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Effects of lindane(δ- isomer) on adrenal glands in mice (*Mus musculus*)

Pimpi Sahu[1], Kumar Kritartha Kaushik[2]
[1]*Department of Zoology, Gauhati University, Guwahati - 781014, Assam*
[2]*Department of MBBT, Tezpur University, Tezpur - 784028, Assam*

ABSTRACT

Lindane is a broad spectrum organochlorine pesticide contains wide range of application such as in crops protection, treatment of lice and others. Lindane given subcutaneously in mice shows decrease in cortisol level and marked regression in zona fasciculata region of adrenal glands.
Keywords: Lindane, cortisol, zona fasciculate and adrenal glands.

INTRODUCTION

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. Lindane is a white, crystalline organic solid. Its formula is $C_6H_6CI_6$ and has a molecular weight of 290.8 Its melting point is at 112.5^0C, boiling point 323^0C, water solubility 7.3 mg/L at 25^0C, vapour pressure 4.2×10^{-5} mm Hg at 20^0C, 4.4×10^{-3} Pa at 24^0C (Source HSBD). Lindane is stable to heat, light, air, carbon dioxide and strong acids. Technical HCH is an isomeric mixture that contains mainly five forms differing only by the chlorine atoms orientation (axial or equatorial positions) around the cyclohexane ring. The five principal isomers are present in the mixture in the following proportions: alpha-hexachlorocyclohexane (53%–70%) in two enantiomeric forms ((+)alpha-HCH and (-)alpha-HCH), beta-hexachlorocyclohexane (3%–14%), gamma - hexachlorocyclohexane (11%–18%), delta-hexachlorocyclohexane (6%–10%) and epsilon-hexachlorocyclohexane (3%–5%).

**Corresponding author's Email: kkritartha@gmail.com*

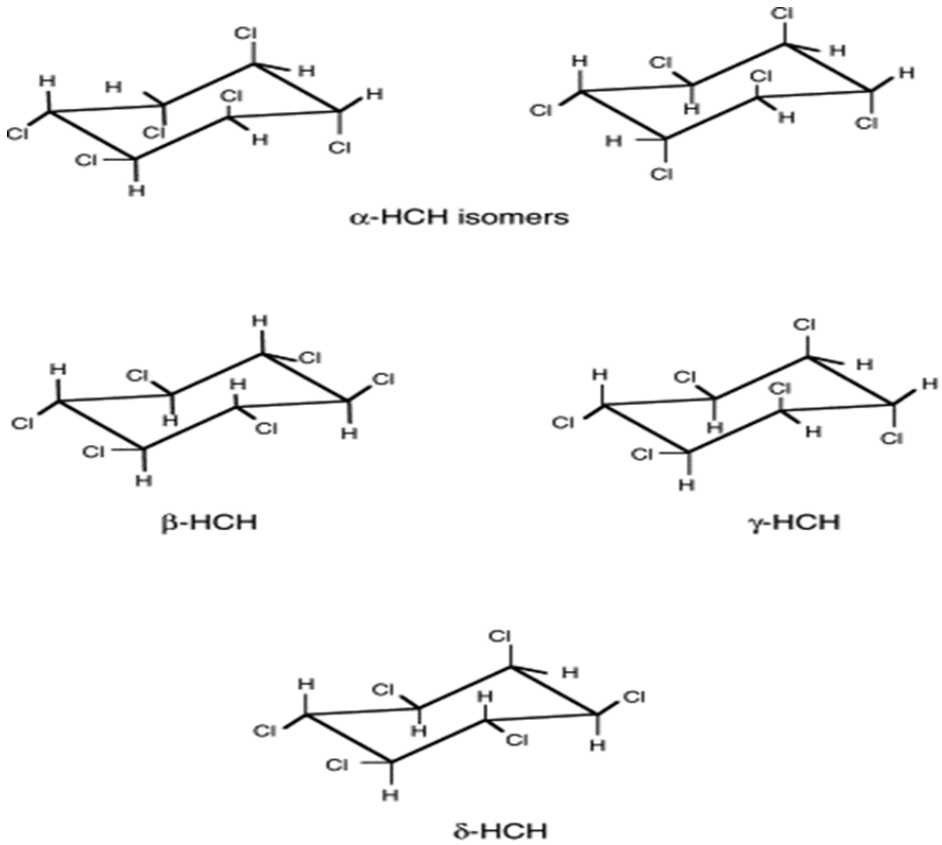

Fig 1. Isomers of lindane (Source:IPOL_STU(2016)571398_EN(1))

Lindane has been reported to induce oxidative stress (Videla, L.A., S.B. Barros and V.B. Janquer, 1990), membrane perturbation (Bhalla,P. and D. Agarwal, 1998) , functional impairment in blood brain barrier disturbance in glutathione homeostatis (Sahoo, A. and G.B.N Chainy, 1998) and alteration in cyt P450monooxygenase enzymes (Parmar, D., S. Yadav, M. Dayal, A. Johri, A. Dhawan and P.K. Seth, 2003)

Aims and objectives:

1. To study the effects of lindane in adrenal gland (cortisol level) in serum of mice.
2. To study the histological change in adrenal glands in mice.

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 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.

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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.

Preparation of experimental doses:

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/kgbw), 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 healthy adult mice were weighted and randomly categorized into ten groups (n=6) in ten properly labelled separate cages with steel mesh as lid. The cages were labelled as control group, vehicle control group, estradiol group,50 mg/kg bw and 100 mg/kg bw 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. After 7 days, 15 mice ie., 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.

Table1. Showing treatment schedule

Experimental Group (n=6)	Treatment (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

Albino mice were taken in 5 different groups (6 animals per group) and treated with two different doses, 50mg/kg bw (considered as low) and 100mg/kg bw (considered as High) 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) l .Another group of animals were treated with oestradiol 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.

Fig 2. Mice weight measurement

Fig 3. Narcotization and pinning of mice

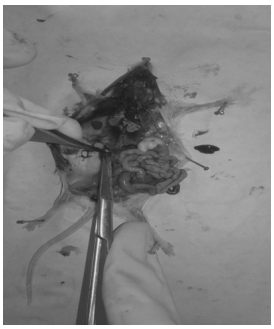

Fig 4. Dissection of adrenal gland

Fig 5. Adrenal gland of mice

Blood and tissue 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. The serum was then collected in newly labelled micro-centrifuge tubes and stored at -20^{0}C for estimating cortisol level.

Following proper laboratory procedure animals were sacrificed one batch on the day 7 and 14 respectively. In the first batch of animals after treatment day 7, all animals were taken in to the laboratory from the animal house. Animals were anesthesised with mild chloroform. Then they were placed on dissecting tray and pinned properly. Adrenal gland was located and taken out to petridishes in normal saline. The weights of the adrenal gland taken using the Sartorius electronic balance(0.1 mg sensitivity). The adrenal gland transferred to Bouin's fluid for histology.

Acute toxicity:

The oral LD50 of mice is 86 mg/kg (PulakLahiri and Sipra Sircar, 1990). The acute dermal LD50 of mice is 896 mg/kg.

Cortisol hormone assay:

By electrochemiluscence technique in "Apex diagonistics , Guwahati -781005, Assam", cortisol hormone assay was done.

Histopathological study:

The sample tisssues of adrenal gland that is kept in Bouin's fluid for 18-24 hours. The fixed specimens were then washed and dehydrated in ascending grades of alcohol(30%, 50%, 70%, 90% and 100%).The specimens were then cleared in xylene, infiltrated and embedded in molten paraffin (60•C),sectioned at 4 micron thickness using microtome(Ernst Leitz Wetzlar GMBH, Germany). The sections were taken in properly affixed slides i.e., fixed with 70% alcohol. The sections were stretched in warm water bath, temperature being maintained at 50-60^0 centrigrade. Proper spreading of the sections done using a hot plate. The sections were then stained with Haematoxylin and eosin (H & E), and mounted with DPX. The slides were examined under light microscope. Photomicrographs were taken in Microscope (Leica).

RESULTS

Following are the findings of effect of lindane on adrenal gland

Table 2. Effect of different doses of lindane on serum cortisol level. Values are expressed in Mean±SD

Experimental group	nmol/L (Mean±SD)
Control	58.26±3.46
Vehicle control (7 days)	56.86±3.27
Vehicle control (14 days)	54.79±2.03
50 mg/kg bw (7 days)	52.92±2.46
50 mg/kg bw (14 days)	51.89±1.86
100 mg/kg bw (7 days)	50.88±1.29
100 mg/kg bw(14 days)	46.04±1.14
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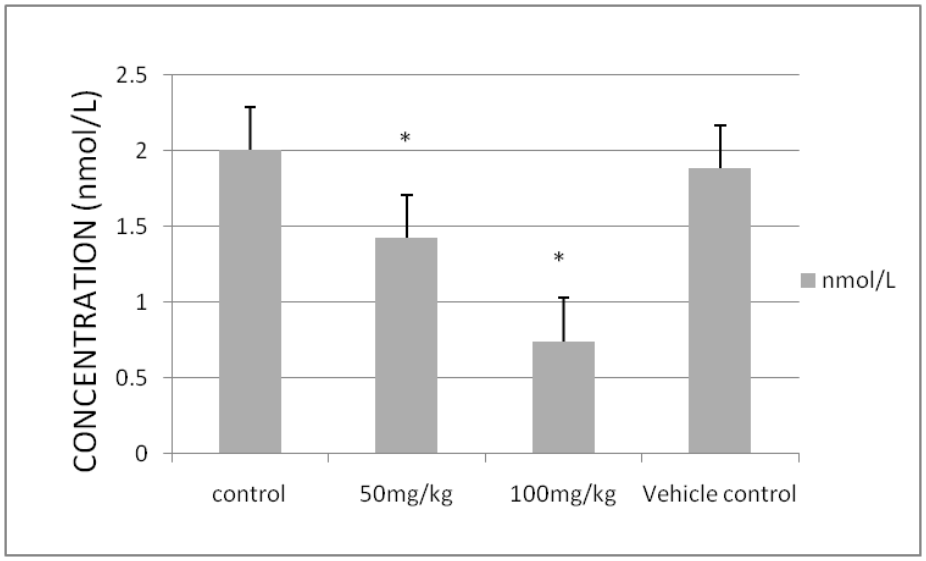

Fig 6. Effect of lindane on the serum cortisol level at 7 days in four animal groups viz., control, 50 mg/kg bw, 100 mg/kg bw and vehicle control. Values expressed in mean ± SD.Valuesare significant at P <0.05(* indicates value is significantly different at p <0.05 level compared to the respective control values determined by one way ANOVA analysis).

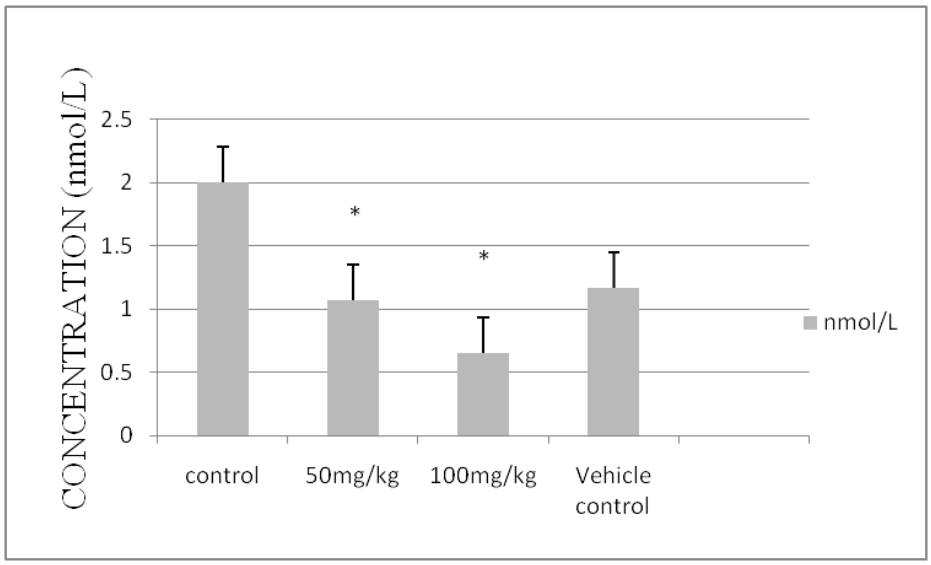

Fig 7 : Effect of lindane on the serum cortisol level at 14 days in four animal groups viz., control,50 mg/kg bw,100 mg/kg bw and vehicle control. Values expressed in mean ± SD.Values are significant at P <0.05(* indicates value is significantly different at p <0.05 level compared to the respective control values determined by one way ANOVA analysis).

Histology

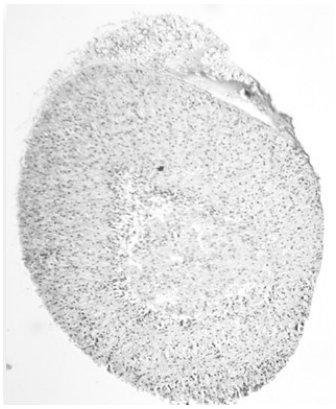

Fig8. T.S control adrenal gland 4x magnification

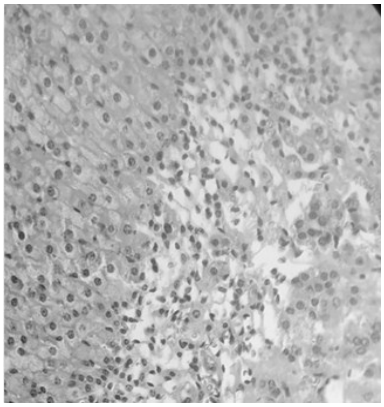

Fig 9:T.S control adrenal gland 10x magnification

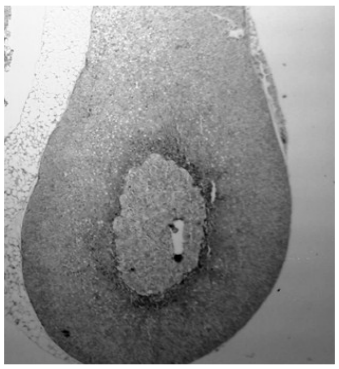

Fig 10:T.S low dose adrenal gland 4x magnification

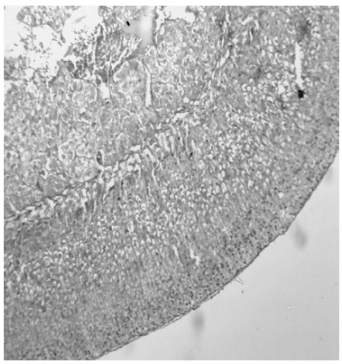

Fig 11:T.S low dose adrenal gland 10x magnification

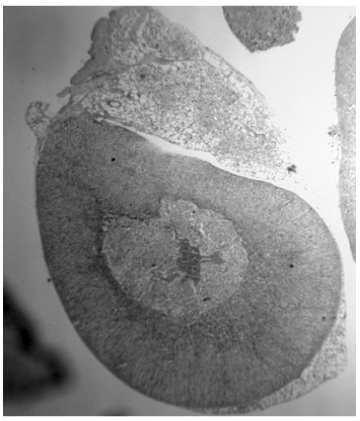

Fig 12: T.S high dose adrenal gland 4x magnification

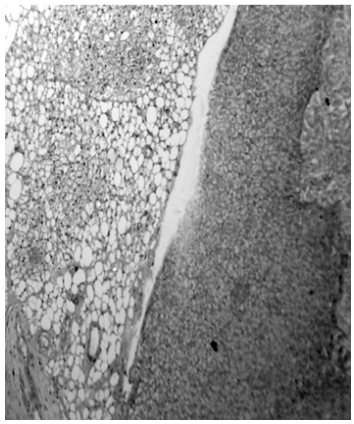

Fig 13: T.S high dose adrenal gland 10x magnification

DISCUSSION

The experimental result of the present study indicates that cortisol secretion from the adrenal gland in the treated group decreases which is represented graphically in fig 6 and fig 7. Compared to the low dose of lindane there is marked decrease of cortosol level in high dose . Similar result found by Agneta Oskarsson et.al (2006), at the highest lindane concentration cortisol secretion was reduced almost to the baseline levels.

It was also observed when the mice were treated with high dose of δ-lindane there is marked regression in the zona fascicula region of the adrenal gland (fig 12 & 13). In low dose of δ HCH no prominent regression was being observed(fig10-11). According to Pulak Lahiri and Sipra Sircar,1990 also fasciculata and reticularies zones markedly regressed when treated with ɣ-lindane.

CONCLUSION

Pesticides are widely used in agriculture mainly to increase crop yields to cater huge supply of food products for increasing world population as well as to protect crops from pests and control insect-borne diseases. Increased use of pesticides result in contamination of the environment and the excess accumulation of pesticide residues in food products, which has always been a matter of serious concern. Pesticide residues in food and crops are directly related to the irrational application of pesticides to the growing crops. Accumulated pesticide residues in food products have been associated with a broad variety of human health hazards, ranging from short-term effects to long term toxic effects.

The present study firmly established that lindane has many deleterious effects on adrenal glands in mice. Lindane is a persistent organochlorine compound which is widely distributed in the environment. Lindane is considered to be highly toxic. People are exposed to lindane mainly from ingestion of foods contaminated with this pesticide. Additional exposure may come from breathing air contaminated with lindane, dermal contact with contaminated soil or in drinking water. Some people, especially children, may also come into contact with lindane through the use of lotions for scabies or lice control. Infants are also contact with lindane through the use of lotions for scabies or lice control. Infants are also exposed to lindane and other HCH isomers via their mother's milk.

As from my experiment and secondary sources of data lindane is found to have health hazards effect so its use must be reduced to prevent from its ill effects.

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