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Ameliorating effect of *emblica officinalis on* testosterone and sperm toxicity of endosulfan induced mice

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ABSTRACT
The ameliorating effect of *Emblica officinalis* was evaluated against changes in sperm and hormone of mice. The animals received aqueous preparation of Endosulfan (Pesticide) once daily for 35 days at the dose of 3 mg/kg body wt by oral gavage method and observed deleterious effect such as of reduction in the number of sperm count and motility. Declination in the level of Testosterone were observed which signify the testicular dysfunctions and finally causes infertility. To ameliorate the deleterious effect made by endosulfan, *Emblica officinalis* was administered to mice @ 100 mg/kg body wt & 150 mg/kg body wt The earlier study have demonstrated potent anti-microbial, antioxidant, adaptogenic, hepatoprotective, anti-tumor and anti-ulcerogenic activities in the fruits of *Emblica officinalis*. *Emblica officinalis* treatment mitigate the fluctuation in sperm count and sperm motility as well as elevated the level of testosterone which was depressed by pesticide treatment.

Introduction

Among pesticides and their related chemicals, organochlorine insecticides have so far drawn the primary attention. Dischlorodiphenyl Trichloroethane (DDT), dieldrin, and toxaphene have instrinsic estrognic activity and are known as possibly endocrine-disrupting chemicals. (Soto et al, 1994).Many attempts have been made to study the toxic effects of pesticides on the reproductive organs of mice (Kumar and Nath 1995, Russel 1995). Endosulfan may cause decrease in semen quality, increase in testicular and prostrate cancer and an increase in defects in male sex organs (Hileman 1994, Solo 1993). Biochemical changes in endosulfan treated testes of rats was observed by (Sinha et al 1995). Endosulfan treatment in pubertal rate inhibits testicular functions (Chitra et al 1999).

Endosulfan is an organochlorine pesticide which is widely used by farmers in India for pest control. It has been well reported that endosulfan causes degradation of testicular cells in laboratory animals (Khan & Sinha 1996, Sinha et al 1997 & Sinha et al 2004). Such chemicals have been found to cause reproductive health problems & apoptosis in both humans & wild life (Murray et al 2001). Testosterone level steadily drops in men (Scelto, 2006).

To evaluate a comparative ameliorating effect, if any, to mitigate the sperm toxicity and hormonal imbalance in mice for which *Emblica officinalis (*medicinal plants) have been taken as curative measure : -

Emblica officinalis leaves and fruit have been used for fever and inflammatory treatments by rural populations in its growing areas. The earlier study have demonstrated potent anti-microbial (Ahmad et al, 1998), antioxidant (Bhattacharya et al, 1999), adaptogenic (Rege et al, 1999), hepatoprotective (Jeena et al, 1999), anti-tumor (Jose et al, 2001) and anti-ulcerogenic activities (Sairam et al, 2002) in the fruits of *Emblica officinalis*, leaf extracts have been shown to posses anti-inflammatory activity (Asmawi et al, 1993; Jhantola-Vormisto et al, 1997).

Material & Methods

In the present investigation, experiments were performed on 3-4 months old healthy male Swiss albino mice, *Mus musculus*. For the optimal growth and reproduction the mice were kept in ideal condition in the laboratory. The oral LD_{50} value of endosulfan for mice was estimated by standard interpolation method, which was 7 mg / kg b.w.

The standard data reference for LD_{50} of endosulfan for mice is 7.36 mg/ b.w. (EXTOXINET, 1996).Endosulfan manufactured by Excel Industries Mumbai (E.C - 35%) was dissolved in distilled water to prepare sublethal dose of 3 mg/kg b.w was administered by gavage method. A vehicle of control group of mice was established and served with equal volume of distilled water by gavage method. The treated and control group were sacrificed on 35th day of treatment. The sperm count was done through cauda epididymis.

Sperm Count

The Cauda epididymis was dissected out and washed thoroughly in normal Saline (0.85 %).

Cauda epididymis was incised at several places in 1 ml distilled water so as to allow the sperm to ooze out. After that, two drops of Eosin Y is mixed well with sperm.

Sperm counts was made using an improved Neubauer's chamber taking a drop of above preparation in it & observed at 450x magnification.

Sperm Motility

Cauda epididymis was dissected out and ruptured on microscopic slide. After covering it with a cover slip, the motility of the spermatozoa was examined.

Estimation of Testosterone Level In Serum of Mice: By Elisa Technique

Blood sample were collected after each sacrifice and there serum were isolated. Using the ELISA method Testosterone kit of LILAC Medicare (P) Ltd., Mumbai was utilized for the experiment.

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Method: The normal range was calibrated and then 25 µl serum samples were taken in the well plates. 100 µl of enzyme conjugate was added in each well. After that, it was left for incubation at 37^0C in incubator for 1 hour. Then, the wells were washed with 300 µl distilled water for at least 3 times and blotted. Then, 100 µl TMB solution was added as substrate in each well plate and was again left for the incubation for 15 minutes for the colour. Finally, 100 µl stop solution was added in each well to stop the reaction Reading was taken at 630nm through Merck ELISA reader in ng/ml value. Reading was taken at 630nm through Merck ELISA reader in ng/ml value.

Calculation of LD_{50} and Maximum Permissible Dose (MPD) of *Emblica officinalis* (Amala) aqueous fruit extract:

For calculating the LD_{50} value of *Emblica officinalis* for mice by standard method was reported by Khandelwal et al (2002), as 1500 mg/kg b.w as LD_{50}. At 250 mg/Kg, 150 mg/Kg and 100 mg/Kg b.w. although there were no death reports but the no side effects were seen at 100 mg/kg b.w, 150 mg/Kg So,100 mg/Kg and 150 mg/Kg b.w. was selected as Maximum Permissible Dose (MPD) for the experiment.

Results & Discussion

After 35 days exposure(in case of mice spermatogenesis cycle is of 35 days.) of endosulfan at the dose of 3 mg/kg b.w. showed significant changes in all the parameters like testosterone level, sperm count & sperm motility. After 35 days of continuous exposure of endosulfan, it was observed that endosulfan at some extent checks the process of spermatogenesis. The level of testosterone reduces to maximum in comparison to normal value that ranges from 6.5± 0.09 to 1.16 ± 0.02. The value for control group with treated group at the dose of 3.0 mg/ Kg b.w in the first week (t= 2.81 : p< 0.05) and 5^{th} week (t = 2.94 : p< 0.05) which are statistically significant. Further changes were observed in the mice sperm count exposed to 3.0 mg/ Kg b.w for 35 days which was about 1.18±0.02($x10^6$/ml) and the sperm motility observed were 10 (%)± 0.65.

The present study indicates that oral administration of sublethal dose of endosulfan (3.0 mg/ Kg b.w) brings about various significant changes in the level of testosterone, sperm count and sperm motility that shows damaging effect on various stages of spermatogenesis.

Reduction in sperm count resulting from adverse effect on spermatogenesis and less mobile defective spermatozoa after endosulfan treatment have also been observed by Panday et al (2003). As in the present investigation, increased evidences of testicular and reproductive defects related to endosulfan exposure was confirmed that that pesticide adversely effects spermatogenesis and causes testicular atrophy. Chitra et al (1999) have also studied the effect of endosulfan on the testis of growing rat. Setchell (2004) and cram et al (2004) have observed the infertility in men and it causes due to hormonal imbalance. Ichihara et al (1993) have observed the effect on testosterone due to chemically caused ageing.

In my investigation regarding sperm count and sperm motility, it has been observed that marked reduction in sperm count ($1.18 \times 10^6 \pm 0.02$) as well as sperm motility (10%) as compared to control which were observed ($5.25 \times 10^6 \pm 0.13$) for sperm count and (68%) for motility.

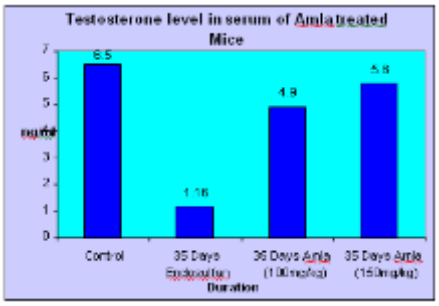
There are reports of testicular toxicity of endosulfan manifested as decreased spermatogenesis and testicular hormone synthesis (Steroidogenesis), as evidenced by a decrease in spermatid count in testes and sperm count in the Cauda epididymis and by changes in marker enzymes for testicular steroidogenesis in adult animals (Chitra et al, 1999; Sinha et al, 1995) whis is in support of my work, Similar study have been observed by Singh and Pandey (1990) that a dose related decrease in testicular testosterone, plasma testosterone, LH, and FSH in groups of male Wistar rats administered endosulfan at 0, 7.5, or 10 mg/ kg / day for 15 or 30 days.

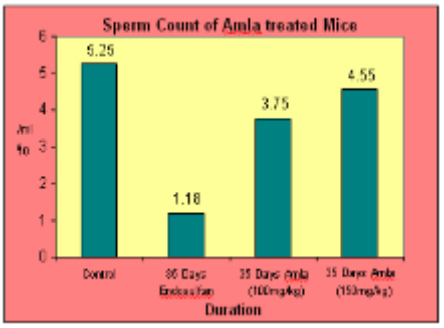
The main theme of present investigation is to evaluate the possible role of *Emblica officinalis* fruit extract to restore the Sperm count, motility and serum hormonal level of mice which have initially been exposed to sublethal treatment of endosulfan pestiside.

The legend mentioned in the graph clearly indicates the testosterone fluctuation in mice, due to pesticide and their restoration towards more or less normalcy after *Emblica officinalis* fruit extract treatment. It was observed that @ 150 mg/kg.b.w the level of testosterone elevated to 4.1± 0.16 which was about to control value.

Sperm counts and motility have been done for all control, pesticide treated and pesticide treated followed by plant extracts treated mice.All the values of sperm count and sperm motility mentioned in text table have been graphically represented in graphical plates. The legend mentioned in each of the graph clearly indicates the fluctuation in sperm count and sperm motility due to pesticide and their mitigating effect *after Emblica officinalis* treatment.

At the dose of 100mg/kg.b.w and 150mg/kg.b.w of amla helped to ameliorate the affect of endosulfan, but at the dose of 150mg/kg.b.w of amla given better result.





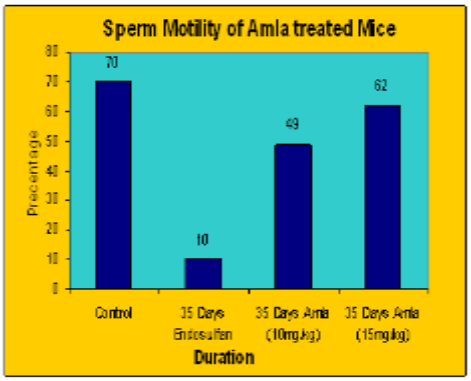


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Table:- 1 Showing serum testosterone level (ng/ml) in control & 35 Days *Emblica officinalis* treatment at the dose of 100mg/kg.b.w and 150mg/kg.b.w followed by Endosulfan treatment.

	Control	Endosulfan treated for 35 Days at dose of 3mg/kg.b.w	*Emblica officinalis* treated at the dose of 100mg/kg.b.w for 35 Days	*Emblica officinalis* treated at the dose of 150mg/kg.b.w for 35 Days
No of observation	10	10	10	10
Mean	6.5	1.16	4.9	5.8
S.D.	± 0.09	± 0.02	± 0.26	± 0.11

Table:- 2 Showing sperm count (10^6/ml) in control and *Emblica officinalis* at the dose of 100mg/kg.b.w and 150 mg/kg.b.w for 35 days followed by Endosulfan treatment.

	Control	Endosulfan treated for 35 Days at dose of 3mg/kg.b.w	*Emblica officinalis* treatment at the dose of 100mg/kg.b.w for 35 Days	*Emblica officinalis* treated at the dose of 150mg/kg.b.w for 35 Days
No of observation	10	10	10	10
Mean	5.25	1.18	3.75	4.55
S.D.	±0.13	±0.02	±0.05	±0.06

Table:- 3 Showing sperm motility (%)in control and *Emblica officinalis* at the dose of 100mg/kg.b.w and 150 mg/kg.b.w for 35 days followed by Endosulfan treatment.

	Control	Endosulfan treated for 35 Days at dose of 3mg/kg.b.w	*Emblica officinalis* treatment at the dose of 100mg/kg.b.w for 35 Days	*Emblica officinalis* treated at the dose of 150mg/kg.b.w for 35 Days
No of observation	10	10	10	10
Mean	68	10	49	62
S.D.	±0.02	±0.65	±0.41	±0.27;