International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2019; 7 (4):701-704
ISSN:2320-7817(p) | 2320-964X(o)

Original Article Open Access

Endosulfan Induced Male Reproductive changes and their Treatment with *Withania somnifera*

Pryuttma[1*] and Singh RP[2]

[1]Department of Zoology, GMRD College Mohanpur (Samastipur), LNMU Darbhanga, India - 848506
[2]Department of Zoology, GB College Naugachia, T.M.B University, Bhagalpur, India – 853204
*Corresponding author E-mail:- pryuttma@gmail.com

Manuscript details:

Received: 13.11.2019
Accepted: 12.12.2019
Published: 30.12.2019

Cite this article as:
Pryuttma and Singh RP (2019) Endosulfan Induced Male Reproductive changes and their Treatment with *Withania somnifera*, *Int. J. of. Life Sciences*, Volume 7(4): 701-704.

Available online on
http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

The ameliorating effect of *Withania somnifera* was evaluated against changes in sperm profile of mice. The animals received Endosulfan (Pesticide) once daily for 35 days at the dose of 3 mg/kg body wt by oral gavage method and observed toxic effect such as of reduction in the number of sperm and sperm motility which signify the testicular dysfunctions and finally causes infertility. To ameliorate the toxic effect made by endosulfan, *Withania somnifera* (Ashwagandh) was administered to mice along with its food @ 150 mg/kg body wt. The potent medicinal power like antioxidant, anti-stress, anti-inflammatory, anti-aging, aphrodisiac, astringent, deobstruent, diuretic, narcotic, sedative, anti-tumor and anti-ulcerogenic activities in the root extract of *Withania somnifera* alleviate the fluctuation in sperm count and sperm motility which was decreased by pesticide treatment.

Keywords: Endosulfan, Sperm profile, *Withania somnifera*, Gavage method.

INTRODUCTION

In most of the developing countries population is increasing tremendously and to fulfill their demand of food, Farmers use improved quality of seed and pesticide to increase their productivity. Use of pesticide increases food production, increased pesticide profits for farmers and prevention of diseases. Pesticides includes wide variety of insecticides, rodenticides, fungicides, herbicides and antimicrobials. All pesticides are generally the most actually (immediately) toxic and basically poisons. Although these pesticides offer shield to the crops in the field but they become major cause of elimination of other beneficial insects and finally threat to human health. (Fawzy, *et al*., 2007). Over 90% of pesticides reach a destination other than their target species, because they are sprayed or spread across entire agriculture fields. (Anon 1984, Sultatos 1994, Gomes, *et al.* 1999). Run off can carry pesticides into aquatic environments. While wind can carry them to other fields, grazing areas, human settlements and undeveloped area, potentially affecting other species. However, widespread use and misuse of pesticides has created an awareness of the potential health hazards and the need to protect the consumer from residue in food (Radwan, et al., 2001 a, b).

Endosalfan is one of the pesticides used widely throughout the world for control of number of insects and mite pests on a variety of food crop field , fruit , Cashew, Tea, Coffee and vegetable crops (Michel, 2003) and on other non food cash crops like tobacco, cotton and timber. Exposure to pesticides causes temporary acute effects like irritation of eyes, excessive salivation to chronic diseases like cancer, reproductive and developmental disorders etc. Population that are usually susceptible to endosalfan include the unborn and neonates, the elderly and people with liver, kidney, immunological hematological defects (Xavier, *et al.*., 2004) on neurological disease (ATSDR, 2000). The most common way to get exposure is by endosalfan eatable and drinking water. The endosanfan may be found in many food particles such as oil and fats, fruits and vegetables, fish, milk products (Campoy *et al.*., 2001, Nag and Raikwar, 2008) Many attempts have been made to study the toxic effects of pesticides on the reproductive organs of mice (Kumar and Nath, 1995 and Russel, 1995). Endosulfan may cause decrease in semen quality, increase in testicular and prostate cancer and an increase in defects in male sex organs (Hileman, 1994; Singh, 2011). Biochemical changes in endosulfan treated testes of rat were observed by (Sinha *et al.*., 1995). Endosulfan treatment in pubertal rat inhibits testicular functions (Chitra, *et al.*., 1999). Endosulfan is an organochlorine pesticide which has been well reported that causes degradation of testicular cells in laboratory animals (Khan and Sinha, 1996; Sinha *et al.*., 1997 and Sinha, *et al.*., 2004). Such chemicals have been found to cause reproductive health problems and apoptosis in both human and wild life (Murray *et al.*., 2001).

The *Withania somnifera*, also known as Ashwagandha, poison gooseberry or Indian ginseng, or winter cherry (Grin, 2011). It is low lying perennial shrub in the Solanaceae or nightshade family. It is used as an herb in Ayurvedic medicine (Gupta et. al., 2011). This species is a short, tender, perennial shrub growing 30–75 cm tall. Tomentose branches extends radially from a central stem. The plants long, brown, tuberous roots are used in traditional medicine (pandit *et al.*., 2013). The roots of the plant are categorized as rasayans (store house of chemicals) which are reported to to promote health and longevity by augmenting defense against disease, arresting, aging process and as an antioxidants also. Double-blind, placebo controlled studies on humans have found have found the remedy to be effective at a number of health-related uses, including anxiety treatment, neuroprotection, long-term memory, anaerobic running endurance, and bodily regulation of cholesterol, cortisol and C-reactive protein are responsible for its antioxidant property (Bhattacharya, *et al.*., 1997). Benefits were seen in healthy as well as non-healthy patients. (Mirjalili *et al.*., 2009). Leaves and roots of this plant are abortifacient, aphrodisiac, diuretic, nervine tonic (Bahr and Hansel, 1982), alterative, narcotic, sedative, astringent, growth promoter and anthelmintic. It has anti-arthritic, anti-bacterial, anti-dote for scorpion sting, anti-stress, anti-tumour and anti-cancer activities.

In the present work an attempt ameliorating effect to mitigate the sperm toxicity in mice for which Withenia Somnifera (medicinal plants) have been taken as remedial measurement.

MATERIALS AND METHODS

Experiments were performed on 6 to 8 week old healthy laboratory–inbreed male Mus musculus weighing about 22-35 gram were obtained from CDRI Lucknow. Mice were reared and maintained at the animal house of University Department of Zoology, T. M. Bhagalpur University, Bhagalpur under standard condition and fed with standard diet. Food and water were given ad libitum. Ambient relative humidity was 55-60%. Rice husk was used as bedding material and changed daily. Male Swiss albino mice- *Mus musculus* was used. Animal handling was performed as per good laboratory practice. The mice were acclimatized for one week before the experiment and then used in experiment at about 12 weeks of age. The oral Ld-50 value of endosulfan for mice was estimated by standard interpolation method, which was 3 mg / kg b. w. Endosulfan manufactured by Excel Industries Mumbai (E.C - 35%) was dissolved in distilled water to prepare sub 3 lethal dose of 3 mg/kg b.w was administered by oral gavage metod. 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 of mice were administered L.D-50 dose of 3mg/kg b.w of endosalfan daily for 35 days. The treated and control group of mice were sacrificed on 7,14,21 and 28 days. Then this endosalfan treated group were ameliorated by giving *Withania somnifera* daily with L.D-50 dose of 150 mg/kg b.w for next 35 days and then mice were sacrificed on 7,14,21 and 28 days. The sperm count was done through cauda epididymis.

The experimental mice were divided into four groups of 10 animals.
Group-I (Control)
Group-II (Endosulfan treated)
Group-III (Endosulfan treated mice fed with root extract of *Withania somnifera*)

The sperm count methodology was followed as suggested by Rastogi and Levin (1987). Animals treated with endosulfan with alcoholic extract of *Withania somnifera* for 35 days were sacrificed by quick cervical dislocation. The caput epdidymis of the both sides were dissected out and kept in normal saline. Both the caput epididymis was then cut into small pieces in a watch glass containing 2 ml of normal saline. Small pieces were macerated with the help of forcep and curved needle. The whole content was then sieved through a metallic net of mesh-size 100 µm to avoid debris. The filtrate was thereafter taken in a RBC pipette and five sperm–counts were made with the help of neubauer haemocytometer, using its RBC-counting chamber. This process of counting was repeated 5 times for each animal. Mean count was taken from these 10 readings for 10 animals in each experimental group and the control. Mean count/ml was calculated.

RESULTS AND 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 sperm parameters like, sperm count and sperm motility. After 35 days of continuous exposure of endosulfan, it was observed that endosulfan at some extent checks the process of spermatogenesis. Further changes were observed in the mice sperm count exposed to 3.0 mg/ Kg b.w for 35 days which was about 8.48 million / ml, the sperm motility observed were 53.3, the sperm mortality were recorded 44.1.

The present study indicates that oral administration of sub lethal dose of endosulfan (3.0 mg/ Kg b.w) brings about various significant changes in the level sperm count, sperm motility and sperm mortality 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 and Ratna (2003). As in the present investigation, increased evidence 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 mice. Setchell (2004) and Cram, *et al*., (2004) have observed the infertility in men and it causes due to hormonal imbalance. In present investigation regarding sperm count and sperm motility and sperm mortality, it has been observed that marked reduction in sperm count was ameliorated by using *Withania somnifera* 150 mg/kg b.w. mixed with food showed increased value in all the parameter.

Table1: Endosulfan induced male reproductive changes and their treatment with *Withania somnifera* (150mg / kg bw) for 35 days on sperm parameter

Sperm Parameter	Group of Mice		
	GROUP- I	GROUP- II	GROUP-III
Sperm count (million /mm	18.14	8.48	17.15
Sperm motility (%)	80.9	53.3	77.4
Sperm mortality (%)	19.7	44.1	22.5

N=10 Values are given as mean ±SEM for groups of six mice. Values are statistically a significant ($p<0.01$); b highly significant ($p<0.001$).

Sperm count (17.15 million/mm) as well as sperm motility (77.4 %) and sperm mortality (22.4%) as compared to control which were observed (18.14 x 10million/mm) for sperm count and 80.9 % for motility and 19.7 % was recorded for sperm mortality. 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) this is in support of present work.

The main theme of present investigation is to evaluate the possible role of *Withania somnifera* root extract to restore the Sperm count, motility and sperm mortality of mice which have initially been exposed to sublethal treatment of endosulfan pestiside.

Conflict of interest

The author declares that there is no conflict of interest.

REFERENCES

Ahmed J and Mehmood F (1998) Screening of some Indian medicinal plant for their antimicrobial property. J. Ethanopharmacology .62:183-193.

Anon (1984) Environmental Health criteria 40, Endosulfan. IPCS (International programme on Chemical Safety – WHO, Geneva).

Asmawi MZ, Kankaanranta M, Moilanen E and Vapaataslo NH (1993) Anti-inflammetry activity of Emblica officinalis Gaertn Leaf Extract. J pharmacy and pharmacology. 45.581-584.

ATSDR (2000) Toxicological Profile for Endosulfan. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Bhattacharya A, Chatterji A, Ghosal S and Bhattacharya SK (1999) Antioxidant activity of tannoid principles of Emblica officinalis(Amla). Indian J. Exp. Biol, 37:676-680.

Campoy C, Jimenez M, Olea-Serrano MF, Moreno-Frias M, Canabate F, Olea N, Bayes R and Molina-Font JA (2001) Analysis of organochlorine pesticides in human milk: preliminary results. Early Human Development. 65: 183–190.

Chitra KC, Lat Choumycandane C and Mathur PP (1999) Chronic Effect of endosulfan on the testicular function of rat. Asian Journal Of Andrology, 21-6431,1833-109.

Cram D, Lynch MO, Bryan MK, Salvado C, Mc Lachlan RI and De Krester DM (2004) Genetic screening of infertile men. Reprod. Fertil. Dev. 16 (5): 573-580.

Fawzy I, Iman, Z, Hamza A and Ihab A (2007) The Effect of an Organophosphorous Insecticide on the Hepatic, Renal and Pulmonary Tissues of Mice Fetuses. Egypt J. Med. Lab. Sci. 16(2): 99 -113.

Gomes J, Dawodu AH, Lloyd O, Revitt DM and Anilal SV (1999) Hepatic injury and disturbed amino acid metabolism in mice following prolonged exposure to organophosphorus insecticides. Hum Exp Toxicol. 18(1): 33-7.

Hileman B (1994) Environmental estrogens linked to reproductive abnormalities, Cancer, C & EN: 19-23.

Ihantola-V ormisto A, Summanen J, Kankaanranta H, Vuorela H, Ashwani ZM and Moilanen E (1997) Antiinflammatory activity of extracts from leaf of Phyllanthus emblica. Plant medica. 63: 518- 524.

IPCS/CEC (1999) International chemical safety cards: 0742, Endosulfan (Mixed isomers)

Jeena KJ, Joy KL and Kuttan R (1999) Effect of Emblica officinalis, Phyllantus amarus and Picrorhizia kurroa on NNitrosodiethyl amine induced hepatocarcinogenesis. Cancer letter 136:11-16.

Jose JK, Kuttan Y and Kuttan R (2001) Antitumor activity of Emblica officinalis. J. Ethanopharmacology. 75:65-69.

Khan PK and Sinha SP (1996) Ameliorating effect of vitamin con murine sperm toxicity induced by three pesticides (endosulfan, phoshphamidon and mancozeb). Mutagenesis, 11:33-36..