



AN ACUTE ORAL TOXICITY STUDY OF *CYCAS CIRCINALIS* L AND *IONIDIUM SUFFRUTICOSUM* (GING) IN WISTER ALBINO RATS

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ABSTRACT

Cycas circinalis L. (Cc) and *Ionidium suffruticosum* Ging., (Is) were used in Siddha system of medicine (Indian Medicine) for years together to improve the fertility of male. Both the herbs have aphrodisiac activity. The study was carried out to evaluate the acute toxic effect of the ethanolic extract of both the herbs Cc and Is and to determine the LD₅₀. The acute toxicity study (Main test) was done as per the guide lines of Organization for Economic Cooperation and Development (OECD) guideline 425. As the herbs were used for treating male infertility by traditional practitioners for years together, the toxicity study was also proposed to study in male albino rats. About 18 male albino rats of three groups having six rats in each group of Control, Experimental I and Experimental II. The ethanolic extracts of Cc and Is were administered by oral gavage. The distilled water was administered to control rats, *Cycas circinalis* extract for Experimental I and *Ionidium suffruticosum* extracts for Experimental II in single dosages of 175, 550, and 2000mg/kg bodyweight with 48hrs of interval between the dosages as recommended in OECD guidelines 425. The weight of rats, Wellness parameters, mortality, hematological parameters, biochemical markers, and histopathological analysis of all vital organs were observed to know the acute toxic effect of the drugs Cc and Is. All the parameters of the study do not show any significant changes between the control and experimental groups. There was no mortality among the experimental groups. The high no-observed adverse effects level (NOAEL) value of 2000 mg/kg bodyweight implies that the drug could be safe and the LD₅₀ value of both Cc and Is was found to be more than 2000 mg/kg bodyweight by this acute toxicity study.

Keywords: *Cycas circinalis*, *Ionidium suffruticosum*, Acute toxicity, Wister Albino rats, OECD Guidelines, Siddha system of Medicine.

INTRODUCTION

"For every disease there is a plant that cures it", said by Hippocrates. The herbs are safe to consume when its toxic dose is determined. The determination of Biosafety level of the drugs, the harmful effect of a compound and its level are very much important for its safe usage.¹ *Cycas circinalis* and *Ionidium suffruticosum* both are unrevealed herbs to the public which is used in Siddha system of Medicine (Indian system of Medicine) for years together.² *Cycas circinalis* L. is a gymnosperm tree which belongs to the family Cycadaceae and grows to a height of 5 meter, commonly grown in western Ghats and hilly regions of southern peninsula, in the states of Kerala, Karnataka, Tamilnadu and the south of Maharashtra and also in other countries like China, Japan and South Africa.³ The male cone of the tree has aphrodisiac activity and commonly known as Madana Kama Pu, Salapari and Entha Panai in Tamilnadu.^{2,3} *Ionidium suffruticosum* Ging. is a seasonal, sporadic, rare ethno botanical herb, a perennial herb widely distributed in Africa, Madagascar, Srilanka, China, New Guinea Australia and India.⁴ In India commonly grown in south India and called as Ratna purush in India which belongs to the family Violaceae,. Both the plant has medicinal effect particularly in improving male fertility. The effect of the herb and its medicinal value was not scientifically proved even though under traditional practice, so an effort was made first to study the acute toxic effect of both herbs on male Wister albino rats followed by the aphrodisiac activity study

which is ongoing. The study was done to determine LD₅₀ of both the herbs and to constitute a baseline data for the safe usage of the herbs. The acute oral toxicity study was done as per the guide lines of Organization for Economic Cooperation and Development (OECD) guideline 425.⁵

MATERIALS AND METHODS

Extraction of Plant Material

The *Cycas circinalis* dry male cones specimen was identified as per Siddha literature, authenticated and collected from the trees directly and air dried in shadow for 10 days and simultaneously the *Ionidium suffruticosum* plant was also identified as per Siddha literature, authenticated and collected, washed with distilled water and air dried for 10 days. The dried male cones of Cycas and Ionidium (whole plant) was powdered using pulveriser and passed through sieve to get fine powder. The 50 grams of powder of plant material of Cc and Is were successively extracted with 500ml of absolute ethanol solvents by hot continuous percolation method in Soxhlet apparatus for 24 hrs.⁶ The extracts were concentrated by using hot water bath and subjected to drying in an hot air oven.¹ The percentage yield of Cc and Is extracts was found to be 7.14 and 8.42 respectively.¹

Experimental animals

A total of 18 healthy young adult male albino rats of Wister lineage, weighing about 180- 200 g were procured



from Biomedical Research Unit and Laboratory Animal centre (BRULAC) of Saveetha University, Chennai (Reg.no-865/ac/04/CPCSEA). The experiments were performed in accordance with the CPCSEA guidelines and the study was approved by Institutional Animal Ethical Committee of Saveetha University, approval number-ANAT.005/2012. Male rats were selected because the literature of conventional LD₅₀ tests shows that usually there is little difference in sensitivity between sexes². As the herbs were used for treating male infertility by traditional practitioners for years together, the toxicity study was also proposed to study in male albino rats. In the present study male rats were selected for toxicity study. The animals were distributed into control (C) (n=6) and Experimental groups E-I (n=6) and E-II (n=6) respectively by random allocation method. The animals were housed in cages 2 rats per cage. The rats were fed with standard pellets and given fresh water, acclimatized on a 12 hour light & 12 hour dark schedule.⁷ The cages were labeled with group, weight of the animal and day of drug administration. About one week was given to the rats for acclimatization. Separate record was maintained for each animal in all the groups including control animals.

Evaluation of Acute toxicity

The animal was fasted overnight but allowed water ad libitum before drug administration.⁵ The weight of the fasted animal was taken and drug dosages were calculated. The extracts were orally administered in a single dose by oral gavage. The volume given was 2ml/100gm body weight. After administration of drug the animal was withheld from food for 3-4 hours. Simultaneously the control animals were given distilled water orally. Single animals were dosed in sequence usually at 48hrs intervals¹. Dosing was initiated at 175mg/kg, then 55 mg/kg, and proceeded with

2000mg/kg as recommended in OECD Guidelines 425.⁵ Signs of toxicity and mortality were observed for the first 30 min followed by 1,2,4,6 and 24 hours and thereafter twice daily until 2nd-14th day.⁸

Body weight of the animals

The initial weight of all the animals (C, E-I, E-II) were noted on day 1 and followed by drug administration, the weight of animals at the end of first week (7th day) and as well as at the end of second week (14th day) were noted and tabulated to compare between control and experimental animals.¹

Mortality and signs of toxicity

The wellness parameters such as changes in the skin, fur, eyes and mucous membranes and behavioral pattern with attention given for observation of tremors, convulsion, salivation, diarrhea, lethargy, sleep, coma and mortality were recorded for each animals.^{1,9}

On the 15th day all rats were anesthetized using Ketamine and Xylazine and blood was collected from the jugular vein for analyzing hematological parameters and biochemical markers.⁸⁻¹² The organs such as brain, lung, heart, liver, stomach, spleen, kidney, testis were removed, processed, sections of 5 μ thickness were taken, stained by Haematoxylin and Eosin, then Histopathological analysis was done.^{11,12}

Statistical Analysis

The data's were collected, calculated and tabulated as Mean \pm SD and significance among the 3 groups were analyzed by one way ANOVA using SPSS software and the P values taken to find the significance.

Table 1: Body Weight of Rats

Groups	Day 1	Day 7	Day 14
Control (gm)	134.12 \pm 2.76	136.33 \pm 4.13	175.33 \pm 4.5 ^{ns}
Experimental I (gm)	134.41 \pm 2.61	134.50 \pm 5.6	172.12 \pm 4.2 ^{ns}
Experimental II (gm)	136.11 \pm 2.80	137.33 \pm 5.1	177.6 \pm 5.31 ^{ns}

Values are expressed as Mean \pm SD (gm), n = 6, ns – non significant

Table 2: wellness Parameter observation for main test at 2,000 mg/kg body weight¹

Parameters	30 min			4hrs			24hrs			1 st Week			2 nd Week		
	C	EI	EII	C	EI	EII	C	EI	EII	C	EI	EII	C	EI	EII
Skin & Fur	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mucous Membrane	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Respiratory rate	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Heart rate	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Salivation & Lacrimation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Piloerection	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Urinary incontinence	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Defecation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sleep & Gait	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Tremors and Convulsion	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

N – Normal, C – control, EI – Experimental I (Cc extract), EII – Experimental II (Is extract).



Table 3: Hematological and biochemical parameters^{9, 12}

Parameters	Control	Experimental I	Experimental II
Hb (mg/dl)	13.62 ± 0.56	13.78 ± 0.61	14.21 ± 0.74 ^{ns}
RBC (×10 ⁶ /μl)	8.31 ± 0.72	8.29 ± 0.34	8.42 ± 0.73 ^{ns}
WBC (×10 ³ /μl)	3.77 ± 0.48	3.68 ± 0.34	3.98 ± 0.82 ^{ns}
Platelet (×10 ³ /μl)	4.98 ± 0.37	5.71 ± 0.41	5.43 ± 0.32 ^{ns}
Total Protein (g/dl)	5.77 ± 0.71	5.94 ± 0.72	5.64 ± 0.64 ^{ns}
Urea (mg/100ml)	32.64 ± 0.33	38.82 ± 0.45	29.63 ± 0.21 ^{***}
Creatinine (mg/dl)	0.32 ± 0.04	0.41 ± 0.07	0.39 ± 0.41 ^{ns}
AST (U/l)	39.41 ± 4.31	42.81 ± 5.27	41.68 ± 4.84 ^{ns}
ALP (U/l)	36.42 ± 2.21	32.6 ± 2.28	33.72 ± 1.28 ^{**}
Cholesterol (mg/dl)	42.4 ± 0.60	47.71 ± 0.91	41.91 ± 0.54 ^{***}

Values are expressed as Mean ± SD, n = 6, ns – non significant, * - significant, P value *P<0.05, **P<0.01, ***P<0.001

RESULTS AND DISCUSSION

There were no Cc and Is extract treatment related mortality until a dose of 2000mg/kg body weight. The lethal dose will be above 2000 mg/kg body weight. The weight of the animals on day 1 and subsequently day 7 and day 14 were also tabulated (table 1). The weight of C, E I and E II on day 1, day 7 and day 14 do not show any significance (P value – 0.41, 0.61, 0.16 respectively). The weight gain of rats was gradual in all groups.

The wellness parameters such as skin looked for pigmentation and discoloration and fur loss, nasal and oral mucous membrane for any ulceration, respiratory rate, heart rate, salivation, lacrimation, lethargy, piloerection, urinary incontinence, defecation, sleep, gait, tremors, convulsion and mortality were all observed, recorded for each animals and compared between control and experimental groups and found to be normal in all the animals (Table 2).

The Hematological parameters includes hemoglobin concentration, red blood cell count, White blood cell count, platelet count of all the rats were found out and data's were tabulated as Mean ± SD (Table 3) and compared between control and experimental rats by one way ANOVA and P value for all hematological parameters were insignificant and show only less difference among the groups and the values are approximately within same range to the study done by Akila et al 2012 for some Siddha formulations.¹² The Biochemical parameters such as total protein, creatinine, Alkaline Phosphatase, were within same ranges with a slight difference between control and experimental rats. P value for all markers was not significant among the groups. Urea, Aspartate aminotransferase and cholesterol were highly significant among the groups. The data's are in the same range as that of study done by Patrick et al 2012 on baker cleanser bitters.¹²

The histopathological examination of different organs such as brain, lung, heart, kidney, liver and testes of both control and experimental groups were compared with the help of pathologists and no abnormal architecture, morphological disturbances, desquamation, disorganization, infiltration and destruction were noted.¹¹

Traditional Medicine has gained its popularity all over the world and the use is rapidly on the increase.¹⁴ Though there are many traditional herbal medicines available, only few were proved scientifically by clinical trials, but still their efficacy and safety are questioned by consumers.¹⁵ The lethal value (LD50) of both the herbs Cc and Is were determined to be higher than 2000mg/kg and also regarded as safe within the dose. Any compound or drug with oral LD50 estimates greater than 1000 mg/kg bodyweight is considered to be of low toxicity and safe.¹⁶ There are also various factors such as animal species, gender, diet, bedding, temperature, caging condition and time all these affects the LD50, so it cannot considered to be a biological constant.¹⁷

CONCLUSION

The present study proves that ethanolic extract of Cc and Is when given orally at concentration of 2000mg/kg body weight did not exhibit any toxicological effect and proved to be safe until 2000mg/kg. Further the study is extended at present with chronic toxicity study with a detailed analysis of all hematological, biochemical parameters, histopathological findings at cellular level.

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