglobinometer and RBC, WBC, platelets, differential count of lymphocytes, neutrophils and eosinophils were performed by using Neubauer haemocytometer.

Statistical analysis

Data are represented as mean ± S.E.M and statistically evaluated by one-way analysis of variance (ANOVA) followed by student's t - test to identify the differences between treated groups and control. P values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Oral toxicity assessments form the basis of the safety of any drug formulation. Acute oral toxicity studies using the extracts of *Didemnum psammathodes* supplemented with (+) and without (-) vitamin E revealed that the extracts are safe and practically nontoxic in single dose of 2000 mg/kg body weight to Swiss albino mice. This may imply that the components present in the extract may be of low toxic nature, poorly absorbed by the alimentary canal or that they are quickly broken down to nontoxic metabolites. To assess the morphological and physiological changes in the vital organs it is essential to evaluate the sub acute toxic profile. Sub acute oral toxicity studies showed that the animals treated with 500 mg/kg body weight of extract and above indicated irritability, tremor, labored breathing, staggering and convulsion but not mortality (Table-1). In the present study, it was noted that the batches which received the extract supplemented with vitamin E showed lesser changes in gross behaviour. Free radicals released during toxic conditions induce oxidative stress which in turn damages the cells as well as tissues of vital organs. Antioxidants play a major role by reducing the generation of ROS or by scavenging them. This can be attributed to the antioxidant nature of the vitamin supplement.

Table 1: Effect of *Didemnum psammathodes* supplemented with (+) and without (-) vitamin E on gross behavioral changes.

Group/ Dose mg/kg	Signs and symptoms (No of animals)		Score	
	+	-	+	-
I-saline	Irritability (0), Tremor (0), Laboured breathing (0), Staggering (0), Convulsion (0), Death (0)	Irritability (0), Tremor (0), Laboured breathing (0), Staggering (0), Convulsion (0), Death (0)	I	I
II-100	Irritability (0), Tremor (0), Laboured breathing (0), Staggering (0), Convulsion (0), Death (0)	Irritability (0), Tremor (0), Laboured breathing (0), Staggering (0), Convulsion (0), Death (0)	I	I
III-500	Irritability (0),Tremor (2),Laboured breathing (1), Staggering (2), Convulsion (1), Death (0)	Irritability (2), Tremor (3, Laboured breathing (4), Staggering (4), Convulsion (2), Death (0)	II	III
IV-1000	Irritability (2), Tremor (2), Laboured breathing (2), Staggering (2), Convulsion (2), Death (0)	Irritability (4), Tremor (6,)Laboured breathing (5), Staggering (6), Convulsion (5), Death (0)	IV	V
V-2000	Irritability (2), Tremor (3), Laboured breathing (2), Staggering (4), Convulsion (2), Death (0)	Irritability (5), Tremor (6), Laboured breathing (6), Staggering (6), Convulsion (6), Death (0)	V	V

I - Normal; II - low effect; III - medium effect; IV - high effect; V - Very high effect.

Liver function test showed a moderate elevation in the level of bilirubin, ALT, AST and ALP in the groups treated with a dose of 500 mg/kg body weight and above compared to control (Table-2). Elevated bilirubin are an indication of altered liver functions and a small elevation is an important indicator of liver damage in laboratory animals or could be a sign of biliary duct obstruction.[23] Determination of plasma protein like albumin can act as a criterion for analyzing the synthetic capacity of the liver.[24] Heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reaction caused by toxicants.[25] In the present study, the serum biochemical parameters related to kidney functions demonstrated significant differences with respect to control group at a dose of 500 mg/kg of the extract and above only, it can be inferred that higher dose of extracts can affect the normal renal functions (Table-3).

Analysis of the hematological parameters in the groups which received a dose of 500 mg/kg body weight and above of the extract recorded a decrease in the content of hemoglobin, platelets, lymphocytes; increase in WBC, neutrophils (Table-4). In both the batches of group

II, treated with 100 mg/kg of the extract, the values for all the haematological parameters were well within normal limits. Haematological parameters are most sensitive and while evaluating the toxicity of medicines in humans and animals a blood profile normally gives important information on the reaction of the body to damage or stress.[26] It is suggested that the tested extract may not have harmful effects on bone marrow function at a dose range below 500 mg/kg body weight thus justifying the fact that it does not induce anaemia or other serious effect on the hemopoietic system making it safe for further *in vivo* pharmacologic studies in Swiss albino mice.

Table 2: Effect of *Didemnum psammathodes* on liver function.

Group/ Dose mg/kg	Total bilirubin mg/dl		Total protein g/dl		Albumin g/dl		Globulin g/dl		ALT (U/L)		AST (U/L)		ALP (U/L)	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-
I-saline	0.73±0.02	0.76±0.01	7.93±0.13	7.81±0.22	4.56±0.18	4.38±0.11	3.37±0.11	3.48±0.32	11.48±0.27	12.84±0.13	10.84±0.13	12.13±0.27	118.16±2.15	126.16±1.92
II-100	0.76±0.02	0.84±0.02	7.88±0.11	7.62±0.15	4.46±0.31	4.18±0.18	3.42±0.24	3.44±0.11	19.46±0.92	21.16±0.73	26.18±1.13*	29.13±1.54*	141.16±3.16*	154.22±4.26
III-500	1.06±0.01	1.26±0.04	7.94±0.39	7.91±0.26	4.31±0.27	4.26±0.13	3.63±0.18	3.65±0.27	46.54±1.81**	53.16±1.74***	51.16±1.18***	60.27±1.54***	184.16±3.27***	194.22±3.92**
IV-1000	4.81±0.84*	4.94±0.27*	8.04±0.18	8.02±0.91	4.69±0.27	4.73±0.16	3.35±0.13	3.39±0.18	54.18±2.71***	61.16±2.37***	68.22±1.13***	79.16±1.26***	191.54±3.21***	193.16±1.81**
V-2000	4.93±0.73*	5.89±0.56*	8.12±0.16	8.54±0.27	4.54±0.18	4.39±0.27	3.58±0.41	3.55±0.24	68.31±2.86***	71.92±2.16***	82.16±1.92***	88.96±2.81***	216.16±3.92***	224.18±2.84***

Data represented as mean ±SEM, (N=6). Significance between control and extract treated groups.*p <0.05; **p <0.01; ***p <0.001.

Table 3: Effect of *Didemnum psammathodes* on kidney function.

4	Sodium m Mg/L		Potassium m Mg/L		Bicarbonate m Mg/L		Chloride m Mg/L		Urea mg/dl		Creatinine mg/dl	
	+	-	+	-	+	-	+	-	+	-	+	-
I-saline	113.54±2.91	126.36±3.84	4.01±0.12	4.21±0.14	24.56±0.21	23.16±0.15	102.16±2.54	99.56±1.31	13.46±0.61	16.22±0.73	0.69±0.01	0.74±0.07
II-100	121.16±1.84	132.54±2.16	4.12±0.21	4.34±0.13	21.54±0.21	20.81±0.27	106.31±2.16	102.16±2.54	24.11±1.06	26.56±1.51	1.06±0.27	1.31±0.15
III-500	132.84±2.16	138.54±1.84	3.36±0.54	3.56±0.31	20.16±0.16	18.61±0.18	104.11±1.91	102.54±2.62	56.92±2.05**	68.31±2.16**	2.16±0.18*	3.16±0.73*
IV-1000	146.92±3.88*	153.92±4.84*	3.24±0.18	2.92±0.13*	19.15±0.92	18.36±0.21	98.54±0.84	99.56±2.84	68.16±1.13***	76.81±1.65***	3.18±0.67**	3.92±0.81**
V-2000	142.84±2.61*	154.88±3.92*	3.04±0.21	2.86±0.14*	18.56±0.67*	18.08±0.16*	93.31±1.16	97.31±1.06	74.16±1.91***	82.16±1.91***	3.93±0.31**	4.11±0.96**

Data represented as mean ±SEM, (N=6). Significance between control and extract treated groups.*p <0.05; **p <0.01; ***p <0.001.

Table 4: Effect of the extract of *Didemnum psammathodes* on haematological parameters.

Group/ Dose mg/kg	Hemoglobin %		RBC million/mm^3		WBC 10^3 cells/ mm^3		Platelet 10^5 cells/mm^3		Differential count					
									Lymphocytes		Neutrophils		Eosinophils	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-
I-saline	12.96±0.91	12.31±0.54	4.16±0.11	4.11±0.21	8.4±0.11	8.13±0.18	381.46±10.27	373.16±11.27	51.27±1.56	54.16±1.37	41.16±1.16	38.16±1.27	6.31±0.16	7.18±0.54
II-100	11.61±0.54	11.56±0.94	3.91±0.27	3.90±0.12	9.56±0.26	9.84±0.27	373.54±9.56	361.54±7.96	46.25±1.31	50.31±1.84	49.81±1.34	39.21±1.36	3.86±0.29	9.54±1.08
III-500	11.26±0.18	11.31±0.21	3.82±0.16	3.81±0.12	10.91±0.16	10.86±0.31	356.11±10.27	343.16±12.16	40.43±1.68	44.18±1.27	51.86±1.18	50.16±1.93	7.26±1.06	5.81±0.96
IV-1000	10.16±0.21	10.56±0.18	3.54±0.27	3.61±0.27	10.54±0.27	10.16±0.17	324.16±7.92*	316.53±9.23**	38.16±1.12*	40.84±1.48*	56.12±1.84*	54.26±1.24**	5.93±1.86	4.86±0.27**
V-2000	10.02±0.01	10.16±0.26	3.26±0.18	3.11±0.18	10.31±0.16	10.56±0.16	304.17±9.54**	294.16±7.96**	35.80±1.08*	37.16±1.67**	60.27±1.13**	56.96±1.65**	3.16±0.96**	5.96±0.86**

*Data represented as mean ±SEM, (N=6). Significance between control and extract treated groups.*p <0.05; **p <0.01.*

CONCLUSION

The extract of *Didemnum psammathodes* was safe and nontoxic to mice at a dose below 500 mg/kg body weight and therefore could be well considered for further investigation for its medicinal and therapeutic efficacy.

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REFERENCES

1. Tamba B, Foltea G, Leon M, Iliesu R. Determination of drug toxicity in animals. 2013; http://webmail.umfiasi.ro/
2. [http://webmail.umfiasi.ro/Cercetare/CentreDeCercetare/Documents/Carte%20Experimental%20models%20in%20rodents/15.pdf.]
3. Meenakshi VK, Gomathy S, Chamundeswari KP. Acute and sub chronic oral toxicity of *Microcosmus exasperatus* Heller, 1878. Journal of Microbiology and Biotechnology Research., 2012; 2(4): 94-98.
4. Meenakshi VK, Shanmuga Priya D, Gomathy S, Paripooranaselvi M, Senthamarai S, Chamundeswari KP. Studies on the toxicity of crude methanol extract of *Phallusia nigra* Savigny, 1816. International Journal of Chemical and Pharmaceutical Sciences., 2013; 4(2): 64-68.
5. Kohila Subathra Christy HK, Jothibai Margret R, Meenakshi VK. Studies on toxicity of *Phallusia arabica* Savigny, 1816. International Journal of Pharmacy & Therapeutics., 2014; 5(5): 353-357.
6. Stella Packiam C, Jothibai Margret R, Meenakshi VK. Evaluation of the safety profile of *Ascidia sydneiensis* to wistar albino rats. World Journal of Pharmacy and Pharmaceutical sciences., 2016; 5(4): 1740-1748.
7. Sankaravadivu S, Jothibai Margret R, Meenakshi VK. Assessment of acute and sub chronic oral toxicity of *Ecteinascidia venui*, Meenakshi, 2000. World Journal of Pharmacy and Pharmaceutical Sciences., 2016; 5(7): 1225-1234.

8. OECD (Organization for Economic Co-operation and Development). 2001. OECD guideline for testing of chemicals: Guideline 423, Acute oral toxicity- Acute toxic class method. OECD, Paris, France.
9. OECD (Organization for Economic Co-operation and Development). 2008. OECD guideline for testing of chemicals: Repeated dose 28-day oral toxicity study in rodents - TG 407. OECD, Paris, France. [https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecdtg407-2008.pdf.]
10. Quigley JJ. Quantitative estimation of total bilirubin in serum. *Analytical Chemistry.*, 1952; 24(11): 1859-1860.
11. Lowry OH, Rosenbrough NJ, Farr AL, Randal RJ. Protein measurement with the folin's phenol reagent. Journal of Biological Chemistry., 1951; 193(1): 265-275.
12. Rodkey FL. Direct spectrophotometric determination of albumin in human serum. Clinical Chemistry., 1965a; 11(4): 478-487.
13. Rodkey FL. Separation and determination of the total globulins of human serum. Clinical Chemistry., 1965b; 11(4): 488-494.
14. Reitman S, Frankel S. A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. American Journal of Clinical Pathology., 1957; 28: 56-58.
15. King EJ, Armstrong AR. A convenient method for determination of serum and bile phosphatase activity. Canadian Medical Association Journal., 1934; 31(4): 376-381.
16. Eric GB, Joseph FS. A study of the estimation of sodium in blood serum. Journal of Biological Chemistry., 1936; 113: 661-674.
17. Taylor FHL. The determination of potassium in blood serum. Journal of Biological Chemistry., 1930; 87: 27-32.
18. Charles WB, John BF. Determining serum bicarbonate. California Medicine., 1953; 79(6): 420-421.
19. Wilson DW, Ball EG. A study of the estimation of chloride in blood and serum. Journal of Biological Chemistry., 1928; 79: 221-227.
20. Barker SB. The direct colorimetric determination of urea in blood and urine. Journal of Biological Chemistry., 1944; 152: 453-463.
21. Owen JA, Iaggo B, Scandrett FJ, Stewart CP. The determination of creatinine in plasma or serum, and in urine; a critical examination. Biochemistry Journal., 1954; 58(3): 426-437.

22. Rajesh Kumar NV, Kuttan G. Induction of apoptosis in mouse and human carcinoma cell lines by *Emblica officinalis* polyphenols and its effect on chemical carcinogenesis. Amala Research Bulletin., 2001; 21: 114-126.
23. Saravanan N, Nalini N. *Hemidesmus indicus* protects against ethanol-induced liver toxicity. Cell and Molecular Biology Letters., 2008; 13: 20-37.
24. Woodman DD. Assessment of hepatotoxicity. In Evans GO, editor, Animal Clinical Chemistry: a Primer for Toxicologist, London: Taylor & Francis., 1996; 71-86.
25. Dybing E, Doe J, Groten J, Kleiner J, O'Brien J. Hazard characterization of chemicals in food and diet: dose response, mechanism and extrapolation issues. Food Chemistry and Toxicology., 2002; 42: 237-282.
26. Liju VB, Jeena K, Kuttan R. Acute and sub chronic toxicity as well as mutagenic evaluation of essential oil from turmeric (*Curcuma longa* L.). Food and Chemical Toxicology., 2013; 53: 52-61.