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ACUTE TOXICITY STUDY OF HYDROETHANOLIC LEAF EXTRACTS OF LAWSONIA INERMIS AND MANGIFERA INDICA L. ON *MUGIL CEPHALUS* L.

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ABSTRACT:

The study was carried out to investigate the toxicity of hydroethanolic leaf extracts of *Lawsonia inermis* and *Mangifera indica* L. on *Mugil cephalus* L. The experiment was carried out under laboratory conditions for 96 hours with 10 fishes in each group (3 replicates for each group). *Mugil cephalus* L. treated with each of the six graded concentrations (0 mg/300ml, 150mg/300ml, 300mg/300ml 450mg/300ml, 600mg/300ml and 750mg/300ml) of hydroethanolic leaf extracts of *Lawsonia inermis* and *Mangifera indica* L. The study showed that the higher the concentration of the extract the higher the mortality of *Mugil cephalus* L. Fifty percent mortality of the fingerlings was recorded for 600mg/300ml and 750mg/300ml concentrations of extracts respectively. The results also showed that there were significant differences in 450mg, 600 mg and 750mg ($P < 0.05$) when compared with the control group. The treated *Mugil cephalus* L. with the hydroethanolic leaf extracts of *Lawsonia inermis* and *Mangifera indica* L. were found to have only low toxic effects further studies has to be carried out on other parts of *Lawsonia inermis* and *Mangifera indica* L. to detect their effect on different fish species.

Keywords: *Lawsonia inermis, Mangifera indica* L. *Mugil cephalus* L. Acute toxicity.

INTRODUCTION

Lawsonia inermis of family Lythracae commonly known as Henna is a Perennial shrub. It was believed to have originated in North Africa (Egypt Arid area perhaps Ethiopia) and has naturalized and cultivated in the tropics of America, Egypt, India and part of Middle East. It is also known as El- Henna, Egyptian priest, and Mignonette tree. Henna is a large shrub reaching a height of up to 6 meters. It has spreading lateral branches with opposite leaves (Simon *et al.,* 1984). Leaves of henna contain Lawsone the chief active ingredient responsible for its dye properties. The dried leaves paste is used as cosmetic for decoration of hand, feet and body on different festivals and religious occasions in India, as hair dye and hair conditioner to improve their lustre (khem chand *et al.,* 2003) and also extensively used as a dye in silk and wool industries. In India, Henna is mostly grown in the states of Rajasthan, Gujarat, Madhya Pradesh and Punjab. Rajasthan henna farms normally produce body art quality henna while Punjab henna is mostly used for the purpose of hair dye (Khandelwal, 2002).

Mangifera indica L. is a large evergreen tree, long living, 10-45 m high with a strong trunk and heavy crown. Native from tropical Asia, it has been introduced wherever the climate is sufficiently warm and damp and is now completely naturalized in many parts of tropics and subtropics (Ross 1999). Native from Southern Asia, especially Eastern India, Burma and the Andaman Islands, *M. indica* has been cultivated, praised and even revered in its homeland since ancient times. In this day and age, *M. indica* resides in most tropical biotopes in India, Southeast Asia, Malaysia, Himalayan regions, Sri Lanka, Africa, America and Australia (Calabrese 1993; Kirtika and Basu 1993). In all the regions of *M. indica* distribution, one of main organs used is the bark. Based on ethnopharmacological knowledge, a standardized aqueous extract of *M. indica* L. stem bark with antioxidant, anti-inflammatory and immunomodulatory properties has recently been developed in Cuba. This extract is proposed as a nutritional supplement (antioxidant) and an anti-inflammatory, analgesic and Immunomodulatory treatment to prevent disease progress or increase the patient's quality of life in gastric and dermatological disorders, AIDS, cancer and asthma (Nuñez-Selles 2005).

Mullet, any of the abundant, commercially valuable schooling fishes of the family Mugilidae (order Perciformes). Mullets number fewer than 100 species and are found throughout tropical and temperate regions. They generally inhabit salt water or brackish water and frequent shallow, inshore areas, commonly grubbing about in the sand or mud for microscopic plants, small animals, and other food. They are silvery fishes 30–90 cm (1–3 feet) long, with large scales; relatively stocky, cigar-shaped bodies; forked tails; and two distinct dorsal fins, the first containing four stiff spines. Many have strong, gizzard-like stomachs and long intestines capable of handling a largely vegetarian diet. The common, or striped, mullet (*Mugil cephalus*), cultivated in some areas because of its rapid growth rate, is a well-known species found worldwide.

Acute toxicity tests provide information on the time and/or concentration causing a significant effect or detectable response in 50% of the exposed population of test organisms. The tests are considered ecologically significant and legally defensible, simple and cost effective (Buikema *et al.,* 1982). Acute toxicity studies can provide fast and valuable information and indicate whether further toxicity studies should be conducted. Toxicity tests have traditionally been performed with a variety of freshwater and saltwater species representing algae, fish and invertebrates. Although the initial aquatic toxicity tests were carried out using bacteria, invertebrates and other groups, they can in no way replace the actual test performed on fish, which is the last chain in the aquatic food cycle (Castano *et al.,* 1996).

The objectives of this study included toxicity evaluation of *Lawsonia inermis* and *Mangifera indica* L. The survival of the fish exposed to the test substance was compared with the survival of the fish in an appropriate control over a fixed period of time. Behavioral changes of the above mentioned species were also observed.

MATERIALS AND METHODS

Collection of plant material

To study the phytochemical constituents, the collected *Lawsonia inermis* and *Mangifera indica* L. leaves were air-dried in shade and ground into fine powder using grinder. Different extract (Ethanol, chloroform, butanol, petroleum ether and aqueous) samples were prepared by soaking 100 gm of dried powdered sample in 200 ml of

corresponding solvent and water for 12 h of duration and subjected to soxhlet extraction (14 cycles). Then the extracts were kept for evaporation and resulted brown crystal powder was used for phytochemical analysis to identify the Tannins, Flavanoids, steroids, glycosides and alkaloids (Harborne 1973; Sofowara, 1993).

Test species (*Mugil cephalus* L.)

Mugil cephalus L. used in this study were obtained from a local breeder and transported immediately to the laboratory within 25 minutes in appropriately aerated plastic bags. In the laboratory a total of 160 fishes were kept in 100 liter glass aquaria (width of 50 cm, depth of 40 cm and a length of 100 cm) containing filtered tap water (pH 6.2-6.4, dissolved oxygen concentration 7.3-8.5 mg L^{-1}, and ammonia < 0.5 mg L^{-1}), the aquaria were equipped with a water-cycling device by which the water was continuously aerated 1 week before putting the fish inside it, to remove chlorine. Fish were acclimated for 14 days with continuous aerating and the water renewed every 24 h.

The fish were fed with commercial fish food Aquadene® once daily. Feeding was terminated 48 h prior the initiating of the experiment to reduce metabolic wastes. The temperature was maintained at 25±1°C and the photoperiod was set at 12 h of light and 12 h of dark during the entire experiment. Care was taken in order to keep the mortality rate less than 5% in the last 5 days before the experiments was started. *Mugil cephalus* L. with mean total length 3.83±0.19 cm (The total length of the species was measured using a milimetric ruler on fish measurement plate) and wet body weight 0.92±0.24 g (was weighted in a balance with 0.1 g accuracy), were used for acute toxicity tests in this study.

Acute toxicity test

Acute toxicity test with *Mugil cephalus* L. was performed according to the APHA (APHA, 1998), OECD (OECD, 1993) and EPA (US EPA, 1998) recommendations. Groups of 10 fish of similar size were randomly sampled and transferred with the help of small hand net from the acclimation tank into a suitable test chambers (i.e. aquarium) of 20 L capacity avoiding any possibility of mechanical injury to the test fish. Each aquarium was stocked with fish with a ratio of 1.0 g 1.0 L^{-1}water.

The treatments (concentrations in mg/300ml of water) were assigned to the experimental units (Aquaria) at random; using a table of random numbers as described by Akindele (1994). Aquarium A and its replicates received no treatment and served as control throughout the experiment. Aquaria B, C, D, E, F and their replicates were treated with the 150mg, 300mg, 450mg, 600mg, and 750mg of hydroethanolic leaf extracts derived from the *Lawsonia inermis* and *Mangifera indica* L. respectively. The aquaria were covered with netting materials to prevent the fish from jumping out. The mortality of fingerlings in each aquarium was monitored at 4 hours interval for 96 hours, the percentage mortality and mean mortality per treatment were determined. The behavioural pattern of the fish after introduction of the extract was critically observed.

A flexible silicone tube attached to a regulated air supply was inserted through the test chambers and remained in its place throughout the test period to supply a sufficient amount of dissolved oxygen, the amount of dissolved oxygen was observed to be not less than 5 mg L^{-1}. Physiochemical parameters (pH, Dissolved oxygen and Temperature) were measured daily in each aquarium throughout the experiment. The water temperature was maintained at 24±1°C. No food was given to the fish during the experiments. During the experiment, dead fish were removed immediately because such mortality in static bioassays may deplete the DO, affecting tolerance limits (Kirchen and West, 1976). Data collected were analysed using percentages and tables.

RESULTS AND DISCUSSION

The presence of phytochemicals such as tannin, phenols, alkaloids and flavonoids were confirmed in the aqueous and ethanolic leaf extracts of *Lawsonia inermis* and *Mangifera indica* L. when compared to other solvent extracts. Thus hydroethanolic extracts of the plants were taken for further studies (Table 1).

Tables 2 and 3 depicts the percentage mortality for different exposure periods at different concentrations of *Lawsonia inermis* and *Mangifera indica* L. LC50 value of *Lawsonia inermis* and *Mangifera indica* L. for the fish *Mugil cephalus* L. was determined by the simple percentage tables. *Mugil cephalus* L. was stressed progressively with various concentration of *Lawsonia inermis* and *Mangifera indica* L. The pattern of mortality was similar for various concentrations of the two botanicals. At higher concentration (450, 600 and 750 ppm), rate of mortality

significantly (P< 0.05) increased from 43% to 57% at 96-h for both plant extracts. 10 to 13% of mortality in the control group was (0.0 ppm) observed. It may be due to the other reasons. The stressful behaviour of *Mugil cephalus* L. tend to show feelings of respiratory impairment due to the mild toxic effects of *Lawsonia inermis* and *Mangifera indica* L. on the gills. Statistical observations revealed that the significant difference at 95% confidence level with control group was observed in 45, 60 and 75 mg/L concentrations (Table 4, 5 & 6). The 50% of mortality was observed at 60 mg/L concentration for both the plant extracts.

CONCLUSION

In this study it is concluded that the leaf extracts of *Lawsonia inermis* and *Mangifera indica* L. was not found to be toxic to the fish *Mugil cephalus* L. The constituents of the extract are biodegradable and thus diminish within a short period after exposure. Since a mild toxicity was observed in higher concentrations of the plant extracts. Further studies were planned to use this plants for herbal dyeing.

Table-1: Phytochmical analysis in the various solvent leaf extracts of *Lawsonia inermis* and *Mangifera indica* L.

S.No	Phytochmicals	*Lawsonia inermis*					*Mangifera indica* L.				
		E	C	B	P	A	E	C	B	P	A
1	Carbohydrate	+	-	+	-	+	+	-	+	-	+
2	Proteins	+	+	+	-	+	+	+	-	-	+
3	Aminoacids	+	+	-	-	+	+	-	-	+	+
4	Phenols	+	+	-	+	+	+	-	-	+	-
5	Tannin	+	+	-	-	-	+	-	+	-	+
6	Alkaloids	+	-	-	+	-	+	-	-	-	-
7	Flavonoids	+	+	-	-	-	+	-	-	-	-
8	Glycosides	+	-	-	-	+	+	-	-	-	+

E – Ethanol, C – Chloroform, B – Butanol, P – Petroleum ether, A – Aqueous.

Table -2: Mortality of *Mugil cephalus* L. subjected to different concentrations of hydroethanolic leaf extract of *Lawsonia inermis* for 96 h.

C	Time (in hours)																									T.F	TM	M%
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72	76	80	84	88	92	96			
0	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	-	30	3	10%
150	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2	-	-	2	1	-	-	2	-	2	-	30	10	23.3%
300	-	-	-	-	-	-	2	-	-	-	3	-	-	1	-	-	2	-	-	1	1	-	-	-	2	30	12	40%
450	-	-	-	3	-	-	-	-	-	-	-	4	-	-	-	-	1	-	2	2	-	1	-	-	-	30	13	43.3%
600	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	4	4	2	1	-	-	2[a]	-	30	15	50%
750	-	-	-	-	-	-	-	-	-	-	5	4	1	-	-	-	-	-	3	-	-	1	-	-	2[a]	30	16	53.3%

a –Time duration for 50% mortality, Each group consist 10 fishes, C – Concentration in m g/300 ml, T.F - Total Number of Fishes Studied,
TM – Total Mortality, M% - Percentage of Mortality .

Table -3: Mortality of *Mugil cephalus* L. subjected to different concentrations of hydroethanolic leaf extract of *Mangifera indica* L. for 96 h.

C	Time (in hours)																									T.F	TM	M%
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72	76	80	84	88	92	96			
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	2	-	30	4	13.3%
150	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	2	-	-	-	-	-	-	-	2	-	30	6	20%
300	-	-	-	-	-	-	2	-	-	-	3	-	-	-	-	-	1	-	-	-	-	-	3	-	2	30	11	36.6%
450	-	-	-	-	-	-	-	2	-	-	-	-	4	-	-	-	4	-	-	-	1	-	2	-	-	30	13	43.3%
600	-	-	-	-	-	-	-	-	-	-	-	-	2	1	2	3	-	-	-	4	-	-	-	3[a]	1	30	16	53.3%
750	-	-	-	-	-	-	-	-	3	4	3	-	-	-	-	2	-	-	3[a]	-	2	-	-	-	-	30	17	56.6%

a –Time duration for 50% mortality, Each group consist 10 fishes, C – Concentration in mg/300 ml, T.F - Total Number of Fishes Studied,
TM – Total Mortality, M% - Percentage of Mortality .

Table 4: Statistical Analysis of Variance (ANOVA) for Mortality of *Mugil cephalus* L. in Relation to the Treatment with Hydroethanolic Leaf Extract of *Lawsonia inermis* for 96 h.

S.No		Sum of Squares	Mean Squares	Fisher F- value
1	**Between Groups**	4.380	0.876	0.876
2	**Within Groups**	144.062	1.00	---
3	**Total**	148.442	---	---

Table-5: Statistical Analysis of Variance (ANOVA) for Mortality of *Mugil cephalus* L. in Relation to the Treatment with Hydroethanolic Leaf Extract of *Mangifera indica* L. for 96 h.

S.No		Sum of Squares	Mean Squares	Fisher F- value
1	**Between Groups**	5.553	1.111	1.122
2	**Within Groups**	142.500	0.990	---
3	**Total**	148.053	---	---

Table-6: Mean Mortality of *Mugil cephalus* L. Subjected to Different Concentrations of Hydroethanolic Leaf Extracts of *Lawsonia inermis* and *Mangifera indica* L. for 96h.

S.No	Different concentrations	Various time durations	
		Lawsonia inermis	***Mangifera indica* L.**
1	0	0.12 ± 0.33^{a}	0.16 ± 0.47^{a}
2	150	0.40 ± 0.76^{a}	0.24 ± 0.59^{a}
3	300	0.48 ± 0.87^{a}	0.44 ± 0.96^{a}
4	450	0.52 ± 1.08^{b}	0.52 ± 1.19^{b}
5	600	0.60 ± 1.22^{c}	0.64 ± 1.18^{c}
6	750	0.64 ± 1.38^{d}	0.68 ± 1.28^{d}

Values with different alphabets differ significantly from control

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