

The Effect of Herbicide Paraquat and Organophosphate Pesticide Malathion on Changes of Sex Hormones in Female Rats

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ABSTRACT

Paraquat is an herbicide with a liquid formulation and dark green color. It is used in lands as a general, contact and leaf-poisoning. Herbicide paraquat "Gramaxon" has high toxicity after preparation and is absorbed through the skin. Malathion is a contact insecticide which is effective on pests through digestive and respiratory systems. Since both materials are abundantly used in agriculture today, and agricultural products are daily used by human, so, the toxins directly and indirectly have adverse influence on different body systems, especially on reproductive system. So, this study is aimed to investigate herbicide paraquat and organophosphate pesticide malathion on changes of LH, FSH, estrogen, and progesterone hormones in female rats. A number of 80 adult female Wistar rats were used in this study. They at first divided into control, blank, experimental paraquat, and experimental malathion groups. Each of the experimental groups were subdivided then into 3 groups of 10 each including, experimental P1 (daily received 1 mg/kg paraquat), experimental P2 (daily received 2 mg/kg paraquat), and experimental P3 (daily received 4 mg/kg paraquat); and experimental M1 (daily received 10 mg/kg malathion), experimental M2 (daily received 20 mg/kg malathion), experimental M3 (daily received 40 mg/kg malathion). Malathion and paraquat toxins were injected using insulin syringe. Variations of LH, FSH, estrogen, and progesterone hormones were measured by ELISA test. Hormonal measuring results in groups receiving paraquat indicate that the concentrations of FSH hormone in all experimental groups have significant increase compared with the control group. Progesterone hormone levels in experimental P1 and P2 groups have significant reduction and significant increase compared with the control group, respectively. Obtained results in groups receiving malathion show that LH hormone levels in different groups have no significant change compared with the control group ($p < 0.05$), however FSH hormone levels in these groups have significant increase compared with the control group. Estrogen hormone levels in experimental M1 and M2 groups have significant decrease and there is no significant change in the levels of estrogen hormone in experimental M3 group. Progesterone hormone levels show significant decrease in the experimental groups compared with the control group ($p < 0.05$). The results of this research indicate that herbicide paraquat and organophosphate pesticide malathion by production of free radicals, probably have adverse effects on sex hormones of female rats and oogenesis process.

Key words: Paraquat, Malathion, Sex Hormones, Rats.

INTRODUCTION

By production of chemical pesticides, human decides to control own environment to protect agricultural product cultivate for food supply, against pests. The antiquity of chemical opposition with pests and herbal diseases returns to more than a thousand years before Christ¹. Paraquat is an herbicide in the category of bipyridylum². As a powerful herbicide due to unique characteristics, paraquat has remarkable consumption and production in all around the world. Its chemical formula is N, N'-dimethyl-4, 4'-bipyridinium dichloride. Paraquat is mainly used as herbicide and causes serious lung damage and other injuries in mammals.

This herbicide is highly toxic for human (especially with delayed toxicity mechanism to human) and animals. This kind of toxicity is associated with mitochondrial and microsomal oxidation and reduction systems³. The mechanism of paraquat toxicity is often related to production of superoxide anions, which can lead to production of large amounts of reactive oxygen species (ROS), such as hydrogen peroxide and superoxide anions. The two free radicals are considered as two major toxicants. These radicals are highly toxic and are intensively combined with macromolecules and cause serious damages to various organs^{4, 5}. As a strong peripheral toxic compound, paraquat affects on the growth and development of amphibians. Followed by orally absorption, paraquat accumulates in all important body organs, particularly the lungs (more than 10 times higher than plasma) as an optional member and due to the high volume of distribution. So that in case of severe poisoning, death occurs from respiratory failure due to pulmonary edema and fibrosis⁶. The impact of paraquat and its mechanism in reproductive system of male rats is decreased weight of male reproductive systems (includes testes, epididymis, seminal vesicles, and prostate), as well as reduced number of sperms, cell division, and spermatogonial cells⁷. Researches performed on bacteria, mice and human revealed that paraquat has no mutagenic and carcinogenic effects. However, it weakens the immune system and endometriosis. Reduced number of sex cells and considerable shrinkage in Bulb/c race of mice

is caused by paraquat, as well⁸.

Malathion is introduced in 1950 as the first phosphorous compounds of mammals. Rapid decomposition in body into non-toxic compounds followed by consumption is the important prominence of organophosphorous compounds. Unlike chlorinated organic pesticides, this compound does not accumulate in body tissues of living organisms and biological cycles¹. Its scientific name is *malathion* or *carbofos* and its trade name is malathion and malathiosol¹. Malathion is derived from phosphorodithioic acid¹. Its utilization for a certain period may be toxic for some cells such as T cells⁹. Liver tissue is a candidate for receiving of malathion most effects¹⁰. This toxic compound also causes disturbance of DNA in body¹¹, induction of free radicals production and oxidative stress in the brain, and increased activity of anti-oxidant enzymes¹². Use of lethal and sub-lethal dose of this poison can also increase fatty acids, glycerol, and lipase activity in tested tissues and this can have a negative impact on reproductive activity and causes decline in the population¹³.

By inhibition of acetylcholinesterase enzyme, steroidogenesis process is inhibited by malathion, too¹. Followed by absorption of organophosphorus (OP) pesticides in body, they undergo numerous bio-transformation reactions. Since OP compounds are lipophilic, they permeate to skin readily. The bio-transformation reactions are mainly derived toward formation of polar compounds to achieve the ability of exertion from kidneys. OP compounds in biological terms may convert to some metabolites which their toxicity changes determinately¹⁵.

Given to the above as well as widespread use of paraquat and malathion and various direct and indirect effects of these toxins on reproductive system, so this study is aimed to investigate the impact of an herbicide (paraquat) and an organophosphate pesticide (malathion) on changes of sex hormones in female rats.

METHODS

This study was conducted in vitro and completely random. All ethical use of animals was

observed in this study. A total number of 80 adult female Wistar rats weighing 200-250 g and at 100-120 days of age were produced from Razi Vaccine and Serum Research Institute of Shiraz. The rats were then transported to Jahrom and kept for 2 weeks in Animal House of Islamic Azad University under in vitro condition, including 212 °C and a cycle of 12 hours of light and 12 hours of darkness. Malathion insecticide and paraquat pesticide were prepared from Moshkfam and Alafkosh Companies, respectively. The toxins were injected intraperitoneally for 14 days. Since malathion and paraquat are soluble in saline, so malathion and paraquat lethal doses were determined 80 mg per kilogram of body weight and 8 mg per kilogram of body weight, respectively. The rats were then divided randomly as follows:

Control

The rats in this group were kept in normal condition and without receiving any medications.

Blank

The rats in this group received saline intraperitoneally.

Paraquat receiving groups

Experimental P1: the rats in this group intraperitoneally received paraquat at 1 mg/kg body weight.

Experimental P2: the rats in this group intraperitoneally received paraquat at 2 mg/kg body weight.

Experimental P3: the rats in this group intraperitoneally received paraquat at 4 mg/kg body weight.

Malathion receiving groups

Experimental M1: the rats in this group intraperitoneally received malathion at 10 mg/kg of body weight.

Experimental M2: the rats in this group intraperitoneally received malathion at 20 mg/kg of body weight.

Experimental M3: the rats in this group intraperitoneally received malathion at 40 mg/kg of body weight.

After the injections, all rats were anesthetized and dissected and blood samples were obtained directly from their hearts using insulin syringe (5 cc). Subsequent to separation of blood serum, the concentrations of LH, FSH, estrogen, and progesterone hormones were measured by ELISA method in the laboratory of Jahrom's Medical Science University. One-way analysis of variance (abbreviated one-way ANOVA) was used for comparison between treatments. $P < 0.05$ was considered as significant level. SPSS version 15 was also used for data analysis and to perform statistical tests.

RESULTS

The obtained results related to changes of sex hormones in paraquat receiving groups are as follows. As listed in Table 1, it is clear that the amounts of LH hormone in all the experimental groups do not show significant changes compared with the control group. However, FSH levels in all groups reveal a significant increase compared with the control group ($P < 0.05$). There is no significant change in estrogen levels in all the experimental P groups. A significant decrease is observed in

Table 1: Sex hormones changes in the experimental groups receiving paraquat (the experimental P groups)

Various Groups	LH(mIU/ml)	FSH(mIU/ml)	Estrogen(ng /ml)	Progesterone(ng /ml)
Control	0.07500.00559	0.0150.00250	23.8200.731	19.3202.810
Blank	0.07000.00306	0.010.022	22.2201.62	15.562.082
Experimental P1	0.002800.0813	*0.0550.01	24.050.68	*4.71670.87
Experimental P2	0.06880.0077	*0.0530.007	21.51.68	*27.73.0
Experimental P3	0.0560.00280	0.0620.008	23.50.92	22.11.6

* The numbers with an asterisk are significantly different from the control group ($p < 0.05$).

progesterone hormone levels in the experimental P1 group compared with the control group. However, this amount shows a significant increase in the experimental P2 group compared with the control group and no significant change is observed in the experimental P3 group.

The obtained results related to changes of sex hormones in malathion receiving groups are as follows. According to Table 2, LH hormone levels in all the experimental groups do not show a significant change compared with the control group. FSH hormone levels in all groups show a significant increase compared with the control group ($p < 0.05$). Estrogen levels in the experimental M1 and M2 groups show significant decrease compared with the control group ($p < 0.05$). However, this amount in the experimental M3 group do not show a significant change compared with the control group. Progesterone hormone levels show a significant reduction compared with the control group ($p < 0.05$).

DISCUSSION

A- Interpretation of results related to changes in sex hormones in the experimental groups receiving paraquat (the experimental P groups) With increasing population in human society, preparation of food supplies is impossible without use of advanced scientific farming. In modern agriculture, people have to use pesticides, herbicides and chemical fertilizers, as well. All of these pesticides, herbicides and chemical fertilizers while serving humans are somehow cause problems. One of the toxins is paraquat which its

biochemical parameters and mechanisms have not been identified accurately.

Studies have shown that oxidative stress plays an important role in pathogenesis of several diseases such as cancer, diabetes, atherosclerosis, and lung disease and cataracts¹⁶. Oxidative stress is caused by free radicals and mitochondria are known as the main location for production of free radicals¹⁷. Mitochondria are targets of choice for paraquat toxicity in animal and herbal tissues¹⁸. On the other hand, lipids peroxidation increases adverse effects such as increased stiffness of membrane, reduction of intracellular materials, decreased in fluidity of membrane, and osmotic fragility¹⁹. Paraquat also reduces the amounts of glutathione and glucose 6-phosphate dehydrogenase, while increases superoxide glutathione, glutathione peroxidase catalase, and superoxide. This reflects defense and impact on antioxidant system of rats²⁰.

Performed studies have shown till now some impacts of paraquat such as histopathological changes in rats²¹ and ovarian and testicular atrophy²². Paraquat impacts investigation on different generations of particular race of hen during certain periods revealed that paraquat caused reduced fertility, and ovaries and fallopian tubes weight loss, as well as impairment in newborn baby chicks²³. Other performed studies on paraquat and glyphosate effects on ovaries and testes of amphibians found that paraquat inhibits 17-beta estradiol, resulting impairment in steroidogenesis pathway. This may be caused by oxygenic reagents or reactive oxygen species (ROS)²⁴. So it can be

Table 2: Sex hormones changes in the experimental groups receiving malathion (the experimental M groups)

Various Groups	LH(mIU/ml)	FSH(mIU/ml)	Estrogen(ng/ml)	Progesterone(ng/ml)
Control	0.07500.00559	0.01500.00250	23.8200.731	19.3202.810
Blank	0.07000.00306	0.02250.01000	22.2201.620	15.5602.082
Experimental M1	0.07290.00597	*0.07040.00637	*16.661.221	4.0330.711
Experimental M2	0.07710.00208	*0.07040.00549	*16.7501.622	*8.7830.692
Experimental M3	0.08750.00323	*0.09330.01188	22.3332.254	*5.3330.545

* The numbers with an asterisk are significantly different from the control group ($p < 0.05$).

concluded that if an ovarian tissue is damaged, then it will face with impairment in the secretion of estrogen and progesterone hormones. This impairment appears in the form of decreased or increased levels of the hormones. On this basis, significant reduction of progesterone hormone levels in the experimental P1 group compared with the control is quite logical. In this study, the estrogen levels increase in the experimental P1 group and decrease in the experimental P2 and P3 groups, however these changes are not significant compared with the control group. Changes of LH and FSH hormones may initially seem to be due to increased amount of GnRH which increases blood serum FSH, but since these changes have no effect on blood serum levels of LH, they could not be effective in increase of GnRH. FSH levels show significant increase in all the experimental P groups compared with the control group ($p < 0.05$). According to the researches of Hemayatkhah Jahromi *et al.* in 2008, the concentration of these hormones reduced in adult male rats. This is contrary to what we have achieved in this study which could be due to the released free radicals and lipid peroxidation (1-captopril). This causes tissue changes and as a result impairments in secretion of hormones. This difference also may depend to gender of rats.

B- Interpretation of results related to changes in sex hormones in the experimental groups receiving malathion (the experimental M groups) So far, most researches have been done in the terms of the effect of organophosphorous pesticides with various doses on different organs and on different animals and each of which obtained different results. The results obtained in this research that show malathion effect on ovaries of rats can complete available data about the effects of this pesticide on various species investigated previous.

Organophosphorous pesticides have alkylation properties and thus can affect on nuclear DNA²⁵. Malathion increases malondialdehyde levels in ovarian tissue that may be caused by release of free radicals as a result of body lipid

metabolisms. On the other hand, malathion causes ovarian tissue damages and weight loss; with increased doses, malathion toxicity reduces healthy follicles and increases the number of atretic follicles. It also changes corpus luteum²⁶. Other studies show that increased levels of malondialdehyde and lower antioxidant defense system in body can lead to destruction of cells²⁷. So this conclusion that levels of estrogen and progesterone hormones significantly decreased is quite logical since these two hormones secrete from ovaries and any tissue damages to ovaries impairs the secretion of these hormones. Studies also reveal that malathion does not significantly change levels of FSH and 17-beta estradiol in dairy cows. However, progesterone level was significantly decreased after injection of this pesticide. Malathion induces oxidative stress and production of free radicals and increases the activity of antioxidant enzymes²⁸. Malathion effects on concentrations of acetylcholinesterase (AChE) and 5-delta, 3-beta hydroxysteroid and phosphate dehydrogenase were investigated in a study. This pesticide induces chromosomal aberrations in somatic cells (bone marrow) and early sexual cells (primary spermatocytes)^{30,31}. It should be noted that ovarian is the secretion site of estrogen and progesterone hormones, while gonadotropin hormones are released from pituitary gland. There is no research about malathion impact on pituitary tissues. On the other hand, ovarian hormones influence on secretion of pituitary gonadotropin secretion during the estrous cycle. Of course, the main role of hypothalamus should not be ignored.

CONCLUSION

According to this study, it can be told that malathion pesticide and paraquat herbicide can have devastating effects on female rats reproductive indicators and these effects may be extended in lesser extent to human. Of course, in this case the use of pesticides as well as the amount of exposure to it is very important. So care must be taken in generalizing of this research findings to human and possible experiments should be carefully done.

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