Full Length Research Paper

Ameliorating effect of *Withania somnifera* on acephate administered male albino rats

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This study was performed to investigate the effects of *Withania somnifera* (family: Solanaceae) on the antioxidant status and hormonal level in acephate administered rats. Oral administration of acephate (75 mg/kg body weight/day) for 15 and 30 days caused a significant decrease in serum testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentration when compared with control group. Serum testosterone, LH and FSH concentration were increased in group IV and group V indicating a positive influence of *W. somnifera* on acephate administered rats. The changes in the antioxidant parameters were accompanied by an increase in testicular lipid peroxidation and reduction in glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activity. The level of lipid peroxidation was reduced whereas GSH content, SOD and catalase activity were elevated after treatment with *W. somnifera* at the dose level of 100 mg/kg body weight/day. In conclusion, this study showed that acephate apart from being a hormonal disrupter also causes oxidative stress which contributed to reproductive toxicity in the male rats. The protective effects of *Withania* on reproductive toxicity and oxidative stress have also been shown as evidenced by a clear attenuation of acephate-induced hormonal imbalance and oxidative stress.

Key words: Testosterone, antioxidant, lipid peroxidation and oxidative stress.

INTRODUCTION

Human reproductive health is a major health problem, indicating the decline in male reproductive health and increase in population of infertile males. The global magnitude of infertility is that 60 to 80 million couples suffer infertility each year and this highlights the need for research in this field to study the decline in reproductive health and effect of environmental antiandrogens (WHO, 1996).

Pesticides are the most common xenobiotics present in the environment and causing toxicity. Prolong exposure of pesticides affected the normal functioning of different organ system and produced many clinical effects (Azmi et al., 2006; Sharma and Singh, 2010). They also alter the reproductive function by altering sperm count and sperm shape, alter sexual behavior or increase infertility in animals and human beings (Chang et al., 2004; Okamura et al., 2005; Jensen et al., 2006; Joshi and Sharma, 2011; Joshi et al., 2011).

Organophosphate pesticides belong to a group of insecticides that act by inhibiting acetylcholinesterase (AChE) activity in insects and mammals (Spassova et al., 2000; Cabello et al., 2001). Besides inhibiting cholinesterase (ChE), oxidative stress has been recently proposed as a main toxicity mechanism for organophosphorus (OPs) both in acute and chronic poisoning cases (Mostafalou et al., 2012). There have

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been increasing concerns about the effects of various organophosphate insecticides in humans and animals. These include cholinergic and non-cholinergic biological disturbances (Quistad et al., 2001; Bomser et al., 2002; Gordon and Mack, 2003). Acephate is an organo-phosphate insecticide used to kill insects by direct contact or ingestion and disrupts their normal nervous system functions by phosphorylating the active site of the acetylcholinesterase enzyme, rendering it inactive (Kumar, 2004). It is used worldwide because of easy availability. A number of studies were conducted on the toxicity of acephate on different organisms and indicated that it is a potent neurotoxic, mutagenic, carcinogenic, and cytotoxic compound (Singh and Jiang, 2002).

Herbal medicines have been widely used all over the world since ancient times and have been recognized by physicians and patients for their better therapeutic value as they have fewer adverse effects as compared with modern medicines (Goldman, 2001; Jasuja et al., 2012). *Withania somnifera* has been in use for over 2500 years to treat all kind of diseases and human ailments (Bhattacharya et al., 2001). Various investigators reported useful phytochemical constituents in *W. somnifera* possess antiserotogenic, anticancer, anabolic activity and beneficial effects in the treatment of arthritis, geriatric problems and stress (Asthana and Raina, 1989; Grandhi et al., 1994; Gupta and Rana, 2007; Bairwa et al., 2011; Barkatullah et al., 2013). Further, various medicinal plants were found to be strong radical scavengers, anti-inflammatory, antitumour, antistress, rejuvenating, immunomodulatory, hematopoietic and antioxidant properties (Gautam et al., 2004; Ahmad et al., 2005; Rasool and Varalakshmi, 2006; Steenkamp et al., 2013) which could compromise the medicinal use of these plants in folk medicine (Samy et al., 2013).

Since the plant is of considerable importance in traditional medicinal systems, the objective of the present study was to evaluate effects of oral administration of methanolic extract of *W. somnifera* on the hormonal level and antioxidant parameters in rats administered with acephate.

MATERIALS AND METHODS

Acephate (Figure 1) (Chemical name: O, S-dimethyl acetylphosphoramidothioate; Trade name: Orthene; Chemical family: Organophosphate) (KR exports pvt. Ltd., Jammu, India) was used as a chemical dissolved in olive oil and administered orally via gavage.

W. somnifera (Registration Number - RUBL20910, Department of Botany, University of Rajasthan) used in the present study belongs to family Solanaceae and popularly known as ashwagandha. The plant was obtained from the National Institute of Ayurveda, Jaipur. Leaves and roots were powdered and extracted with 70% methanol for 24 to 36 h by soxhlet extraction method. Then, methanol was separated under reduced pressure to obtain solid mass.

This study was carried out on male albino rats breeds that have been proven fertile (*Rattus Norvegicus*) of Wistar strain weighing 150 to 200 g. They were housed in a hygienic, well-ventilated room
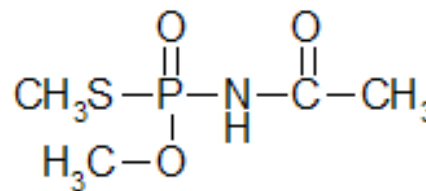

Figure 1. Acephate.

with natural light and dark cycles (12 h dark/12 h light) with relative humidity of 55±5%. They were individually housed in clean polypropylene cages (12"×10"×8") with sawdust bed and covered with stainless steel wire lids. They were fed on standard commercial pellet feed procured from Ashirwad Food Industries Ltd., Chandigarh (Punjab) and fresh water was provided *ad libitum* throughout the study. The rats were divided into 6 groups (n=6) mentioned in Table 1. All animal experiments were carried out as per the guidelines of Department of Zoology, University of Rajasthan, Ethical Committee. Experimental design are as shown in Table 1.

At the end of experimental period (30 days), the animals were weighed and euthanized under light ether anaesthesia and blood samples were collected by cardiac puncture in preheparinized tubes. Serum was separated by centrifugation at 3000 rpm and stored at -20°C to carry out biochemical parameters, FSH, LH and testosterone assay. Testis was dissected out and frozen for the biochemical estimations. They were also fixed in Bouin's fixative for at least 48 h, processed by the paraffin wax impregnation method and sections (5 μm thick) were cut using a rotary microtome. The sections were mounted on clean slides, then stained with haematoxylin and eosin (H&E) and examined by light microscopy for histopathological changes. Microphotographs of sections were taken.

The following parameters have been estimated in serum and testis-testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) were estimated in serum through chemilumine-scence in fully automatic Advia Cemtaus Immuno Assay System. Oxidative stress and antioxidant parameters, that is, lipid peroxidation (LPO) (Ohkawa et al., 1979), catalase (Claiborne, 1985), superoxide dismutase (SOD) (Das et al., 2000) and glutathione (GSH) (Carlberg and Mannervik, 1975) were performed in testis. Histopathological examination of the testis was performed by fixing them in Bouin's fixative. Further, they were cut into pieces and processed through ethanol-xylene series. The tissues were then embedded in paraffin and bee wax (3:1) (M.P. 55-62°C). Sections were cut at 5 μm thickness and stained with Harris haematoxylin and eosin (H&E).

The data obtained from the aforementioned experiments were subjected to statistical analysis. Data were represented as mean±standard error (SE). The differences were compared for statistical significance by "t- test" by using Statistical Package for Social Sciences (SPSS) software (16.0 version) and they were considered non significant at $p \leq 0.05$, significant at $p \leq 0.01$ and highly significant at $p \leq 0.001$. Graphical representation of data has been done using Microsoft Excel 2007.

RESULTS

A significant decrease ($p \leq 0.01$ and $p \leq 0.001$) was observed in serum testosterone, FSH and LH concentration of acephate administered rats at the dose level of 75 mg/kg body weight/day for 15 and 30 days except FSH value in group II which was non-significant.

Table 1. Experimental design (The rats were divided into 6 groups as summarized).

Group	Treatment
I	Control received vehicle (olive oil) only
II	Acephate 75 mg/kg body weight/day for15 days
III	Acephate 75 mg/kg body weight/day for 30 days
IV	Acephate 75 mg/kg body weight/day for 15 days, then acephate withdrawn and treated with *W. somnifera* extract (100 mg/kg body weight/day) for next 15 days, that is, from day 16 to 30
V	Acephate 75 mg/kg body weight/day + *W. somnifera* extract (100 mg/kg body weight/day) from day 1-30 (Concurrent treatment)
VI	Acephate 75 mg/kg body weight/day for 15 days, then no treatment for next 15 days (that is, from day 16 to 30)

Table 2. Effect of acephate and *W. somnifera* on Sex hormones levels in male albino rats.

Treatment	Serum testosterone (ng/ml)	Serum LH (mIu/ml)	Serum FSH (mIu/ml)
I Control (Vehicle only)	3.10 ± 0.09	1.92 ± 0.05	0.65 ± 0.04
II Acephate for 15 days	$2.58^* \pm 0.07$	$1.48^* \pm 0.09$	$0.45^{ns} \pm 0.05$
III Acephate for 30 days	$1.56^{**} \pm 0.06$	$0.81^{**} \pm 0.06$	$0.30^{**} \pm 0.04$
IV Acephate from day 1 to 15+*W. somnifera* extract for next 15 days	$2.78^* \pm 0.04$	$1.65^{**} \pm 0.03$	$0.50^* \pm 0.02$
V Acephate+*W. somnifera* extract from day 1-30 (Concurrent feeding)	$2.71^* \pm 0.08$	$1.60^* \pm 0.06$	$0.49^* \pm 0.03$
VI Acephate from day 1 to 15+No treatment for next 15 days	$2.68^{ns} \pm 0.10$	$1.54^{ns} \pm 0.11$	$0.48^{ns} \pm 0.04$

Mean ± 6 animals, Acephate: 75 mg/kg body weight/day; *W. somnifera*: 100 mg/kg body weight/day. Groups II, III, IV, V, VI compared with I: $^{ns}p \leq 0.05$ (Non significant); $^*p \leq 0.01$ (Significant); $^{**}p \leq 0.001$ (Highly significant).

Hormonal concentration was increased in groups IV and V indicating a positive influence of *W. somnifera* on the serum testosterone, FSH and LH in acephate administered rats (Table 2).

At dose level of 75 mg/kg body weight/day for 15 and 30 days, significant decrease in the catalase, SOD and GSH level of testis were noticed in acephate exposed rats in comparison to control animals. Statistically significant elevation ($p \leq 0.01$ and $p \leq 0.001$) was observed in catalase, SOD and GSH level of testis in *W. somnifera* treated rats (Figure 2). As a biomarker of lipid per oxidation, malondialdehyde (MDA) levels were measured in testis homogenates. MDA level was significantly increased in the acephate administered rats in comparison with the control one, sustaining the impaired antioxidant status of these animals. Moreover, lipid peroxidation was significantly decreased as indicated by the lower levels of MDA in testis after treatment with *W. somnifera* when compared with acephate administered rats. Non-significant decrease was observed in no treatment group (Figure 2).

The histology of testis of control rats showed normal histoarchitecture of seminiferous tubule with all successive stages of spermatogenesis. Sertoli cells were present. Irregular seminiferous tubules with inhibited spermatogenesis were seen in testis of rats treated with acephate for 15 and 30 days. Microphotograph of testis

treated with *W. somnifera* depicts slight improvement. Inter tubular space has also reached near to normal. Testis of concurrent group also maintain almost normal histoarchitecture, but not as recovered as treatment group. Lumen was filled with spermatozoa. In untreated group, testis was showing loosened tunica propria and degenerated Leydig cells. Lumen was filled with cellular debris (Figure 3).

It was found that acephate administration to rats for 15 and 30 days caused a significant reduction in the activity of catalase, SOD and GSH contents whereas an increase in thiobarbituric acid reactive substances (TBARS; measurement of LPO) activity of testis was observed. The level of LPO was reduced whereas GSH content and catalase activity were elevated after the treatment with 70% methanolic extract of *W. somnifera*. In this study, acephate administered rats had significantly lower levels of GSH contents in testis.

SOD and catalase are the major enzymes dealing with reactive oxygen species (ROS) in most cells. Both enzymes play an important role in elimination of ROS derived from the redox process of xenobiotics in the liver tissue. SOD catalyses dismutation of superoxide anions into hydrogen peroxide (H_2O_2). Hydrogen peroxide is the end product of SOD dismutation, while degradation of H_2O_2 and O_2 is catalyzed by catalase and glutathione peroxidase (GSH-Px) (Bansal and Jaswal, 2009). Since
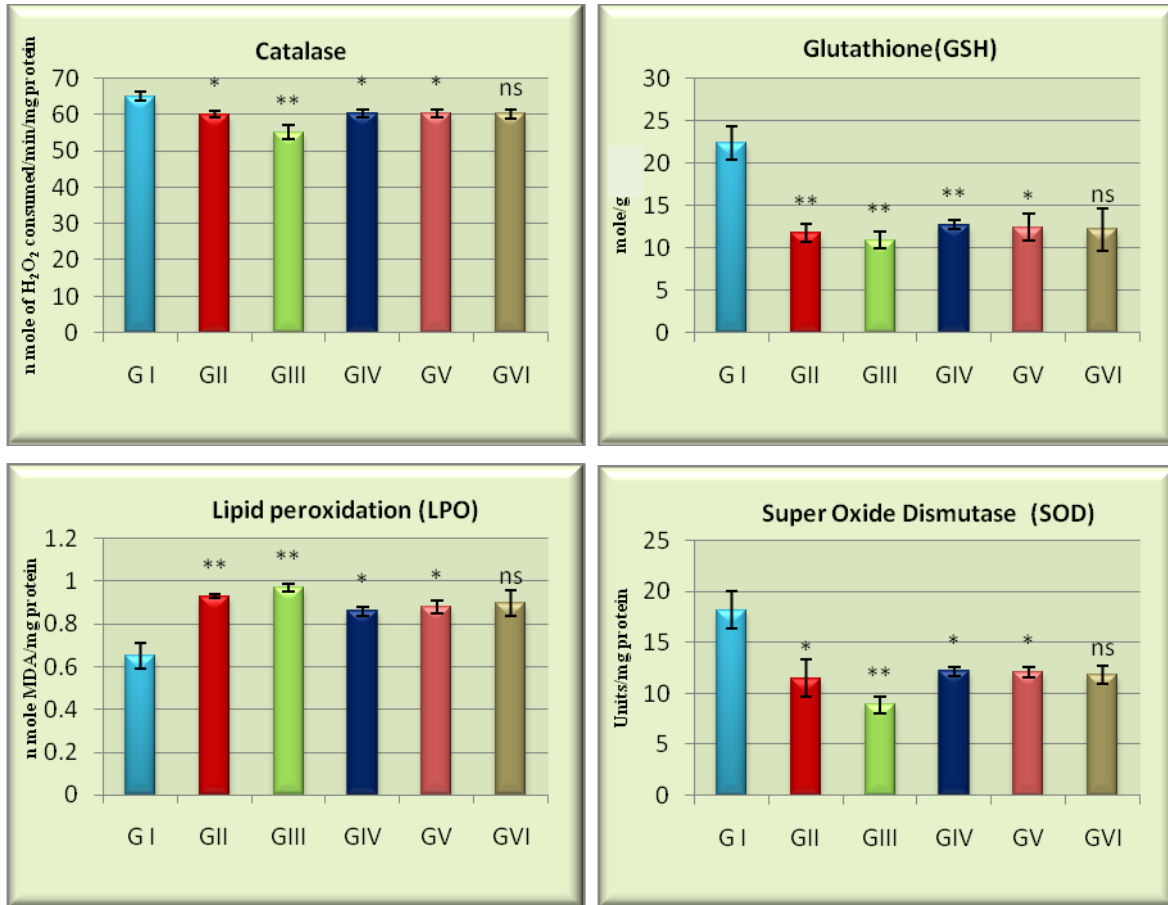
Figure 2. Testicular antioxidnt and oxidative stress parameters (Acephate 75 mg/kg body weight/day). Mean ± 6 animals (G II; G III; G IV; G V; G VI compared with G I); [ns]$p \leq 0.05$ (Non significant); *$p \leq 0.01$ (Significant); G: group.

the end product of SOD is substrate for catalase catalyzation. Studies have shown that pesticides diminish the antioxidant defense system and decrease the activity of SOD and CAT, thereby elevating the lipid peroxide content.

Co-treatment with acephate and *W. somnifera* attenuated spermatogenic/testicular damage induced by acephate treatment as shown by the return of sperm count, motility and normal morphology toward normal control values. The restoration of testosterone, LH, and FSH levels to normal after administration with *W. somnifera* might have stimulated the production of quantitatively and structurally normal sperm.

DISCUSSION

This study described that pesticides may disrupt the hormonal function of the male reproductive system and fertility. The spermatozoa, in common with all cell types have developed an elaborate antioxidant defense system consisting of enzymes such as catalase, SOD and reduced GSH that scavenge and suppress the formation

of ROS. Estimation of end products of LPO such as MDA is an index of the extent of oxidative damage to cellular structures (Sharma and Agarwal, 1996). Increased LPO is thought to be a consequence of oxidative stress which occurs when the dynamic balance between pro-oxidant and antioxidant mechanism is impaired (Nur Azlina and Nafeeza, 2007). Administration of *W. somnifera* reduced the lipid peroxidative markers in the tissues. This indicates that *W. somnifera* extract react with peroxyl radicals including the inhibition of LPO chain propagation (Kulisic et al., 2004). Attenuated level of LPO in extract treated animals is suggestive of the antioxidant nature of this plant. El-Demerdash (2011) also observed that insecticide mixture resulted in a significant increase in TBARS, which might be associated with decreased levels of reduced GSH, SOD and catalase activities. Apart from enzymatic antioxidants, non-enzymatic antioxidants such as GSH play a vital role in protecting cells from oxidative damage. GSH, a reactive, intracellular, non-protein (tripeptide) thiol in living organisms, performs a key role in coordinating innate antioxidant defense mechanism. It is involved in the maintenance of normal structure of cell, probably through redox and detoxification reactions
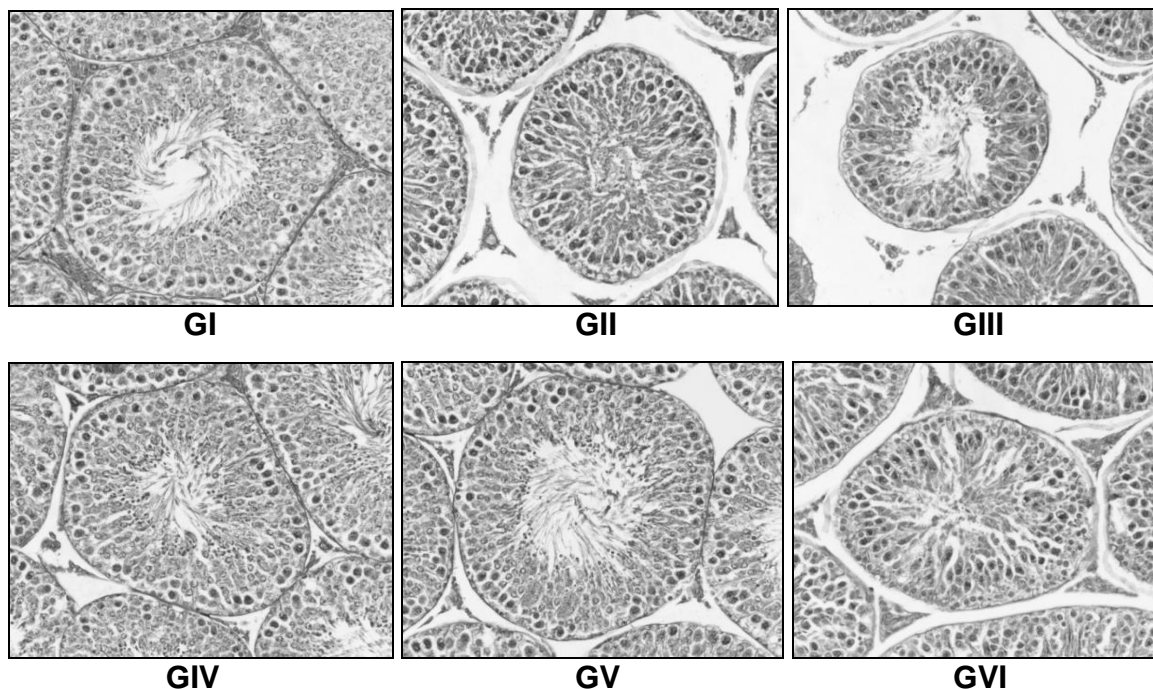
Figure 3. Photomicrograph of rat testis (X200). GI: Normal testis histoarchitecture of a control rat; GII: Administered acephate for 15 days; GIII: Administered acephate for 30 days; GIV: Acephate from day 1 to15+*W. somnifera* extract; GV: Acephate+*W. somnifera* extract from day 1 to 30 (Concurrent feeding); GVI: Acephate from day 1 to 15+No treatment for next 15 days.

(Kharb, 2010). Depletion in GSH content might be resulted from intoxication with acephate (Cereser et al., 2001). *W. somnifera* extract treated animals, showed a significantly elevated level of GSH. It is possible that extract might have reduced the extent of oxidative stress, leading to lesser GSH degradation or increase in the biosynthesis of GSH (Prasanna and Purnima, 2011). Supplementation of *W. somnifera* along with acephate (concurrent groups) decreases the free radicals may be by quenching and lowering oxidative stress (Deepa and Anuradha, 2011). Decrease in the SOD and the catalase levels in the acephate administered animals are again attributed to increased oxidative stress in these animals. It is well known that flavonoids and polyphenols are natural antioxidants but have also been reported to significantly increase SOD and catalase activities. The currently noted elevated levels of both SOD and catalase levels could be due to the influence of flavonoids and polyphenols of *W. somnifera* (Fang et al., 2002; Visavadiya and Narasimhacharya, 2005). The decrease in testosterone concentration of acephate treated rats may occur due to the reduced levels of LH (Kerr and Sharpe, 2006) as circulating LH is responsible for maintaining normal plasma testosterone concentrations. Spermatogenesis requires LH and FSH for initiation and maintenance in male rats, LH stimulates Leydig cells to secrete testosterone, normal testicular function is dependent on FSH and testosterone is absolutely required for normal spermatogenesis.

Conclusively, this study demonstrated that oral administration of *W. somnifera* extract could prevent or be helpful in reducing the complications of reproductive toxicity associated with oxidative stress.

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