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Dose-Dependent Effect of Short-Term Repeated Exposure to Amitraz on Some Reproductive Parameters in Male Albino Rats

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Abstract

Amitraz is a pesticide used in agriculture and public health to control insects, weeds, animals, and vectors of disease. Although the use of pesticides is of benefit in general, abuse of the pesticides is harmful due to their potential toxicity to humans and animals. Present study examined the effect of the pesticide on the male reproductive parameters of the male albino rats. 20 rats were grouped into four. Group 1 was control, Group 2 was given low dose Amitraz, Group 3 was median dose, and Group 4 was high dose. The administration was for a period of 21 days. Results showed that Amitraz has dose dependent toxicity effect on the reproductive parameter of the rat. Amitraz is toxic to the reproductive parameters of the albino rat, and could lower sperm concentration, sperm motility, and sperm morphology. By implication, Amitraz is toxic to the reproductive system and could cause infertility in male.

Keywords: Infertility; Sperm Concentration; Toxicity; Pesticide; Repeated Exposure

Introduction

A pesticide is 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 processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies [1]. They are widely used for preservation across the world.

Over the past years, estrogenic activities of pesticides have been the focus of research. However, not much attention has been paid to anti-androgenic effects of the pesticides for the observed degeneration in male reproductive health. This is the aim of this study; to determine their effects on male reproductive system.

Amitraz is one of the commonest pesticides used my man. Amitraz [N-methylbis (2,4-xylyliminomethyl) amine] is an insecticide of the formamidine group, initially synthesized in England in 1969, that is used as an acaricide and tickic albino ide in Veterinary medicine [2]. Its varied uses include treatment and control of generalized demodicosis in canines, ticks and mites on cattle and sheep, psylla infection of pears and also for control of red spider mites on fruit crop [3]. The formulations available for commercial use contain 12.5–50% of Amitraz in an organic solvent like xylene and it is an effective treatment of feline scabies and demodicosis [4] is a very popular product, cheap and readily available, [5] and it is diluted with water before use [6].

In mammals, the main mechanism of action consists of activation of alpha 2- adrenoceptors similar to the mechanism of action of xylazine and clonidine, agonist alpha 2-adrenergics, and in the inhibition of the enzyme monoaminoxidase (MAO) [7].

Amitraz is rapidly and well absorbed after oral administration and eliminated from most of the tissues within few days. Amitraz is rapidly metabolized and excreted, mainly formamidine and 2, 4, dimethyl formanilide. These metabolites still contain the 2, 4 dimethylaniline (2,4DMA) moiety. The end product is 4-amino-3-methylbenzoic acid which is rapidly conjugated and excreted. This metabolic pattern is qualitatively similar in the rat, mouse, cat, dog, boboon, cow and human. In urine of these species 4-amino-3-methylbenzoic acid (free and conjugated form) is most predominant (>70%). Other metabolites like N-(2,4dimethylpheny1) N-methyl1 formamidine and 2,4, dimethyl formanilide constitute not more than 10% each.

Oral LD50 of Amitraz in the rats is the acute oral medical lethal dose for the rats which is 523- 800mg/kg body weight and the LC50 (6 hours) is 65mg/L of air [8,9]. LD50 (Lethal Dose) of Amitraz is the dose which when administered to the rats will cause the death of half the population. LC50 (Lethal Concentration) of Amitraz is the concentration of the pesticide in air or water that will cause the death of half the population of the rats.

According to Amitraz Risk Characterization Document Volume 1 released by the Department of Pesticide Regulation (DPR), Carlifornia Environmental Protection Agency on December 12, 1995, a DPR review of the toxicology studies on the effects of Amitraz had identified adverse responses in human and animal studies.

Amitraz exerts toxic effects on ectoparasites by interaction with the octopamine receptors of arthropods. Although mammals do not have octopamine receptors, Amitraz exerts side effects in mammals through activation of α-2-adrenoceptors [10]. It is classified as a Group C possible human carcinogen [11].

Evidence from animal studies suggests that Amitraz is a potential reproductive toxicant. Moreover, Amitraz exhibits toxic effects in the human reproduction cells in vitro and inhibits the production of the steroid hormone progesterone [12].

Amitraz has been shown to induce cytochrome P450-dependent monooxygenases in the liver of treated rats and decrease hepatic glutathione activity in mouse [13,14]. In bovine seminal vesicle, amitraz inhibited prostaglandin E2 synthesis. At high dose levels, amitraz can cause tumours in female mice. In dogs, following a topical application, amitraz increased plasma glucose and inhibited insulin secretion [10].

In humans, amitraz intoxication has been reported, and exposure effects include CNS depression, hypothermia, bradycardia, hypotension, hyperglycemia, glycosuria, vomiting and respiratory failure [15,16].

Due to its excellent miticidal activity, amitraz is widely used in apiculture for the obligatory annual control of Varroa destructor. In beehives, amitraz is used in the form of smoke or vapour. This acaricide is easily hydrolyzed to toxic 2,4-dimethylaniline (2,4-DMA) and various products containing the 2,4- DMA moiety [17].

According to a DPR toxicology review, Central nervous system (CNS) effects in humans and dogs have been detected within hours of amitraz exposure. The effects seen in humans included paleness, dry mouth, drowsiness, disorientation, light headed feeling, slurred speech, and loss of consciousness. Toxic effects associated with subchronic exposure were indicated in a variety of studies. The most prevalent effects included decreases in body weight gain and abnormal CNS responses. The toxic effects of chronic exposure to amitraz included CNS depression, depressed growth rate, a reduction in food intake, hyperplastic nodules, and hyperkeratosis of the forestomach. Chronic exposure to amitraz has also been associated with oncogenicity [18].

Exposure to amitraz would be expected to pose a greater hazard to pet owners, agricultural workers and beekeepers because of the continual exposure to this

acaricide. Therefore, it is very important to evaluate possible reproductive effects of amitraz in view of health protection [18].

Target Organs on Exposure to Amitraz

Amitraz have been shown to alter thyroid gland function and to reduce circulating thyroid hormone levels. Reduction in thyroid hormone levels can compromise the catalytic activity of hepatic cytochrome P450 monooxygenases, resulting in an altered hepatic androgen metabolism [17].

Amitraz decreases respiratory rate. Respiratory depression induced by Amitraz is possibly due to the central alpha 2-adrenergic action of this acaricide [18].

In the CVS, bradycardia and hypotension experienced after administration of Amitraz may be due to activation of central presynaptic alpha 2 receptors, decreasing release of dopamine and noradrenaline and reducing sympathetic tonus [19].

The hyperglycemia caused by Amitraz occurs by the action of Amitraz and its active metabolite, by alpha 2-adrenergic-receptor-mediated inhibition of insulin secretion [20]. This leads to glycosuria.

In the renal system, Amitraz leads to polyuria due to alpha 2 adrenoceptor stimulation that decreases antidiuretic hormone (ADH) and renin secretion, inhibits ADH effect, and enhances diuresis by increased glomerular filtration rate [21].

In the liver, Amitraz is a potent hepatotoxic drug acting by decreasing hepatic glutathione activity. Amitraz causes abdominal distension as well as inhibit Prostaglandin E2 synthesis [21].

Method

Twenty male rats obtained at the age of 2 weeks old and kept in the laboratory for a period of 14 days to acclimatize to the new environment. Animals were well fed with rat feeds and given access to water and hygienic environment throughout the duration of experiment.

When the rats attained an average weight of 160g they were the randomly assigned into groups consisting of five rats each.

The LD 50 of Amitraz was 800mg/kg

Grouping of Animals and Administration of Amitraz

Control Group: Rats in this group were given feeds and distil water

Group 1: Rats in this group were give feeds and Amitraz was dissolved in distil water at a concentration of 1/10 of the LD 50. This represented the low dose group.

Group 2: Rats in this group were give feeds and Amitraz was dissolved in distil water at a concentration of 1/8 of the LD 50. This represented the median dose group.

Group 3: Rats in this group were give feeds and Amitraz was dissolved in distil water at a concentration of 1/5 of the LD 50. This represented the high dose group. The administration which lasted for 21 days was by gavage. The rats were thereafter weighed, cervically dislocated and then sacrificed. The male reproductive organs were harvested ad weighed.

Blood Collection

At the end of the 21 days, just before the cervical dislocation, blood samples were collected from the ocular vein using capillary tube. Blood samples were then allowed to clot at room temperature to obtain serum for hormonal assay.

Determination of Sperm Concentration

The sperm concentration was done by diluting the sperm suspension, after which a drop was delivered into Neubaure hemocytometer in each side of the counting chamber. The cytometer was allowed to stand for five minutes to allow equilibration. The sperm cells were counted in each of the chambers with the aid of microscope [22,23].

Determination of Sperm Motility

Motility of sperm was evaluated directly after mincing in drop of sperm suspension microscopically. Non motile sperm numbers were first determined followed by counting of total sperm. Sperm motility was expressed as percentage of motile sperm of the total sperm counted [24].

To evaluate grade of motility, at least **5** microliter (diluted if concentration of the sperm is greater than 60 million/mL) of semen was placed on a standard microscopic slide, a cover slip, a cover slip was placed over the drop, and under high power objective number of

motile sperm per field were counted. The grade of sperm motility was recorded on a scale of 0 to 4 [25].

0: no motility

1(+) sluggish activity, minimal forward progression,

2(++); poor to fair motility

3(+++); Good motility with tail movement visualized

4 (++++); Excellent forward progression, tail movement difficult to visualize

Determination of Sperm Morphology

After liquefaction and prior to staining, and aliquot of semen was washed with Quinn's Sperm Washing medium, SAGE and centrifuged at 300g for 10 minutes. The supernatant was removed was removed and 0. **5** mL of Quinn's medium added to the remaining pellet. 10 microlitre of washed semen was then spread onto a glass slide, fixed and air dried. The smear was washed with distilled water and stained with spermac stain. Strict criteria were applied for the evaluation, according to which a spermatozoon was normal if it has an oval head, 4.0 to 5.0 micrometer long and 2.5 to 3.5 micrometer wide. The length to width ration between 1. 50 to 1.75 as measured with an ocular micrometer.

The normal spermatozoon had a well-defined acrosome that covers 40 to 70 percent of the head. The mid piece was thin, less than 1 micro 1 micometer wide, about 1. Five times longer than the head. Cytoplasmic droplets were not less than half of the head width. The tails were thin, uniform, uncoiled and about 45 micro meter long. According to this classification system, all borderline forms are considered as abnormal [26].

Tissue Weight Determination

On sacrificing the rats, the weights of their testes, seminal vesicles, and epididymis were taken using scale.

Body Weight Determination

All 20 rats were weighed before the first administration of Amitraz and after the final administration of at the end of 21 days.

Determination of Serum Testosterone Concentration

Testosterone determination was performed by using the coat A count technique (radioimmunoassay) at the National Hospital, Abuja, Nigeria.

Statistical Analysis

Data obtained was presented as 4 group mean ±SED and analyzed by one way ANOVA. The data were presented in tables of Parameters (the six parameters of study) against the four groups. At $p < 0.05$, data was considered significant.

Results

Change in Body Weight

Table 1 show the changes in body weight in each group after the study period. Only Groups 3 and 4 body weights showed a statistically significant change, though generally, there was decrease in mean body weight with increase in dosage.

Group	Before administration	After 21 days administration	Difference in body weight
1	154.00 ± 2.45	196.00 ± 2.45	42.00 ± 3.74
2	154.00 ± 2.50	192.00 ± 3.74	38.00 ± 4.90
3	154.00 ± 2.45	180.00 ± 3.16*	26.00 ± 4.00*
4	154.00 ± 2.45	164.00 ± 2.45*	10.00± 0.00*

Values are mean ± SEM

*Represents a significantly ($p < 0.05$) lower means value as compared with the control group

Table 1: Mean Body Weight in Grammes (mean ± SEM).

Change in Organ Weight

As shown in table 2, the organ weights of Group 3 and 4 shows statistically significant changes when compared to control group. However, the mean weight of Group 3 is greater than the mean weight of Group 4, and the mean weight of Group 1 is greater than the mean weight of Group 2.

Group	Testes	Seminal vesicles	Epididymis
1	0. 65 + 0.03	0.10 + 0.01	0.76 + 0.01
2	0. 62 + 0.04	0.08 + 0.01	0.73 + 0.02
3	0. 57 + 0.01	0.04 + 0.01*	0. 58 + 0.06*
4	0. 39 + 0.08*	0.03 + 0.00*	0.27 + 0.01*

Values are mean ± SEM
*Represents a significantly ($p < 0.05$) lower means value of the parameter as compared with control group
Table 2: Mean Organ/Body Weight Ratio of the Reproductive Organs (mean ± SEM).

Change in Serum Testosterone Concentration

Table 3 shows the changes in serum testosterone level in each group after the study period. Group 3 and 4 showed statistically significant changes in serum testosterone level.

Group 4 had the lowest mean serum testosterone level, Group 3 had the next lowest mean serum testosterone level, while Group 2 showed no statistically significant change in serum testosterone level, though its mean value was lower than that of the control group.

Group	Serum Testosterone Level (ng/mL)
1	12.13 + 1.83
2	7.05 + 1.46
3	3.94 + 0.94*
4	1.49 + 0.39*

Values are mean + SEM
*Represents a significantly ($p < 0.05$) lower means value of serum testosterone as compared with control group
Table 3: Serum Testosterone Level (mean ± SEM).

Changes in Sperm Morphology, Motility and Concentration

The results of sperm examination as shown in table 4 indicated that the mean of abnormal spermatozoa increased with Amitraz dosage; the mean of abnormal spermatozoa of the control group was lowest. However, only Group 3 and 4 showed statistically significant difference from the control group.

The results of Sperm examination as reported in table 4 showed that the mortality of the spermatozoa decreased with increasing dosage of Amitraz. The mean of abnormal spermatozoa of the control group was the lowest, while its motility grade was the highest. Groups 3 and 4 showed statistically significant difference from the control group.

Results also showed (table 4) that the Sperm Count decreased with increasing Amitraz dosage. Group 2 had a mean lower than control, Group 3 had a mean lower than 2, while Group 4 had a mean lower than Group 3. However, only Groups 3 and 4 showed statistically significant difference from the control group.

Group	Morphology (abnormal)	Motility	Concentration
1	6.80 + 1.24	74.00 + 4. 00	159.12 + 1. 05
2	7.20 + 0. 58	74.00 + 2. 45	153.49 + 3. 14
3	10.00 + 0.84*	52.00 + 7. 35*	59.94 + 2. 39*
4	13.00 + 1.05*	28.00+ 4. 90*	15.29 + 0.47*

Values are mean + SEM
*Represents a significantly ($p < 0.05$) lower means value of the parameter as compared with control group
Table 4: Sperm motility, morphology and concentration (mean ± SEM).

Discussion

Results show that short term repeated oral administration of Amitraz caused reproductive toxicity in dose-dependent manner. This was evident with the decreasing sperm motility and sperm concentration from group 1 to group 4. This may imply that exposure to Amitraz affects the interstitial cells of Leydig that are responsible for the production of testosterone. Results also showed increase in the number of abnormal sperm cells (morphology) in dose-dependent manner as show in table 4. This agrees with a previous work in which it was documented that chlorimeform, a formamididine insecticide caused decrease in testosterone concentration resulting in infertility. It was also reported that a log time administration of Amitraz caused infertility [24].

Semen toxicity was also evident with decreasing serum concentration of sperm cells from low to high doses of Amitraz (Table 3).

There was also suppression of body and organ weights as observed in table 1and 2. These are also general responses to toxicity [27,28].

There were clinical signs such as increased nasal discharge and nervousness that may have resulted from exposure to alpha-2-adreergic effect of Amitraz, while other clinical sigs such as reddish tear, fur staining, and dull fur suggest stress as a result of exposure to the test chemical [29,30].

Conclusion

Short-term repeated exposure to Amitraz toxic to the body, and particularly the reproductive system and could therefore cause infertility in male. Therefore, male working in such companies that produce or apply Amitraz must take extra and deliberate precaution to reducing exposure.

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