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EVALUATION OF ACUTE AND SUBCHRONIC TOXICITY OF *LAGENARIA SICERARIA* AERIAL PARTS

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ABSTRACT

Lagenaria siceraria is traditionally being used in many countries in the treatment of various diseases including diabetes. The aim of the present study was to evaluate the safety of the methanol extract of *L. siceraria* aerial parts (MELS) through acute and subchronic toxicity study in mice. For acute toxicity study 500-2000 mg/kg MELS were administered orally and obvious toxic symptoms and mortality was studied upto 72 h. In subchronic study, effect of multiple weekly dosing of 400 mg/kg (one-fifth of the maximum tolerated dose) of MELS was investigated in mice for six weeks and the evaluation was done by the studies of hematological parameters, biochemical estimations of hepatorenal parameters, antioxidant status, and histological observations of the tissue. The extract was found to be well tolerated upto 2g/kg in acute toxicity study. In subchronic toxicity study it showed no significant alteration on any of the parameters, however an improvement in the lipid profile was observed in the treated group of animals. Hence the results suggest that methanol extract of *L.siceraria* aerial parts is quite safe and can be used in the treatment of the chronic diseases like diabetes without any toxicity.

INTRODUCTION: Nature has been a source of medicinal agents from the ancient times and medicinal plants, especially have formed the basis of the wide variety of traditional medicines used in various countries worldwide. Present estimates indicate that about eighty percent of the world's population relies on traditional medicine for health care delivery [1, 2]. The exclusive use of herbal drugs for the management of variety of ailments continues due to easy access, better compatibility and for economic reasons. Studies of medicinal plants using scientific approaches showed that various biological components of medicinal plants exhibit a variety of properties and can be used to treat various ailments. However, a number of studies have reported the toxic effects of herbal medicines [3, 4]. Hence a systemic scientific study of a medicinal plant should include a thorough toxicity study before the recommendation for its use in the treatment of any disease or disorder.

Lagenaria siceraria (Mol.) Standley, commonly known as bottle-gourd (in English), belongs to cucurbitaceae family. It is a climbing or trailing herb, with bottle or dumb-bell shaped fruits. Both of its aerial parts and fruits are commonly consumed as vegetable. The plant is widely available in India, China, European countries, Brazil and Hawaiian island and traditionally is used as medicine for its cardiotonic, general tonic, and diuretic properties [5]. Further, antihepatotoxic, analgesic and anti-inflammatory, hypolipidemic, antihyperglycemic, immunomodulatory and antioxidant activities of its fruit extract have been evaluated [6-10]. *Lagenaria siceraria* fruits are good source of vitamin B complex,

ascorbic acid, fibers, proteins, cucurbitacins, saponins, fucosterols and compesterols, polyphenolics, flavones-C-glycoside [8, 10-13]. Methanol extract of its leaves showed the presence of sterols, polyphenolics, flavonoids, saponins, proteins and carbohydrates [14]. A novel protein, Lagenin has also been isolated from its seeds and it possesses antitumor, immunoprotective and antiproliferative properties [15]. Despite of the popular use, exploring various medicinal importances of the various parts of the plant, there is no report on the toxicity study of its aerial parts. The present investigation was therefore carried out to study the acute and subchronic toxicity of the methanol extract of *L. siceraria* aerial parts (MELS) in mice.

MATERIALS AND METHODS:

Plant material: The aerial parts of *L.siceraria* was collected in November 2008, from Madanpur, West Bengal, India and identified by the Botanical Survey of India, Howrah, India. A voucher specimen (P/LS/1/08) was retained in our laboratory for further reference.

Preparation of plant extract: The aerial parts were dried under shade and powdered in a mechanical grinder. The powdered material was extracted with methanol using soxhlet apparatus. This extract was filtered and concentrated in *vacuo* in a Buchi evaporator, R-114 and kept in a vacuum dessicator until use. The yield was 18.13% w/w with respect to dried powder. Aqueous suspension of MELS was prepared using 2 % (v/v) Tween-80 and used for oral administration.

Animals: Healthy Swiss albino mice (20 ± 2 g) were used for the present study. They were maintained at standard laboratory conditions and fed with commercial pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory condition for one week before commencement of experiment. The experiments were performed based on animal ethics guidelines of University Animals Ethics Committee.

Phytochemical analysis: Preliminary phytochemical screening of the extract was carried out using standard methods [16].

Acute toxicity study: Healthy Swiss albino mice (20 ± 2 g) of either sex, starved overnight, were divided into five groups (n=6). Group I-IV animals were orally fed with MELS in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 g/kg, while group V (untreated) served as control. The animals were observed continuously for first 2 h for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again at 24 h , 48 h and 72 h for any lethality or death. One-fifth of the maximum safe dose of the extract tested for acute toxicity was selected for the subchronic toxicity experiment [17].

Subchronic toxicity study: Sixteen mice were randomly divided into two groups of eight mice in each. Group I (normal control) animals received 2% Tween 80 solution (0.5 ml, p.o.) and Group II animals received MELS (400mg/kg, p.o., ie., one-fifth of the maximum tolerated dose) every 72 h for six weeks [18, 19]. During the experimental period, the animals were weighed every three days and food and water intake were monitored daily. At the end of the experiment, after 24 h of the last dose and 18 h fasting, animals were sacrificed and blood was collected intracardially and taken into heparinized tube for hematological studies and non-hepainized centrifuge tube for biochemical estimations. Liver tissue was collected from the animals for the evaluation of *in vivo* antioxidant status and part of the liver tissue was taken for the histological studies.

Hematological studies: RBC, WBC counts using of Neubauer hemocytometer and estimation of hemoglobin using Sahli's Hemoglobinometer were carried out by standard procedures from the blood obtained intracardially [20, 21].

Biochemical estimation: The effect of MELS treatment on the biochemical parameters of the experimental mice were evaluated by the estimation of serum biochemical enzymes such as serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities by the method of Reitman and Frankel [22], alkaline phosphatase (ALP) activities by Kind and King method [23], total bilirubin [24], total protein [25], urea [26], uric acid [27], creatinine [28], glucose, total cholesterol, triglyceride, HDL and LDL

cholesterol [29, 30]. All the analysis was performed by standard enzymetic methods using commercially available kit from Span Diagnostics Ltd.

***In vivo* antioxidant assay:** The antioxidant assay was performed with the liver tissues of the experimental animals and evaluation of the antioxidant status was carried out by measuring the level of lipid peroxidation [31] and the amount of enzymatic (Catalase: CAT) and nonenzymatic antioxidant system (reduced glutathione: GSH) by the methods of Luck [32] and Ellman [33] respectively.

Histological studies: After sacrificing the mice, parts of liver tissues were collected for the histological studies. The tissues were washed in normal saline and fixed immediately in 10% formalin for a period of at least 24 h, dehydrated with alcohol, and embedded in paraffin, cut into 4-5µm thick sections and stained with hematoxylin- eosin dye for photomicroscopic observation.

Statistical analysis: Values were presented as mean ± S.E.M. Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test using SPSS software. $P<0.01$ were considered as statistically significant.

RESULTS: Preliminary phytochemical screening of MELS revealed the presence of polyphenolics, flavonoids, glycosides, triterpenoids, saponin and carbohydrates. In acute toxicity study, MELS did not show any mortality or toxic effect upto the dose of 2 g/kg during the observational period of 72 h. It did not produce any significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses, and gastrointestinal effects in male and female mice. These results showed that in single dose, there are no adverse effects of MELS, indicating that the medium lethal dose (LD_{50}) is higher than 2000 mg/kg for both male and female mice. Accordingly one-fifth of the maximum tolerated dose ie, 400 mg/kg was considered as the high dose of MELS and used for the subchronic toxicity study in the present investigation.

In sub-chronic toxicity study, MELS administration did not show any significant effect on water and food intake and body weight of the treated animals (data not shown).

Effect of MELS on hematological parameters has been presented in **Table 1**. RBC and WBC count remained unaltered in MELS treated animals, hemoglobin content was slightly decreased in group II mice; however, it was within normal range.

The normal levels of hepatic biomarker enzymes (SGPT, SGOT and ALP), total bilirubin and protein in serum and the unaltered values of renal biochemical parameters (urea, uric acid and creatinine), as shown in **Table 2**, indicate that subchronic treatment with MELS does not posses any significant adverse effect on hepato-renal functioning of the animals.

TABLE 1: EFFECT OF METHANOL EXTRACT OF *LAGENARIA SICERARIA* AERIAL PARTS (MELS) ON HEMATOLOGICAL PARAMETERS OF CONTROL AND TREATED MICE

Groups	Hemoglobin (g %)	RBC (million/cu.mm)	WBC (thousand/cu.mm)
Normal Control (2% Tween 80)	13.03±1.00	6.70±0.65	3.47±0.29
MELS (400 mg/kg)	12.20±1.65	6.45±0.45	4.03±0.94

Values are mean ± SEM, (n=8), * $p<0.01$ for MELS treated group *vs.* normal control group

TABLE 2: EFFECT OF METHANOL EXTRACT OF *LAGENARIA SICERARIA* AERIAL PARTS (MELS) ON BIOCHEMICAL PARAMETERS FOR HEPATORENAL FUNCTIONS IN CONTROL AND TREATED MICE

Groups	Hepatic Biochemical parameter					Renal Biochemical parameter		
	SGOT (IU/dl)	SGPT (IU/dl)	SALP (IU/dl)	Total Bilirubin (mg/dl)	Total Protein (g/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Normal Control (2% Tween 80)	52.52±1.68	45.25±4.02	88.26±1.48	1.10±0.13	7.25±0.46	40.16±2.50	6.06±0.65	0.90±0.29
MELS (400 mg/kg)	60.30±2.02	54.92±3.58	89.05±2.90	0.99±0.22	7.77±0.52	45.08±5.05	7.15±0.81	1.31±0.76

Values are mean ± SEM, (n=8), * $p<0.01$ for MELS treated group *vs* normal control group

Table 3 explores the lipid profile and blood sugar level of normal and MELS treated animals after the six week experimental period. The results revealed that the extract does not adversely alter the lipid profile and blood sugar level of the animals after subchronic treatment, however there was a tendency of the increase in HDL cholesterol with subsequent decrease in LDL cholesterol level after treatment.

No significant difference in case of endogenous antioxidant status among the normal control animals and extract treated mice were observed (**Table 4**).

TABLE 3: EFFECT OF METHANOL EXTRACT OF *LAGENARIA SICERARIA* AERIAL PARTS (MELS) ON LIPID PROFILE AND GLUCOSE LEVEL IN CONTROL AND TREATED MICE

Groups	Lipid profile (mg/dl)				Glucose (mg/dl)
	Triglyceride	Total cholesterol	HDL	LDL	
Normal Control (2% Tween 80)	90.66±3.26	120.45±2.41	76.60±1.06	26.98±3.05	82.25±3.04
MELS (400 mg/kg)	100.09±4.98	123.00±4.71	79.99±1.00	23.98±2.66	80.65±2.66

Values are mean ± SEM, (n=8), * p<0.01 for MELS treated group *vs.* normal control group

TABLE 4: EFFECT OF METHANOL EXTRACT OF *LAGENARIA SICERARIA* AERIAL PARTS (MELS) ON ANTIOXIDANT SYSTEM OF CONTROL AND TREATED MICE

Groups	LPO (nM/mg wet tissue)	GSH (µg/mg wet tissue)	CAT (µM of H_2O_2 decomposed/min/mg wet tissue)
Normal Control (2% Tween 80)	98.26±6.50	34.92±3.45	71.90±6.66
MELS (400 mg/kg)	100.30±2.55	40.02±1.88	77.80±3.66

Values are mean ± SEM, (n=8), * p<0.01 for MELS treated group *vs.* normal control group

Histological observation of the liver tissue of both normal control mice as well as extract treated mice (**Fig. 1A and 1B**) showed normal cellular architecture with prominent central vein.

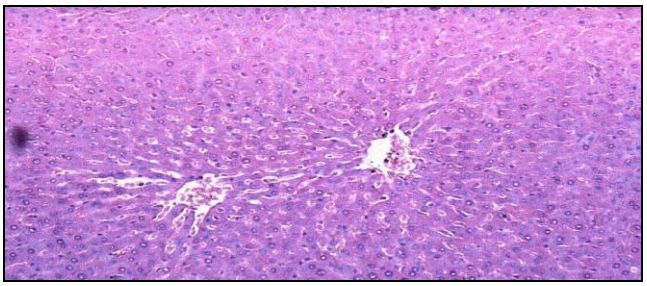

FIG. 1A: PHOTOMICROGRAPH OF LIVER SECTION OF NORMAL MICE

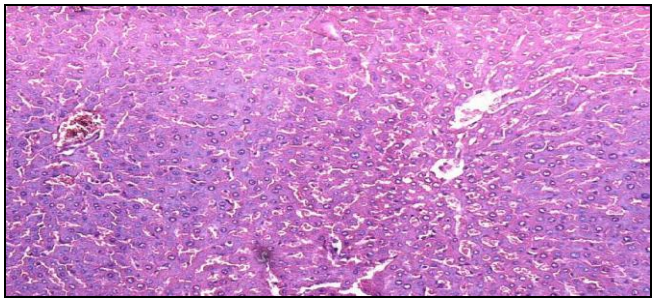

FIG. 1B: PHOTOMICROGRAPH OF LIVER SECTION OF MICE TREATED WITH MELS 400mg/kg

FIG. 1: HISTOLOGICAL OBSERVATION OF THE LIVER TISSUES OF NORMAL MICE AND TREATED MICE WITH METHANOL EXTRACT OF *LAGENARIA SICERARIA* AERIAL PARTS (MELS)

DISCUSSION: Herbal medicines have received a great attention as alternatives to synthetic pharmaceutical products in recent times, leading to the increase in their demand [34]. Experimental screening method is therefore important to ascertain the safety and efficacy of these herbal drugs.

The lack of mortality or toxicity at oral treatment of over 2000 mg extract/kg body weight obtained suggests that the methanol extract of *L. siceraria* aerial parts is practically nontoxic at single dose. However in case of subsequent use in the treatment of the chronic diseases like diabetes whether it will be safe that can be clear from its sub chronic toxicity study.

The effect on hemoglobin concentration and RBC count indicated the unlikelihood of the extract to induce anaemia. Insignificant change in WBC count was probably due to normal response to foreign bodies or stress associated with the chronic toxicity studies [35, 36]. Increase in the level of SGPT, SGOT and ALP reflects the structural and functional dysfunction of hepatocellular membrane or cell rupture, and thereby indicates liver damage. Bilirubin is formed from degeneration of hemoglobin by the action of reticuloendothelial systems throughout the body. Increased bilirubin level reflects the depth of jaundice

[19, 37]. The normal value of the hepatic biochemical parameters reveals the safety profile of the extract on liver function even on its chronic use. The normal values of the renal biochemical parameters, including urea, uric acid and creatinine suggest that the extract does not produce any sort of disturbance in the kidney function, as has been found in case of various plant extracts [18] and hence is safe on its chronic use in various diseases.

Although, the extract possesses antidiabetic property, however it does not affect adversely normal blood glucose level. The tendency to improve the lipid profile in the present study indicates its hypolipidemic potential, which may be beneficial in further studies. The endogenous antioxidant status after the chronic use of the extract was found to be quite equivalent to that of the normal mice. Free radicals generated either exogenously or endogenously in our body have been implicated in causation of several diseases such as liver cirrhosis, inflammation, atherosclerosis, diabetes, cancer, neurodegenerative diseases and so forth. The link between free radicals and diseases has led to considerable research into nontoxic drug that possesses antioxidant property and can scavenge the free radicals.

Thus, in present investigation the improved antioxidant status in the extract treated animals indicates that it may be beneficial in face of the oxidative stress in case of various diseases and disorders [6, 38]. Histological observations correlate the other results showing the normal cellular architectures in the treated group of animals, without any necrosis or fatty infiltration, which can substantiate the safety profile of the extract clearly. The present study thus, provides evidence for the total safety profile of the methanol extract of the aerial parts of *L. siceraria*, suggesting its safe use in single dose treatment as well as for long term use for the treatment of various chronic diseases, without producing any toxic effects. Hence further phytopharmacological studies on the basis of its ethnobotanical use can help to explore and establish the bioactive constituents which can be used safely for the treatment of various diseases and disorders in future.

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REFERENCE:

1. Akah PA: Indigenous Knowledge and Medical practice. In: Ethnopharmacology. Akah PA. (eds) Research Signpost, Kerela, India, 2008: 1-13.
2. Kamboj VP: Herbal Medicine. Current science 2000; 78(1): 35-39.
3. Jaouad EH, Israilli ZH and Lyoussi B: Acute toxicity and chronic toxicological studies of Ajugaiva in experimental animals. Journal of ethnopharmacology 2004; 91: 43-50.
4. Kalplowitz N: Hepatotoxicity of herbal remedies: Insight into the intricacies of plant-animal warfare and cell death. Gastroenterol 1997; 113: 1408-1412.
5. Kirtikar KR and Basu BD: Indian Medicinal Plants. Vol V, Edition 2, Dehradun, Uttaranchal, India: Oriental Enterprises, 2003: 1551-1554.
6. Deshpande JR, Mishra MR, Meghre VS, Wadodkar SG and Dorle AK: Antioxidant activity of *Lagenaria siceraria* (Mol) Stand. Fruit. Natural Product Radiance 2007; 6: 127.
7. Deshpande JR, Choudhari AA, Mishra MR, Meghre VS, Wadodkar SG and Dorle AK: Beneficial effects of *Lagenaria siceraria* (Mol) Stand. fruit epicarp in animal models. Indian Journal of Experimental Biology 2008; 46: 234-242.
8. Ghule BV, Ghante MH, Upganlawar AB and Yeole PG: Analgesic and anti-inflammatory activities of *L. siceraria* (Mol) Stand. fruit juice extract in rats and mice. Pharmacognosy Magazine 2006a; 2: 232.
9. Ghule BV, Ghante MH, Saoji AN and Yeole PG: Hypolipidemic and antihyperlipidemic effects of *Lagenaria siceraria* (Mol) fruit extracts. Indian Journal of Experimental Biology 2006b; 44: 905.
10. Shirwaikar A and Sreenivasan KK: Chemical investigation and antihepatotoxic activity of the fruits of *Lagenaria siceraria*. Ind J Pharm Sci 1996; 58: 197.
11. Baranawska MK and Cisowaski W: High performance chromatographic determination of flavones-C-glycosides in some species of cucurbitaceae family. The Journal of Chromatography A 1994; 675: 240.
12. Duke JA: Handbook of phytochemical and constituents of GRASS herbs and economic plants. Florida: CRC press. 1999: 98.
13. Sonja S and Herman SS: Analysis of cucurbitacins in medicinal plants by HPLC-MS. Phytochemical Analysis 2000; 11: 121.
14. Shah BN and Seth AK: Pharmacognostic studies of the *Lagenaria siceraria* (Molina) Standley. International Journal of Pharm Tech Research 2010; 2 (1): 121-124.
15. Wang HX, Ng TB: Lagenin, a novel ribosome inactivating protein with ribonucleolytic activity from bottle-gourd (*Lagenaria siceraria*) seeds. Life Science 2000; 67: 2631.
16. Kokate CK: Preliminary phytochemical screening, Practical pharmacognosy. New Delhi: Vallabh Prakashan. 1994: 107-113.
17. Ghosh MN: Fundamentals of Experimental Pharmacology, Edition 2, Calcutta: Scientific Book Agency. 1984: 192-194.
18. Mazumder UK, Gupta M, Pal D and Bhattacharya S: Chemical and toxicological evaluation of methanol extract of *Cuscuta*

reflexa Roxb. stem and *Corchorus olitorius* Linn. Seed on hematological parameters and hepatorenal functions in mice. Acta Poloniae Pharmaceutica- Drug research 2003; 60(4): 317-323.

19. Pieme CA, Penlap VN, Nkegoum B, Taziebou CL, Tekwu EM, Etoa FX and Ngongang J: Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Senna alata* (L.) Roxb (Ceasalpiniaceae). African Journal of Biotechnology 2006; 5 (3): 283-289.
20. D'Armour FE, Blood FR and Belden DA: In: "The Manual for laboratory work in mammalian physiology", Edition 3, Illinois Chicago, The University of Chicago Press. 1965: 4-6.
21. Wintrobe MM, Lee GR, Boggs DR, Bithel TC, Athens JW and Foerester J: Clinical Hematology, Edition 5, Philadelphia, Les and Febiger, PA. 1961: 326.
22. Reitman S and Frankel AS: A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am J Clin Path 1957; 28: 53-56.
23. Kind PRN and King EJ: Estimation of plasma phosphatase by determination of hydrolysed phenol with antipyrin. J Clin Pathol 1954; 7: 322-331.
24. Malllay HT and Evelyn KA: Estimation of serum bilirubin level with the photoelectric colorimeter. J Biol Chem. 1937; 119: 481-484.
25. Lowry OH, Rosebrough NJ, Far AL and Randall RJ: Protein measurement with Folin Phenol reagent. J Biol Chem 1951; 193: 265-275.
26. Wooton IDP: In: Microanalysis in Medical Biochemistry, Edition 5. J & A Churchill Ltd., London. 1964: 138.
27. Caraway WT: In: Standard methods of clinical chemistry, Seligson D. (ed) Vol. 1, Academic press, New York and London. 1963: 239.
28. Bartels H, Bohmer M and Heierli C: Serum creatinine determination without protein precipitation. Clin Chem Acta 1972; 37:193-197.
29. Foster LB and Dunn RT: Stable reagents for determination of serum triglycerides by colorimetric hantzsch condensation method. Clinical Chemistry 1973; 19: 338-340.
30. Wasan KM, Najafi S, Wong J and Kwong M: Assessing plasma lipid levels, body weight, and hepatic and renal toxicity following chronic oral administration of a water soluble phytostanol compound FMVP4, to gerbils. J. Pharm. Sci. (www.ualberta.ca/~csps) 2001; 4(3): 228-234.
31. Ohkawa H, Oishi N and Yagi K: Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.
32. Luck H: Methods of Enzymatic Analysis ed. Bergmeyer HV, vol. III, Academic press, New York. 1963: 886-888.
33. Ellman GL: Tissue sulphydryl groups. Arch Biochem Biophys 1959; 82: 70-72.
34. Mythilypriya R, Shanthi P and Sachdanandam P: Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation on rats. J. Health Sci 2007; 53(4): 351-35.
35. Ilodigwe EE, Akah PA and Nworu CS: Evaluation of the acute and subchronic toxicities of ethanol leaf extract of *Spathodea campanulata* P. Beauv. International Journal of Applied Research in Natural Products 2010; 3 (2): 17-21.
36. Kelly WR: Veterinary Clinical Diagnosis. Balliere Tindall, London. 1977: 271-282.
37. Tedong L, Dzeufiet PD, Dimo T, Asongalem EA, Sokeng SN, Flejoy J, Callard P and Kamtchouing P: Acute and subchronic toxicity of *Anacardium occidentale* Linn (Anacardiaceae) leaves hexane extract in mice. Af. J. Trad. Comp. Alt. Med 2008; 4(2):140-147.
38. Halliwell B and Gutterridge JMC: Free radicals in Biology and Medicine. Oxford, Clarendon Press. 1985: 107-170, 627-630.

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