Estrogenic Nature and Effects of Endosulfan in White Albino Mice

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Abstract- An invisible assault on life is underway. The indiscriminate and injudicious use of organochlorine pesticides for better agricultural productions and protection of crops respectively invariably leads to their accumulation in crop products and thereby affect the whole environment. Endosulfan is one of the most commonly used organochlorine pesticides with moderate toxicity. In the present study two doses of endosulfan were administered (s.c) to ovariactomised albino mice. The first dose of the chemical 500 µg/kg/BW showed increase in uterine weight (normal 27.5 mg, treated 105.3mg) decrease in serum cholesterol level (normal-459.57mg/100ml, treated-182.97mg/100ml), increase in uterine protein concentration (normal-234 µg/gm, treated-1136.33 µg/gm). The initiation of oestrous cycle in overiactomized female mice in the treated group also indicated significant biological effect. That was a clear indication of oestrogenic nature of Endosulfan. The above-mentioned effects were found to be dose dependent. Nevertheless, in dose concentration 1000µg/kg/BW there was a trend towards the decrease in uterine weight. In male mice reduction in epidydymal sperm count (normal 30500/µl, treated-5750/µl), decrease in testes weight (Normal-0.08745gm, treated-0.04953gm) were observed. The oestrogenic activity of endosulfan in the present study was found to be about 10,000 fold less than that of 17B-oestradiol, i.e. the endogenous estrogen. This study established the estrogenic nature and effects of endosulfan in mice.

Index Terms- environmental oestrogens, estrogenic nature, effects of endosulfan, white albino mice, effects of organochlorine pesticides on mice, endocrine disruption

I. GENERAL INTRODUCTION

For a number of years concern has been growing over changes in the health and fecundity of both humans and wildlife which may be associated with the disruption of the hormonal system by environmental chemicals. The issue of environmental disruptors has become a focus of considerable media attention through out the world and is now on the agenda of many experts groups, panels and steering committees of governmental organizations, Industries in USA, Europe and Japan. The major findings driving this interest are derived from experimental and epidemiological studies on humans and wildlife, particularly those pertaining to effects on reproductive health, which may result from exposure to endocrine disruptors early in life.

The mechanisms through which endocrine disruptors interfere with the hormonal system are complex and not yet well understood. The extent of disruptors effect on human beings is still being debated, as many factors limit research and prevent researchers from leading conclusions as easily as they do for wildlife. Since the endocrine system is sensitive to perturbation, it is likely to target for disturbance. In contrast to natural hormones found in animals plants some of the components and by product of many manufactured organic compounds that interfere with the endocrine system are persistent and undergo bio magnification in the food web, which makes them of greater concern as endocrine disruptors.

Man made chemicals range across all continents and oceans. This endodisruptors are found in native population from the Arctic to the tropics, as because of their persistence in the body, can be passed from generation to generation. The seriousness of the problem is exacerbated by the externally by the low levels of hormones produced naturally by the endocrine system which are needed to moderate and induce appropriate responses. In contract many endocrine disrupting chemicals even if less potential than the natural products, are presents in living tissues at concentrations millions of times higher than the natural hormones. Wild life, laboratory animals and human exhibit adverse health effects at contemporary environment concentration of man made chemicals that act as endocrine disrupt.

The main hypothesis of my study includes as Organochlorine pesticides especially Endosulfan is a such harmful group of compound that bind to the hormone receptors and it can induce inappropriate endocrine system responses and have oestrogenic activity. It can bind to progesterone receptors, inhabits synthesis of androgen leading to reproductive health effects.

II. REVIEW OF LITERATURE

The indiscriminate and injudicious use of chemicals, apart from the occupational hazard in the developing world, is today posing a serious threat to human heart causing disruption of the endocrine system. Deformity and embryo mortality in birds and fish caused by exposure to organochlorine insecticides, depressed thyroid and immunofunction in fish eating birds, feminization of fish near municipal effluent outlets. Source: Environmental and endocrine disrupting substances in the Environment 2000.

A literature review reported an another research by scientist when male fetouses were inadvertently exposed to abnormal doses of synthetic estrogens such as diethyl stilbestrrol, observe a decrease in sperm count increase in disease in male genital organs. These results have blazed a trail for the world scientific community, which is now studying the issue of active compounds in the environment in relation to the human population. Over the past years many countries have witnessed an increase in diseases related to most functions of the endocrine system. In Canada, Ontario has experienced an 2% increase each year in the Endocrine of testicular cancer (60% over 30 years)

Some studies indicate that most of the studied products appear to have at least some Expects on mammals. Other studies
suggested that the effects of endocrine disruptions are seen parentally. A Danish study of 18 organochlorine products has linked the incidences of breast cancer to pesticide and has shown that some organochlorines may have slight estrogenic effects.

Scientists agree on at least the current data on endocrine disruption are inadequate. More comprehensive studies of the endocrine system and toxic substances must be conducted to establish clearly the scope of the problem and to develop strategy for prevention and intervention. Hopefully, this short review has illustrated that there necces chemicals which are still not studied due to inadequate data and raises uncertainty if these estrogenic chemicals causes damage to the health or might be beneficial to the endocrine system.

The expanding definition and hypothesis of Endocrine disruption:

- Originally, the concern over endocrine disruption was based on perceived effects on the reproductive system and it was usual to refer to the chemicals concerned as oestrogen or oestrogenic chemicals. Later chemicals were found that could block oestrogen responses or androgenic responses and it was soon recognized that chemicals could effect other elements of the endocrine system via interaction with hormone receptors. The term endocrine disruptor is now preferred because it allows inclusion of health effects thought to result from interference with any part of the endocrine system, including thyroid; thymic acid, pituitary hormones.

- In order to establish concensus on the scope of the endocrine disruptor issue, it is essential to agree a precise definition of an endocrine disruption (ED). According to a major European Workshop “An Endocrine disruption is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, subsequent to changes in endocrine function. The U.S. environmental Protection Agency (EPA) risk assessment forum has stated in the following manner:

  "An endocrine disruption is an exogenous agent that interferes with synthesis, secretion, transparent, binding, action on elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproductive development and behavior."

- Hypothesis:

  The hypothesis of environmental endocrine disruption may be stated in the following manner:

  “Environmental pollutants that bind to hormone receptors are able to induce inappropriate or disproportionate endocrine system responses, thereby causing adverse disruption of normal physiologic function.”

III. TOXICITY OF ENDOSULFAN

A. Classification

- Primary use - Insecticide
- Secondary use - Acaricide
- Chemical Group - Organochlorine compound.

B. Chemical Identity

Technical grade endosulfan contains at least 94% of 2 pure isomers, and endosulfan (Mair –Bode 1968,NRCC 1975 ). The and isomers of endosulfan are present in the ratio of 7:3, respectively.

Pesticide Action Network (PAN), an international network focused on protecting community health and the environment applauds the recent recommendations by Government chemical experts that politicians include the toxic chemical endosulfan on the Prior Informed Consent (PIC) list of the Rotterdam Convention in 2008.

“Communities should not have to suffer from exposure to Endosulfan when so much is known about its dangers”. Endosulfan is acutely toxic, is known to disrupt the hormone system, can damage the human reproductive system and has been linked to breast cancer among other health effects.

C. Acute toxicity to wild life

Endosulfan is acutely toxic to wildlife, cats, dogs, housefly, honfly, birds, amphibians, fish, aquatic insects, crustaceans, alligators, crocodiles, turtles, soil microorganisms, and arthropods.

- It has caused excessive fish kills in numerous countries, including Germany, Canada, USA, and decline of amphibians.

Endocrine Disruption - Endosulfan is known to interface with hormonal mechanism at any low level of concentration and existing levels of environmental contaminations pose a threat to the long term viability of animal population, and of chronic illness and health in humans.

- It can blind to progesterone receptors, increasing the risk of miscarriages. It also inhibits testicular synthesis of Androgen and alters sex ratio. Impacts on male reproductive health impacts include –Reduce sperm count, testicular damage, delayed sexual maturity.

D. Chronicle effects

- Damages blood cells, thyroid, kidneys, liver, and muscles. It is hepatotoxic, genotoxic, mutagenic, inhibits immune function.
- It has produced malignant neoplasm and lymph sarcomas in rats.

E. Persistence

- It is volatile and persistent and also has evidence of wide spread in human, environment and food chain contamination mood of world.
- Residues of endosulfan have been detected in the environment in areas far distant from where it has been used, in air in the arctic and Mount Everest regions, lichen, snow-water, lake-water, snow samples in California Mountains.

F. Bioaccumulation

The US EPA considers endosulfan as having high potential to bioaccumulate in fish, and hence may affect animals higher up the food chain. It has been found in trout from lakes in North America, and in fish in Benin, Nigeria and Uganda.

- Again residues were found in human umbilical cord blood, placental tissue, breast milk, fat, blood, urine-in Columbia, Japan, India, Spain.

IV. RESEARCH METHODOLOGY

A. Study of oestrous cycle (Ref-Allen’s method)
10 mice were taken for this experiment driven from a colony maintained, by a grant from the Animal house of the zoology department. 4 groups were made for the study- Control group ii) Concentration -1 iii) concentration-2 IV) Vehicle group

With the help of loop the vaginal smear was taken in the slide, when dried methanol is poured and lastly stained with methylene blue. After staining with methylene blue for about 15 minutes, slides were washed, dried and examined. Thus for a week continuous cycle was studied.

B. Ovariactomy of Mice
Ketamine dilution - 1:9 ratio.200mg/kg (ref. Lamb ,W, and Jones E1984,vet, Anasthesia, 2nd ED-K.M.Verghese and co, Bombay,pg-435), taken 350 mg/kg

Xylazine, 50 mg/kg.=10mg/ml.1:9 dilution.

Thus the mixture of 2 ml katamine and 1 ml xylazine were injected to the mice and after 2 minutes mice are ovariactomized. Both ovaries are removed from the body. Now with the help of thread and needle incision portion is stitched , newspaper is applied and kept for 14 days After 14 days,

C. Concentration of Endosulfan ie.5 mg/kg BW and 1 mg/kg BW were prepared.
G1 - Treated with oestradiol (2) Animals =.1ml
G2 - Concentration - 1.5mg /kg BW (3) animals =500 mg/kg BW
G3 - Concentration - 2.1mg /kg BW (3) animals=1000mg/kgBW
G4 - Vehicle group = Olive oil =.1 ml.

Chemicals were applied for 1 week.

D. Determination of Protein from Uterine tissue
Frozen tissue is grinded using Mortes, and pested adding liquid
N2 - Homogenized to make 1% Homogenate with DH2O - 1 ml of 1% homogenate taken in a test tube - 1ml of cold 10% TCA added and stand for 10 minutes - Centrifugal at 400 RPM for 10 minutes - Supernatant disordered and 5% TCA (2.5) ml. added to the precipitate - Centrifugal at 1000 RPM for 5 minutes - Supernatant dishoarded and precipitate in 2.5 ml 95% ethanol - Centrifugal at 1000 RPM for 5 minutes - The ppt mixed with 2-3 ml of alcoholic ethane (3:1) - Centrifugal twice at 3000-4000 rpm for 5 minutes. Supernatant dishoarded and to be containing pellet inverted on a tissue paper for 30 minutes 3ml of .1N NaOH added to the tubes and kept at 30c for 3-4 hrs.

Black Standard Unknown
1ml of DH2O taken – 5 no’s of different concentration .1ml of sample in one test tube.
S1 - 100ml+.9ml of DH2O .9 ml of DH2O
S2 - 200ml+.8 ml of DH2O
S3 - 400ml+.6 ml of DH2O
S4 - 800ml+.2 ml of DH2O
S5 - 1ml .5ml of solution D added and allowed to stand for 15 minutes -.5ml of Folino-phenal reagent for 30 minutes - O.D. measured at 670 mm.

E. Estimation of Serum Cholesterol levels

jejidymal sperm is collected and then diluted with .5ml of saline. Now from the diluted solution , 1ml is poured over j

F. Sperm count

From the sacrificed 1ml of epidymal sperm is collected and then diluted with .5ml of saline. Now from the diluted solution, 1ml is poured over the slide and covered with cover slip and observed under microscope.

V. RESULTS AND DISCUSSION

A. Case Study 1 - Study of the estrous cycle after treatment

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Fig - Study of estrous cycle

Conclusion - Endosulfan has estrogenic activity which induces estrous cycle. Initiation of estrous cycle was observed in the ovariactomized mice of the treated group whether in the normal group there was no trace of estrous cycle. In the G1 NO-2 estrous cycle completed in 7 days .G2 NO-2 showed completion in 7 days .Thus it can be concluded that endosulfan has estrogenic activity which induces estrous cycle.

B. Case Study 2 - Determination of cholesterol level in female group

Serum Cholesterol Level in Female Group

Fig - Level of cholesterol in the female mice
Conclusion - From the above analysis it can be concluded that endosulfan decreases the serum cholesterol level in the treated groups of mice.

C. Case Study 3 - Estimation of Uterine protein

From the analysis it is found that the protein concentration increases suddenly when the first dose concentration was applied but remarkably decreases again with the increase of the dose concentration.

Conclusion - It can be concluded the protein level increases to a optimum level but decreases afterwards with the increase of endosulfan dose.

D. Case Study 4 – Sperm count using haemocytometer

Fig - Sperm count from epididymal fluid

E. Case Study 5 – Uterine weight after treatment

Fig - Estimation of uterine weigh after treatment

Conclusion - From the above analysis it is seem that use of normal group sperm count is very high. But suddenly it decreases drastically in the treatment group. Infect the no of sperm decreases with the increase of the dose concentrations.

F. Case Study 6 - Determination of testes weight after treatment

Fig - Determination of testes weight

Conclusion - The calculation of values also indicate that at dose concentration of 500ml/kg, the weight of testes reduces than the normal mice. But after getting an optimum value at concentration 1000 ml/mg little amount of weight increases and remains this.
shows that endosulfan has effects on testes structure. It degrades the testes histo architecture also.

VI. CONCLUSION

We are living in a newly made sea of estrogenic chemicals and similar toxic substances. There is growing evidence that risk from exposure to these toxic chemicals have been grossly underestimated. As a result the foundation of our health interaction between endocrine, immune and nervous system is being undermined by modern chemicals.

We are told the amounts of these toxicants entering our bodies are so tiny like a few grains of sand on the beach how can they hurt? Because of the exquisite complexities of life are designed to respond to natural hormones in the parts per quadrillion ranges. And now we are learning that this endo disrupting chemical exists at similar levels in our everyday environment and are assaulting our intricate biological system.

In the concluding part of the study there is a humble quote by Madam Rachel Carson “It is not my contention that chemical insecticides must never be used. I do contend that we have put poisons and biologically potent chemicals indiscriminately into hands of persons largely or wholly ignorant of their potentials for harm”.

From the study of my project work it is seen that endosulfan is acutely toxic substance causing serious health hazards and there should have more research on the topic.

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