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Acute toxicity of Ethanolic Extract of Luffa acutangula and Syzygium samarangense in Wistar Rats

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ABSTRACT:
Syzygium samarangense commonly known as Wax apple which belongs to the family [Myrtaceae]. Several antioxidant compounds believed to have great potential benefits for human health. It has also been used in traditional medicine for variety of illness such as anti-microbial, diuretic, and anti-inflammatory. Luffa acutangula commonly known as Ridge gourd which belongs to the family [Cucurbitaceae]. It is a popular vegetable in India and other Asian countries. It is reported to contain many phytochemicals such as flavonoid, anthraquinone glycoside, and carotenoid. The ethno botanical survey revealed its use to protect jaundice, dysentery, swollen hemorrhoids and traditionally it is used in the treatment of constipation, asthma and to boost immune system. The study investigated the acute toxicity effects of Syzygium samarangense and Luffa acutangula as per OECD guidelines 425 (Acute Oral toxicity: Up and Down Procedure). For acute toxicity no evidence of toxicity was observed i.e. (inflammation, fever, irritation, rashes, mortality). A significant weight gained ($p<0.02$) in rats that received 2000mg/kg of both the extracts and observed, however gross examination of internal organ revealed no detectable inflammation. Depending upon the toxicity study the subsequent low dose (400mg/kg) and high dose (800mg/kg) was decided for the further study

KEYWORDS: Syzygium samarangense, Luffa acutangula, acute toxicity test, OECD guidelines

I. INTRODUCTION

Plant-based medicines are used in all civilizations and cultures and, hence, plants have always played a key role in health care systems worldwide. In most developing countries, the original modes of herbal treatment are a part of the culture and the dominant method of healing therapy. Plants used in traditional medicine, therefore, have a critical role in the maintenance of health all over the world [1]. Due to the adverse side-effects, and also the development of resistance against synthetic drugs, the uses of plant-based drugs are becoming popular in developed countries. However, the latest surveys have indicated many medicinal plants also showed negative effects. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used preclinically, is a crucial part of its assessment of potential toxic effects [2].

Taking into account the both ethno botanical and pharmacological uses the study were carried out to record clinical observation and to evaluate safety of crude ethanolic extract of S. samarangense and L. acutangula by oral route in the case of acute toxicity in Wistar rats.[2,3] Toxicity is the degree to which a chemical substance or a particular mixture of substance that produce adverse effects in biological system.

The objective of toxicity study is to make sufficient information for estimation of toxicological properties of chemicals and to find out whether the substance is safe or not. [4]

II. MATERIALS & METHODS

PROCUREMENT AND AUTHENTICATION OF PLANT MATERIAL

The dried leaves were procured from local source Manoj Ayurvedic Shop No 2/3 Charkop Mangalmurti C.H.S Ltd Sector No. 4 Kandivali (West) Mumbai - 400067 and both the plant powders were authenticated by Harshad.M. Pandit (Department of Botany).

PREPARATION OF PLANT EXTRACTS

Ethanolic extract of S. samarangense and L. acutangula were prepared by using Soxhlet apparatus. Later, the extracts were concentrated using rotary evaporator at low temperature and reduced pressure. Then the extracts were preserved

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in air tight container, labelled and stored in desiccator.

ANIMAL

Adult female Wistar albino rats weighing 150-200g were selected as model for the present study. The experiments were performed in accordance with the principles and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India. The study protocol was approved by Institutional Animal Ethics Committee of Bharati Vidyapeeth's College of Pharmacy Navi Mumbai. The protocol number BVCP/IAEC/02/2019. Adult Male Wistar albino weights 150-200g were selected as model for the present study. Animal were procured from Bharat Serum and Vaccine pvt Ltd. Wagle Industrial Estate Road No: 27, Thane 400604. These animals were housed in polypropylene cage and maintained under the standard laboratory condition (12hrs light/12hrs dark cycle) with standard diet and water and were acclimatized a week before starting the experiment.

PROCEDURE

Acute Oral Toxicity study was performed as per OECD guideline 425 (Acute Oral Toxicity: Up and Down Procedure) [8]. Wistar albino rats (n=12/each dose) selected randomly. The animals were fasted for 12hrs with free access to water only. Following the period of fasting, animals were weighed and the test extracts of L. acutangula and S. samarangense were administered orally at dose 2000mg/kg. After the administration of the test extracts, food for the animal (n=6) were withheld for 2hrs. As no toxic symptoms were observed within 48hrs there after another dose of 2000mg/kg of test extracts were administered to next set of animals (n=6) and the animals were observed. On the 14th day the rats were sacrificed and their six vital organs (lungs, kidney, liver, heart, ovaries, and spleen) were sent for histopathological evaluation [5, 6].

HISTOPATHOLOGY

Liver, kidney, lungs, heart, ovaries and spleen were isolated and stored in 10% v/v formaldehyde solution and sent for histopathological evaluation. It was observed at 100x magnification power. The microscopic images showed no difference between control and treated groups [7].

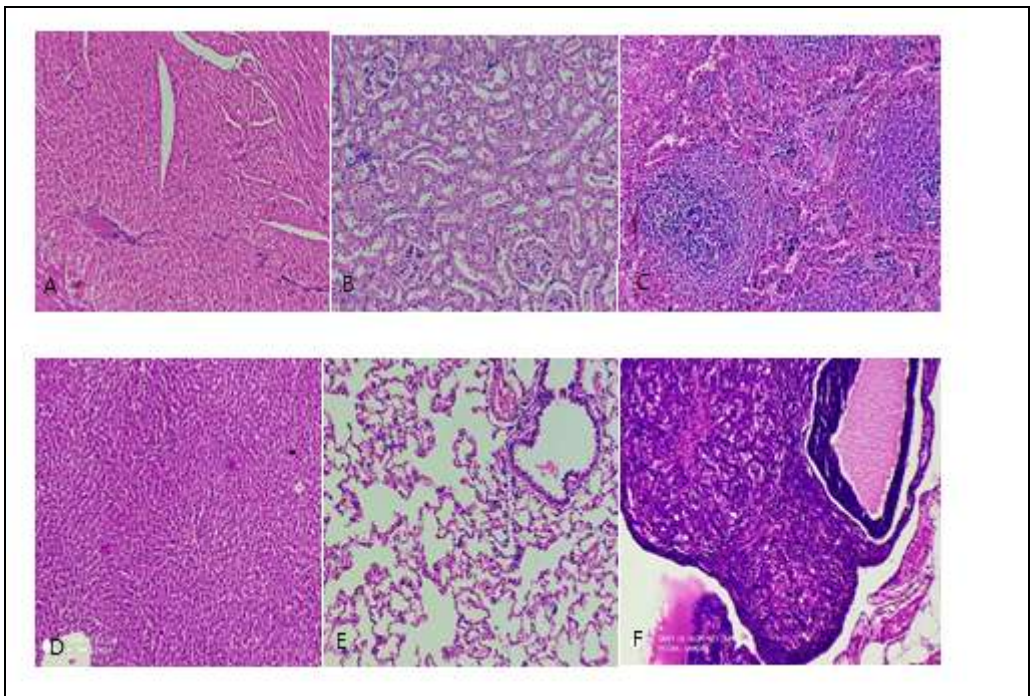

FIGURE1: Histopathological images of six vital organs of S. samarangense at 100x a) Heart b) Kidney c) Spleen d) Liver e) Lungs f) Ovaries

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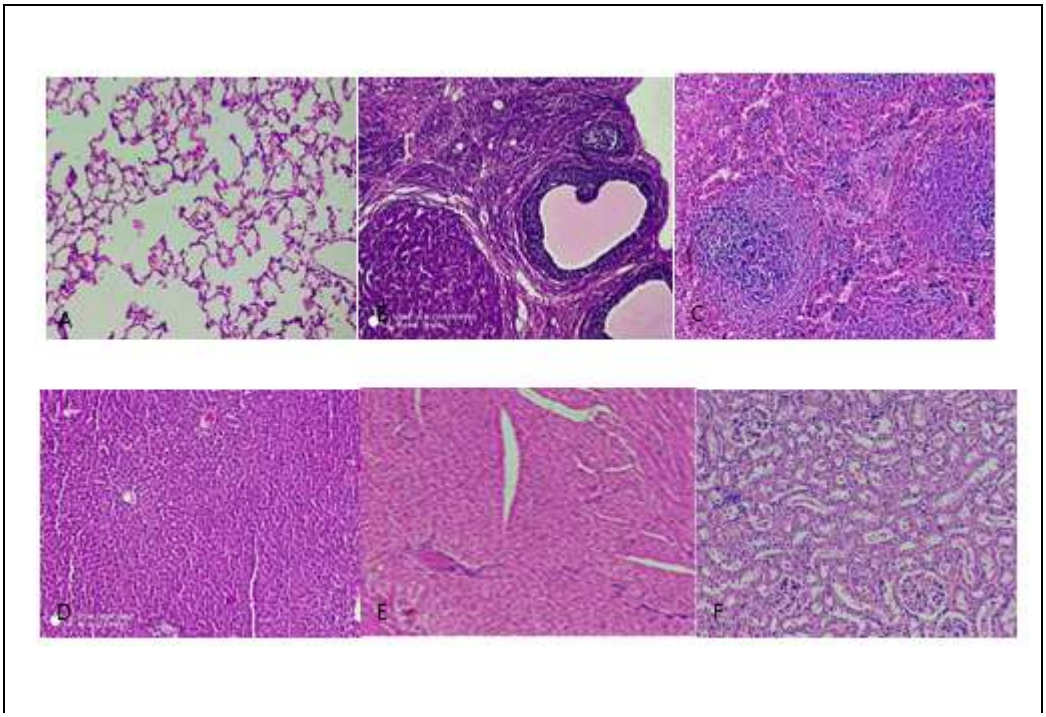

FIGURE 2: Histopathological images of six vital organs of L. acutangula at 100x a) Lungs b) Ovaries c) Spleen d) Liver e) Heart f) Kidney

III. RESULTS

The result of acute toxicity studies revealed that the oral administration of ethanolic extracts of both the plants i.e., S. samarangense and L. acutangula to rats up to 2000mg/kg body weight resulted in no toxic evidence and mortality of any test animal during the observation period of 14 days. All animals survived over the study period and there were no significant loss of fur and skin lesion. Animals did not show any signs of aggression or abnormal behavior during handling. Eyes appeared clear normal and there was no lethargy, convulsion tremor and salivation.

IV. STATISTICAL ANALYSIS

Results are expressed as Mean ± SD. Significance difference between control and experimental groups were assessed by ANOVA test followed by Newman Keuls test supported by Graph Pad Prism Inc. software version 5.00 at a significance level of $p \leq 0.05$.

TABLE NO 1: Effect of Ethanolic extracts of S. samarangense and L. acutangula leaves on body weight of rats at 2000 mg/kg after 14 days.

GROUPS	Treatment	Body weight (Grams)	
		Before Treatment (Mean ± SD)	After Treatment (Mean ± SD)
Control	1% CMC solution	184 ± 6.90	189 ± 5.95
Treatment 1	2000 mg/kg Ethanolic extract of S. samarangense	185 ± 6.80	190 ± 5.87
Treatment 2	2000 mg/kg Ethanolic extract of L. acutangula	185 ± 6.25	190 ± 5.75

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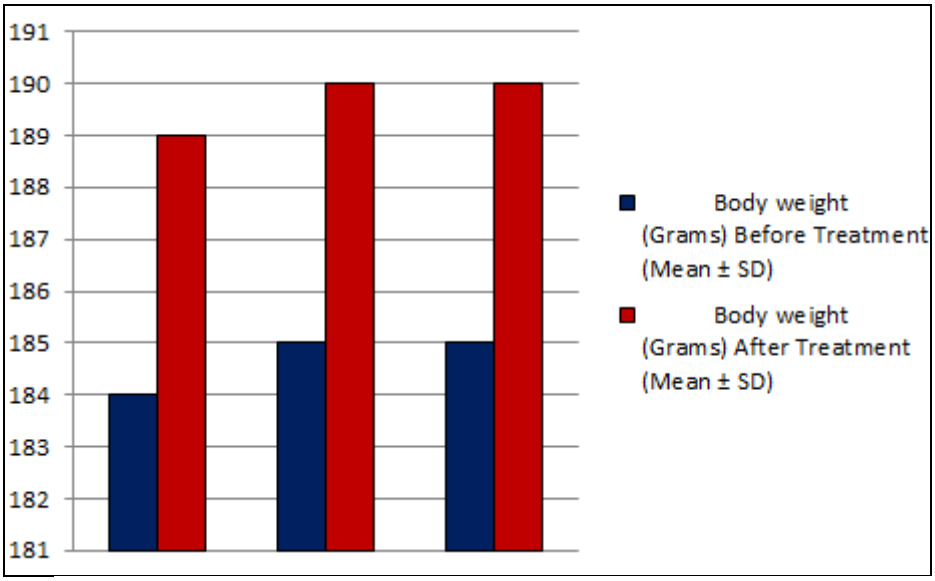

GRAPH NO 1: Effect of Ethanolic extracts of S. samarangense and L. acutangula leaves on body weight of rats at 2000 mg/kg after 14 days.

TABLE NO 2: General appearance and behavioral observations of acute oral toxicity study for control and treated group

Observation	30 mins			4 hours			8 hours			24 hours			48 hours			7th day		14th day		
	S.s	*L.a*	C	*S.s*	*L.a*	C	*S.s*	*L.a*	C	*S.s*	*L.a*	C	*S.s*	*L.a*	C	*S.s*	*L.a*	C	*S.s*	*L.a*
Food intake	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Body weight	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Changes in skin fur	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Tremor	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Convulsion	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Lethargy	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Diarrhoea	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hyperactivity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

S.s – Syzygium samarangense, L.a- Luffa acutangula, C- Control groups, N- Normal, AB- Absent

V. MORTALITY

No mortality was exhibited at 2000mg/kg body weight of S. samarangense and L. acutangula ethanolic extracts and the results were tabulated in table no. 3

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TABLE NO 3: Mortality records of Ethanolic extracts of S. samarangense and L. acutangula leaves on rats for 14 days.

Group	Control	Ethanolic extract of S. samarangense	Ethanolic extract of L. acutangula
Hour 1	Nil	Nil	Nil
Hour 2	Nil	Nil	Nil
Hour 3	Nil	Nil	Nil
Hour 4	Nil	Nil	Nil
Hour 24	Nil	Nil	Nil
Day 2	Nil	Nil	Nil
Day 3	Nil	Nil	Nil
Day 4	Nil	Nil	Nil
Day 5	Nil	Nil	Nil
Day 6	Nil	Nil	Nil
Day 7	Nil	Nil	Nil
Day 8	Nil	Nil	Nil
Day 9	Nil	Nil	Nil
Day 10	Nil	Nil	Nil
Day 11	Nil	Nil	Nil
Day 12	Nil	Nil	Nil
Day 13	Nil	Nil	Nil
Day 14	Nil	Nil	Nil

VI. DISCUSSION

The result of the acute toxicity study showed that the ethanolic extracts of S. samarangense and L. acutangula revealed to be safe up to the dose of 2000 mg/kg, and it had no adverse effects on the treated rats upto 14 days of observation.

Similarly, no significant changes in the weight of the heart, liver, lung, spleen and kidney were noticed. The protocol of weighing relative organs in toxicity studies includes their sensitivity to predict toxicity and it corresponds with histopathological changes. The results of this study showed no significant changes in the relative organ weight of control and treated groups which showed that none of the organs were negatively affected, nor showed any signs of toxicity all over the study.

Lethal dose (LD_{50}) can be decided based on the OECD guideline 425: Acute Oral Toxicity (Up and Down Procedure).

Since no toxic effects were found during the acute toxicity, the extracts of S. samarangense and L. acutangula can be used for the further studies.

VII. CONCLUSION

The acute toxicity study of the ethanolic extracts of S. samarangense and L. acutangula leaves did not produce any adverse effects on the behavior and gross pathology of the rats at treated doses. Therefore, the oral LD_{50} of the ethanolic extracts of the leaves of S. samarangense and L. acutangula was greater than 2000 mg/kg. Both the plant extracts did not adversely affect the body weight. There were no signs of toxicity observed in the kidney, liver, heart, lungs, ovaries of the treated rats. The outcome of the acute toxicity study carried out as per OECD guideline 425 (Acute Oral Toxicity: Up and Down Procedure) revealed that there were no mortality and no evidence of any visible toxic symptoms in rats. Therefore, the extracts were found to be safe.

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