Vol 2|Issue 1| 2012 | 29-33.

e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

International Journal of
Current Pharmaceutical & Clinical Research
www.ijcpcr.com

TOXICOLOGICAL STUDIES ON ETHANOL EXTRACT OF *LUFFA ACUTANGULA* IN ALBINO WISTAR RATS

A. Arunachalam[1*], S. Selvakumar[2], S. Jeganath[3]

[1]Department of Pharmaceutics, A.M.Reddy Memorial College of Pharmacy, Narasaraopet, Guntur, Andhra Pradesh, India.
[2]Department of pharmaceutical chemistry, Jagan's college of pharmacy, Nellore, Andhra Pradesh, India.
[3]Department of Pharmaceutics, Saastra College of Pharmaceutical education and research, Varigonda, Gudur, Nellore, Andhra Pradesh, India.

ABSTRACT

Luffa acutangula (Family: Cucurbitaceae) is commonly known as Ridge gourd. It is a widely growing vegetative climber. The fruits are base ball club shaped. Various pharmacological activities include hepatoprotective activity, antidiabetic activity, antioxidant activity, fungistatic property, CNS depressant activity etc. The present investigation was carried out to evaluate the safety of ethanol extract of *Luffa acutangula* (ELA) whole plant by determining its potential toxicity after acute and chronic administration in rats. Study on acute toxicity of extract found to be safe at the doses 2000mg/kg body weight orally as per OECD guidelines No.423. General behavior adverse effects and mortality were determined for up to 14 days. In the chronic toxicity study, the ELA was administered orally at doses of 100, 200 and 400 mg/kg once in a week for 6 weeks to rats. Biochemical and hematological parameters were determined after 6 weeks. In the acute study in rats, there was no toxicity/ death was observed at the dose of 2000mg/kg b.w. The onset of toxicity and signs of toxicity also not there. In the chronic toxicity study, no significant treatment-related changes in the levels of haematological, hepatic and renal parameters such as SGOT, SGPT, cholesterol, creatinine, urea, uric acid, protein and glucose, and serum ALP activities were observed at the termination of the study. It suggests that the ethanol extract of *Luffa acutangula* does not appear to have significant toxicity. In view of the dose of *Luffa acutangula* consumed in traditional medicine, there is a wide margin of safety for the therapeutic use of the ethanol extract of *Luffa acutangula* whole plant.

Key words: *Luffa acutangula*, Traditional Medicine, Acute and Chronic Toxicity, Heamatological Parameters, Biochemical Parameters.

INTRODUCTION

Luffa acutangula (Family: Cucurbitaceae) is commonly known as Ridge gourd. It is a widely growing vegetative climber. The fruits are base ball club shaped. Various pharmacological activities include hepatoprotective activity, antidiabetic activity, antioxidant activity, fungistatic property, CNS depressant activity etc. Its chemical constituents were found to be carbohydrates, carotene, fat, protein, phytin, aminoacids, alanine, arginine, cystine, glutamicacid, glycine, hydroxyproline, leucine, lectin, serine, tryptophan, pipecolic acid [1-6]. And also whole plants are used for the treatment of ulcers and sores In spite of the use of *Luffa acutangula* in traditional medicine and its potential for toxicity, systematic evaluation of its toxic effects is lacking. Therefore, the aim of the present study was to investigate the acute and chronic toxic effects of ethanol extract of *Luffa acutangula* in rodents.

MATERIALS AND METHODS

Plant material

The whole plant of *Luffa acutangula* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

Corresponding Author :- **A. Arunachalam** Email:-harisarun1985@gmail.com

Preparation of plant extract

The collected whole plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with ethanol (90%) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment. The percentage yield of prepared extract was around 10.5%w/w.

Animals Used

Albino rats (180–200 g) of either sex were maintained in a 12 h light/dark cycle at a constant temperature 25 ◦C with free access to feed (Sai durga feeds and foods, Bangalore) and water. All animals were fasted prior to all assays and were allocated to different experimental groups ELAh of 6 rats. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute toxicity study of *Luffa acutangula* extract in rats

The procedure was followed by using OECD 423 (Acute Toxic Class Method) [7]. The acute toxic class method is a step wise procedure with three animals of a single sex per step. Depending on the mortality or moribund status of the animals and the average two to three steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use number of animals while allowing for acceptable data based scientific conclusion. The method used to defined doses (2000, 1000, 500, 50, 5 mg/kg body weight, Up-and-Down Procedure). The starting dose level of ELA was 2000 mg/kg body weight p.o as most of the crude extracts posses LD 50 value more than 200 mg/kg p.o. Dose volume was administered 0.2ml per 100gm body weight to overnight fasted rats with were *ad libidum*. Food was withheld for a further 3-4 hours after administration of ELA and observed for signs for toxicity. The body weight of the rats before and after administration were noted that changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted for 14 days. The onset of toxicity and signs of toxicity also noted. Hence, $1/20^{th}$ (100mg/kg), $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study.

Study of Chronic Toxicity of *Luffa acutangula* extract in rats

Design of Treatment

Animals were divided into 5 groups of six rats ELAh.

Group I - Normal saline (0.9%, NaCl, 5ml/kg, p.o) once in a week for 6 weeks.

Group II- Vehicle 1% SCMC (5ml/kg, p.o) once in a week for 6 weeks.

Group III-V- Ethanol extract of *Luffa acutangula* whole plant at the dose of 100, 200 and 400 mg/kg, p.o respectively.

Animals from ELAh group were sacrificed at the 6^{th} week, after the last dose. Different haematological and serum biochemical tests were then performed.

Collection of blood and serum samples

Paired blood samples were collected by cervical decapitation from diethyl ether anaesthetized rats into heparinised bottles for haematological studies and clean non-heparinised bottles and allowed to clot. The serum was separated from the clot and centrifuged into clean bottles for biochemical analysis.

Methods for estimation of haematological parameters

Estimation of Hemoglobin [9], RBC count [9], WBC count [9], different leucocytic count [8], Elongation time [8] and ESR [10]were determined according to the standard procedures.

Determination of serum biochemical parameters

Blood Glucose, [11] Serum Bilirubin [12], Serum Gluconate – Oxaloacetate Transaminase (SGOT) [12], Serum Glutamate – Pyruvate Transaminase (SGPT) [12], Serum Alkaline Phosphatase (ALP) [12], Blood Cholesterol [11], Blood Urea [11] , Serum Uric Acid [11], Blood Creatinine [11] and Serum protein[11] were estimated by standard procedures.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

RESULTS

Acute toxicity study

The body weight of the rats before and after administrations were noted that there is slightly increased the body weight. But there are no changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also no sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of

toxicity also not there. In this study there was no toxicity/ death were observed at the dose of 2000mg/kg b.w. The acute toxicity study in rats showed that at 2000 mg/kg dose, the plant is safe for consumption and for medicinal uses (Table 1).

Chronic toxicity study

The chronic oral administration of ethanol extract of *Luffa acutangula* whole plant caused no noticeable change in the general behaviour of the rats and, compared to the control group (saline and vehicle), no significant changes in body weight, food intake and utilization of food in the ELA treated rats. Both the control and treated rats appeared uniformly healthy at the end and throughout the six weeks period of study.

Effect of ethanol extract of *Luffa acutangula* whole plant on the haematological and biochemical parameters of rats

In the chronic toxicity study, the haematological parameters, hemoglobin concentration, clotting time, neutrophils, easinophils, lymphocytes, monocytes, red and white blood cells in the treated rats did not differ significantly ($P > 0.01$) from that of the control group (Table 2) and all the values remained within normal limits throughout the experimental period. As shown in Table 3 & 4, no significant treatment-related changes in the levels of hepatic and renal parameters such as SGOT, SGPT, cholesterol, creatinine, urea, uric acid, protein and glucose, and serum ALP activities were observed at the termination of the study.

Table 1. Acute toxicity study of ethanol extract of *Luffa acutangula* (ELA) in rats

S.No	Groups	Dose/kg b.w, p.o	Weight of animals		Signs of Toxicity	Onset of Toxicity	Duration of study
			Before Test	After Test			
1	ELA	2000 mg	175 g	180 g	No signs of Toxicity	Nil	14days
2	ELA	2000 mg	180 g	190 g	No signs of Toxicity	Nil	14days
3	ELA	2000 mg	165g	175 g	No signs of Toxicity	Nil	14days
4	ELA	2000 mg	195 g	205 g	No signs of Toxicity	Nil	14days
5	ELA	2000 mg	210 g	215 g	No signs of Toxicity	Nil	14days
6	ELA	2000 mg	205 g	215 g	No signs of Toxicity	Nil	14days

Table 2. .Effect of ethanol extract of *Luffa acutangula* (ELA) on heamotological profiles in rats

Design of treatment	Group I Saline(0.9 % W/V)	Group II Vehicle (1%SCMC)	Group III ELA	Group IV ELA	Group V ELA
Dose mg/kg	5 ml/kg,p.o	5 ml/kg,p.o	100mg/kg,p.o	200mg/kg,p.o	400mg/kg,p.o
Neutrophil (%)	23.2± 0.32	26.2 ± 0.24	33.7 ± 0.43[a]	37.8 ± 0.42[a]	39.2 ± 0.47[a]
Eosinophil (%)	1.2 ± 0.04	0.8 ± 0.24	1.4 ± 0.04[a]	0.7 ± 0.04[a]	0.7 ± 0.02[a]
Lymphocyte(%)	72.4 ± 0.27	72.4 ± 0.3	64.4 ± 2.17[a]	59.4 ± 2.12[a]	53.6 ± 2.47[a]
Monocyte (%)	3.4 ± 0.67	2.7 ± 0.44	2.4 ± 0.22[a]	2.6 ± 0.44[a]	1.9 ± 0.47[a]
Clotting time (seconds)	77.3 ± 2.47	80.2 ± 2.74	93.2 ± 2.72[a]	97.7 ± 2.69[a]	100.4 ± 2.72[a]
Haemoglobin (gm%)	14.4 ± 0.67	14.2 ± 0.42	13.7 ± 0.22[a]	12.6 ± 0.22[a]	12.3 ± 0.24[a]
RBC cells (cu.mm)×10^9(%)	8.3 ± 0.74	7.4 ± 0.4	7.7 ± 0.9[a]	6.7 ± 0.22[a]	7.7 ± 0.12[a]
WBC cells (cu.mm)×10^9(%)	7.9 ± 0.36	7.7 ± 0.29	7.8 ± 0.22[a]	8.4± 1.21[a]	10.2± 1.07[a]

a- Group I & II Vs group III, IV &V. $P < 0.01$ when compared to control group
ELAh value represents the mean ± S.E.M six rats in ELAh group

Table 3. Effect of ethanol extract of *Luffa acutangula* (ELA) on hepatic parameters in rats

Groups	Design of treatment	Dose Mg/kg	Glucose Mg/dl	Bilirubin Mg/dl	SGOT 1 Unit/L	SGPT 1 Unit/L	ALP 1 Unit/L	Cholestrol mg/100ml
I	Saline(0.9 % W/V)	5 ml /kg,p.o	87 ± 3.4	0.4 ± 0.001	40.7 ±0.7	32.1 ±0.7	7.3 ±0.37	60.7 ±1.9
II	Vehicle (1% SCMC)	5ml/kg,p.o	97 ± 3.2	0.6 ±0.001	57.2 ±0.4	34.1 ±1.2	7.7 ±0.33	66.6 ±1.4
III	ELA	100mg/kg,p.o	99 ± 3.7[a]	0.4 ± 0.001[a]	53.1 ±0.2[a]	34.2 ±0.6[a]	10.2 ±0.32[a]	54.2 ±1.7[a]
IV	ELA	200mg/kg,p.o	104 ± 3.4[a]	0.6 ± 0.001[a]	54.2 ±0.2[a]	37.6 ±0.4[a]	11.1 ±0.32[a]	59.1 ±1.6[a]
V	ELA	400mg/kg,p.o	106 ± 3.1[a]	0.7 ±0.001[a]	57 ± 0.6[a]	37.0 ±0.6[a]	12.1 ±0.2[a]	70.2±1.2[a]

a- Group I & II Vs group III, IV &V. $P < 0.01$ when compared to control group
ELAh value represents the mean ± S.E.M six rats in ELAh group

Table 4. Effect of ethanol extract of *Luffa acutangula* (ELA) on renal parameters in rats

Groups	Design of treatment	Dose mg/kg	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl	Protein gm/dl
I	Saline(0.9 % W/V)	5 ml/kg,p.o	20 ± 0.54	4.1 ± 0.7	0.9 ± 0.001	6.7 ±0.10.
II	Vehicle (1%SCMC)	5 ml/kg,p.o	21 ± 0.41	4.3 ± 0.4	1.2 ± 0.004	6.9 ±0.12
III	ELA	100mg/kg,p.o	24 ± 0.41[a]	3.9 ± 0.6[a]	1.1 ±0.002[a]	6.8 ± 0.14[a]
IV	ELA	200mg/kg,p.o	27 ± 0.44[a]	3.7±0.7[a]	1.2 ±0.001[a]	7.1 ± 0.1[a]
V	ELA	400mg/kg,p.o	28 ± 0.16[a]	3.7 ±0.6[a]	1.4 ±0.001[a]	7.4± 0.31[a]

a- Group I & II Vs group III, IV &V. $P < 0.01$ when compared to control group
ELAh value represents the mean ± S.E.M six rats in ELAh group

DISCUSSION AND CONCLUSION

A Word Health Organization survey indicated that about 70–80% of the world's populations rely on non-conventional medicine, mainly of herbal source, in their primary healthcare [13,14]. Although medicinal plants may produce several biological activities in humans, generally very little is known about their toxicity and the same applies for Luffa acutangula. Because safety should be the overriding criterion in the selection of medicinal plants for use in healthcare systems [15]. To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dose in humans [16]. One should, in addition to the use of historical documentation on Alocassia macrorhiza, also have formal toxicological evaluations of this plant to optimize its safe use as a medicine. The ethanol extract of *Luffa acutangula* used in the present study offers several advantages as a form of the *Luffa acutangula* medicine [17]. But before such evaluation can be fully justified in humans, the preclinical evaluation of the safety of the *Luffa acutangula* is required.

In this study, the ethanol extract of *Luffa acutangula* was found to be non-toxic in rats when administered orally in doses up to 2000 mg mg/kg, p.o. The onset of toxicity and signs of toxicity also not there. In this study there was no toxicity/ death were observed at the dose of 2000mg/kg b.w. Based on this animal study, may be described as being practically non-toxic.

In the six weeks chronic toxicity study, the ELA at the doses of 100, 200 & 400mg/kg did not appear to affect the bodyweight or the behaviour of the rats and caused no significant changes in their food intake and utilization of food indicating normal metabolism in the animals and suggesting that, at the oral doses administered ELA did not retard the growth of rats. After six weeks treatment, there were also no treatment related changes in the haematological parameters (i.e. hemoglobin concentration, clotting time, neutrophils, easinophils, lymphocytes, monocytes, red and white blood cells) between control and treated groups indicating that the ELA was not toxic to the circulating red cells, nor interfered with their production. Hematopoiesis and leucopoiesis were also not affected even though the haematopoietic system is one of the most sensitive targets for toxic compounds [18] and an important index of physiological and pathological status in man and animals [19].

In addition, most of the hepatological and renal parameters (i.e. Glucose, creatinine, Bilirubin, SGOT, SGPT, ALT, urea, uric acid, protein and cholesterol,) were also unchanged by the doses of ELA 100, 200 & 400mg/kg. The lack of significant alterations in the levels of ALP, creatinine, Bilirubin, SGOT, SGPT and

cholesterol, good indicators of liver and kidney functions, respectively [20]. The transaminases (SGOT and SGPT) are well known enzymes used as biomarkers predicting possible toxicity [21]. Generally, damage to the parenchymal liver cells will result in elevations of both these transaminases [22]. The transaminases were not significantly increased at the doses of ELA 100, 200 & 400mg/kg. It suggests that chronic ingestion of ELA did not alter the hepatocytes and kidneys of the rats, and, furthermore the normal metabolism of the animals. The relevance of this result may be associated with the biological value of the plant Luffa acutangula.

In conclusion, the present investigation demonstrates that at doses consumed in the traditional medicine, the ethanol extract of *Luffa acutangula* may be considered as relatively safe, as it did not cause either any lethality or changes of in the general behavior in both the acute and chronic toxicity studies in rats. Studies of this type are needed before a phytotherapeutic agent can be generally recommended for use.

REFERENCES

1. Brenner M. Drugs for neurodegenerative diseases. *Pharmacology review*, WB Saunders company. 104-106.
2. Jadhav VB, Thakare VN, Suralkar AA, Deshpande AD, Naik SR. 2010, 48, 822-829.
3. Shekhawat N, Soam PS, Singh T, Vijayvergia R. 2010, 5(4), 298-301.
4. Dixit SN, Tripathi SC. *Nat. Acad. Sci.* Lett, 1 , 1975, 287.
5. B. Kandlakunta,B.Rajendran, L. Thingnaganing, *Food chem*. 2008, 106.
6. V. Anantharam, S.R. patanjali, M.J. Swamy, A.R. Sanadi, I.J.Goldstein, A. Surolia, 1986, 261(5), pp 14621-14627.
7. OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organisation for economical co-operation and development, Paris, June, 2000.
8. Crossland J. In: Lewis Pharmacology**,** 5th edn, Churchill Livingston, Edinburgh, 1980, 656.
9. Dacie JV, Lewis SM. In: Practical Hematology**,** J and A Churchill Ltd. London, 1958, 38.
10. Wintrobe MM, Lee GR, Goggs DR, Forester J. In: Clinical Hematology**,** Lee and Febiger, Philadelphia, 7th ed, 1994, 49.
11. Oser BL, Tata Mc- Graw Hill. In: Hawk's Physiological Chemistry Book, Company Ltd. New Delhi, 14th ed, 1965, 1035, 1040, 1045, 1048, 1052, 1053, 1062, 1088.
12. Bergmeyer HU. In: methods of enzymatic Analysis, HU Berbmeyer (Ed.) Academic Press, NewYork, 3rd ed., 1965, 783, 837, 840.
13. Chan K, Some aspects of toxic contaminants in herbal medicines. *Journal of Chemosphere*,52, 2003, 1361–1371.
14. Dyson A, Discovering Indigenous Healing Plants of the Herb and Fragrance Gardens at Kirstenbosch National Botanical Garden. National Botanical Institute, Printing Press, Cape Town 1998.
15. Tomlinson TR, Akerele O. Medicinal Plants their Role in Health and Biodiversity. University of Pennsylvania Press, Philadelphia 1998.
16. Olson H, Betton G, Robinson D et al. Concordance of toxicity of pharmaceuticals in humans and in animals. *Regulatory Toxicology and Pharmacology*, 32, 2000, 56–67.
17. Roberts M, Indigenous Healing Plants. Southern Book, South Africa 1990.
18. Harper HA. Review of Physiological Chemistry, 14th ed. Lange Medical Publications, California, 1973.
19. Adeneye AA, Ajagbonna OP, Adeleke TI, Bello, S.O. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of Musanga cecropioides in rats. *Journal of Ethnopharmacology*, 105, 2006, 374–379.
20. Hilaly EJ, Israili ZH, Lyoussi B. Acute and chronic toxicological studies of *Ajuga Iva* in experimental animals. *Journal of Ethnopharmacology*, 91, 2004, 43–50.
21. Rahman M., Siddiqui MK, Jamil K. Effects of Vepacide (*Azadirachta indica*) on aspartate and alanine aminotransferase profiles in a sub chronic study with rats. *Journal of Human and Experimental Toxicology*, 20, 2001, 243–249.
22. Wolf PL, Williams D, Tsudaka T, Acosta L. Methods and Techniques in Clinical Chemistry. John Wiley & Sons, USA. 1972.