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Study on mitigating role of fenugreek aqueous extract against gonadotoxic induced by permethrin on male swiss albino mice (*Mus musculus*).

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Abstract- The aims of the present study were to assess the mitigating role of Fenugreek extract against gonadotoxicity induced by permethrin on Male *Mus musculus*. For the research experiment, model animals *Mus musculus* of the same age group were selected for the research experiment and divided into four experimental variant groups. After the completion of treatment, Sperm counting and Wt. of reproductive organs parameter were included in this research to investigate the mitigating role of Fenugreek extract against gonadotoxicity induced by permethrin. The data obtained from the control and treated group are expressed as mean \pm SE and 't' test was used to determine by Excel Software and the level of significance is $p < 0.05$. The result of sperm count was 175.4 ± 3.5 (C), 90.02 ± 2.8 (PM), 188.6 ± 3.3 (FG) and 133.1 ± 2.2 (PM + FG) and result of reproductive organ wt. of testis - 0.14 ± 1.3 (C), 0.12 ± 0.8 (PM), 0.17 ± 1.0 (FG) and 0.13 ± 1.2 (PM + FG), Seminal vesicles wt. was - 0.41 ± 0.3 (C), 0.36 ± 0.5 (PM), 0.43 ± 0.2 (FG) and 0.39 ± 0.3 (PM + FG) and Vas deferens wt. was 0.21 ± 0.4 (C), 0.17 ± 0.3 (PM), 0.22 ± 0.2 (FG) and 0.23 ± 0.3 (PM + FG) and it is a significant difference to control and treated groups at $p < 0.05$.

Key words: Gonadotoxicity, *Mus musculus*, Sperm count, Seminal vesicles, Testis, Vas deferens.

INTRODUCTION

Different fertilizers such as fungicides, pesticides, herbicides and insecticides have long been used in agricultural practices as they are effective and quick.^{1,2} There are several definitions of pesticides; the Food and Agriculture Organization (FAO) of the United Nations (UN) defines pesticide as, "any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production,

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processing, storage or marketing of food, agricultural commodities, wood and wood products or animal food stuffs or which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies".^{3,4} Although these man-made chemicals are playing a pivotal role in meeting the food, cotton fibre and tobacco demand of escalating population and control of vector-borne diseases, most of the applied pesticides get dispersed in the environment and affects the health of unprotected agricultural and industrial workers.⁴ Several studies have described the adverse effects of pyrethroids on the liver, gastrointestinal, respiratory, neurological, and immunological systems, as well as other organ systems.

Pyrethroids are metabolized in the liver and their mechanism of action is by acting on voltage-sensitive sodium channels to prolong the closure time, with consequent reduction in action potential threshold and repetitive firing.⁵ Studies in rats showed that permethrin affects male reproductive functions by directly reducing the motility of mature sperm cells.⁶ Chronic exposure to pesticides containing pyrethroids may also cause disturbances in endocrine functions relating to androgen action, as they interact competitively with human androgen receptors and sex hormone binding globulin.⁷ The use of different medicinal plant extract both ethanolic and aqueous used for the treatment of adverse effects is as old as humanity. The main benefits of herbal-based medications include wide availability, their low price, and no incidences of serious harmful effects. According, to the number of experimental research work on natural remedies and species of medicinal plant that could protect both humans and animals against fertilizers toxicity has increased.

water to get rid of extraneous matter, air-dried, and grind into a fine powder in a mixer. The powder of fenugreek seed will be mixed with distilled water (25 gm fenugreek seed powder 500ml distilled water) and then centrifuged at 10,000 rpm and the supernatant will be collected in a test tube. The supernatant will be used as the final aqueous extract for feeding the animals. This freshly prepared Fenugreek aqueous extract will be administered to mice at a dose level of 100 mg/kg body weight accordingly.

MATERIALS & METHOD

(a) Maintenance of experimental model animals -

For the research experiment, 15 adult Male Swiss albino mice of same age and average weight of 28-35 gm body weight were kept in a polypropylene cage under hygienic conditions in a well-ventilated room and were divided into equal number of experimental animals in three groups one group was considered the control (C) group and the other two were considered treated (PM, FG & PM + FG) groups.

(b) Preparation of Plant Aqueous Extract -

Fenugreek seeds will be washed with distilled

METHODOLOGY

Sperm counting - After the completion of 20 days of treatment, the control and treated group mice were sacrificed by cervical dislocation and epididymal parts were exposed into watch glass. Each epididymal part minced with forceps and needle in 1 ml of normal saline and were sieved by a metallic filter to avoid the tissue debris in seminal content. Sperm count was done with a Neubauer haemocytometer⁸ at 40X under compound microscope.

Weight of reproductive organ - Experiment mice were sacrificed after completion of treatment and exposed the reproductive part (Testis, Cauda epididymis, Caput epididymis, Vas deferens, Seminal vesicles) and weight was done by digital balance.

STATISTICAL ANALYSIS

Data were analyzed using excel 2019 software. In each experimental variant obtained data from the control and treated groups are expressed as Mean ± SE and for the comparison of data between the control and treated groups unpaired t-test was used to determine at significant level $p < 0.05$.

Table 1- Effect of Permethrin and Fenugreek at different concentration on weight of reproductive organs of *Mus musculus*.

Experimental variant	Dose (20 Days)	Testis	Seminal vesicles	Vas deferens
Control	Distilled water	0.14 ± 1.3	0.41 ± 0.3	0.21 ± 0.4
Permethrin (PM)	130mg/kg. B.wt.	0.12 ± 0.8	0.36 ± 0.5	0.17 ± 0.3
Fenugreek (FG)	100 mg/ kg. B. wt.	0.17 ± 1.0	0.43 ± 0.2	0.22 ± 0.2
Permethrin (PM) + Fenugreek (FG)	130mg/kg. B.wt. + 100 mg/ kg. B.wt.	0.13 ± 1.2	0.39 ± 0.3	0.23 ± 0.3

Values of the result expressed as Mean ± SE and statistical analysis of p value ($p < 0.05$) were analysed using Unpaired 't' Test for multiple comparison.

Table 2- Effect of Permethrin and Fenugreek at different concentration on sperm count of *Mus musculus*.

Experimental variant	Dose(20 Days)	Sperm count
Control	Distilled water	175.4 ± 3.5
Permethrin (PM)	130mg/kg. B.wt.	90.02 ± 2.8
Fenugreek (FG)	100 mg/ kg. B. wt.	188.6 ± 3.3
Permethrin (PM) + Fenugreek (FG)	130mg/kg. B.wt. + 100 mg/ kg. B.wt.	133.1 ± 2.2

Values of the result expressed as Mean ± SE and statistical analysis of p value ($p < 0.05$) were analysed using Unpaired 't' Test for multiple comparison.

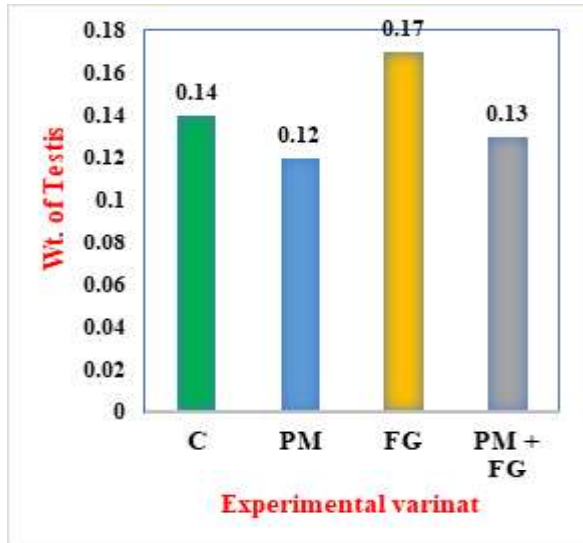


Fig. A

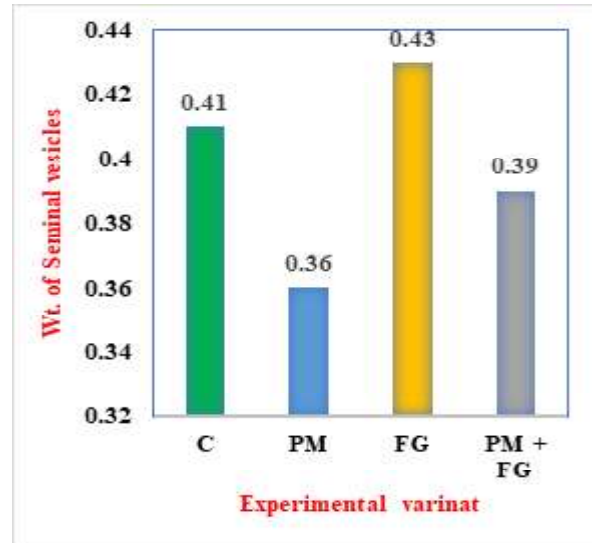


Fig. B

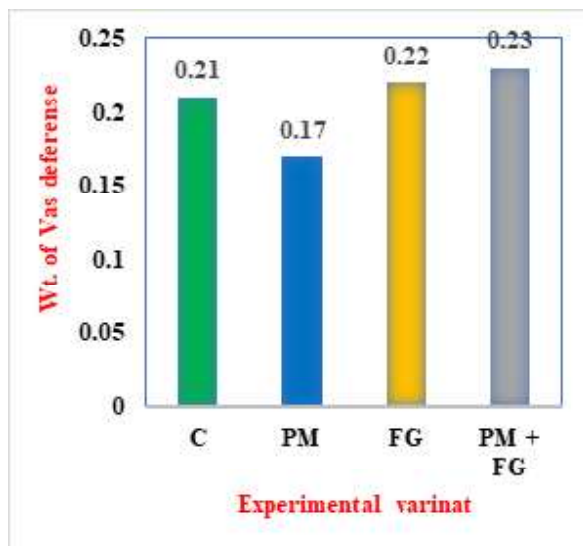


Fig. C

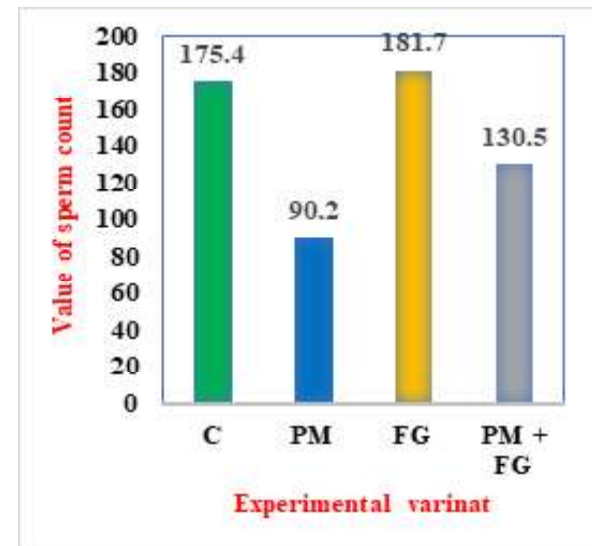


Fig. D

Figure A, B, C and D - Histogram represents mean value of sperm count in caput epididymis and wt. of reproductive organs in 20 days of treatment duration.

RESULT & DISCUSSION

Experimental animals treated (Group III) with Permethrin (130 mg/kg B.wt./day) resulted in decrease the wt. of reproductive organ and sperm count and there was a statistically highly significant difference between these Group III (Permethrin) and Group I (Control). Experimental animals treated with Fenugreek extract (100 mg/kg B. wt./day) resulted in increase the wt. of reproductive organ and sperm count as compared to the control group and there was not a statistically significant difference between Group II (FG) and Group I (C). Experimental animals treated with Permethrin + Fenugreek extract (130 + 100 mg/kg/day)

resulted in an increase the wt. of reproductive organ and sperm count and there was a statistically significant difference between Group II (FG) and Group IV (PM + FG). These results are summarized in Tables: 1, 2, and Figure A, B, C, D.

The Wt. of testes, accessory sex organs, caput and cauda epididymis are the primary indicators of a possible alteration in androgen status.⁹ Furthermore, according to Maina (2008)¹⁰ testicular weights are considered valuable in toxicity studies because the changes in the testis weight reflect changes in seminiferous tubules or interstitial edema.

Testicular weights also reveal their sensitivity to toxicity due to perturbations in rapidly dividing cells, physiology and hormones.¹¹ Reduced organ weights of testis, caput and cauda epididymis, seminal vesicle and vas deferens were also observed in the present study. Spermatogenesis is the process by which mature spermatozoa develop from germ cell inside seminiferous tubule. Damage to the spermatozoa or their precursors can result in reversible or irreversible impaired spermatogenesis, depending on the stage of differentiation affected by the chemical.¹² Damage to spermatogonia causes impaired sperm production and decreased fertility because of changes in the cell number, structure, motility, or viability of spermatozoa.¹³ However, little is known about plant extract acting as a mitigating agent against pesticides induced gonadotoxicity. Administration of Fenugreek extract with Permethrin pesticides treatment clearly restored the gonadotoxic effect. Accumulating evidence suggests that the protective effect of plant materials could be attributed to their anti-oxidative properties.¹⁴ It is clear from the obtained results that Fenugreek aqueous extract has the potency to mitigate the gonadotoxicity induced by the pesticides Permethrin on Reproductive organs of Male *Mus musculus* (Swiss albino mice).

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