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RESEARCH ARTICLE

EFFECT OF LINDANE (Δ-ISOMER) ON LIVER ENZYMES IN MICE (*MUS MUSCULUS*) AND HEPATOPROTECTIVE ACTIVITY OF *MIMOSA PUDICA*

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ABSTRACT

Lindane is an organochloride pesticide which has immense vicinity of function such as in protection of crops from pest, lice treatment and others. Our study investigated that how lindane can cause harm to liver enzymes i.e, AST and ALT in mice and we further investigated the hepatoprotective effects of ethanoloic extract of leaves of *Mimosa pudica* against the increase level of liver enzymes.

INTRODUCTION

Lindane is used as an insecticide on fruit and vegetable crops. Lindane (1,2,3,4,5,6-hexachlorocyclohexane) is the only stereoisomer with insecticidal efficacy and has a variety of applications, including protection of crops, prevention of insect borne diseases such as malaria, diseases removal of ectoparasites such as lice and mites, and treatment of human pediculosis. The widespread use of insecticides has caused the scientific community and the public at large to consider more seriously the influence of these agents as environmental pollutants and their possible effects on wildlife and human health. Lindane (g-HCH) organochlorine pesticide extensively employed for public health and agricultural purpose in developing countries. Liver ataxia is an extremely developing disease all over the world. Liver is an important organ which can eliminate the harmful chemicals and purifies the blood which enters over body. Liver anomalies may lead to death. Till yet no other can substitute the function of liver. *Mimosa pudica* called as Chuimui or Lajwanti (Chauhan *et al.*, 2009) has been traditionally used for its various medicinal properties like hepatoprotective (Ei di A *et al.*, 2012), hypolipidemic (Rajendran *et al.*), anticonvulsant, anti-inflammatory and analgesic (Chandrashekhar *et al.*, 2010 and 2012) and many more. In the leaf of *Mimosa pudica* the compound like flavonoids, alkaloids, phenols, tannins, terpenoids, saponins and coumarin are found (Sharma *et al.*, 2010).

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Aims and objectives

- To study the effect of lindane on serum level of liver enzymes (ALT and AST) in mice.
- To study the hepatoprotective nature of *Mimosa pudica*

MATERIALS AND METHODS

Animals: Adult albino mice weighing 28-35 gm and approximately 8 weeks of age were procured from Animal House Facility of Department of Zoology, Gauhati University, Assam, India. The animals were housed in properly labelled steel mesh plastic cages with solid bottom containing saw dust and maintained under uniform condition of natural photoperiod (12 hr light and 12 hr dark), relative humidity, 75%-87% and temperature, 27-30^{0}C. Animals were acclimatized to normal environmental conditions in the laboratory for two weeks before use. Standard diet (pellet diet) and water at libitum were supplied regularly.

Chemicals: Lindane (δ-HCH) is being procured from Zenith India Guwahati, Assam, India. The analytical grade alcohol and distilled water is being supplied by the Department of Zoology, Gauhati University.

Collection of plant material: *Mimosa pudica* leaf and stem were collected from Nagaon, Assam from the road side areas.

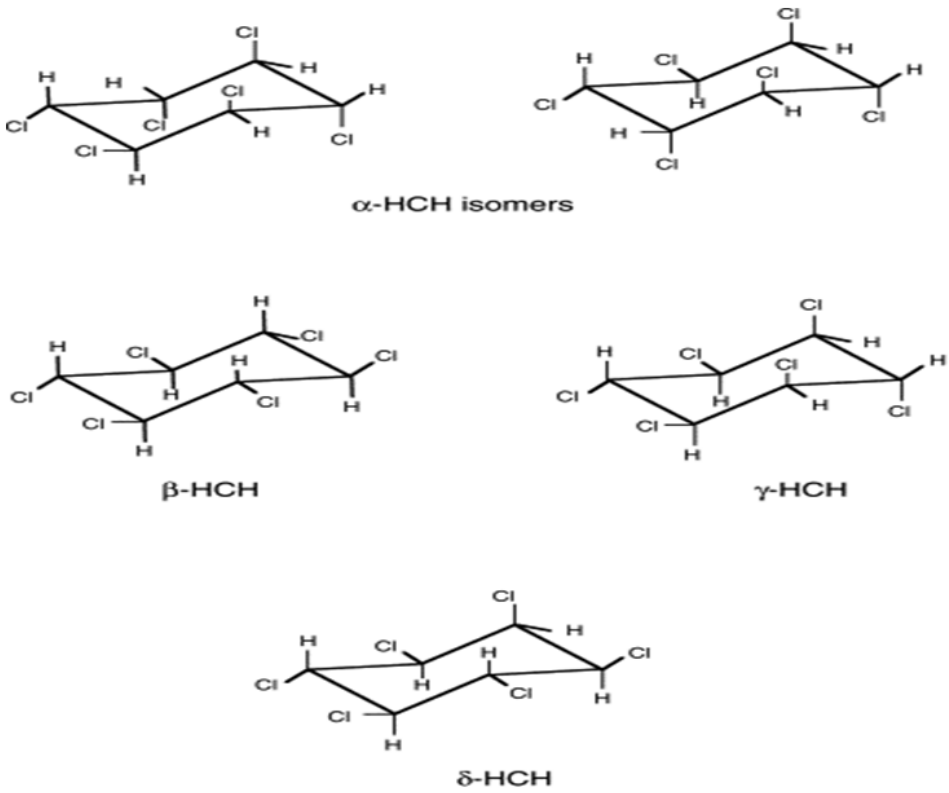

Fig 1. Isomers of lindane

Preparation of plant extract: Absolute alcohol (99.9% v/v) was used for the extraction process as almost all the components of *Mimosa pudica* is soluble in ethanol solvent. The leaves were shade dried and powdered. The powder was subjected to extraction with absolute alcohol in Soxhlet Apparatus at 45^0C for a period of 4 hours. The extract was collected and filtered using What man No. 1 filter paper and the filtrate was then subjected to evaporation under reduced pressure until a soft semi solid mass was obtained. The sample was stored in the refrigerator as a stock solution dissolved in liquid paraffin in 1:2 ratio (sample**:** liquid paraffin) in an airtight container at a temperature of below 10^0C (Purkayastha *et al.*).

Preparation of experimental doses of lindane: Two doses 100 mg/kg bw and 50 mg/kg bw of Lindane were preparedand used in the study. Initially two stock solutions were prepared. For high dose (100mg/kg bw), of test chemical (lindane) was prepared by adding 1 ml of ethanol (Analar Grade) and 9 ml of distilled water to 0.1 ml of the above mentioned doses were injected once daily with the help of 1 ml syringe of 29G (Romson syringe) for 7 and 14 days.

Experimental grouping of animals: Thirty six healthy adult mice were weighted and randomly categorized into six groups (n=6) in nine properly labelled separate cages with steel mesh as lid. The cages were labeled as control group, vehicle control group, estradiol group, 50 mg/kg bw , 100 mg/kg bw and leaf extract treatment respectively. 0.1 ml of the above mentioned doses were injected subcutaneously once daily in the morning around 9 am to 10 am by 'Romson syringe' (29 G) for 7and 14 days. In both low and high doses of lindane in 3 mice lindane is subcutaneously injected and in the other 3 mice lindane as well as the leaf extract of *Mimosa pudica* is subcutaneously injected.

After 7 days, 15 mice i.e., 3 mice from the each of the groups were sacrificed for estimation of effect lindane on various parameters, while the rest 15 mice were dissected after 14 days to check the effect of lindane along with the mice treated with plant extract.

Table 1. Showing treatment schedule

Experimental Group (n=6)	Treatment with lindane and plant extract(mg/kg bw/animal/day)	Volume Administered (ml)	Duration of treatment (Days)
Control			7 and14
Vehicle control (Ethanol:water: 1:9 v/v)		0.1	7and 14
Estradiol 17β	0.1	0.1	7 and 14
Low dose	50	0.1	7 and 14
High dose	100	0.1	7 and14
Plant extract dose	20	0.1	7 and 14

Albino mice were taken in 6 different groups (6 animals per group) and treated with three different doses, 50mg/kg bw of lindane (considered as low) and 100mg/kg bw of lindane (considered as high) and 20 mg/kg bw of plant extract respectively for consecutive 7 and 14 days. A control group was maintained without any treatment. A vehicle control was given ethanol: water (1:9 v/v) Another group of animals were treated with estradiol 17β (considred as positive control). Treatment schedule: 0.1 ml of test chemical was administered subcutaneously to animals daily in the morning hr daily regularly.

Blood collection: Blood samples were drawn by using 2ml Nypro syringe (26G) using cardiac puncture procedure. Approximately 200 µl of blood was collected and kept separately in micro-centrifuge tubes.

The blood samples were then subjected to centrifugation (Eppendorf mini spin centrifuge) at 5000 rpm for 15 min to obtain clear serum.

Fig 2. Mice weight measurement

Fig 3. Narcotization and pinning of mice

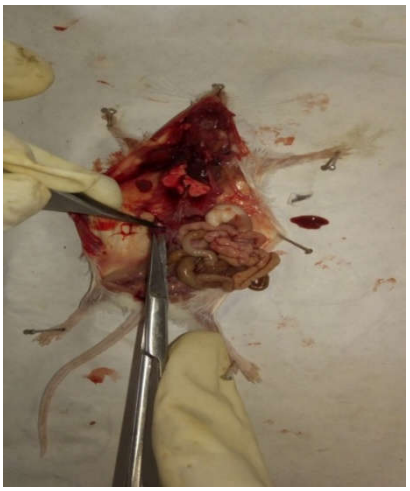

Fig 4. Dissection of mice

The serum was then collected in newly labelled micro-centrifuge tubes and stored at – 20^0C for estimating AST and ALT level.

Acute toxicity: The oral LD50 of mice is 86 mg/kg (Pulak Lahiri and Sipra Sircar, 19889 and 1990). The acute dermal LD50 of mice is 896 mg/kg.

Toxicity study: Liver function tests were performed by taking blood serum from the sacrificed animal (as described earlier). SGPT and SGOT level in serum were detected to observe whether there was any alteration of liver functions. SGPT test; Serum glutamic pyruvate transaminase (SGPT) is also known as alanine transaminase (ALT) is an enzyme present in liver cells. The test is used to detect any injury to the liver and is considered as one of the most important liver tests.

This test is more specific than the SGOT level test. SGOT level test; Serum glutamic-oxaloacetic transaminase (SGOT) or aspirate transaminase (AST) is another enzyme found in the liver cells. SGOT test not only helps in management and detection of liver diseases but also works for cardiac problems. ALT and AST in serum samples were measured using the commercial kits of Aspen Laboratories

Estimation of serum AST: Principle: The oxidation of NADH in this reaction could be measured as a decrease in the absorbance of NADH at 340 nm which is proportional to AST activity.

Procedure: 1000 µl working reagent was mixed with 100 µl serum of test sample. Mixed well and then aspirated. Two readings were taken at 340 nm at 60 seconds of interval.

Estimation of serum ALT: Principle: The transfer of amino group from L- Alanine to 2- oxoglutarate with the formation of pyruvate and L- glutamate is catalyzed by SGPT. The pyruvate so formed is allowed to react with NADH to produce L-lactate. An indicator reaction coupled with LDL in the presence of NADH (nicotinamide adenine dinucleotide) regulates this rate of reaction. A decrease in the absorbance of NADH at 340 nm is a direct measure of the oxidation of NADH in this reaction which in turn is proportional to the activity of SGPT.1000 µl working reagent was mixed with 100 micro litre serum of test sample. Mixed well and then aspirated. Three readings were taken at 340nm at 60 seconds of interval.

RESULTS

Table 2 .Mean biochemical indices as compared to controls (AST)

Experimental group	U/L (Mean±SD)
Control	66.87 ± 1.2
Vehicle control (7 days)	70.47 ± 1.25
Vehicle control (14 days)	73.63 ± 1.51
Estradiol treated group (7 days)	75.06 ± 1.02
Estradiol treated group (14 days)	70.01 ± 1.70
50 mg/kg bw (7 days)	112.98 ± 2.62
50 mg/kg bw (14 days)	151.86 ± 3.65
100 mg/kg bw (7 days)	147.01 ± 3.5
100 mg/kg bw (14 days)	173.31 ± 2.81
Treated with plant extract 20 mg/kg bw (7 days)	105.39±2.06
Treated with plant extract 20 mg/kg bw (14 days)	67.77±1.30

Table 3.Mean biochemical indices as compared to controls (ALT)

Experimental group	U/L (Mean±SD)
Control	47.62±2.45
Vehicle control (7 days)	55.67±3.77
Vehicle control (14 days)	62.64±4.29
Estradiol treated group (7 days)	52.37±3.43
Estradiol treated group (14 days)	63.24±4.64
50 mg/kg bw (7 days)	148.38±5.18
50 mg/kg bw (14 days)	145.04± 6.08
100 mg/kg bw (7 days)	170.36±5.34
100 mg/kg bw (14 days)	164.73±8.27
Treated with plant extract 20 mg/kg bw (7 days)	79.08±2.87
Treated with plant extract 20 mg/kg bw (7 days)	50.23±2.68

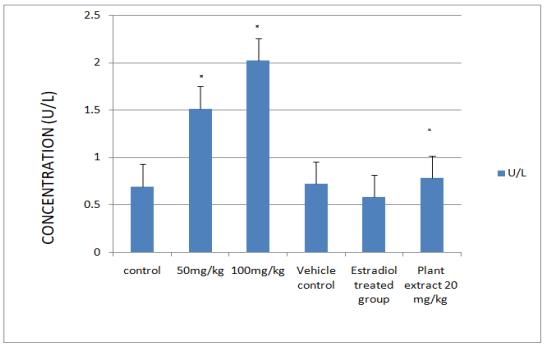

Fig 5. Graph showing effect of lindane and plant extract on the AST level at 7 days in five animal groups viz., control, 50 mg/kg bw,100 mg/kg bw , plant extract 20 mg/kg bw vehicle control and estradiol treated group Values expressed in mean ± SD.Values are significant at P <0.05(* indicates value is significantly different at p <0.05 level compared to the respective control values determined by one way ANOVA analysis).

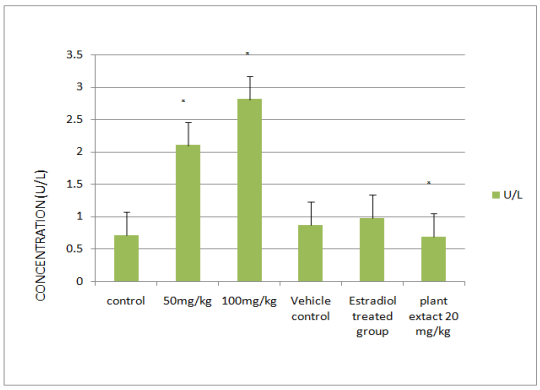

Fig 6. Graph showing effect of lindane and plant extract on the AST level at 14 days in five animal groups viz., control,50 mg/kg bw,100 mg/kg bw , plant extract 20 mg/kg bw vehicle control and estradiol treated group Values expressed in mean ± SD. Values are significant at P <0.05(* indicates value is significantly different at p <0.05 level compared to the respective control values determined by one way ANOVA analysis).

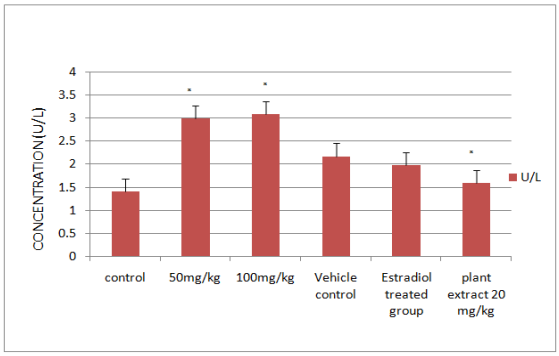

Fig 7. Graph showing effect of lindane and plant extract on the ALT level at 7 days in five animal groups viz., control, 50 mg/kg bw, 100 mg/kg bw , plant extract 20 mg/kg bw vehicle control and estradiol treated group Values expressed in mean ± SD. Values are significant at P <0.05(* indicates value is significantly different at p <0.05 level compared to the respective control values determined by one way ANOVA analysis).

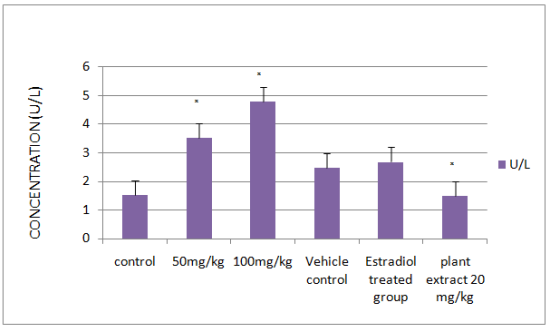

Fig 8.Graph showing effect of lindane and plant extract on the ALT level at 14 days in five animal groups viz., control, 50 mg/kg bw, 100 mg/kg bw, plant extract 20 mg/kg bw vehicle control and estradiol treated group Values expressed in mean ± SD.Values are significant at P <0.05(* indicates value is significantly different at p <0.05 level compared to the respective control values determined by one way ANOVA analysis).

DISCUSSION

When in mice subcutaneously lindane was injected at low and high dose i.e. 50 mg/kg bw and 100 mg/kg bw respectively the AST and ALT level increases in compare with the control group. When in the low dose 3 mice were only injected with lindane and the other 3 mice along with lindane plant extract of 20 mg/kg bw was injected subcutaneously then the mice which was given the plant extract the level of AST and ALT gradually comes in the range of control group. When the plant extract was given for 14 days the effectiveness to gradually normalise the AST and ALT level comparatively more than the plant extract given for 7 days.

Conclusion

From the above discussion we may conclude that the plant extract of *Mimosa pudica* is effective to cure the abnormal increase of liver enzymes i.e, AST and ALT. Future advanced research in this field might revolutionize the treatment of liver disorders.

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