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Acetamiprid, Insecticide-Induced Oxidative Damage on Reproductive Parameters Male Rats

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ABSTRACT

Acetamiprid is a neoncotinoid insecticide that acts as agonist to the nicotinic acetylcholine receptor used as insecticide in crops and control fleas on dogs and cats. The objective of this study was to evaluate the acetamiprid toxicity of oral administered on the reproductive system, to assess the role of oxidative stress inducing damage to the testes in male rats. The low dose equal 1/4 of LD_{50} and high dose equal 1/2 of LD_{50}. Doses of acetamiprid adjusted according to rat's body weights. The results revealed that acetamiprid at high dose significantly ($P<0.05$) increased thiobabituric acid reactive substances (TBARS) resulting in significantly ($P<0.05$) decrease reduced glutathione (GSH), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) in testes homogenate. Also, administratim of acetamiprid significantly increased catalase while, there was insignificant change in superoxide dismutase (SOD). Acetamiprid at low and high doses significantly ($P<0.05$) decreased plasma testosterone level, sperm count, viability, motility, fructose level, and significantly ($P<0.05$) increased abnormal sperms, altered acrosome and abnormal DNA. Acetamiprid induce oxidative stress leading to decrease antioxidant enzymes and damage sertoil cells resulting in decreasing testosterone level and effect on fertility. Moreover, the histological investigation revealed that exposure to acetamiprid disorganized stratification of spermatogonia, primary and secondary spermatocytes in some tubules sheeding of sperm cells into lumina, necrosis in tubules and reduction of the primary and secondary spermatids. In conclusion, it seems that acetamiprid induces adverse effect at high dose level but the low dose level may not and short-term exposure make adverse effect. So, it can recommend using this insecticide as long as the recommended dose.

1. INTRODUCTION

Acetamiprid (ACP) is a member of the neonicotinoid insecticide family is the most highly effective and largest selling group of worldwide for the controlling aphids, beetles, moth, leafhopper, pests on crops and leafy vegetables, along with fleas infesting livestock and pet animals. Moreover, acetamiprid being highly water soluble indicates a high potential for the compound to leach in soil or to run off in surface water (Singh *et al.*, 2012).

Widespread use of acetamiprid is causing pesticide entry into the food chain, which in turn causing toxicity to man and animal, acetamiprid is rapidly and almost completely absorbed and is widely distributed into tissues, being found at highest concentrations in gastrointestinal tract, adrenal gland, liver and kidney, the major route of elimination was via the urine and bile (Mondal *et al*., 2014). Adverse clinical effects include cardiovascular symptoms, central nervous symptoms, respiratory symptoms, low body temperature, muscle weakness, miosis, and dry mouth, suggesting a wide variety of possible clinical symptoms associated with ACP poisoning (Terayama *et al.*, 2016).

Because acetamiprid has a neurotoxic mode of action, early studies mainly focused on its acute toxicity to the mammalian nervous system. Inhalation of acetamiprid can cause dizziness, headaches, vomiting, and nausea. Acetamiprid can have damaging effects on other body systems. Decrease in plasma testosterone concentration and

sperm quality after exposure to acetamiprid. At the same time, acetamiprid increased malondialdehyde and nitric oxide (NO) levels of Leydig cells. Acetamiprid exposure also decreases cytochrome P450, and testicular mRNA levels, which are cAMP-dependent proteins that are essential for steroidogenesis. Electron microscopy indicated mitochondrial membrane damage in the Leydig cells of the testes of exposed rats. Also indicated that acetamiprid causes oxidative stress and mitochondrial damage in Leydig cells. Acetamiprid disrupts subsequent testosterone biosynthesis by the rate of conversion of cholesterol to testosterone and by preventing cholesterol from the mitochondria within the Leydig cells. These effects caused reproductive damage to the rats (Kong et al., 2016).

Plasma testosterone concentration and sperm quality decreased as the levels of luteinizing hormone (LH) decreased after acetamiprid exposure. Testosterone is the body's major androgen. Its action on cells of the male reproductive system is essential for spermatogenesis and the maturation of spermatogonia to spermatozoa. Disrupting testosterone productions adversely affect male reproductive function (Sgro et al., 2014).

Acetamiprid increased malondialdehyde and nitric oxide (NO) levels of Leydig cells. Further analysis showed that acetamiprid reduced the adenosine triphosphate (ATP) and cyclic adenosine monophosphate (cAMP) production of Leydig cells. Many studies have illustrated that the cyclic adenosinemonophosphate (cAMP) and protein kinase A signaling pathways are the major signaling mechanisms of steroidogenesis, so ROS produced by acetamiprid may be inhibited the signal pathway and reduced testosterone secretion acetamiprid abnormally increased the ROS level in mouse testes, and found that this was mainly due to acetamiprid inhibiting antioxidant enzyme including catalase, glutathione peroxidase (GPx), superoxide dismutase (SOD) activity in testes (Manna and Stocco, 2005)

Zhazng et al. (2011) reported that, acetamiprid decreased the body weight and the weight of testosterone responsive organs, such as the testes, epididymis, seminal vesicle, and prostate. Furthermore, acetamiprid also reduced the plasma testosterone concentration, and decreased sperm count, viability, motility, and the intactness of the acrosome.

2. MATERIALS AND METHODS

2.1. Animals, Experimental Design and Sampling

This study was approved by the Ethical Committee of the Institutional Animals Care and Use, Alexandria, Egypt and met all guidelines for their use.

Acetamiprid (98%) was obtained from central agriculture pesticide laboratory. Eighteen healthy adult male albino rats with average weight of 180±10g, were obtained from animal house, Faculty of Medicine, Alexandria University, and acclimated for two weeks prior to the experiment. They were assigned to 3 groups and housed in Universal galvanized wire cages at room temperature (22-25 °C) and in photoperiod of 12h/day. Animals were provided with balanced commercial diet containing, 18% crude protein, 14% crude fiber, 2% fat and 2600 Kcal DE/Kg feed.

Animals were divided randomly into 3 groups (6 animals each). Animals were maintained on food and water *ad libitum*. Doses of acetamiprid were prepared by dissolving in distilled water and adjusted according to rat's body weights and given orally by gavages approximately at the same time each morning, 3 times per week day after day for 4 weeks. Group I (control) was orally administered with saline. Group II was orally administered with a dose equal 55 mg/Kg body weight of acetamiprid (1/4 LD50). Group III was orally administered with a dose equal 110 mg/Kg body weight of acetamiprid (1/2 LD50). The doses were choosen based on the previous study (Chakroun *et al*., 2016). At the end of treatment period rats were sacrificed, blood was collected from the heart vena cava, in heparinized tubes, and was centrifuged at, 1,000 xg for 15 min. Blood plasma was separated in Eppindorff tubes and stored at -80˚C till further investigations. Testes were isolated, weighed, then washed with saline and kept at -80 ˚C for further biochemical studies. Parts of testes used for histological studies kept in formalin (10%).The crude homogenates of the testes were prepared according to Greer *et al.* (2003).

2.2. Testes homogenate biochemical assays and blood plasma hormonal assay

Thiobarbituric acid reactive substances (TBARS) were measured as described by Tappel and Zalkin (1959). Reduced glutathione (GSH) was determined according to the method described by Ellman (1959), glutathione-s-transferase (GST) (GST; EC 2.5.1.18) activity was assayed according to the method of Habig *et al*. (1974), glutathione peroxidase (GPx) activity (GPx; EC 1.1.1.9) activity was determined according to the method described by Pagila and Valantine (1967) and glutathione reductase (GR) (E.C.1.6.4.2) was determined according to the method described by Goldberg and Spooner (1983). Superoxide dismutase (SOD) (SOD,

EC 1.15.1.1) was assayed according to the method described by Nishikimi *et al*. (1972). Catalase (CAT; EC 1.11.1.6) was determined according to the method described by Sinha (1972). Fructose was determined according to Foreman *et al.* (1973). Enzyme linked immunosorbent assay (ELISA) of testosterone was determined according to (Nash *et al*. (2000).

Biochemicals and testosterone kits purchased from BioSystems Company

2.3. Sperm collection and analysis

Immediately after decapitation, both rat's testes and epididymis were removed, cleaned of accessory tissue and weighed and sperm collection was performed according to Trošić *et al.* (2013). Sperm viability was assessed by the eosin Y stain and the motility of sperm was assayed by the number of sperm that could move in a line. The percentage of viable sperm and the motility of sperm were calculated according to Wyrobek and Bruce, (1975). The integrity of the acrosome was assessed using Tejada acridine orange Method (Tejada *et al*., 1984; Erenpreiss *et al.*, 2001).

2.4. Histological examination

Parts of testes were fixed in 10 % formaldehyde solution, embedded in paraffin wax, and cut with microtome for 5µ thick sections. The sections were stained by Hematoxylin and Eosin (H&E) stains and microscopically studied to evaluate its morphology (Drury and Wallington, 1980)

2.5. Statistical Analysis

The data were analyzed using a one-way analysis of variance (ANOVA) followed by Duncan multiple comparison. P<0.05 was statically significant according to Norušis (2006).

3. RESULTS

3.1. Effects of acetamiprid on thiobarbituric acid reactive substances, enzymatic and non-enzymatic antioxidants in testes homogenate of male rats

The present study, showed that, treatment of rats with ACP-d2 significantly (P<0.05) increased TBARS when compared to the control group. Treatment of rats with ACP-d2 significantly (P<0.05) decreased GSH, GPx and GR when compared to the control group, while in the group treated with ACP-d1, GSH significantly (P<0.05) increased when compared to control group. Treatment with ACE-d1 and ACP-d2 significantly (P<0.05) decreased GST, while significantly (P<0.05) increased catalase activity when compared to the control group, treatment of rats with acetamiprid showed insignificant change in SOD activity (Table1).

3.2. Effects of acetamiprid on testosterone in blood plasma and sperm quality

Results revealed that, acetamiprid (ACP), significantly (P<0.05) decreased plasma testosterone. Also, ACP significantly (P<0.05) decreased sperm motility, viability and significantly (P<0.05) increased the number of total abnormal sperm, altered acrosome and abnormal DNA when compared to the control group (Table2).

Table 1: Effects of acetamiprid on thiobarbituric acid reactive substances (TBARS), super oxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione-s-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) in testes homogenate of male rats

Parameters	Groups		
	Control	ACP-d1	ACP-d2
TBARS (µmol/g tissue)	1.42±0.05^{b}	1.45±0.05^{b}	1.77±0.04^{a}
SOD (IU/g tissue)	1232.39±17.38^{a}	1167.61±45.10^{a}	1230.68±14.66^{a}
CAT (IU/g tissue)	991.80±49.13^{b}	1762.78±36.66^{a}	1675.71±82.97^{a}
GSH (mg/g tissue)	23.49±0.32^{b}	28.02±1.15^{a}	16.90±1.54^{c}
GST (IU/g tissue)	26.55±0.82^{a}	19.40±0.326^{b}	19.95±1.23^{b}
GPx (IU/g tissue)	42.25±2.42^{b}	72.01±4.59^{a}	23.61±1.66^{c}
GR (IU/g tissue)	12.14±0.50^{a}	12.50±0.78^{a}	8.68±0.67^{b}

Results expressed as Mean±SE, n=6

In Tables, the values denoted by different letters within same row represent significant differences (P<0.05).

ACP-d1, ACP-d2: acetamiprid dose-1 (55mg/Kg) and acetamiprid dose-2 (110mg/Kg), respictively

Table 2: Effects of acetamiprid on testosterone in blood plasma and sperm characteristics of male rats

Parameters	Groups		
	Control	**ACP-d1**	**ACP-d2**
Testosterone (µg/dL)	3.93±0.114[a]	3.20±0.093[b]	3.06±0.089[b]
Motility (%)	76.20±0.58[a]	59.20±3.18[b]	53.80±2.33[b]
Viability (%)	71.00±0.55[a]	53.40±3.37[b]	47.20±2.33[b]
Abnormal sperms (%)	5.30±0.20[c]	6.80±0.37[b]	10.40±0.68[a]
Altered acrosome (%)	5.40±0.25[c]	6.80±0.37[b]	10.60±0.51[a]
Abnormal DNA (%)	6.20±0.58[b]	7.20±0.58[ab]	9.20±0.80[a]

Results expressed as Mean±SE, n=6
In tables, the values denoted by different letters within same row represent significant differences (P<0.05).
ACP-d1, ACP-d2: acetamiprid dose-1 (55mg/Kg) and acetamiprid dose-2 (110mg/Kg), respictively

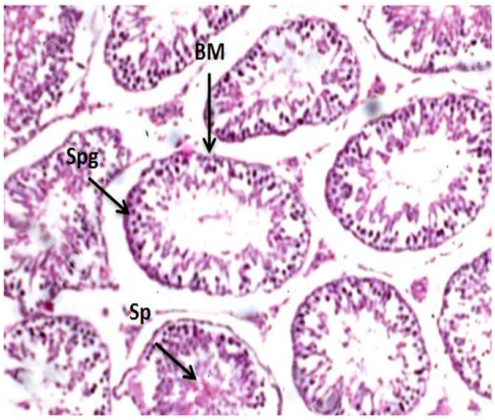

Figure (1): Photomicrographs of testes section of male rats control group **(H&EX200)**

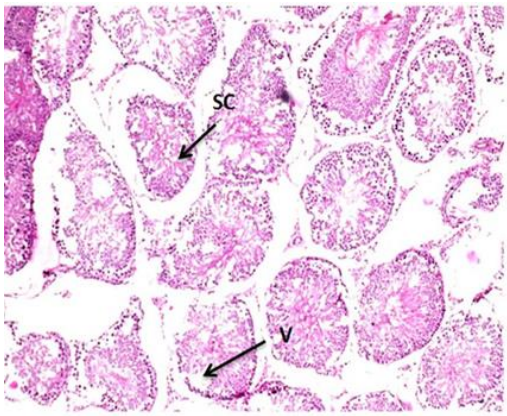

Fig. 2. Photomicrographs of testes section of male rats treated with ACP-d1 (H&EX100)

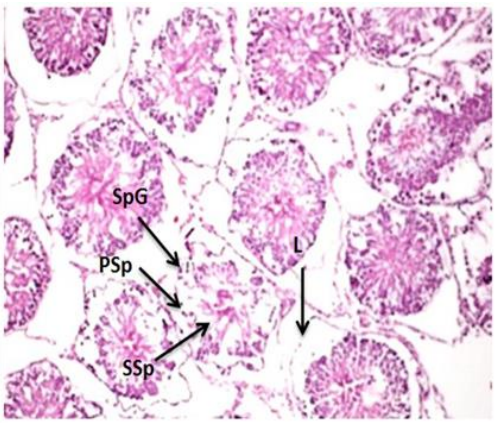

Figure (3): Photomicrographs of testes section of male rats treated with ACP-d2 (H&EX100)

3.3. Effects of acetamiprid on histological changes in testes

Microscopic examination of control testes of male rats showed; normal testicular structure, normal spermatogonium cells (Spg) with its normal basophilic differentiated nuclei cells, normal spermatogenic series start from the basement membrane (BM) giving abundant number of spermatids (Sp) in the center of the testes tubuli (Figure 1). On other hand, testes tissue of male rats treated with ACP-d1 (55 mg/kg); revealed, large basal vacuoles (V) in cytoplasm of Sertoil cells (Sc) (Figure 2). Testes tissue of male rats treated with ACP-d2 (110mg/kg); illustrated disorganized stratification of spermatogonia(SpG), primary and secondary spermatocytes in some tubules sheeding of sperm cells into lumina (L) (Figure 3).

4. DISCUSSION

The antioxidant enzymes are considered the first body line defense against free radicals. The decrease in the antioxidant enzymes activities may be due to lipid peroxidation. The increasing of catalase activity may due to free radicals inducing the production of catalase enzyme (Chakroun *et al*., 2016).

The production of reactive oxygen species (ROS) has been associated with a reduction in sperm motility, decreased capacity for sperm-oocyte fusion and infertility. Excessive ROS are toxic to spermatozoa due to their high polyunsaturated fatty acid content lead to the loss of membrane structure and function. Nicotine may cause a decreasing in sperm function. Acetamiprid and its metabolites induced oxidative stress leading to loss testicular and spermatogonia tissues (Keshta *et al*., 2016)

Kong *et al*. (2016) study corresponds with the present results, rats which administered with acetamiprid (30mg/kg/body weight for 35 days) showed: seminiferous tubules were severely impaired and had vacuolization. The number of Interstitial Leydig cells decreased. Keshta *et al*. (2016) showing sloughing of spermatogenic cells, areas of edema, reduced number of sperms and congestion in rat testes treated with ACP (1/10 LD50/30 days).

After exposure to acetamiprid, the decreased plasma testosterone concentration in rats would lead to a decreased sperm number and motility. Similar results have been reported in male rats exposed to cypermethrin, fenvalerate, and other synthetic pyrethroids. Acetamiprid induce oxidative stress which disrupt lipid bilayer in cell membrane leading to reduce testosterone biosynthesis which cause decreasing sperm motility, viability and also increasing abnormal sperm, altered acrosome and abnormal DNA. An increase in lipid peroxides indicates serious damage to the cell membrane, inhibition of several enzymes, cell function and cell death (Gasmi *et al*., 2016).

The decrease of testosterone might be responsible for the decreased sperm counts and motility and also morphological abnormality of testes. It is also suggested in earlier studies that insecticides may cause mitochondrial membrane impairment in Leydig cells and disrupt testosterone biosynthesis by diminishing the delivery of cholesterol into the mitochondria and decreasing the conversion of cholesterol to pregnenolone in the cells, thus reducing subsequent testosterone production. Acetamiprid-fed rats had fewer Leydig cells than normal diet fed rats which may be contributed to the reduction in testosterone biosynthesis (Desai *et al.*, 2016).

Nicotinic acetylcholine receptor (nAchRs) could be acted with acetamiprid and suppressed gonadotropins production that caused the hormonal imbalance of luteinizing hormone (LH) causing adverse effect on sperm production (Ngoula *et al.*,2007)

Conclusion

Exposure to acetamiprid was found to induce toxicity in testes by induction of lipid peroxidation and depletion of antioxidant activities and this confirmed by histological changes in testes. Also, acetampirid showed negative impacts on sperms characteristics which in turn affects testosterone and fructose levels.

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REFRENCES

Chakroun, S., Ezzi, L., Grissa, I., Kerkeni, E., Neffati, F., Bhouri, R., Najjar, M.F., Hassine, M., Mehdi, M., Haouas, Z., Cheikh, H.B. 2016. Hematological, biochemical, and toxicopathic effects of subchronic acetamiprid toxicity in Wistar rats. Environ. Sci. Pollut. Res. 23(24): 25191-25199.

Desai, K.R., Moid, N., Patel, P.B., Highland, H.N. 2016. Evaluation of Deltamethrin induced reproductive toxicity in male Swiss mice. Asian Pac. J. Reprod. 5(1): 24-30.

Drury, R.A.B., Wallington, E.A.1980. Preparation and fixation of tissues. Carleton's histological technique. 5: 41-54.

Ellman, M. 1959. A spectrophotometric method for determination of reduced glutathione in tissues. Anal. Biochem. 74: 214-226.

Erenpreiss, J., Bars, J., Lipatnikova, V., Erenpreisa, J., Zalkalns, J. 2001. Comparative study of cytochemical tests for sperm chromatin integrity. J. androl. 22(1): 45-53.

Foreman, D., Gaylor, L., Evans, E., Trella, C. 1973. A modification of the Roe procedure for determination of fructose in tissues with increased specificity. Anal. Biochem. 56(2): 584-590.

Gasmi, S., Rouabhi, R., Kebieche, M., Salmi, A., Boussekine, S., Toualbia, N., Taib, C., Henine, S., Bouteraa, Z., Djabri, B. 2016. Neurotoxicity of acetamiprid in male rats and the opposite effect of quercetin. Biotechnol. Ind. J. 12(7): 113.

Goldberg, D.M., Spooner, R.J. 1983. Methods of enzymatic analysis. Bergmeyer HV. 3: 258-265.

Greer, J. P., Foerster, J., Lukens, J. N. 2003. Wintrobe's Clinical Hematology(Vol.2). Lippincott Williams and Wilkins.

Habig, W.H., Pabst, M.J., Jakoby, W.B. 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249(22): 7130-7139.

Keshta, A.T., Hataba, A.A., Mead, H.M., El-Shafey, N.M. 2016. Oxidative stress and biochemical changes induced by thiamethoxam and Acetamiprid insecticides in rats. World J. Pharm. Sci. 5(6): 44-60.

Kong, D., Zhang, J., Hou, X., Zhang, S., Tan, J., Chen, Y., Yang, W., Zeng, J., Han, Y., Liu, X., Xu, D. 2016. Acetamiprid inhibits testosterone synthesis by affecting the mitochondrial function and cytoplasmic adenosine triphosphate production in rat Leydig cells. Biol. Reprod. 96(1): 254-265.

Manna, P.R., Stocco, D.M. 2005. Regulation of the steroidogenic acute regulatory protein expression: functional and physiological consequences. Curr Drug Targets Immune Endocr. Metabol. Disord. 5: 93–108.

Mondal, S., Ghosh, R.C., Karnam, S.S., Purohit, K., 2014. Toxicopathological changes on Wistar rat after multiple exposures to acetamiprid. Vet. World. 7(12): 1058-1065.

Nash, J.P., Davail-Cuisset, B., Bhattacharyya, S., Suter, H.C., Le Menn, F., Kime, D.E. 2000. An enzyme linked immunosorbant assay (ELISA) for testosterone, estradiol, and 17, 20β-dihydroxy-4-pregenen-3-one using acetylcholinesterase as tracer: application to measurement of diel patterns in rainbow trout (Oncorhynchus mykiss). Fish Physiol. Biochem. 22(4): 355-363.

Ngoula F, Watcho P, Dongmo MC, Dongmo MC, Kenfack A, Kamtchouing P, Tchoumboue J. 2007. Effects of pirimiphos-methyl (an organophosohate insecticide) on the fertility of adult male rats. Afr. J.Health Sci. 7: 3-9.

Nishikimi, M., Rao, N.A., Yagi, K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Bioph. Res. Co. 46(2): 849-854.

Norušis, M.J., 2006. SPSS 14.0 guide to data analysis. Upper Saddle River, NJ: Prentice Hall.

Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. lab. Clin. Med. 70(1): 158-169.

Sgro, P., Romanelli, F., Felici, F., Sansone, M., Bianchini, S., Buzzachera, C.F., Baldari, C., Guidetti, L., Pigozzi, F., Lenzi, A., Luigi, L.2014. Testosterone responses to standardized short-term sub-maximal and maximal endurance exercises: issues on the dynamic adaptive role of the hypothalamicpituitary-testicular axis. J. Endocrinol. Invest. 37: 13–24.

Singh, T. B., Mukhopadhayay, S. K., Sar, T. K., Ganguly, S. 2012. Acetamiprid induces toxicity in mice under experimental conditions with prominent effects on the hematobiochemical parameters. J. Drug. Metab. Toxicol. 3(6): 134-151.

Sinha, A.K. 1972. Colorimetric assay of catalase. Anal. Biochem. 47(2): 389-394.

Tappel, A.L., Zalkin, H. 1959. Inhibition of lipide peroxidation in mitochondria by vitamin E. Arch. Biochem. and Biophys. 80(2): 333-336.

Tejada, R.I., Mitchell, J.C., Norman, A., Marik, J.J., Friedman, S., 1984. A test for the practical evaluation of male fertility by acridine orange (AO) fluorescence. Fertil. steril. 42(1): 87-91.

Terayama, H., Endo, H., Tsukamoto, H., Matsumoto, K., Umezu, M., Kanazawa, T., Ito, M., Sato, T., Naito, M., Kawakami, S., Fujino, Y. 2016. Acetamiprid accumulates in different amounts in murine brain regions. Int. J. Environ. Res. Public Health. 13(10): 937.

Trošić, I., Mataušić-Pišl, M., Pavičić, I., Marjanović, A.M. 2013. Histological and cytological examination of rat reproductive tissue after short-time intermittent radiofrequency exposure. Arh. Hig. Rada. Toksicol. 64(4): 513-519.

Wyrobek, A.J., Bruce, W.R. 1975. Chemical induction of sperm abnormalities in mice. Proc. Natl. Acad. Sci. 72(11): 4425-4429.

Zhang, J. Y., Xiang. H. L., Wang, X., Zhang, J. 2011. Oxidative stress: role in acetamiprid-induced impairment of the male mice reproductive system. Agric. Sci. China. 10: 786–796.