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EFFECT OF DIAZINON ON GONADOTROPINS AND TESTOSTERONE LEVELS IN SERUM OF MALE RAT

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ABSTRACT

Diazinon is insecticides widely used in agriculture and pest control in the environment, which can be highly toxic. DZN is bioactived by cytochrome P450 enzymes through desulphuration to its corresponding Oxon derivative. The aim of present study was to evaluate the effect of Diazinon on gonadotropins and testosterone levels in serum of male Rat. In this study, 14 male Wistar rats were divided into 2 equal groups: group1; normal control which were received standard diet during the experiment, group 2 received Diazinon with corn oil at a dose of 75mg/kg (1.4LD50) daily besides of standard diet for 6 weeks. Diazinon was given to animals through gastric gavage. After mentioned periods, blood samples were obtained and serum was isolated through centrifuge at the 2000 RPM for 15 minutes. The mentioned parameters were measured using the available kits (Pishtazteb Ltd.) and ELISA methods. It shows that there is significant difference among groups in term of the serum value of FSH and Testosterone ($P<0.05$) but not in LH ($p>0.05$). Data showed that FSH decreased significantly in treatment group; contrary, testosterone increased. In summary, diazinon has adversely affected the reproduction organ in male rats. The present results indicate that exposure to DZN has direct effects on rat's testis and the imbalance of circulating testosterone and gonadotropins may reduce fertility.

Keywords: Diazinon, Testosterone, LH, FSH, Rat

INTRODUCTION

Diazinon is a thiophosphoric acid ester developed in 1952 by Ciba-Geigy, a Swiss chemical company. It is a nonsystemic organophosphate insecticide formerly used to control cockroaches, silverfish, ants, and fleas in residential, non-food buildings. Diazinon was heavily used during the 1970s and early 1980s for general-purpose gardening use and indoor pest control. A bait form was used to control scavenger wasps in the western U.S. Diazinon is used in flea collars for domestic pets in Australia and New Zealand. Residential uses of diazinon were outlawed in the U.S. in 2004 but it is still approved for agricultural uses. An emergency antidote is atropine **(Budavari, 1996)**.

The structure of diazinon contains a thiophosphoric ester. The phosphorus center is the reactive site of the chemical. However, no known mechanisms currently exist. A novel mechanism does exist, which proposes that the sulfur is protonated in acidic medium via a hydronium ion which ultimately delivers a hydroxide group to the phosphorus center and can react readily.

The form of diazinon varies widely as it can be in dusts, granules, liquids, concentrates, microencapsulations, wettable powders, and seed dressings. Its appearance varies depending on purity, ranging from a dark brown (industrial grade), to a colorless liquid (pure). Indicative of its functionality of a thiophosphoric ester, the chemical has a pronounced smell similar to that of diethyl ether **(Sharom *et al*., 1980)**.

Diazinon is relatively stable under standard ambient temperature and pressure but is susceptible to form toxic phosphine gas upon heating. Furthermore, diazinon can oxidize into toxic phosphorus oxides if in the presence of a strong oxidizing agent.

Diazinon functions as an acetylcholinesterase (AChE) inhibitor. This enzyme breaks down the neurotransmitter acetylcholine (ACh) into [choline] and an acetate group. The inhibition of the AChE causes an abnormal accumulation of ACh in the synaptic cleft. When Diazinon enters the body, it is oxidatively degenerated to diaxozon, an organophosphate compound that is much more poisoning than diazinon and causes mainly the inhibition of AChE. The activation of diazinon is located in the liver microsomal enzyme system and requires O2 and NADPH. Additionally it can also be degenerated via oxidation in the microsomal enzyme system. Both reactions are possible, and likely are catalyzed nonspecifically by the same mixed function oxidase **(Hansch *et al*., 1995; Geller *et al*., 2003)**. Once formed diazoxon is biotransformed again as it is degenerated.Diazoxon is degenerated due to

the very effective hydrolyases in the microsomal and other sub cellular factions within the liver. Mammalian specie degenerate diazoxon at a much slower rate though (with a half-life of 2 to 6 weeks). Insects lack this hydrolysis step which allows for the toxic species to accumulate rapidly. The detoxification of diazoxon is processed through the mircosomal mixed function oxidase system. Although not fully known, it is believed that this is the cause for the selectivity of diazinon against insects. After the hydrolysis or oxidation diazinon is further degenerated. The aim of present study was to evaluate the effect of Diazinon on gonadotropins and testosterone levels in serum of male Rat.

MATERIALS AND METHODS

In this study, 14 male Wistar rats (200±2.1g and 2-3 month age) were selected for the study and were purchased from Animal House, Islamic Azad University and randomly divided into 2 equal groups: group1; normal control which were received standard diet during the experiment, group 2 received Diazinon with corn oil at a dose of 75mg/kg (1.4LD50) daily besides of standard diet for 6 weeks. Diazinon was given to animals through gastric gavage.

Animal care and experiments confirmed with the Guide for the Care and Use of Laboratory Animals of China and approval of the ethics committee of Islamic Azad University was obtained before the commencement of the study. The animals were housed under standard environmental conditions (23±1°C, with 55±5% humidity and a 12 h light/12 h dark cycle) and maintained with free access to water and a standard laboratory diet *ad libitum*. After mentioned periods, blood samples were obtained and serum was isolated through centrifuge at the 2000 RPM for 15 minutes. The mentioned parameters were measured using the available kits (Pishtazteb Ltd.) and ELISA methods.

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. The data obtained were tested by ANOVA followed by T-test. $P<0.05$ was considered statistically significant.

RESULTS

Data related to measurement of FSH, LH and testosterone in both groups are presented in **Table 1**.

Table 1: serum value of FSH, LH and testosterone in animals before and after Diazinon administration

Group	FSH (mIu/ml)	LH (mIu/ml)	Testosterone (ngr/ml)
Control	0.2±0.02	1.01±0.16	1.22±0.06
Treatment	0.1±0.015	0.96±0.08	1.5±0..49
p-value	<0.05	>0.05	<0.05

As seen in **Table 1** it shows that there is significant difference among groups in term of the serum value of FSH and Testosterone ($P<0.05$) but not in LH ($p>0.05$). Data showed that FSH decreased significantly in treatment group; contrary, testosterone increased.

DISCUSSION AND CONCLUSION

Some studies have showed that DZN wascapable of inducing structural and functionalchanges **(Wesseling *et al*., 1999)**, and some biochemical alterations inthe ovaries and testes **(Dutta *et al*., 2003)**. Our results demonstratethat administration of Diazinon can adversely effects in reproductive function by increasing the mass of testis and testosteroneblood levels, but decreasing the concentrations ofcirculating gonadotropins.

Some histopathological studies in animals haveshowed that diazinon treatment may inhibitspermatogenesis and make destruction of all kindof seminiferous tubules. Maintaining LH serumlevels is very important for initiating andupporting spermatogenesis, hence degeneration ofSertoli cells and germinal cells may be due to highconcentrations of circulating LH (**Shan *et al*., 1995; Sarkar *et al*., 2000)**. OP causemicrotubule disruption of epithelium **(Nakai *et al*., 1995)**, andfinally leads to tubular atrophy **(Hess *et al*., 2000)** Also, spermatogenesis disruption might be due toincreases in the serum LH concentration which isdetrimental to the germinal cells (having a veryimportant role in the first steps of spermatogenesisprocess) **(Boekelheide and Schoenfeld, 2001; Izumi *et al*., 2005)**.

Toxic effects of diazinon might be mediated by stimulation of lipid peroxidation, changes in the actions of antioxidant enzymes, DNA damage, and free radicals which involved in cell death **(Izumi *et al*., 2005; Ibrahim and El-Gamal, 2003)**.

Several studies have showed increased frequencies of sperm aneuploidy observed in individuals occupationally exposed to DZN **(Narayana *et al*., 2006)**. Also several OPs have been extensively studied as genotoxic chemicals in mammalian germinal cells and spermatids **(Recio *et al*., 2001; Cakir *et al*., 2005)**.

Prolonged IP exposure to the DZN, decreased the body weight in consensus with the earlier reports, and that effect was due to diminished food and water intake in treated mice of DZN group **(Prashanthi *et al*., 2006)**.

DZN has also been described as genotoxic, mutagenic carcinogenic agents mutagenic agent using in vivo and in vitro models **(Aluigi *et al*., 2005; LeBlanc *et al*., 1997)** probably by sharing the alkylation properties ofmany other OP compounds.

DZN exposure also has caused reduction in the Leydig cells.

There are several possible mechanisms for the antigonadal actions of organophosphates; they may exert a direct inhibitory action on the testis; they may affect the pituitary, causing changes in gonadotrophins concentrations and thus subsequent spermatogenic impairment; or they may change the concentration of neurotransmitter **(Pidoux *et al.*, 2007)**.

Antiandrogens can disrupt male differentiation by several mechanisms, including antagonism of receptor binding, or by inhibition of the production, transport, or metabolism of androgens **(Chattopadhyay *et al.*, 2005)**. The damage may have occurred by direct toxic effects of DZN on cells or tissue, but it might also occur because of imbalanced hormones levels. DZN may affect directly upon testis tissue or by entering into the pituitary gland which could cause changes of gonadotropins arise. In these actions of DZN, serum LH and FSH levels increased probably due to suppression of feed-back inhibition of anterior pituitary. The suppression of feedback inhibition may secondarily increase the LH and FSH secretion. The results of the present study indicate that DZN has a direct effect on pituitary, which lead to increase in circulating LH levels. Diazinon increase serum FSH levels after injection in mice. FSH has important effects on sertoli cells. Inhibin and other factors secreted by Sertoli cells cause increase of circulating FSH levels by feedback on pituitary. Increase of LH levels has toxic effects on testis tissue.

In summary, diazinon has adversely affected the reproduction organ in male rats. The present results indicate that exposure to DZN has direct effects on rat's testis and the imbalance of circulating testosterone and gonadotropins may reduce fertility.

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