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Acute toxicity study of *Aponogeton natans*- An important folklore medicine

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ABSTRACT

The biological efficacy of the herbal drug is derived by aiming acute toxicity test on experimental animals. Acute toxicity test on experimental animals is used in information regarding hazard feature and dispensation of the drug. The acute toxicity study of Pet. ether, benzene, chloroform and methanol extracts for *Aponogeton natans.* Linn. were evaluated in Wistar albino rats. All the extracts i.e Pet. ether, benzene, chloroform and methanol were administered orally at the maximum dose of 2000mg/kg/p.o. The adverse toxic symptoms of the animals were observed up to 24 hours. All the extracts were also tested at the dose of 2000 mg/kg/p.o for 14 days toxicity study. The animals were sacrificed on the 15th day, the biochemical and blood parameters were measured. Vital organs histopathalogical interpretations were pretended further. The results of the test groups administered with pet ether, benzene, chloroform and methanol showed that, there were no adverse effects in the general behavior and mortality when compared with the control group. Further, the test groups administered with pet ether, benzene, chloroform and methanol did not showed any significant changes in food, water intake, body weight, kidney and liver function test and hematological parameters. Acute toxicity screening revealed no adverse motor or neurological changes, gastrointestinal tract disorder, respiratory or locomotors effect or changes in any of the animals, when the extracts were administered at 2000mg/kg per day for 14 days.

KEY WORDS: Acute oral toxicity, *Aponogeton natans* Linn. (Engl & Krause), acute toxicity, hematological parameters, biochemical parameters.

1. INTRODUCTION

Acute toxicity survey is performed to know information on the biological effectiveness and instrumentality of action of the drug. The information obtained by the test is used in risk management of the drugs (Walum, 1998). Conventional drugs sometimes can have exquisite adverse effects. So traditional medicines are in worldwide search. Traditional medicine- is defined as (WHO) 'A plant derived material with healing or other human health profits which contains either raw or prepared ingredients from one or more plants. Some traditions, materials of inorganic or animal origin may also be present.' The Indian systems of medicine and folklore medicines make use of thousands of plant-based formulations (Verma and Singh, 2008). The principle behind the use of more than one plant/plant product in these formulations is that they may produce harmonious and/or additive effects, or one may counterpoise the toxic effect of another, which is otherwise therapeutic in the given context (Li, 2007).

Aponogeton natans (Linn.) Engl. & Krause.belongs to Aponogetonaceae family. *Aponogeton natans* (Linn.) Engl. & Krause occurs in plains and marshy places in Asia, Australia, India and Srilanka. Traditionally leaf pastes are taken with hot water to cure cuts & wounds (Britto and Mahesh, 2007)**.** Fresh tuber paste is boiled with 200 ml of coconut oil and applied on hair before bath for three days to cure fungal infection (Jeyaprakash, 2011)**.** *Aponogeton natans* (Linn.) Engl. & Krause is a important ingredient in preparation of an important Ayurvedic preparation Useerasava. This asava is useful in haemothermia, anaemia, impurity of blood and diabetes (http://www.bdu.ac.in). A perusal of existing reports reveals that the no detailed acute toxicity study had been done earlier. The study has been planned to investigate the acute toxicity study of various extracts of *Aponogeton natans* (Linn.) Engl. & Krause. leaf with leafstalks in experimental animals.

2. MATERIAL AND METHODS

Experimental animals: Wistar albino rats of both the sex weighing 140-160g were used for the toxicological studies. The animls were kept in standard conditions of day and night cycles at 22ºC in polypropylene cages. The animals were given on standard pellet's as food (Hindustan Lever Pvt Ltd., Bangalore) and water ad libitum. The rats were acclimatize to laboratory conditions by housing them in propylene cages prior to the experiments for one week. The experiment was conducted in Institute of Pharmacy & Technology, Salipur, Cuttack and CPCSEA recognized local ethical committee approved the protocol bearing No. 19/IAEC-IPT/13.

Acute oral toxicity study: As per the OECD Guidelines 423 (acute class toxic class method), acute oral toxicity was performed. The toxicity study is a stepwise procedure with 3 animals per step. These stepwise procedures results in the minimum number of animal use. 15 wistar albino rats weighing 140-160 grams divided in to 5 groups with 3 rats in each group. All the animals were fasted overnight, but allowed water and libitum. Animals in Group I received 1ml of 1% tween 80 and served as control. *Aponogeton natans.* Linn. Pet ether, benzene, chloroform and methanol extracts were suspended in 1% tween 80 and administered by stomach tube at single dose (2000 mg/kg) to Groups II, III, IV and V respectively at the dose of 2000mg/kg/p.o. which served as test groups. Since the leaf extracts is

nontoxic, therefore highest dose of 2000 mg/kg/p.o. was used in the acute toxicity study. All the animals were taken into observation and observed at the time interval of 1, 2, 4, 6 & 24 hr & from 2-14th day. The animals were then sacrificed on 15th day for pathological examination of different organs including heart, spleen, lungs, livers, kidneys, stomach. The animals were also observed for behavioral activity. 1.5 ml blood was collected in a vial containing 2.5 µg of ethylene diamine tetra acetic acid (EDTA) as an anticoagulant for haematological assay and 2.5 ml of the blood was collected for biochemical studies. The blood was centrifuged at 500 rpm for 15 minutes and serum collected and stored at –80ºC until assayed for biochemical parameters (OECD, 2001).

Effect of *Aponogeton natans* Linn. extracts on organ weights in rats: Organs like spleen, liver, kidney and stomach were excised, fat and connective tissue was trimmed out, blotted to dry and weighed. Body weight of the rats was taken before and on 14th day of experiment. The organ-to-body weight index was calculated as the ratio of organ weight and the animal body weight (at the end of the experiment) x 100.

Effect of *Aponogeton natans* Linn. extracts on haematological parameters: Red blood cell count (RBC), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), total white blood cell count (WBC), neutrophils, eosinophils, monocyte and lymphocytes counts were determined by an auto-analyzer (Sysmex XT-2000 L CELL-DYN 1700, Abbot Diagnostics Division, Abbot Laboratories, Abbot Park, Illinois, USA).

Effect of *Aponogeton natans* Linn. extracts on the serum biochemistry: The different parameters related to liver enzymes such as creatinine, cholesterol, total protein, ALB, ALP, SGPT, SGOT and glucose were also determined. Automatic analyzer ATAC 8000 Random Access Chemistry System (Elan Diagnostics, Smithfied, RI, USA) was considered to determine all the parameters.

Statistical analysis: The data obtained from each experiment were subjected to one way ANOVA followed by Dunnet's t test.

3. RESULTS AND DISCUSSIONS

Acute Toxicity: A single oral dose of *Aponogeton natans* Linn. Pet ether (ANPE), benzene (ANBE), chloroform (ANCE) and methanol (ANME) extracts had not caused any deaths neither did the rats showed any signs of toxicity at dose at (2000 mg/kg).

There was no sign of toxicity or changes of general behaviour found in the acute toxicity study. When the extracts were given at 2000 mg/kg per day for 14 days, rats remained alert with no motor or neurological changes. There was no adverse gastrointestinal tract disorder, respiratory disorder or locomotor changes observed in any of the animals. The extracts appeared to have slight diarrheal properties upon continuous administration to the rats as stools from rats treated with the extract were slightly more hydrated than that from the control animals.

Effect of *Aponogeton natans* Linn. extracts on body and some target organ weight in rats: It was found that there were no significant changes in the body weight of rats that were survived at the end of the experiment. The relative weights of some selected organs were also not affected which are presented in **(Table 1)**.

Table.1. Effect of *Aponogeton natans* Linn. extracts on the organ weights of albino rats

Organ	Relative organ weight				
	CONTROL	ANPE	ANBE	ANCE	ANME
Liver	3.2600±0.32	2.6060±0.20	3.0930±0.25	3.1320±0.26	3.253±0.47
Kidneys	0.7098±0.12	0.5822±0.05	0.6223±0.03	0.8513±0.08	0.871±0.04
Spleen	0.2058±0.04	0.2742±0.04	0.2578±0.02	0.2656±0.06	0.2533±0.02
Stomach	1.0600±0.46	0.7246±0.01	0.8367±0.06	1.1250±0.07	1.2666±0.04
Heart	0.4104±0.06	0.3624±0.02	0.4003±0.01	0.3719±0.02	0.3616±0.05

ANPE*: Aponogeton natans* pet. Ether extract, ANBE: *Aponogeton natans* benzene extract, ANCE: *Aponogeton natans* chloroform extract, ANME: *Aponogeton natans* methanol extract

Table.2. Effect of *Aponogeton natans* Linn. extracts on the haematological indices of albino rats

Heamatological Parameters	CONTROL	ANPE	ANBE	ANCE	ANME
RBC x 10^6/mm^3	6.75 ± 1.26	6.80 ± 0.34	6.83 ± 0.095	6.94 ± 0.74	6.03±0.676
HGB (g/dl)	11.93 ± 2.19	12.70 ± 0.30	12.15 ± 0.05	12.85 ± 1.35	12.61±0.557
MCV (fl)	55.63 ± 0.74	57.35 ± 2.45	56.35 ± 1.15	56.00 ± 0.90	55.56±1.040
MCH (pg)	17.60 ± 0.15	18.70 ± 0.50	17.80 ± 0.30	17.95 ± 0.05	18.16±0.606
MCHC (g/dl)	31.60 ± 0.60	32.65 ± 0.55	31.50 ± 0.00	32.00 ± 0.60	32.91±0.200
PLT (×1000)	907.3 ± 99.49	964.0 ± 100	984.0 ± 99.00	986.0 ± 90.00	982.20±83.26

ANPE*: Aponogeton natans* pet. Ether extract, ANBE: *Aponogeton natans* benzene extract, ANCE: *Aponogeton natans* chloroform extract, ANME: *Aponogeton natans* methanol extract

Table.3. Effect of *Aponogeton natans* Linn. extracts on the differential white blood cell count in albino rats

Group	Total WBC count (×1000)	Differential White Cell Count (%)			
		Neutrophil	Eosinophil	Monocyte	Lymphocyte
CONTROL	3.903±1.87	31.73±9.42	0.9667±0.167	6.20±2.91	71.10±11.20
ANPE	2.87±0.30	30.35±1.55	1.75±1.75	3.50±1.20	64.25±1.15
ANBE	4.95±0.63	28.00±1.60	1.00±0.10	3.55±1.45	87.45±3.15
ANCE	3.23±1.38	35.15±20.55	0.90±0.10	5.30±10.10	69.35±10.65
ANME	3.56±1.071	33.73±11.092	0.92±0.1131	4.66±1.397	75.28±1.064

ANPE*: Aponogeton natans* pet. Ether extract, ANBE: *Aponogeton natans* benzene extract, ANCE: *Aponogeton natans* chloroform extract, ANME: *Aponogeton natans* methanol extract

Table.4. Effect of *Aponogeton natans* Linn. extracts on the serum biochemistry in albino rats

	CONTROL	ANPE	ANBE	ANCE	ANME
Cretinine (mg/dl)	0.75±0.263	0.86±0.11	0.92±0.25	0.85±0.13	0.74±0.04
Cholesterol (mg/dl)	115.70±19.34	98.00±16.00	112.0±3.00	114.5±9.5	115.33±4.248
Total Protein (gm/dl)	5.83±0.617	6.90±0.40	6.40±0.20	5.65±0.25	5.73±0.507
ALB (gm/dl)	3.50±0.306	4.40±0.30	3.90±0.10	3.25±0.15	3.31±0.574
ALP (U/L)	270.85±1.28	280.23±1.40	285.25±1.87	272.52±0.98	269.25±1.12
SGPT (U/L)	81.33±10.48	72.50±12.50	73.50±2.50	79.50±14.50	74.33±5.302
SGOT(U/L)	74.70±25.76	73.5±2.5	78.0±5.00	68.5±31.50	68.33±10.365
Glucose(mg/dl)	107.00±2.082	89.50±14.50	111.5±10.50	77.50±11.50	80.41±9.557

ANPE*: Aponogeton natans* pet. Ether extract, ANBE: *Aponogeton natans* benzene extract, ANCE: *Aponogeton natans* chloroform extract, ANME: *Aponogeton natans* methanol extract

Effect of *Aponogeton natans* Linn. extracts on haematological parameters in rats: The effect of acute oral administration of *Aponogeton natans* Linn. On the haematological and differential white blood corpuscles parameters is presented in Tables.2 and 3, respectively. Haematological values of extracts treated rats were not significantly different from the control group for all parameters measured at 2000mg/kg, (Table 2). The differential white blood cell counts in the treated groups were not different from that of the untreated or control group administered with 1% tween 80 solution either **(Table 3)**.

Effect of *Aponogeton natans* Linn. extracts on the serum biochemistry in rats: Biochemical profiles of the extracts treated group animals are represented in Table.4. The acute oral administration of *Aponogeton natans* (2000 mg/kg/p.o.) did not cause any significant variation in the creatinine, cholesterol, total protein, bilirubin, ALB, ALP, SGPT, SGOT and Glucose from those of the untreated or control group for all parameters at same dose level.

Histopathological study of liver and kidney: Livers and kidneys from the saline treated (control) group had normal appearance and histology. Rat liver multiple sections were observed, which showed preservation of normal lobular architecture. Hepatocytes appear normal and arranged in single cell cords radiating away from central vein. No sign of non specific lobular hepatitis was seen at the test dose level of the extracts. There was no sign of bile stasis, granuloma, dysplasia or malignancy. Multiple sections of kidney were studied, which showed normal size and shape of glomeruli, tubules, interstitium and blood vessels. There was no sign of acute tubular necrosis or glomerular changes.

In the acute toxicity study, all the four extracts of *Aponogeton natans* (Linn.) Engl. & Krause at doses of 2000 mg/kg, p.o. did not showed any significant variation in the body weight increment, indicating that it did not have any adverse effects on body weight, which was used to assess the results to therapy of drugs and to indicate the adverse effects of a drugs . The organs weight of the extracts treated groups remained normal, indicating that plants extracts were non-toxic to vital organs. there were no significant variations in various haematological parameters compared to the control or untreated group, which indicates that test extracts may not be toxic and do not affect circulating red cells, hematopoiesis or leucopoiesis. Furthermore, there were no significant variations observed in any biochemical parameters as compared to the control or untreated group. The results of the histopathology study revealed no specific sign and symptoms of damage on the isolated organs under study and therefore, it may be considered safe at the tested dosage regimen of the extracts under study.

4. CONCLUSION

From our observation, it can be concluded that all the four extracts i.e. ANPE, ANBE, ANCE and ANME of *Aponogeton natans* Linn. at 2000mg/kg/p.o as a single dose orally at 2000mg/kg/p.o. once orally for 14 days is well tolerated by adult wistar rats. In conclusion, acute toxicity screening revealed no adverse motor or neurological changes, gastrointestinal tract disorder, respiratory distress or locomotors changes in any of the animals, when the four extracts were administered to rats at a dose of 2000mg/kg/p.o. per day for 14 days hence $1/10^{th}$ of the dose i.e 200 mg/Kg/p.o. was taken as effective dose for further pharmacological studies.

It further confirmed that above mentioned that *Aponogeton natans* Linn. extracts is safe and can be considered to be used for further scientific and pharmacological evaluation.

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