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DIAZINON ADVERSELY AFFECTS THE SPERM COUNT AND INCREASES THE ABNORMALITY LEADING POSSIBLE INFERTILITY IN ALBINO WISTAR RATS

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ABSTRACT

Toxic effects of diazinon, dose levels 0, 6, 7.5 and 10mg /kg body weight daily single dose for 5 days and one month on male reproductive system of rats was studied. Testis weight, epididymal weight, sperm count and sperm morphology were evaluated with low dose of diazinon based on LD_{50}. Results confirmed that the effects of all doses on body weight, testis weight and epididymal weight were dose dependent. Both the 5 and one month exposure groups showed a significant decrease in sperm count and increase in the number of abnormal spermatozoa. Present study confirms that diazinon alters reproductive parameters and affects fertility. Further studies of fertility determining parameters, gonadal hormone levels, testicular enzymes and seminiferous germ cell architecture and cell cycles are required to confirm the adverse toxicity diazinon.

Key words: - Diazinon, sperm count, spermatozoa, abnormal sperm.

INTRODUCTION

Pesticides are indispensable chemicals to farmers for the production of high yield of food grains and vegetables. Beneficial effects primarily help in hunger alleviation but same time they may cause wide range of health problems from acute and sustainable damage to immune, excretory system, neuronal system cancer and skin disorders[1]. Pesticides enter the food chain then to the animal and human body[2]. Residual amount have been detected in the soil, water bodies, food grains and main food products[3] , an alarming signal of deterioration of male reproductive system. There is an increasing concern about the adverse effects of organophosphates (OP) in living organism as it is used without proper safety guidelines. They cause cholinergic and non-cholinergic functional disturbances[4, 5, 6] inhibiting acetylecholinesterase activity in the target tissues[7, 8]. In pre-clinical toxicity studies, organophosphate compounds resulted in a decrease in sperm motility and sperm count exposed to Malathion, dichlorvos[9] and methyl parathion[10]. D'Souza et al., (2006)[11] confirmed testicular damage on exposure to methyl parathion in rats. Previous study showed that chlorpyrifosmethyl, diazinon and profenofos on testis and seminal vesicles weights was dose dependent as tested pesticides at 50ppm significantly reduced their weights, sperm count and increased abnormal sperm in rat[12]. Few reports have shown close relationship between exposure to pesticide and adverse affect on sperm quality in pesticide workers and laboratory animals[10, 11, 12, 13]. In two decades research reports revealed a large number of pesticides induce genetic lesions in human and animals[14]. Diazinon a commonly used OP by farmers and have adverse effects on reproduction[15, 16]. Diazinon [o, o-diethyl-o-(2isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate] is an organophosphate insecticide for the control of sucking, chewing insects and mites in agriculture and horticulture in entire globe. Less information is available on diazinon toxicity on reproductive system and since there are no reports on the levels of diazinon and its metabolites in environment. Adverse effects on spermatogenesis with low dose exposures based on lethal dose – 50 (LD_{50}) is the need of the hour. Hence, the present study was planned to confirm the diazinon exposure and its adverse effects on reproductive system rats.

MATERIAL AND METHODS

Mature male Wistar rats (13 – 16 weeks old) 200 – 220 gram from the K.V.G. Medical College, Sullia, D.K, animal house were used. The rats were maintained in polypropylene cages (29×22×14cm) with paddy husk bedding in laboratory conditions of 28±2°c temperature and 50±5/ humidity for 15 days for acclimatization. In each cage, three rats were housed to avoid any overcrowding. The rats had access to food and water ad libitum (laboratory pellets; Gold Mohur-Lipton India Ltd.).

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Rats were in good health. Prior to administration of test substance (diazinon), rats were assigned to each group by randomization of body weights. All experimental procedures were done under the guidelines of Institutional Ethics Committee.

TEST CHEMICAL

Diazinon (o, o-diethyl o-2-isopropyl-6methylpyrimidine-4-yl phosphorothioate) of 99% purity kindly provided from Devidayal Agro Chemicals Mumbai. LD 50 of diazinon in rat is 300mg/kg. In this study, the doses used were 1/50th (6mg/kg), 1/40th (7.5mg), 1/30th (10mg/kg) of LD_{50} doses by acute and chronic exposures in adult male wistar rats.

EXPERIMENTAL DESIGN

After 15 days of acclimatization, rats were randomly segregated into 2 groups; Experimental (n=36) and control (n=12) groups. Experimental group were divided into 2 groups, acute (n=18) and chronic (n=18) groups. The acute and chronic groups further divided each into 3 groups (n=6). Each group received diazinon at dose level 0 (control), 1/50, 1/40 and1/30th /kg body weight of LD_{50} daily once for 5 and one month respectively. It was administered orally between 10:00am and 10:30 h. First treatment day with diazinon was considered treatment day 1.The dosage for each rat was corrected for individual body weight each third day by volume adjustment. Control group received 1ml corn oil once in a day. Rats were sacrificed after 24h of last treatment by cervical dislocation. Laporatomy was performed and the reproductive organs were exposed. The epididymis were separated and the right epididymis was immersed in 1ml of phosphate buffer saline (PBS, pH 7.2), medium used to avoid distortion and viability of sperm. Both the testis and left epididymis were removed cleaned of extra tissues and fixed in Bouin's fluid and were preserved for 24h in room temperature and weighed[17].

EPIDIDYMAL SPERM COUNT AND MORPHOLOGY: Epididymis was minced in 1ml PBS, suspension was filterd in a nylon mesh[17] (80 micron). Sperm count was done in the filtrate as per the standard method using Neubaur's chamber[18, 19]. An aliquot from the filtrate (upto 0.5mark) was taken in leucocyte hemocytometer pipette and diluted with phosphate buffered saline upto the mark 11. The suspension was well mixed and infused into Neubauer's counting chamber. Total number of sperm in 8 squares (except central RBC area) of 1mm^2 each was determined and multiplied by 5×10^4 to express the number of sperm/epididymis. For morphological analysis, the filtrate obtained was stained with 1% eosin Y and morphological defects were evaluated in sperm smear[17,18]. Three sperm smear slides were prepared from each sperm suspension. These additional slides were used in confirming any preparation artifact or to have additional sperm to evaluate when sperm count were very low, and also used to verify an increased incidence of detached sperm heads, headless tails were not artifacts of preparation. All the slides were coded to minimize scorer bias. The sperm in the smear were microscopically screened under 40X and any defects of either heads or tails were noted. Two hundred sperm were screened in each slide and total defects were expressed as incidence/200 sperm/rat[18, 19].

STATISTICAL ANALYSIS

Data was expressed as Mean ±SD. Analysed by using one - way ANOVA, followed by Bonferroni's post – hoc test. The statistical tests were obtained from statistical package of social sciences (SPSS) version 16. Values of $P<0.05$ were considered as statistically significant.

RESULTS

There was a decrease in the body weight compared to 6mg/kg, 7.5mg/kg and 10mg/kg dose with control however, there is no statistical significance except in 6mg and 7.5mgs on 15th day ($p<0.05$) and 6mg treated rats on 25day ($p<0.05$). The body weight gain was lower in treated groups compared to control (fig 1) but still was within comparable limits.

There was no significant decrease in the testis weight except in 7.5mg/kg in 5day treatment group compared to control ($p<0.05$). The epididymal weight was significantly decreased in 6mg/kg in 5day group verses control ($p<0.05$) and 6mg acute group compared to 6mg chronic group ($p<0.05$). The sperm count were significantly decreased in 6mg/kg and 10mg/kg ($p<0.001$) and 7.5mg/kg, ($p<0.005$) in 5 day group over control groups. There was a significant decrease in sperm count in all treated 30 day groups compared to control ($p<0.001$) with a dose-dependent response. The sperm count in 10mg ($p<0.05$) and 7.5mg ($p<0.01$) chronic groups

decreased compared to 6mg acute group. The sperm count in 7.5mg treated acute group was less significant compared to 10mg and 7.5mg chronic groups ($p<0.001$). The sperm count was significantly decreased in10mg acute group when compared to 6mg and 7.5mg acute groups ($p<0.001$) and 6mg chronic group ($p<0.05$) (fig 2).

The density of sperm with normal morphology were significantly decreased in acute treatment group over control group ($p<0.001$) and 6mg/kg verses higher doses ($p<0.05$). In all three doses of chronic group the abnormal sperm count increased significantly ($p<0.001$) compared to control. Deformed sperm were increased ($p<0.001$) with higher doses of study and one month treatment groups when compared to 6mg and 7.5mg acute groups. The abnormal sperm count increased in 10mg acute group compared to10mg chronic group ($p<0.001$). The percent abnormal morphology were increased among chronic groups in a dose- dependent manner, i.e,6mg verses 10mg($p<0.001$)and 7.5mg verses 10mg($p<0.01$).

It has induced different types of tail and head abnormalities in sperm. Head anomalies were three or double head; banana shaped, hookless, microcephaly, macrocephaly, amorpous, globular and tubular head sperm. Sperm showed bending at cephalo-caudal junction with or without fibrils projecting out. Both in acute and chronic group diazinon caused the formation of triple or double tailed, coiled or folded, zigzag or wavy tails, broken or tailless sperm (Fig 3).

DISCUSSION

Diazinon, an organophospate compound is routinely employed in agriculture and household purpose. It has negative health effects in living organism. Presence of pesticide residues in the environment, and food products indicate an alarming signal and need to be assessed scientifically at preclinical and clinical levels. In recent years a precipitous drop in semen quality was recorded[20]. Though there are number of studies in literature, pesticide induced fertility decline among human model is difficult to assess and scientific community is heavily dependent on pre-clinical animal study models as these study models contribute a lot of information as all these models mimic a similar body structure, function and metabolism. Since a dose and duration dependent assessment of toxicity reveals a lot of information, application of such data for human wellbeing paves a way for better extrapolation and health care. Diazinon is less studied on its toxicity effects in male animal model, an extensive and systematic dose and time response study based on its lethal dose-50 level, since environmentally available toxic dose is not reported in the literature, minimal multiple doses were selected to systematically assess its adverse effects on spermatogenesis in rats.

In our study sperm number was decreased significantly ($p<0.001$) suggestive of reduction in spermatogenesis. The reason for a dose – dependent decline in sperm count is multifactorial, a direct sperm cell damaging effect of diazinon or its metabolites such as diazoxone on seminiferous epithelial cells by inducing necrosis or apoptosis. Fattahi et al., (2009)[21] showed a decrease in the number of germ cells, spermatocytes and spermatids following 30mg/kg body weight of diazinon in mice. Secondly diazinon being an organophosphorous compound may induce generation of free radicals and a reduction in the natural antioxidants, both having an additive effect on cell damage. Similar reactive oxygen induced toxicity and cell death in testis has been reported earlier with pyrethroid, malathion and chlorpyrifos compounds 3, 12, 23. Measurements of free radical markers such as superoxide dismutase (SOD), glutathione or catalases may further support our hypothesis of cell death on exposure to diazinon and the subsequent decline in sperm count. Thirdly since a decline in count was also observed in chronic groups, a possibility of indirect effect on different hormones such as FSH, LH and testosterone cannot be ruled out. In more recent study, an increase in FSH, LH and a decrease in testosterone levels were reported on exposure to 30mg/kg diazinon in mice[21]. Further, pituitary –gonadal axis response with an increase in FSH, LH and a decline in progesterone levels were observed in female rats on treatment of 50, 100 and 150mg/kg diazinon[22]. These results partly confirm our hypothesis of hormonal imbalance as a possible cause for pesticide induced germ cell atrophy and a subsequent decrease in sperm count. A decrease in weight of the testis also adds to our hypothesis of cell death or cell removal as a possibility of cell sloughing and gradual washing away of cell debris away through the vas deferens there by adding to a decline in sperm number. A decrease in testis weight also may be an outcome of defective cell architecture and cellular harmony.

Measurements of seminiferous tubular dimensions such as tubular diameter, epithelial height may further support our view of diazinon toxicity breaking the cell association and further inducing sloughing of germinal cells and a decrease in sperm count. A decrease in the number of corpus luteum was reported in both the ovaries on exposure to 150mg/kg diazinon in rats supporting the possibility of cell death[23]. Total abnormal sperms were significantly increased in all three tested low doses of diazinon for both acute and chronic exposure groups. This was similar to what was reported by Abd El – Aziz et al (1994)[16]. A varied variety of abnormal sperm morphology was an indication of decline in fertility index. The breaking away of head from flagellum and flexion of head may be due to the impact of the chemical at the neck or connecting piece of the flagellum[24]. The curved/bent or coiled nature of the sperm tail possibly may be due to the action of diazinon on microtubule of the sperm axoneme. Similar findings were reported by Akbarsha et al (2001)[24] in carbendazim, a fungicide which caused disruption of microtubules of sperm axoneme, resulting in weakening of the axoneme and curvature/coiling of the flagellum. They also reported that sticking or fusion of sperm at various points and over short to long distance may be due to the change in the sperm surface protein. An alteration in sperm chromatin condensation and DNA damage after diazinon injection to mice, phosphorylation of proteins and chemical alterations in sperm nuclear proteins in this repair deficient period of late spermatogenesis and epididymal sperm maturation contribute to male reproductive toxicity[14,25]. There is also a possibility of genetic damage and a resultant point mutations must have induced a change in the sperm shape adding to the tail or head abnormality[19, 26]. The most common malformations which were observed in sperm were bent tail, coiled tail, headless and tailless, globular, tubular, banana shape head, double/triple head, double/triple tail, broken sperm, zigzag/wavy sperm and few other unique shaped sperm (Figure 4). Sperm morphology is a useful marker between fertile and infertile males than sperm concentration[21]. Simultaneous chromosomal aberration and molecular DNA studies along with sperm morphology on exposure to diazinon is highly warranted to confirm the present findings in line with genetic toxicity. OPs are known to cause reproductive abnormalities. Broken sperms, cytoplasmic droplets and reduced sperm motility are principal markers of decreased quality due to OP pesticides in rat[13]. Sublethal administration of quinolphos resulted in severe disruption of spermatogenesis with increasing dosage of

Figure:-1

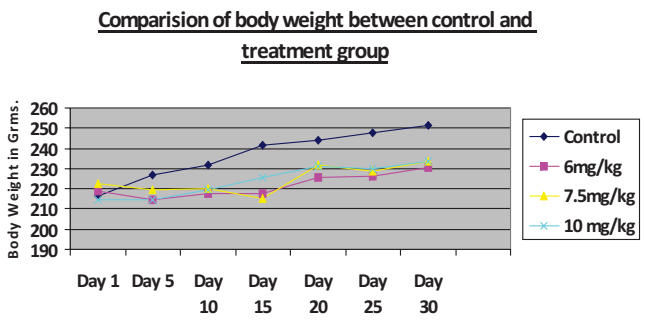

Figure:-2

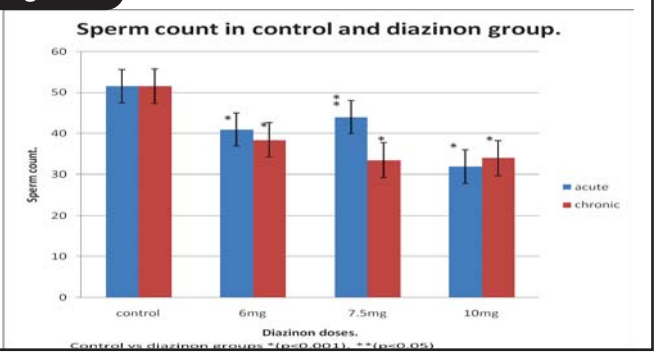

Figure:-3

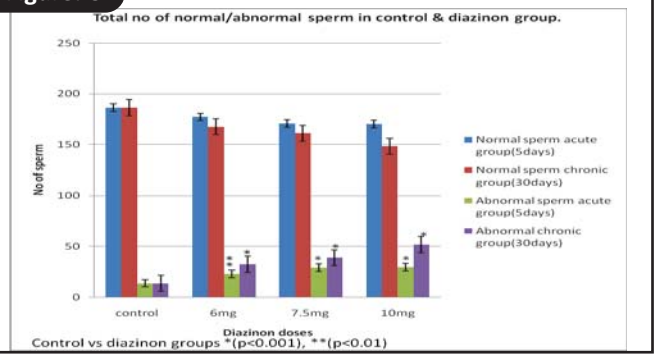

Figure:-4

Normal and different types of abnormal sperm on exposure to diazinon

pesticide[27]. Pedungtod et al (2007)[28] found reduced sperm count in Chinese pesticide factory workers. It was observed that OPs can induce oxidative stress by altering the level of the free radical scavenging enzymes activity. Overall all the three doses of diazinon studied for the acute and chronic exposure period provide an evidence of male reproductive toxicity and impairment in spermatogenesis. Further, measurements of free radical markers, testicular marker enzymes and hormones such as testosterone, LH and FSH may further strengthen our findings.

CONCLUSION

Exposure to diazinon results in impairment of spermatogenesis and induction of abnormal sperm leading to a possible adverse effect on fertility.

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