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Protective Role of Vitamin-C and Resveratrol on Spirotetramat Induced Reproductive Toxicity in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The study was aimed to investigate the potential protective effects of Vitamin-C and Resveratrol on reproductive toxicity induced by spirotetramat in male Wistar rats. To evaluate the protective role of Vitamin–C and Resveratrol, six groups of male Wistar rats ; Group-I (Control), Group-II (Toxicity Control-Spirotetramat 667mg/kgbw), Group-III (Treatment control- Vitamin-C 200mg/kgbw+ Resveratrol 20mg/kgbw), Group IV (Treated 1- Spirotetramat + Vitamin-C 200mg/kgbw), Group-V (Treated 2- Spirotetramat + Resveratrol 20mg/kgbw) and Group-VI (Combination) were framed. The groups were administrated by Spirotetramat (667mg/kgbw), Vitamin-C (200 mg/kgbw) and Resveratrol (20mg/kgbw) for 4 weeks as per the prescribed protocol. Spirotetramat induced testicular toxicity by affecting various parameters of reproductive health. Treatments with the

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Vitamin-C and Resveratrol showed a significant fortification of reproductive system and associated parameters by reducing the ill effects of Spirotetramat thereby enhancing sperm motility and suppressing sperm abnormality. Spirotetramat showed a decline in in Superoxide dismutase (SOD) Reduced Glutathione (GSH) and Catalase (CAT) with elevation in Malondialdehyde (MDA). After Co-administration of Vitamin-C and Resveratrol levels were found to be normalized revealing their potential efficacy against oxidative stress. The results of this study showed that spirotetramat exposure caused significant reductions in sperm count, motility, and viability in male rats. However, treatment with Vitamin-C and Resveratrol was found to mitigate these effects. Specifically, co-treatment with Spirotetramat and Vitamin-C or Resveratrol resulted in significant improvements in sperm count, motility, and viability in male Wistar rats.

Keywords: Reproductive toxicity; resveratrol; spirotetramat; testes; vitamin-c.

1. INTRODUCTION

Spirotetramat is a systemic insecticide that belongs to the chemical class of tetramic acid derivatives. It is used to control a wide range of insect pests in crops such as cotton, citrus, vegetables, and fruits. Spirotetramat works by inhibiting lipid synthesis in insects, leading to the disruption of the insect's energy metabolism and eventual death [1]. Spirotetramat is a systemic insecticide and acaricide that is used in agriculture to control a variety of pests, including aphids, whiteflies, and spider mites [2]. While spirotetramat has been shown to be effective in pest control, it can also be toxic to animals, including rats [3]. While spirotetramat is effective against insect pests, it is important to use the pesticide according to the label instructions and to follow all safety guidelines to minimize the risk of harm to humans and the environment. Overuse or misuse of spirotetramat can lead to the development of resistance in insect populations and can also cause adverse effects on non-target organisms, such as beneficial insects and wildlife. Spirotetramat can be absorbed and converted into metabolites in rats, and that the metabolite residues in various organs and tissues significantly differ [4]. In rats orally fed with Spirotetramat for 7 days, loss of weight and damage to the liver and the genitals has been found [5].

For reproductive toxicity, Spirotetramat has been found to cause DNA (deoxyribonucleic acid) damage by production of Reactive oxygen species (ROS) [6]. It has been observed that the oxidative damage to the testicular cells induced by various xenobiotics, products of abnormal metabolism or ROS can result in testicular dysfunction leading to male infertility [7,8].

Vitamin-C (Ascorbic acid) has been found to be a radical scavenger by acting against lipid peroxidation [9]. It is a water soluble vitamin acting as an antioxidant [10] and protective against oxidative stress [11] by preserving spermatogenesis in an animal model [12] as well as in the medical treatment of male factor infertility [13]. Vitamin-C has been shown to improve sperm motility and enhances semen quality and fertility of rats [14].

Resveratrol (3,5,4′-trihydroxystilbene) is a polyphenolic compound found in the skin of red grapes, red wine and other botanical extracts. It has been found to have various biological activities, including anti-carcinogenic, anti-inflammatory and telomerase enhancing activity, to inhibit cell senescence, protect the cardiovascular system and influence apoptosis [15-18]. It increases sperm production, reduce apoptosis in germinal cells, and protect against environmental toxins [19]. as a potent anti-oxidative effect via prevention of LPO [20]. Resveratrol triggers a variety of established cellular and molecular effectors, the most remarkable of which is the estrogen response systems [21]. Resveratrol modulates the estrogen-response systems and may therefore be involved in male reproduction.

Although Vit-C and Resveratrol has been reported to prevent ill effects of other pesticides but the protective remedies against Spirotetramat induced reproductive toxicity are scare. That is why; this work was designed to study the possible cause of Spirotetramat toxicity and ameliorative role of Vit-C and Resveratrol.

2. MATERIALS AND METHODS

2.1 Chemicals

All the chemicals of high grade purity used in this study were purchased from reputed companies. Spirotetramat was purchased from Bayer India

Limited. Various Kits were used for detection of oxidative stress parameters were purchased from Hi-media India limited. Vitamin-C and Resveratrol were purchased from Sigma Aldrich & Co. and other chemicals were purchased from local suppliers.

2.2 Experimental Model

Male Wistar rats weighing about 200-220g were purchased from Pinnacle Biomedical Research Institute approved wide institutional ethical committee guidelines with registration number 1824/PO/Rc/S/15/CPSCEA. All experiments were carried under prescribed ethical guidelines.

2.3 Experimental Setup, Stress Induction and Dosage

For sub-acute toxicity, a single dose of Spirotetramat of about 667mg/kgbw/day equal to 10000ppm dissolved in water was administrated orally for 4 weeks along with Vitamin-C (200mg/kgbw) and Resveratrol (20mg/kgbw) as per experimental protocol. The experimental setup was divided into six groups with six rats of equal weight in each group and was framed as per the plan Table 1.

2.4 Sample Preparation

Using di-ethyl ether as anesthesia, by asphyxiation rats were sacrificed after 4 weeks and blood was collected from retro orbital plexus. Testes and epididymis were detached and soon after weighted. Relative organ weights were calculated using ratio of organ weight/ body weight x100 formula. 10% buffered formalin was used to fix one testes for histopathological study. Second testes was homogenized using ice-cold KCl_4 (150mM) with a ratio of tissue weight to homogenate equal to 1:10. From this homogenate, serial dilutions were obtained for calculation of concentration of LPO, GSH, Total Protein, SOD and CAT. Serum was obtained after centrifugation at 3000 rpm for 25 mins. at 4°C.

2.5 Sperm Parameters

For determination of sperm count, sperm motility and abnormalities in sperms, epididymus was crushed in 5ml of saline and incubated for half an hour at 37°C for smooth flow of sperms from tubes of epididymus. A warm microscope slide was loaded with one drop of mixture. Using phase contrast microscope, the percentage of motility was determined at a magnification of 400X. Sperms were allowed to dry after removal of coverslip and stained with eosin (1%) to observe the morphological abnormalities along different fields. Neubauer heamoytometer was used to calculate total sperm count [22].

2.6 Assessment of Oxidative Stress, Histopathology and Testosterone

GSH concentration of testes tissue homogenate was calculated for production of a yellow compound 5-thiol-2-nitrobenzoate (Dooran et al. 1978). LPO was determined for production of Malondialdehyde (MDA) to produce pink compound on reacting with thiobarbituric acid (TBA) [23]. The action of SOD enzyme in testis homogenate was revealed [24] by determining the ability of SOD to inhibit the auto-oxidation of epinephrine to adrenochrome and its derivatives. CAT was determined by calculating the exponential disappearance of H2O2 at 240 nm and expressed in units/mg of protein [25]. The total protein was then determined as per the Lowry method [26]. The range of absorbance was observed on Shimadzu spectrophotometer (UV-160). A solution of 10% formalin was used to fix the testes, with 5µm thickened section stained with Hematoxylin and Eosin for microscopic examination. Testosterone level was calculated from serum obtained from overnight blood and interstitial fluid obtained from tunica albuginea centrifuged at the rate of 54xG for 20min using automated Erbachem analyzer by chemiluminescence method.

Table 1. Experimental setup and group distribution

Groups		Remarks
I	Control	Control (Distilled Water)
II	Toxicity Control	Toxicity Control (Spirotetramat 667mg/kgbw)
III	Treatment Control	Vitamin-C (200mg/kgbw)+ Resveratrol (20mg/kgbw)
IV	Treated 1	Spirotetramat and Vitamin-C (200mg/kgbw)
V	Treated 2	Spirotetramat and Resveratrol (20mg/kgbw)
VI	Combination	Vitamin-C (200mg/kgbw)+ Resveratrol (20mg/kgbw) and Spirotetramat (667mg/kgbw)

2.7 Statistical Calculations

Statistical calculations were expresses in terms of Mean ± SD to reveal the results using Students "t' test.

3. RESULTS

3.1 Influence of Spirotetramat on the Reproductive Organ Weights

The relative weight of testes and epididymis on treatment with Spirotetramat was found to be decreased significantly (P<0.05) on calculations. In groups treated with Vit-C and Resveratrol no significant change of weight loss was observed individually and in combination (Fig. 1 and Fig. 2).

3.2 Defensive role of Vitamin-C and Resveratrol

In toxicity control, a significant (P<0.01) reduction in number of sperms/gram of epididymus was reported (Fig. 3) with increased abnormality (P<0.05) and decreased motility (P<0.001). However with the treatment of Vit-C and Resveratrol a significant increase in number was reported up to the mark of normal with enhanced motility and decreased abnormality (Fig. 4) both in combination and individually (Fig. 5). The treatment with Vit-C and Resveratrol has reduced the changes brought about by Spirotetramat induced parameters.

3.3 Oxidative Stress

Reduced Glutathione (GSH) was significantly (P<0.05) found to be in reduced concentrations in testicular tissue after activity of Spirotetramat (Fig. 6). However the production of Malondialdehyde (MDA) was found to be elevated after treatment with Spirotetramat. The levels of Superoxide dismutase (SOD) and Catalase (CAT) were also found to be decreased after induction of treatment but the oral administration of Vit-C and Resveratrol have brought the levels up to the mark of normal to some extent (Fig. 7 and Fig. 8).

3.4 Change in Testosterone

A significant (P<0.001) decline in testosterone levels was estimated in the serum obtained from rats toxicity control group induced with Spirotetramat as compared to control group. However in combination and individually treated groups with Vit-C and Resveratrol, a slight increase in the levels of testosterone was observed possibly due to toxicity of Spirotetramat when compared to control (Fig. 9).

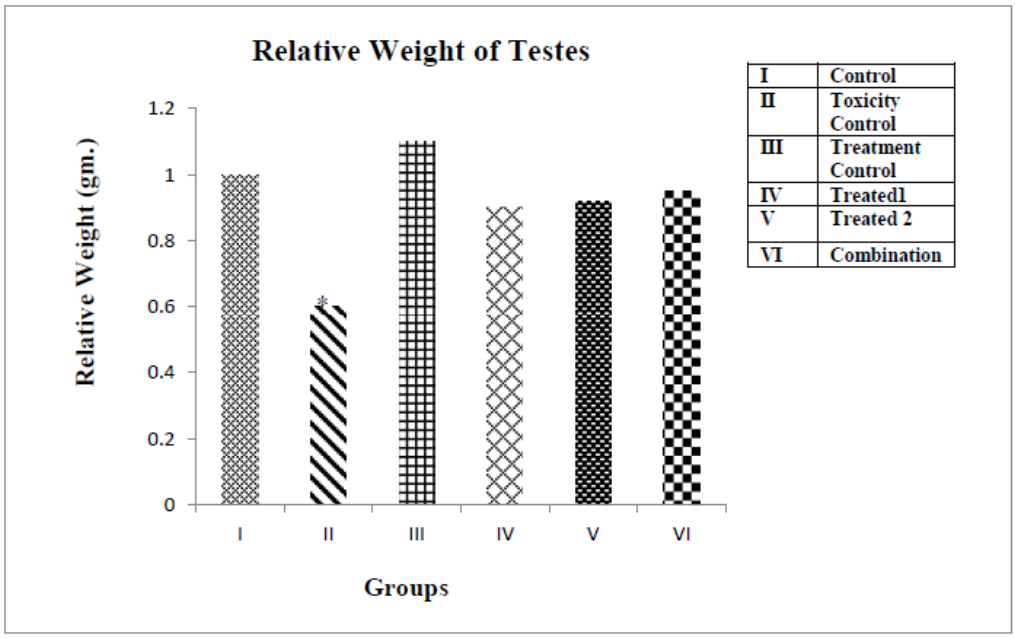

Fig. 1. Effect of Vit-C and Resveratrol on relative weights of testes in Wistar rats induced with Spirotetramat toxicity (mean ± SE *P<0.05 with respect to control

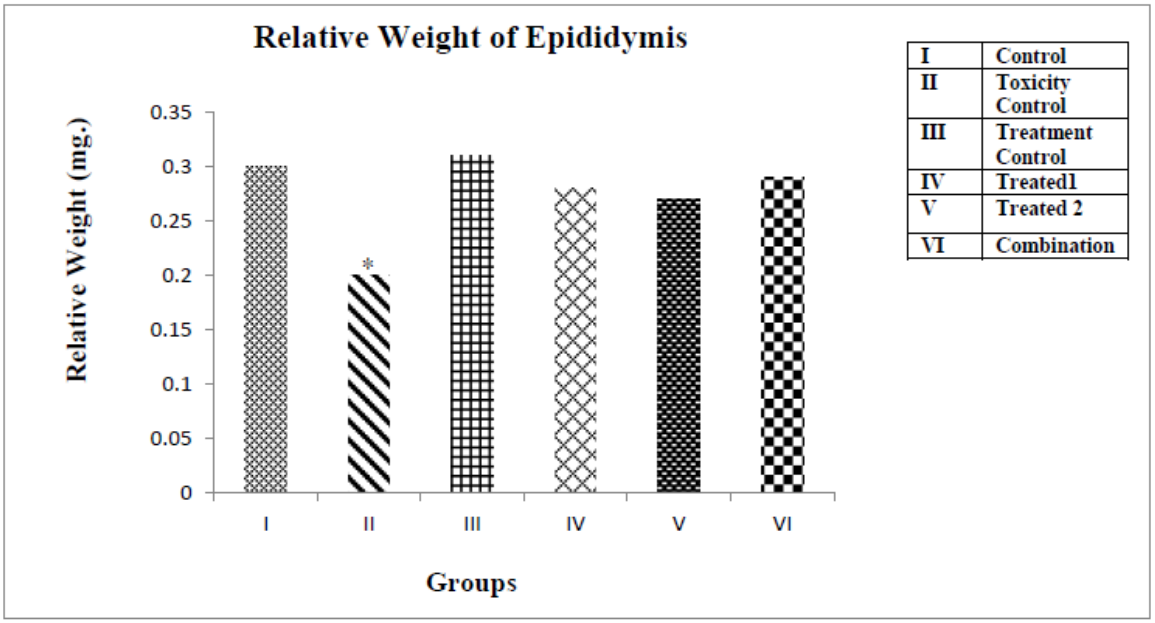

Fig. 2. Effect of Vit-C and Resveratrol on relative weights of epididymis in Wistar rats induced with Spirotetramat toxicity (mean ± SE *P<0.05 with respect to control

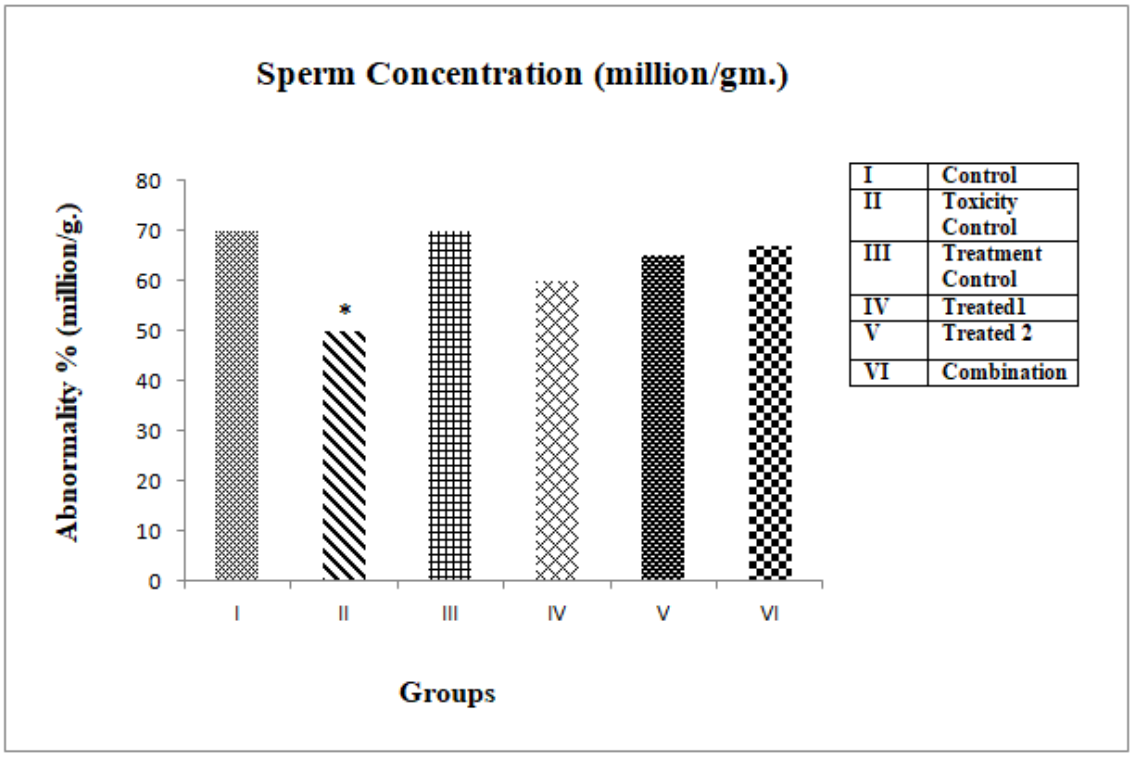

Fig. 3. Change in epididymal Sperm concentration by Vit-C and Resveratrol in Wistar rats induced with Spirotetramat toxicity (mean ± SE *P<0.01) with respect to control

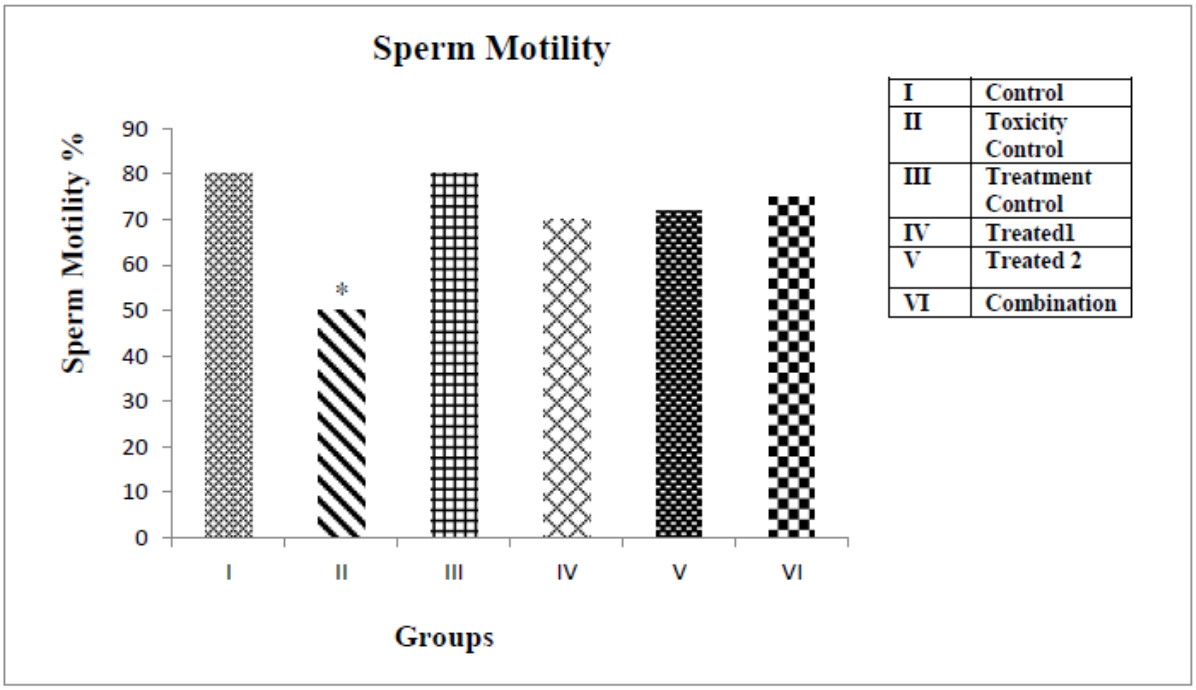

Fig. 4. Change in epididymal Sperm motility by Vit-C and Resveratrol in Wistar rats induced with Spirotetramat toxicity (mean ± SE *P<0.001) with respect to control

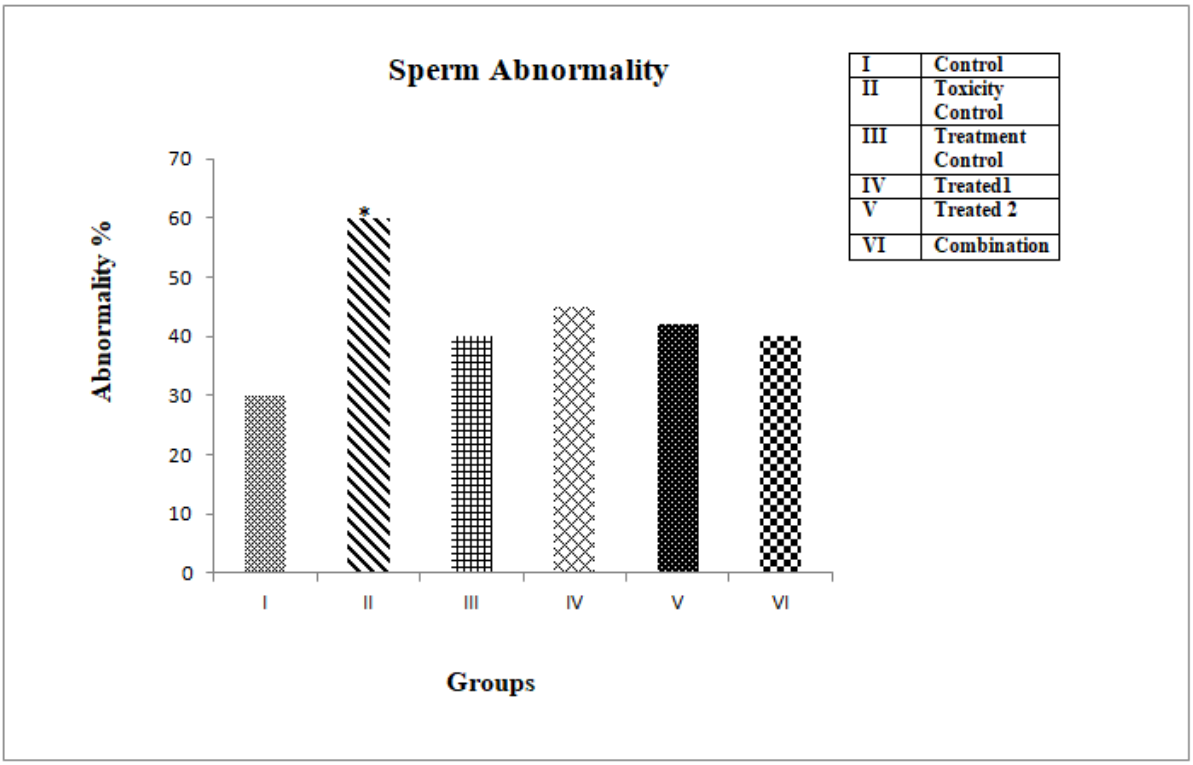

Fig. 5. Change in epididymal Sperm abnormality by Vit-C and Resveratrol in Wistar rats induced with Spirotetramat toxicity (mean ± SE *P<0.05) with respect to control

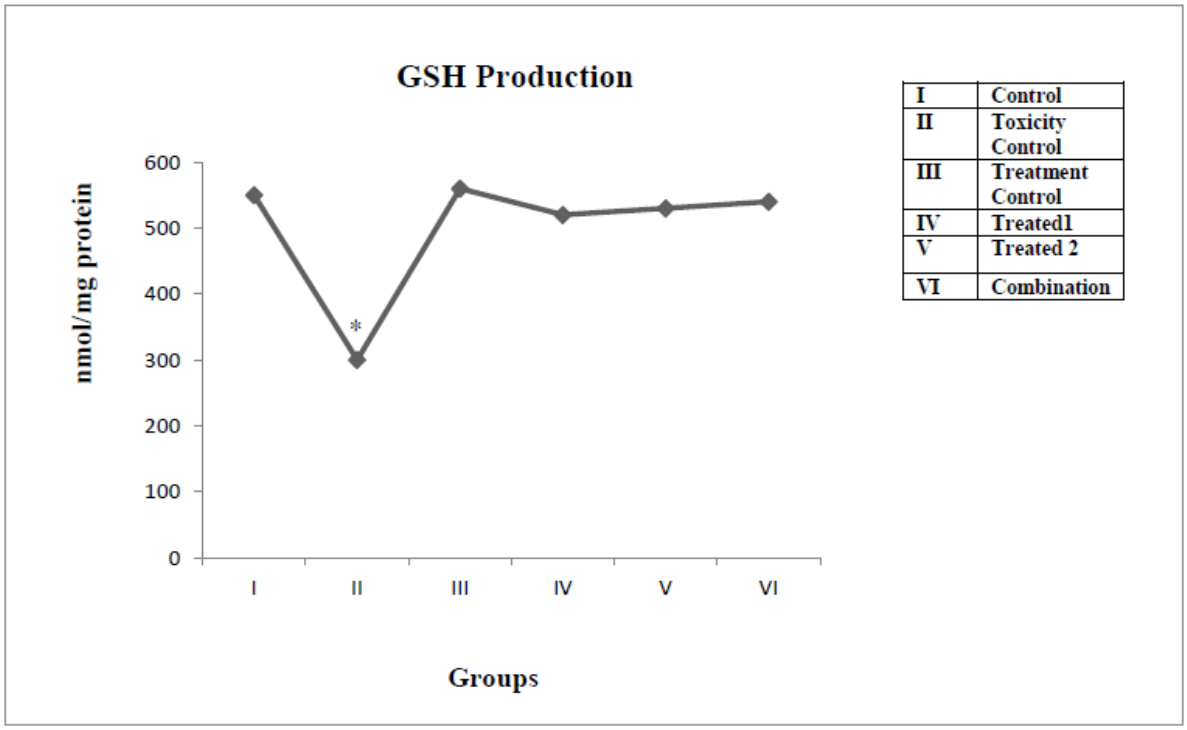

Fig. 6. Change in testicular Production of Reduced Glutathione (GSH) by Vit-C and Resveratrol in Wistar rats induced with Spirotetramat toxicity (mean ± SE *P<0.05) with respect to control

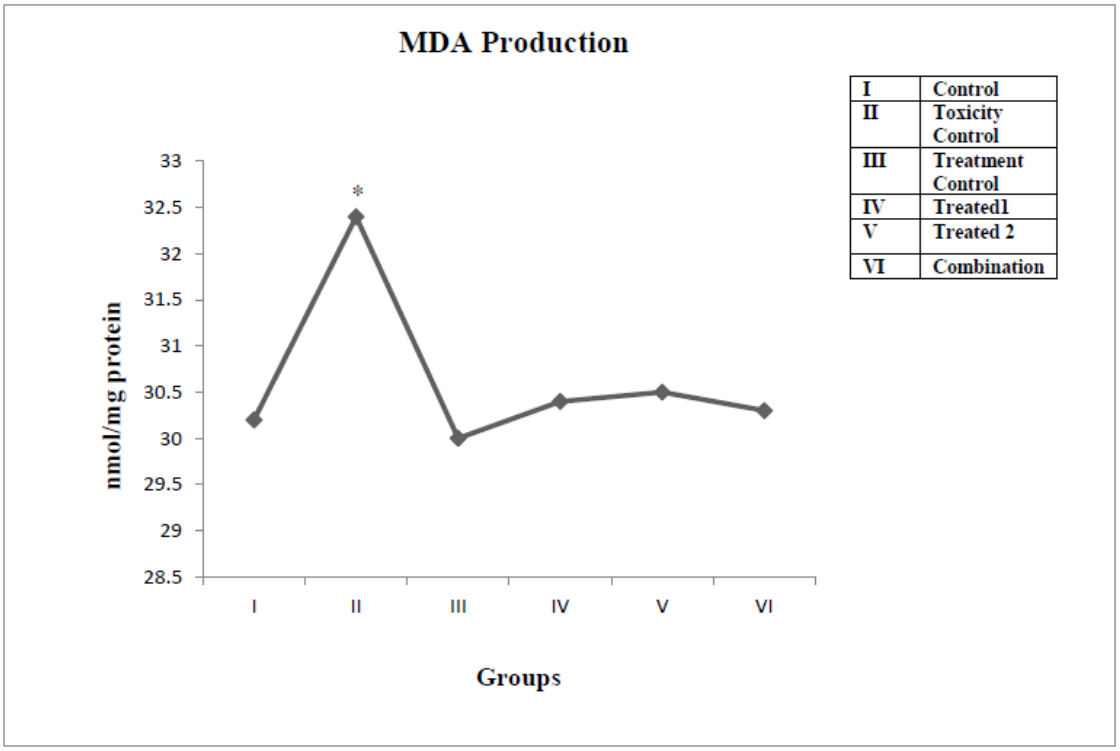

Fig. 7. Change in testicular Production of Malondialdehyde (MDA) by Vit-C and Resveratrol in Wistar rats induced with Spirotetramat toxicity (mean ± SE *P<0.05) with respect to control

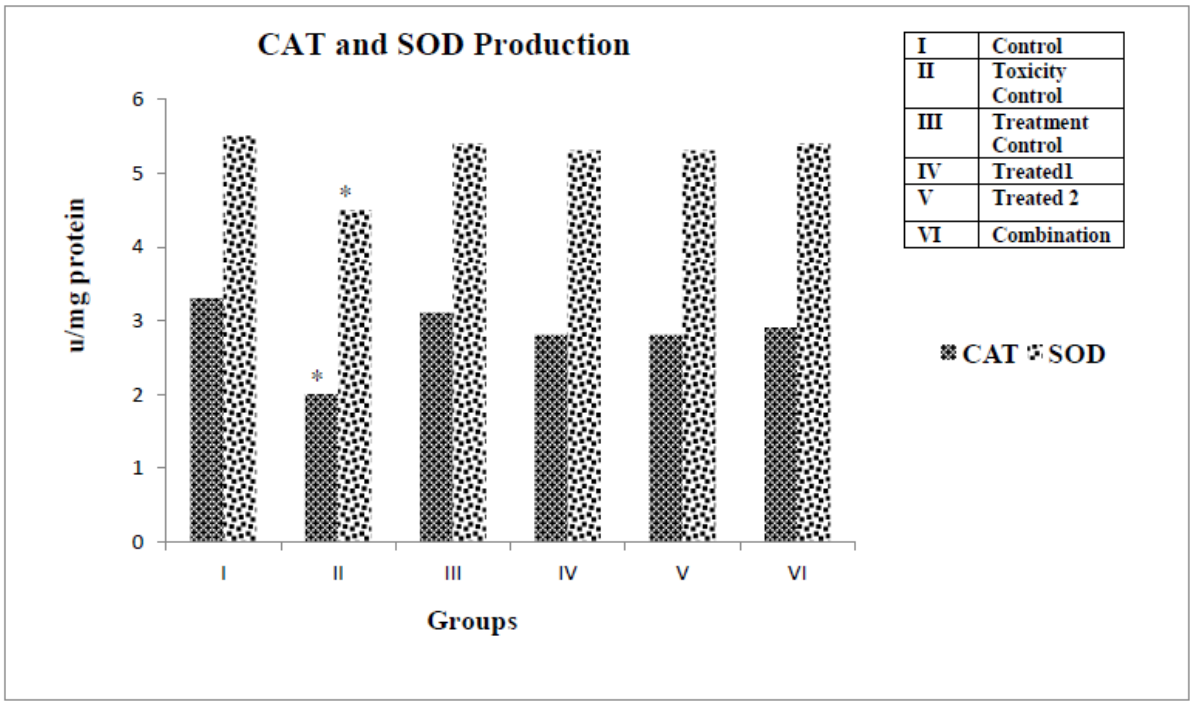

Fig. 8.Change in testicular Production of Catalase (CAT) and Superoxide dismutase (SOD) by Vit-C and Resveratrol in Wistar rats induced with Spirotetramat toxicity (mean ± SE *P<0.05) with respect to control

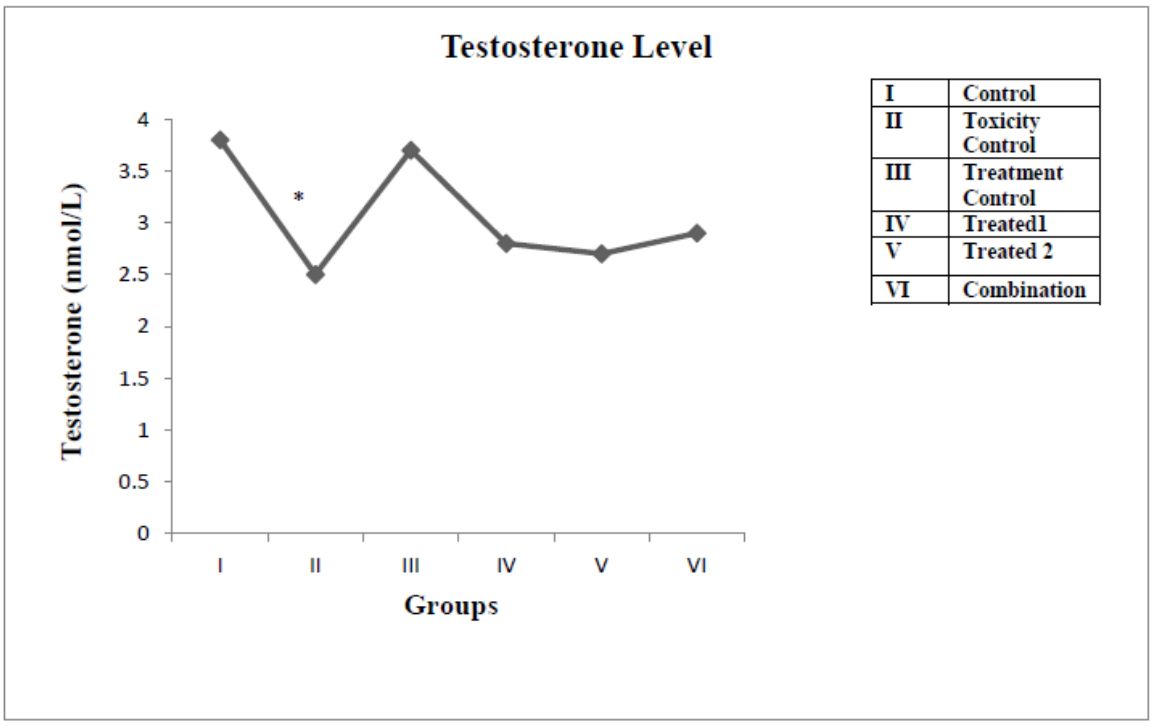

Fig. 9. Change in Testosterone levels by Vit-C and Resveratrol in Wistar rats induced with Spirotetramat toxicity (mean ± SE *P<0.05) with respect to control

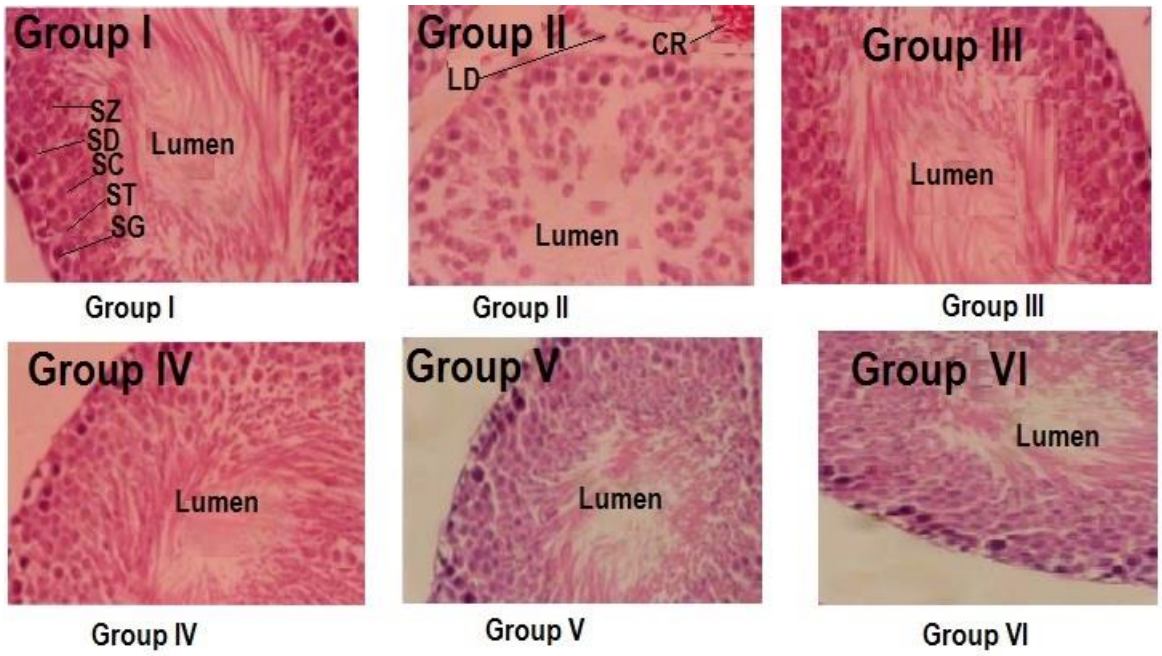

Fig. 10. Exposure of the seminiferous tubules among different groups displaying normal to disrupted organization of germinal epithelium cells at different stages of spermatogenesis. Spermatogonia (SG), Sertoli cell (ST), spermatocytes (SC), spermatids (SD) and spermatozoa (SZ) Leydig cells (LD) and cramping of blood vessels (CR) between the tubules. (H and E, X400)

3.5 Histological Impact of Vitamin-C and Resveratrol

In toxicity control group, a scathing deterioration of seminiferous tubules with diminution and depletion in germinal epithelial cells and leydig cells was reported as clearly visible; possibly due to action of Spirotetramat. Among tubules blood vessels have been found to be in a cramming position with cellular debris in lumen. The groups treated with Vit-C and Resveratrol displayed a standard testicular morphology and spermatogenesis with a trivial relapse of spermatozoa and spermatids (Fig. 10).

4. DISCUSSION

Pesticides are the frontline chemicals used by farmers to battle with pests. They not only affect the target organisms but also leave their impact on non-target organisms their by causing several maladies. Spirotetramat is a newly introduced insecticide that is used deal with the control of variety of agricultural insects like aphids, whiteflies, bugs etc. [27]. Being toxic in nature, Spirotetramat has affected several non-target species like liver and genitals in rats [5]; accumulated as metabolits in various organs [4] and activated acid phosphatases [28]. In humans it causes skin irritation [29]. It has also been found to cause oxidative injury. Therefore, concern over Spirotetramat for reproductive toxicity is increasing and several studies have been now focused on Spirotetramat toxicity to the eco-environment and non-target organisms. Hence, there is a need for exogenous antioxidants to decrease oxidative stress in testes as well as to positively regulate the spermatogenic cycle and steroidogenic function. Therefore, the protective role of Vit-C and Resveratrol came into force.

The present study was thus planned to reveal the role of Vit-C and Resveratrol against testicular toxicity. Results confer that a reduction in relative weight of testes and epididymis with a decreased quality of sperm cells has been occurred due to ill effects of Spirotetramat. These effects may be associated with spermatogenic damage, apoptosis in germ cells, and dysfunction of leydig cells.

The results in this study shows change in morphology, motility and count of sperm cells after treatment with spirotetramat. A significant decrease in testosterone levels and decrease in

caudal sperm storage has been reported. An elevation in MDA levels potentially due to generation of ROS (Reactive oxygen species) may be due to activity of Spirotetramat toxicity. GSH, CAT and SOD were also low in toxicity control group as compared to other groups. Several evidences have suggested that vitamins have a potential role against oxidative stress and production of ROS cause damage to testicular tissue [30]. Co-administration of Vit-C and Resveratrol in Spirotetramat induced testicular toxicity shows decrease in histopathological changes with protective cover against Spirotetramat in bringing down the cell count. The decrease in MDA and restoration of normal parameters has arised due to normalization of antioxidant activity after treatment with Vit-C and Resveratrol. Vit-C by acting on H_2O_2, neutralize it and helps in protection of plasma membrane from LPO [31]. To inference, it may be assumed that oxidative stress contributes to the testicular toxicity induced by Spirotetramat in male wistar rats. Vitamin-C and Resveratrol both have shown a potential protective role to combat with Spirotetramat induced testicular toxicity and oxidative stress in rats. The protective role of Vitamin-C and Resveratrol may be due to their antioxidant activity [32].

5. CONCLUSIONS

The current study reveals that co-administration of Vitamin-C and Resveratrol showed a promising activity against the ill effects of Spirotetramat insecticide and enhanced the reproductive system functioning their by reducing the adopted toxicity. This outcome suggests that for combating reproductive toxicity, alternatives like Vit-C and Resveratrol can be used as alternative therapies for enhancing reproductive functions.

ETHICAL APPROVAL

Pinnacle Biomedical Research Institute approved wide institutional ethical committee guidelines with registration number 1824/PO/Rc/S/15/CPSCEA. All experiments were carried under prescribed ethical guidelines.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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