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Sterility Effect of the Commercial Neem Extract NeemAzal-T/S® (*Azadirachta indica* A. Jus.) on Male Rats (*Rattus norvegicus*)

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Abstract: Numerous studies with experimental animals have shown that neem seed extracts have transient and reversible effects on sterility. NeemAzal-T/S® (1%) was fed to 3 experimental animal groups via stomach intubation (5, 15, and 25 mg/kg) for 6 days and the control group was given only tap water. Hematological parameters were determined on the 4th and 9th days of the experiment. Hemoglobin and MCH levels showed an increase in group 3 on the 4th day, as compared with the control group, and there were no significant differences in the other parameters between these groups, whereas on the 9th day hemoglobin, MCH levels, and WBC count were significantly higher in group 3 than in the control group. Other parameters, including testosterone level, were not significantly different between the experimental groups and the control group. Histopathological changes showed disruption to spermatogenesis in some seminiferous tubules. These changes included derangement of the first layer of spermatogonial cells and necrotic spermatocytes. The time lapse for pregnancy in groups 1, 2, and 3 was 50, 60, and 90 days, respectively, which may have been due to these alterations. It is concluded that NeemAzal-T/S® can be used as a safe sterility agent for the control of rodent pests, and that the most effective dose to cause this sterility and delay reproduction is between 15 and 25 mg/kg.

Key Words: Neem extract, sterility, histopathology, hematology, testosterone, rodent pests

Introduction

Rodents are among the most important agricultural pests, and many studies have been conducted to determine suitable control measures for reducing the damage they cause (Prakash, 1988). Many synthetic chemical compounds have been used to control them. These compounds carry their own risks and dangers, and hence if used recklessly could cause food, soil, air, and surface and underground water pollution. Moreover, residues of these pesticides are harmful to beneficial insects and other non-target organisms, and increase the resistance of pests to these pesticides, which ultimately results in pest outbreaks (Singleton et al., 1999). Presently, there is much concern about the negative environmental effects of synthetic pesticides worldwide; therefore, extensive effort to replace them with safer and less toxic pesticides, such as biopesticides, is ongoing. One such move is the use of plant products to protect crops against pests and diseases. *Meliacea* is among the plant families whose trees have useful medicinal and pesticidal characteristics (Schmutterer, 1995). One of the plants of this family, *Azadirachta indica* (neem), has a number of

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medicinal and pesticidal properties, and has been used widely in Ayurvedic medicine in India (Randhawa and Parmar, 1993). This tree has been called the "wonder tree", because of its numerous properties. Neem compounds have been known for many years, and have been shown to have antiviral (Gogati and Marathe, 1989), antibacterial (Singh and Shastry, 1997), antifungal (Kher and Chaurasia, 1997), anti-inflammatory, and antipyretic properties. Polysaccharides from aqueous extracts of the bark of this tree are reported to have antitumor and interferon induction properties (Fujiwara et al., 1982). The antifertility effects of neem oil were observed by Lal et al. (1986). Numerous studies with experimental animals have shown that neem seed extracts have transient and reversible antifertility, and abortive effects (Mukherjee et al., 1999). Contraceptive tablets made from neem extract are currently being used in India.

Materials and Methods

The study included 24 randomly selected 4-5-month-old male Wistar rats (150-200 g). The rats were divided into 4 groups of 6 rats each—3 experimental groups (groups 1-3) and a control group. The animals were housed in transparent polythene cages with a stainless steel wire line ceiling. The room temperature in which the animals were housed was about 25 °C and a light/dark cycle of about 12 h was maintained. The animals were fed a standard commercial diet (Brook Bond, Lipton) and given tap water ad libitum. The animals were acclimatized to this condition for 1 week prior to any experimental manipulation. The control group was fed plain tap water and the experimental groups were fed 1% NeemAzal-T/S® extract (5 mg/kg, 15 mg/kg, or 25 mg/kg, respectively) by stomach intubations for 6 days. The animals received the doses as follows (Lal et al., 1986; Mukherjee et al., 1999):

Control group: 0.5 ml plain tap water;

Group 1: 0.1 ml NeemAzal-T/S® + 0.4 ml tap water;

Group 2: 0.3 ml NeemAzal-T/S® + 0.2 ml tap water;

Group 3: 0.5 ml NeemAzal-T/S®.

Hematological parameters were determined on the 4th and 9th days of the experiment. For hematological studies, blood samples were collected in small vials containing 1 drop of 2% EDTA to prevent coagulation. Then, WBC (white blood cell) count, RBC (red blood cell) count, Hb (hemoglobin), MCHC (mean corpuscular hemoglobin concentration), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), hematocrit, and lymphocyte count, were determined with a cell counter. Simultaneously, samples were also collected in small vials without EDTA in order to facilitate coagulation to obtain serum. The serum was refrigerated at –20 °C and at the end of the experiments, testosterone levels were determined using a diagnostic kit (DRG Diagnostics) and ELISA reader (Krause et al., 1983; Parshad et al., 1994). On the 10th day 2 animals from each group were sacrificed and dissected in order to histopathologically examine the testes. On the 15th day the experimental animals were housed with fertile female rats to determine the delay in reproduction, if any, caused by NeemAzal-T/S®.

All the animals were weighed on the 1st, 3rd, 5th, 8th, and 10th day of the experiment. Statistical analysis of the data was performed with 2-way ANOVA using SPSS. The Newman-Keuls test was conducted when significant differences were observed. The level of significance was $P < 0.05$ for all the experiments.

Results

Effect of 1% NeemAzal-T/S on Hematological Parameters

On the fourth day of the experiment (first blood sampling day), hemoglobin and MCH levels were higher in group 3 than in the control group ($P < 0.05$), whereas there were no significant differences in the other parameters (Table 1). On the ninth day (second blood sampling day), hemoglobin, MCH levels, and WBC count were significantly higher in group 3 than in the control group ($P < 0.01$, $P < 0.001$, $P < 0.05$), whereas the other parameters were not significantly different between the experimental groups and the control group (Table 2).

Effect of NeemAzal-T/S® on Testosterone Level

Statistical analysis of the serum showed no significant differences between the levels of testosterone in the experimental groups and the control group (Figure 1).

Effect of NeemAzal-T/S® on Body Weight

Statistical analysis showed no significant differences between the weights of the experimental group animals and those in the control group (Table 3).

Table 1. Hematological parameters of the male rats exposed to various doses of NeemAzal-T/S® (4th day of the experiment—1st sampling).

Experimental Groups			Control Group	Hematological Parameters
25 mg/kg	15 mg/kg	5 mg/kg		
8.25 ± 1.40	8.60 ± 1.41	12.97 ± 1.59	9.74 ± 2.62	WBC (M/mm^3)
7.65 ± 0.18	7.61 ± 0.16	7.67 ± 0.12	7.08 ± 0.17	RBC (M/mm^3)
16.50 ± 0.34*	15.27 ± 0.39	15.76 ± 0.39	13.98 ± 0.45	Hb (g/dl)
55.00 ± 1.08	53.11 ± 0.52	53.00 ± 1.03	53.08 ± 0.76	MCV (fl)
21.56 ± 0.43*	20.01 ± 0.24	20.01 ± 0.21	19.76 ± 0.36	MCH (Pg)
39.20 ± 0.40	37.73 ± 0.23	37.93 ± 0.11	37.22 ± 0.19	MCHC (g/dl)
42.00 ± 0.43	40.46 ± 0.91	40.46 ± 0.58	37.60 ± 1.11	Hct (%)
88.10 ± 2.79	67.87 ± 2.60	73.08 ± 2.27	68.60 ± 6.84	Lym (%)

n = 6
*P < 0.05

Table 2. Hematological parameters of the male rats exposed to various doses of NeemAzal-T/S® (9th day of the experiment—2nd sampling).

Experimental Groups			Control Group	Hematological Parameters
25 mg/kg	15 mg/kg	5 mg/kg		
11.87 ± 1.3*	8.78 ± 0.72	9.90 ± 1.60	5.94 ± 1.20	WBC (M/mm^3)
7.43 ± 0.18	7.36 ± 0.17	7.46 ± 0.08	7.20 ± 0.10	RBC (M/mm^3)
15.87 ± 0.26**	14.06 ± 0.35	14.15 ± 0.21	13.56 ± 0.36	Hb (g/dl)
55.32 ± 0.94	52.61 ± 0.56	53.18 ± 0.35	53.80 ± 0.63	MCV (fl)
21.37 ± 0.30***	19.10 ± 0.20	18.45 ± 0.44	18.90 ± 0.47	MCH (Pg)
38.67 ± 0.25	38.90 ± 0.25	35.62 ± 0.30	35.10 ± 0.63	MCHC (g/dl)
41.07 ± 0.44	38.70 ± 0.67	39.68 ± 0.58	37.86 ± 0.63	Hct (%)
85.85 ± 1.23	84.71 ± 1.62	83.35 ± 1.65	83.10 ± 1.74	Lym (%)

n = 6
*P < 0.05, **P < 0.01, ***P < 0.001

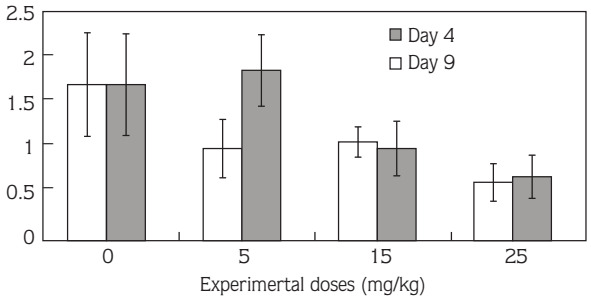

Figure 1. Testosterone Levels in serum of the male rats Exposed to various doses of NeemAzal-T/S®.

Effect of NeemAzal-T/S® on the Testes

Figure 2 shows T.S. of the testes in the control animals, with normal seminiferous tubules and spermatogenesis. Histopathological examination indicated a reduction of spermatozoons and accumulation of blood cells between the interstitial tissue in some seminiferous tubules of the animals in the experimental groups (5 and 15 mg/kg of NeemAzal-T/S®) (Figures 3 and 4). Some tubules showed sperm debris in the center of the tubules. Derangement of the first layer of spermatogonial cells tubule walls, and

Table 3. Weights (mg) of the male rats exposed to various doses of NeemAzal-T/S®.

Experimental Groups			Control Group	Day of the Experiment
25 mg/kg	15 mg/kg	5 mg/kg		
200.00 ± 10.22	200.86 ± 8.45	178.43 ± 6.19	169.50 ± 9.04	1
230.60 ± 12.13	199.57 ± 1.41	194.00 ± 7.23	191.50 ± 8.48	3
227.40 ± 10.47	209.43 ± 11.96	190.29 ± 7.49	188.17 ± 9.53	5
237.00 ± 15.32	176.43 ± 0.49	195.29 ± 9.97	185.83 ± 7.91	8

n = 6

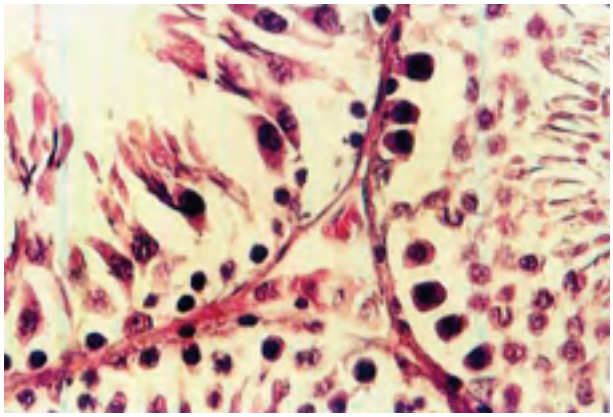

Figure 2. T.S. of the testes in the control animals with normal seminiferous tubules and spermatogenesis (H&E 40×).

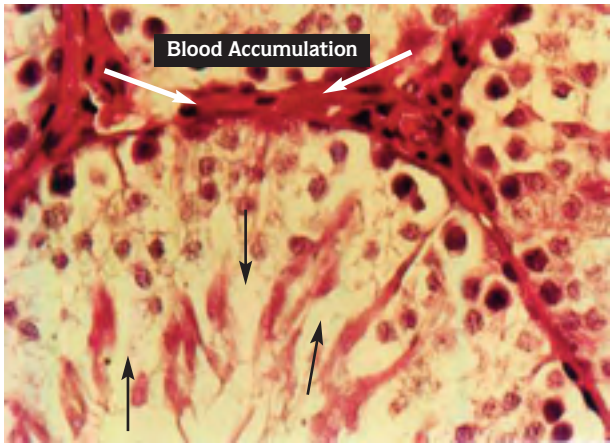

Figure 3. Reduction of spermatozoons (black arrows) cords and accumulation of blood cells between interstitial tissue in some seminiferous tubules of group 1 (5 mg/kg NeemAzal-T/S®) (H&E 40×).

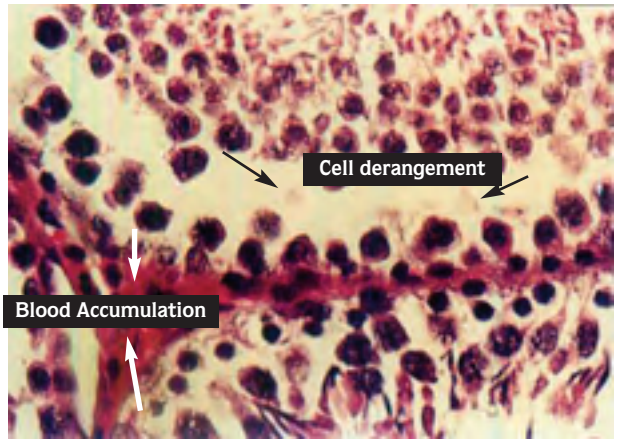

Figure 4. Accumulation of blood cells between interstitial tissue and derangement of the first layer of spermatogonial cells from tubule walls is observed in group 2 (15 mg/kg NeemAzal-T/S®) (H&E 40×).

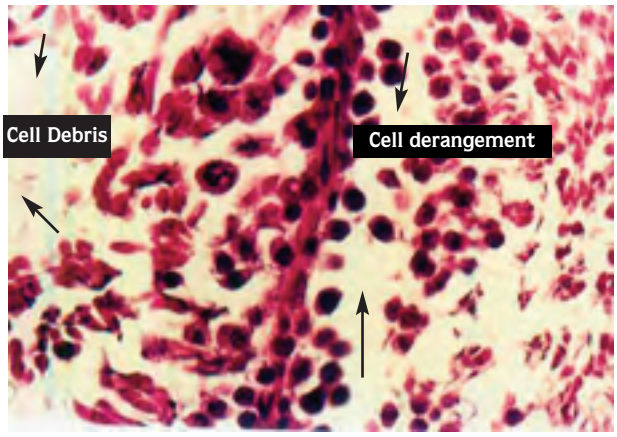

Figure 5. Some tubules show sperm debris in the center of tubules. Derangement of the first layer spermatogonial cells from tubule walls, and inflammation and deformation in the cells indicating cell necrosis are also observed in some tubules in group 3 (25 mg/kg NeemAzal-T/S®) (H&E 40×).

inflammation and deformation in the cells indicative of cell necrosis were also seen in some tubules (25 mg/kg of NeemAzal-T/S®) (Figure 5). The time lapse for pregnancy in groups 1, 2, and 3 were 50, 60, and 90 days, respectively, whilst in the control group pregnancy and reproduction were seen after 23 days (normal reproduction cycle in rats).

Discussion

In the present study the effects of 1% NeemAzal-T/S® on hematological parameters and testosterone levels in male rats treated for 6 days were determined. These parameters were determined on the 4th and 9th days of the experiment. Hb and MCH levels in group 3 on days 4 and 9, and WBC in group 2 on day 9 were significantly different than in the control group (Tables 1 and 2). In a study by Parshad et al. (1994) administration of 0.1%, 0.4%, and 1.6% aqueous neem extract to male rats for 10 weeks caused significant increases in RBC, PCV, Hb, and MCHC levels. They reported that this increase might have been due to the presence of some active compound(s) in the aqueous neem extract that may improve erythropoiesis. In another study Talwar et al. (1997) showed that oral administration of purified extracts of neem to subhuman primates and rodents did not cause any significant changes in hematological parameters. In another study blood analysis of male and female rats after 90 days of oral exposure to azadirachtin did not show any significant changes in Hb, RBC, WBC, or differential leucocytes count (Raizada, 2001). Although in the present study there were no significant changes in testosterone levels on days 4 and 9 of the experiment (Figure 1), Parshad et al. (1994) observed a significant decrease in serum testosterone ($P < 0.01$), with a maximum reduction in rats that received the highest concentrations of neem extract (0.4% and 1.6%). It was also reported that low levels of serum testosterone might have been responsible for decreases in the relative weights of the seminal vesicles in these rats ($P < 0.05$). In contrast, Krause and Adami (1983) did not report any significant differences in serum or testicular testosterone, or in the weights of the seminal vesicles of neem-treated rats. These differences in observations might be due to differences in experimental design, methodology, neem extract formulation and dose, administration route, extraction technique, duration of treatment, and animal species.

In the present study histopathological observations showed that 1% NeemAzal-T/S affected the process of spermatogenesis in some seminiferous tubules and disrupted the production of sperm. The histopathological changes observed include derangement of the first layer of spermatogonial cells, necrotic spermatocytes, disturbances in spermatogenesis, accumulation of spermatozoid debris in the center of the seminiferous tubules (Figures 4 and 5), and reduction in the number of spermatozoons (Figures 3-5). Though there were some tubules in which normal spermatogenesis occurred, there must be a certain number of spermatozoa to ensure male fertility and the observed disturbances may have caused a reduction in the number of sperm necessary for fertility and reproduction. This might have been the cause of the delay in reproduction observed in the experimental groups. Higher doses increase the number of damaged seminiferous tubules and, therefore, the time required for reproduction increases. After the recovery period, the effects of NeemAzal-T/S® remit, reparation of the disturbed cells occurs, and the fertility and reproduction cycle in treated animals returns to normal. Sander-Cramer (1941) and Garge et al. (1994) showed that purified neem extract had potent spermicidal activity. The oil from neem seed kernel is reported to have spermicidal and anti-implantation properties (Sinha et al., 1984). Studies of the testes of albino rats treated with neem leaf extract reported degenerative changes in the germ cells and Leydig cells. Neem extract also reduced sperm counts and motility, and increased the number of abnormal sperm (Shaikh, 1990). Shaikh also reported reductions in weight, epithelial cell height, and nuclear diameter of the ventral prostate and seminal vesicles as indirect evidence of the anti-androgenic action of neem extract. In another study Awasthy (2001) showed that oral administration of a crude ethanolic extract of neem leaves to adult male mice for 6 weeks (1 spermatogenesis duration) increased the incidence of structural changes and synaptic disturbances in meiotic chromosomes, and disrupted meiosis. Neem extract reduced the sperm count and increased the frequency of spermatozoa with abnormal head morphology. It is suggested that at least one of the constituents of the extract may have interfered with DNA.

In the present study NeemAzal-T/S® had no significant effect on hematological parameters, testosterone level, or animal weight. Histopathological changes indicated disruption of spermatogenesis in some seminiferous tubules and reduced sperm counts. These changes

included derangement of the first layer of spermatogonial cells and necrotic spermatocytes, which delayed reproduction in experimental animals. It is concluded that NeemAzal-T/S® could serve as a safe sterility agent for the control of rodent pests and that the most effective dose to cause this sterility and delay reproduction is between 15 and 25 mg/kg of the commercial neem extract NeemAzal-T/S®.

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