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Amelioration of Endosulfan Induced tisticular alterations in mice by *Phyllanthus emblica.*

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Abstract

Endosulfan is an off-potent insecticide cum acaricide that is being phased out globally but is still used in large scale. It has become a highly controversial agrochemical due to its acute toxicity, potential for bioaccumulation, and role as male reproductive disruptor. The present study was carried out to investigate the acute effects of endosulfan on male reproductive parameters like sperm count, testicular weight an changes in seminiferous tubules with respect to germinal epithelium, sertoli cells and spermatogenic cells on male albino mice. Adult male mice were segregated into three groups, each group with 6 animals. Group I, served as the control, was administered with distilled water @ o.1 ml/ 10 g b.w Group II received endosulfan @ 3mg/kg b.w. Group III concomitantly received Phyllanthus emblica (amla) @ 100mg/kg b.w. along with endosulfan through oral gavaging. All the treatments were continued for three months . The toxic effects observed upon endosulfan treatment include reduction in the testicular weight and deformatices in seminiferous tubules with respect to changes in germinal epithelium, sertoli cells and spermatogenic cells, which may be linked to testicular dysfunctions and infertility. On contrast, treatment of extract of P. emblica was found to alleviate the toxic consequences of endosulfan, thereby producing ameliorative effect.

Key words: Endosulfan, Phyllanthus emblica, sperm count, testicular deformities.

INTRODUCTION

The intensive chemization of world's agriculture by indiscriminate and rackless use of chemical pesticides led to the large scale contamination of our living environment (Miller, 2004). Among these Chlorinated pesticides, as they are long-lived and fat soluble in the environment for very long periods, causing their bioaccumulation and

biomagnification which in turn impact toxicity to non-target organisms including human beings. Accordingly, manufacture and use of several chlorinated pesticides, has either been banned or severely restricted.

Endosulfan is a widely used chlorinated hydrocarbon insecticide and acaricide of cyclodien subgroup. Technically endosulfan is a 7:3 mixture of stereoisomers **alfa** and **beta.** Persistence of endosulfan in soil and water have been widely reported by several researchers under difference conditions (Rao and Murthy, 1980; Guerin and Kennedy, 1992) even frequently found in the environment at considerable distance from the point of its original applications owing to in potential transport (Man singh and Wilson, 1995; Miles & Pfeuffer, 1997). The chemical row has been banned worldwide by the Stockhom convention of persistent Organic pollutants (POPs) due to its unacceptable neurological and reproductive risks to farm workers and wildlife, is still used in some developing nations including India.

There is a global concern over the acute toxicity of endosulfan as its ill effects are most pronounced in the male gonads of immature animals (Sinha et al. 1997) whose reproductive system has not yet developed fully (Dalsenter, et al., 1999). Studies have shown that its toxicity can cause histological and functional impairments in male reproductive systems, causing decreased sperm count and testosterone inhibition (Esin, 2008; Hatipoglu et al., 2009); it also reported to interfere in the spermatogenic process (Joshi et al., 2007) and It harms the reproductive system by altering seamen quality and sperm count, by including spermatogonial dysgenesis, sperm head anomalies and other such reprvtonic effects in male sex organs (Khan and Sinha, 1996; Nath and Kumar, 2007; Kumar, et al., 2012). Such chemicals have been found to cause reproductive health problems in both humans and wild life (Marry, et al., 2001).

The Phyllanthus embolica commonly known as Indian gooseberry or Amla, (belongs to deciduous tree of the family Phyllanthaceac). The branchlets are glabrous or finally pubescent, 10-20 cm long, usually deciduous; the leaves are simple, sub sessile and closely set along branchlets, light green, resembling pinnate leaves. The flowers are greenish-yellow. The fruit is nearly spherical, light greenish yellow, quite smooth and hard with six vertical stripes or furrows (Lim, 2002). This plant is of great medicinal importance. It has antiviral and antimicrobial properties (Saeed and Tariq, 2007). There is preliminary evidence in vitro that its extract induces apoptosis and modify gene expression in osteoclasts involved in rheumatoid arthritis and osteoporosis (Penolazzi, et al., 2008). It may prove to have potential activity against some cancers also (Ngamkitidechakul, et al., 2010). The earlier studies have demonstrated that the fruit of this plant has potent anti -microbial (Ahmad et al., 2010), antioxidant (Bhattacharya, et al., 2001), adaptogenic (Rege, et al., 1999), hepatoprotective (Jeena, et al., 1999) and anti-ulcerogenic activities (Siaram, et al., 2002). Phyllanthus emblica leaf extracts have also been shown to possess anti-inflammatory activity (Asmawi, et al., 1993; Jhantola - Vormisto, et al., 1997).

In the present work, ameliorating effect of the fruit extract of Phyllanthus emblica is used to mitigate the sperm toxicity and testicular deformities induced by endosulfan treatment in Swiss albino mice have been studied as a remedial measure.

MATERIAL AND METHODS

Test Animal

Young and sexually mature Swiss albino mice (*Mus musculus*), each with body weight of 2.57 gm, (obtained from DRI Lucknow) were maintained in the animal house of Department of Zoology, T M Bhagalpur University; Bhagalpur. A total of 18 mice segreted regretted into 3 experimental groups were kept separately in cages at 24 ℃ temperature and humidity with 12+1 hr light/dark cycle. Food and water were made available to the animal ad libitum. All the animals were maintained according to the accepted principal for laboratory animal use and care as per the guidelines of CPCSEA. Initially , the mice were acclimatized for two weeks before the start of experiment.

Test Substance as used

The Pesticide used for treatment to animals were endosulfan, purchased from the market under trade name of endocoel 35 EC (Excel industries Pvt. Ltd,, Mumbai). It is a dark brown liquid consisting of 35% w/w endosulfan technical (6,7,8,9,10, 10-hexachloro- 1,5,5a,6,9,9a hexahydro-6, 9-methano-2,4,3-banzo dioxathiepin-3-oxide) widely used as broad spectrum organochlorine insecticide and acaricide.

Treatment Protocol

The 1/8th of the LD50 dose of endosulfan (3.0 mg/kg b.w.) was administered orally for chronic toxicity study. Once daily, for 30 Days by gavage method (Oakberg, 1957).

Similarly, maximum tolerable dose (MTD) of Phyllanthus embolica (100 mg/kg b.w.) was administered for amelioration study at regular interval of 30 days by gavage method.

EXPERIMENTAL DESIGN

a) The animals were divided into three experimental groups with 6 animals in each group.

The Group 1: Animals were maintained as untreated ones (control);

Group 2: Animals were treated with endosulfan only;

Group 3: Animals were treated with endosulfan followed by fed with fruit extract of Phyllanthus emblica.

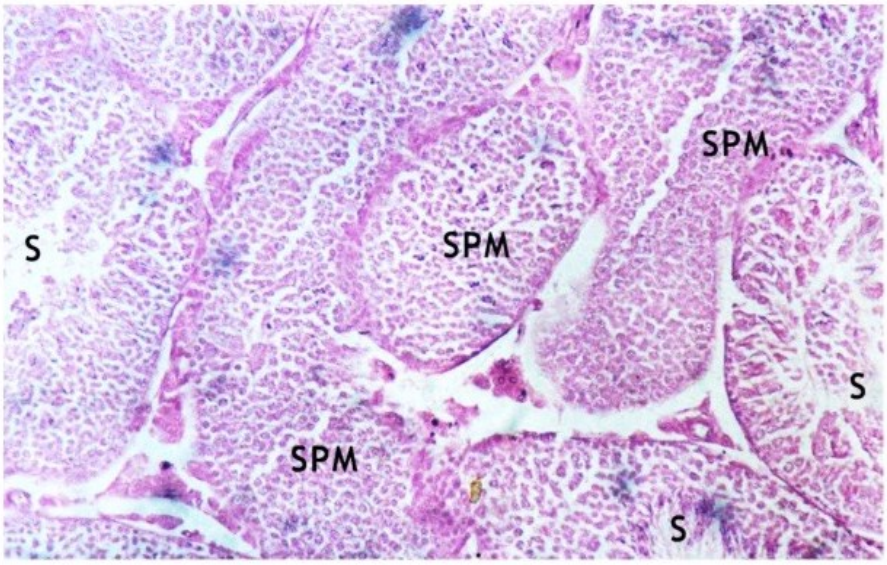
b) Fruit extract of amla (Phyllanthus emblica):-

Ripe fruits of Amla (Phyllanthus emblica) were collected from healthy plants during the winter season in Polythene bags at Bhagalpur and were subsequently washed in running tap water. Collected Fruits were dried in hot air oven at 60 ℃ (Handa et. al.,

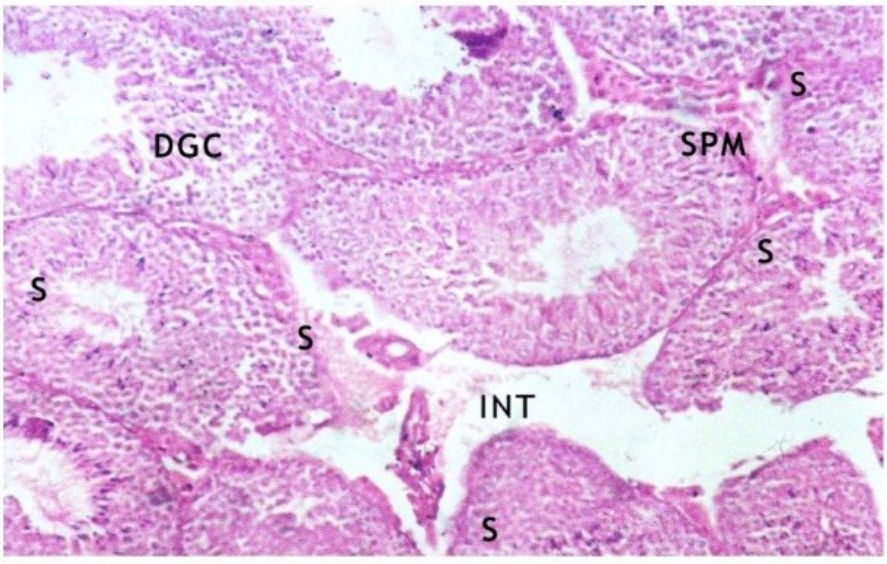
2008) for 5-8 days and then powdered coarsely with the help of glass mortar and pestle. The powder so obtained was sexhlated with ethanol (80% v/v) (Jones and Kingkorn, 2006) at 60-70 ℃ (Handa et. al., 2008;) for 50-90 hrs till the return of the usual shine of ethanol (full extraction).

EFFECT OF FEW MEDICINAL PLANT EXTRACT ON TESTIS OF ENDOSULFAN INDUCED SWISS ALBINO MICE *Mus musculus*

CONTROL



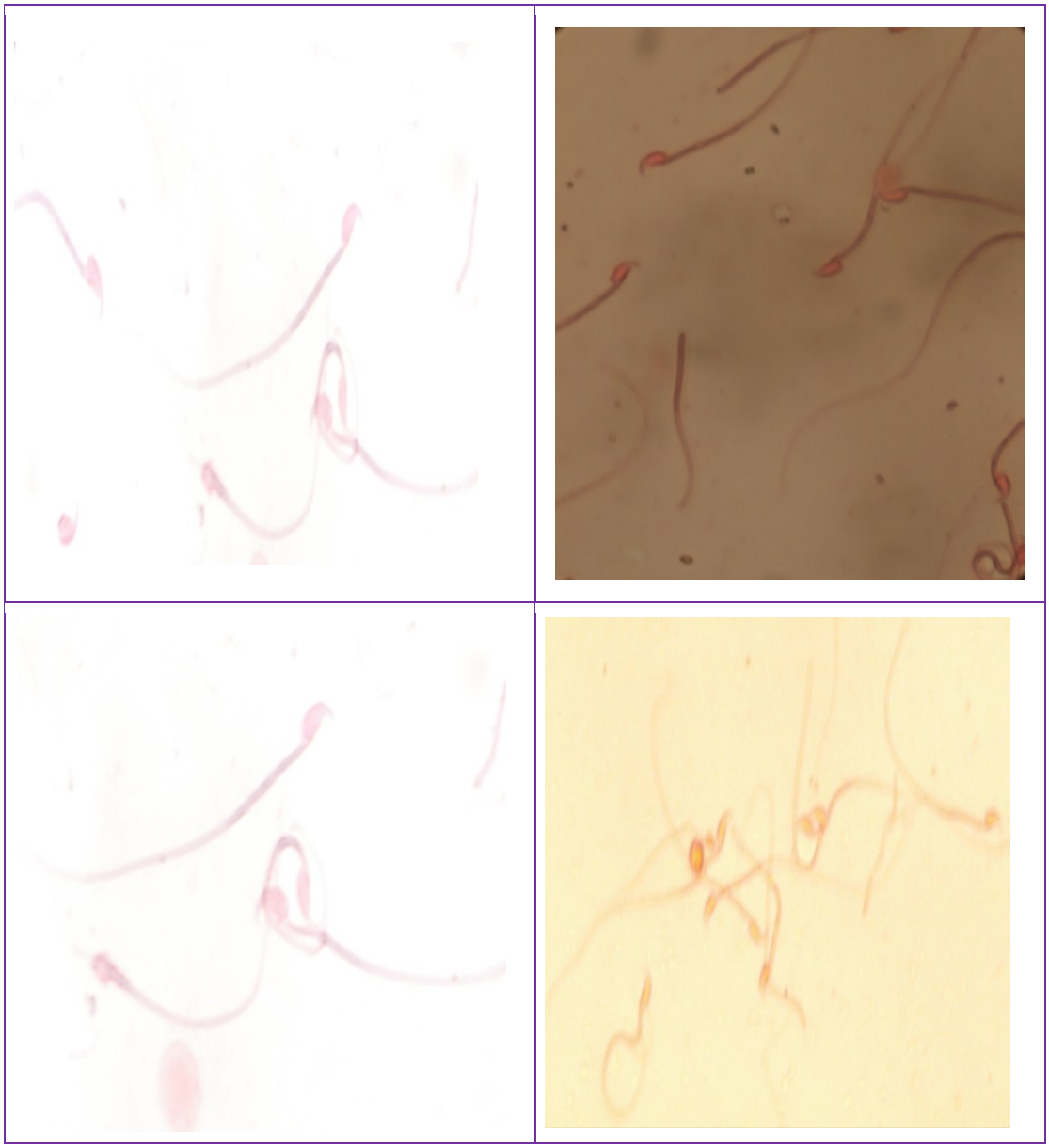
ENDOSULFAN TREATED AFTER 7 DAYS



RESULT AND DISCUSSION

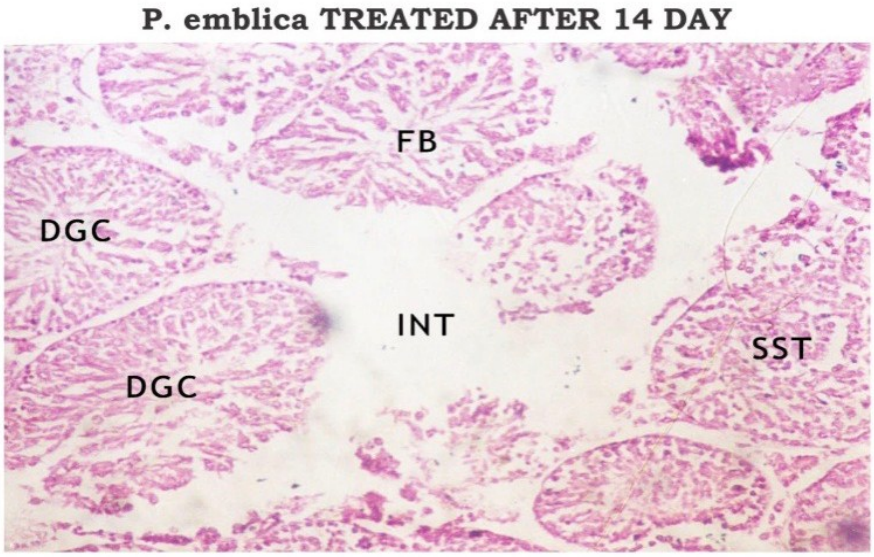
The extent of damage in the testis of mice treated with endosulfan was analyzed and expressed in terms of cellular and histological abnormalities. The degree of damage were then qualified in comparison with those of control male mice as well as mice supplemented with Phyllanthus emblica after endosulfan treatment.

Distinct changes were found in histology of Testis as well as the changes appeared in the head morphology of sperms. (Fig. 1)



(Figure 1)

In the control animals, the seminiferous tubules exhibited normal arrangement of germ line cells, Sertoli cells as well as Leyding cells with different stages of spermatogenesis. Sertoli cells were observed in their good condition. The outline of seminiferous tubules was smooth (Fig.2).



(Figure 2)

In treated group of animals upon endosulfan treatment, the seminiferous epithelium was formed disrupted with emergence of spaces between the various cellular components with the presence of copious vacuoles frequently associated with degenerating germ cells. Spermatogenic cells were found accompanied with the absence of spermatozoa after 14 days endosulfan exposure (Table 1).

Table 1: Endosulfan Induced Male Reproductive Changes and Their Treatment with *Amla-Phyllanthus emblica extract* for four weeks on Sperm Parameter

Sperm Parameter	**Group of Mice**		
	GROUP I	**GROUP II**	**GROUP III**
Sperm count (million /mm^3)	**18.14±0.090**	**8.48±0.04^b**	**15.61±0.13^a**
Sperm motility (%)	**80.9±0.486**	**53.3±0.76 ^{b}**	**71.8±0.65^b**
Sperm mortality (%)	**19.7±0.437**	**44.1±0.92^b**	**27.8±0.740^b**

Similarly, tests after 28 days of treatment with endosulfan showed disorganized spermatogenic cells and reduction in the number of seminiferous tubules, dilatation congestion of blood vessels. Spermatogenesis was found disturbed with reduced cellularity upon extended exposure of endosulfan.

Deviation in the head morphology of sperm was quite prominent and a significant large number of sperm were observed having abnormal head morphology. (Fig. 1)

When endosulfan treated animals were supplemented with the fruit extract of P. emblica. The morphological deformities was ameliorated and normal cytological features of in sperm seminiferous tubules were partially restored (Fig. 2).

In the present study also exhibited endosulfan induced degeneration in spermatogonia as well as in primary and secondary spermatocytes, and the condition was later improved by the fruit extract of phyllanthus emblica.

The cytological alternations observed within the acrosomal cap of sperm in the endosulfan treated mice may interfere with the potential of these cells to mature into functional sperm (Sinha et al., 1995), leading a azospermia and tertespermia as observed in the present study.

The spermato toxic effects documented upon endosulfan treatment led to lower sperm morality and count as well as increased frequency of detached sperm heads as described in case of other environmental toxicants also (Dimethoate - Khagoli et al., 2005; Chlorophyriphos - Ambali et al., 2011; Imidaclopride - Najafi et al., 2010). However, these degenerative effects of endosulfan were found reversed after the supplementation of P. emblica, suggesting the changes induced by endosulfan were mostly reversible.

CONCLUSION

The results of this study supported the reports about P. emblica (100 mg/kg bwt) is having medicinal effect in using endosulfan exposed problems associated with tisticular alterations in the experimental animals. The present work indicates improvement recorded in histoarchitecture of testis and epididymis were successfully ameliorated by P. emblica.

Therefore, P. emblica (100 mg kg-1 bwt) can be effectively exposed patients for therapeutic purpose.

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