Journal of Biology and Today's World

Journal home page: http://journals.lexispublisher.com/jbtw

Received: 24 March 2017 • Accepted: 26 May 2017

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doi:10.15412/J.JBTW.01060503

The Relationship between Diazinon and Malathion Plasma Levels with Follicular Cyst Disease in Dairy Cows

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ABSTRACT

Ovarian cyst is a common disease affecting dairy cows. It is one of the major causes of infertility in dairy cattle. Pathological ovarian cysts are classified into two groups including follicular and luteal cysts. Due to reduced fertility, increased calving interval, and the increase in the culling of cattle as a result of infertility, livestock industry has suffered from abundant financial losses. Hormonal and endocrine disorders are the causes of this disease that may occur through endocrine disrupting pesticides. This study aimed to investigate the relationship between plasma levels of diazinon and malathion with follicular cysts in dairy cattle. A case-control study was conducted on 30 cows. Diagnosis and selection of 15 cows (as the case group) with follicular cysts were done via rectal touch and ultrasonography, and plasma progesterone level was measured by a specialist. Control group consisted of 15 healthy cows that were homogenized with case group. Blood samples were taken from tail vein and were centrifuged, and the plasma was separated and stored at -80° C. Malathion and diazinon pesticides were measured by GC-MS (Gas Chromatography Mass) method in the plasma of the samples with 30ppb resolution. Independent t-test was used for comparing the means in healthy and case independent groups. $P < 0.05$ was considered significant. SPSS 20 was used for statistical analysis. Plasma levels of both pesticides were very low and below the acceptable threshold for GC Mass method used in this study. Since neither of the two toxins was identified in the plasma of healthy nor case groups and no statistically significant difference was observed between the two groups ($P > 0.05$), it can be stated that there is no correlation between diazinon and malathion pesticides, and the risk of follicular cysts in dairy cows.

Key words: Diazinon, Malathion, Organophosphate pesticides, Ovarian cyst, Dairy cows.

Journal of Biology and Today's World is published by *Lexis Publisher*; Journal p-ISSN 2476-5376; Journal e-ISSN 2322-3308.

1. INTRODUCTION

Cystic ovarian disease (COD) is common among milk cows and it rarely affects dry cows and beef cattle. This disease is considered as one of the main reproductive disorders in cows and is one of the main causes of infertility in milk cows (1). Due to the increase in calving interval, the reduction in calving number, milk reduction, increase of cow removal as a result of infertility, the reduction of return on investment, and the increase of managerial and veterinary costs, the disease results in big losses in the livestock industry. Ovarian cysts refer to follicular structures with a diameter over 25mm that are present on the ovary for at least 7-10 days in the absence of the corpus luteum. Recently, with ultrasound works, even the structures over 17 mm can be determined to be cystic.

Pathological ovarian cysts are categorized into two types: follicular and luteal. There is another type of cyst called corpus luteum cyst which is considered as nonpathological and usually exists 30 to 45 days after calving. This cyst is a yellow corpus that can naturally synthesize progesterone and has different sizes. Follicular cysts have a thin wall and little or no luteal tissue. The plasma or milk progesterone level in this case is lower than 1ngml^{-1}. As luteal cysts have a thick wall, their luteal tissue is high. Therefore, the progesterone level of plasma or milk in this case is higher than 1ngml^{-1}. The frequency of follicular cysts is more than that of luteal cysts, and the incidence of ovarian cysts is higher in the right ovary than the left ovary. This indicates the ovary's level of activity (2, 3). The process of cyst formation includes the hypothalamic–pituitary–gonadal axis malfunction, ovarian malfunction, and background factors (such as genetics, amount of milk production, season, age, exogenous estrogen, high stress level, negative energy balance, disease and infection after calving, laminitis, fatness, etc.) (4, 5). Exposure to pesticides play a main role in dysfunction of immune system and susceptibility to autoimmune disorders and endocrine disruption as PCOs (6). The plasma concentrations of dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs) and hexachlorocyclohexane (HCH) could be a factor in the pathogenesis of PCOs by changing the level of hormones (7, 8). Organophosphorus insecticides are a major group of chemical insecticides that are used widely in the world. This type of pesticide is also used in residential areas, in addition to farms and gardens, to control pests and to protect general, industrial, and veterinary health. Diazinon is an organophosphorus insecticide widely used for controlling insects in farms and gardens. This substance can be easily absorbed from the intestine wall, respiratory system, and skin. It can be metabolized into a more toxic substance, diazoxon, in the liver (9).

Diazinon also affects sex hormones, increases FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone) levels, and significantly reduces the testosterone level (10).

Malathion is an organophosphorus pesticide that is commonly used for eliminating insects such as fruit flies, cotton mealybugs, mosquitos, houseflies, and cattle lice. Although poisoning after skin contact and inhalation is possible, the fatal cases have been reported only after the consumption of the poison. If malathion undergoes severe heat, it will emit highly poisonous and dangerous phosphorous oxide fumes. Of course, if water is added to these fumes (or if they are dissolved in trachea and esophagus mucous membrane), phosphoric acid will be created. In addition, recent studies have shown that exposure to low doses of malathion can lead to allergic-like responses in laboratory mice. Although researchers have suggested that malathion effects are not life-threatening, they are not sure about its lack of immunodeficiency-creation impacts (e.g., allergy and immunotoxocity) (11). Organophosphorus insecticides are not just neurotoxins that lead to neurotoxicity symptoms by inhibiting acetylcholinesterase from neuromuscular endings. Rather, different studies have indicated that organophosphorus pesticides also result in ocular toxicity, liver toxicity, cardiovascular toxicity, immunotoxicity, endocrine toxicity, reproductive toxicity, reduction of testosterone, sperm reduction, infertility, miscarriage, weight gain, diabetes, pancreatitis, and competitive thyroid receptor inhibition which are not explained by acetylcholinesterase inhibition. One of the main proposed mechanisms is the activation of oxidative stress paths caused by these pesticides. Scientific reports suggest that the tissue and serum levels of oxidative stress biomarkers increase in animals that are exposed to organophosphorus pesticides. In addition, regarding reproductive and endocrine toxicity, the oxidative stress generated by these poisons results in neurohormonal system disorder, and the levels of reproductive hormones, especially, undergo some changes (12). Endocrine disrupting pesticides, in the body, interfere with hormone metabolism, synthesizing, secretion, transfer, linking, and activity. Pesticides have agonistic and antagonistic activity against aryl hydrocarbon receptors and nuclear membrane receptors (such as retinoic acid receptor, pregnane x, and peroxisome proliferator-activated receptor (PPAR)), and they result in muscular atrophy by anti-androgenic effect. Some organophosphorus materials have estrogenic function and are attached to estrogen receptors (agonist). Thus, they increase the expression of estrogen (13, 14). Some other increase the weight of female reproductive organ by anti-androgenic activity and do not have anti-estrogenic or estrogenic activity. One of the causes of this disease is hormonal and endocrine disorders that ultimately lead to the LH elevation dysfunction and consequently disruption in ovulation and follicular problem that can occur through endocrine disrupting pesticides. As diazinon and malathion are common and widely-used pesticides in Iran, it is possible to cause cystic ovaries by impacting endocrine. As this disease is common in milk cows, it is one of the main causes of infertility and results in big financial losses in the livestock industry in Iran. The present study aimed to explore the level of these pesticides in the plasma of dairy cows with cystic ovary disease and compare them in order to explore the possible link between the disease and the pesticides.

2. MATERIALS AND METHODS

2.1. Sample selection

This case-control study was conducted on 30 Holstein dairy cows in a big dairy farm near Tehran. For this purpose, first the diagnosis and selection of 15 cows with follicular cystic ovary disease were done through rectal touching and ultrasound (2 times with 10 days interval) by a theriogenologist. This group of cows was the case group. Another 15 cows were selected as the healthy group; this group had the same breed, age, weight, milk production level, and calving number record and days in milk (DIM) as those of the case group. This study was approved by the local Ethics Committee of Tehran University.

2.2. Blood Sampling

The blood samples were taken from tail vein, and an amount of the whole blood was kept in heparin tube and transferred to the laboratory for centrifugation. After centrifugation (eppendorf 5810 R) with 3000 rpm for 20 minutes, plasma separation was done, and it was maintained at -80°C. Another amount of the whole blood was kept in tubes containing EDTA at ambient temperature for doing CBC and fibrinogen tests. In order to eliminate other possible diseases, the cases with higher normal ranges of neutrophil and lymphocytes were excluded from the study. At this stage, out of 30 cows, eight cows were excluded from the study, thus the study was carried out on 22 cows (11 healthy cows and 11 afflicted cows). Afterwards, in order to complete the diagnosis of cystic ovarian disease, in addition to rectal touching and ultrasound, plasma progesterone was measured, the level of which in all samples was lower than 1 ng/ml.

2.3. Gas Chromatography - Mass Spectrometry (GC-MS) method

GC-MS device (model Thermoquest trace MS and DB-5 column with a length of 30 m and inner diameter of 250 μm) was used. Helium was used as carrier gas with the flow rate of 1 ml per minute. Three main ions from each pesticide were selected as selected ion monitoring (SIM) (Table 1).

Table 1. Selected ions of the pesticides

Pesticide	selected ions (m/z)		
Diazinon	179	137	304
Malathion	173	127	93

The device's detection limit for these materials was equal to 30 ppb, and organophosphorus malathion and diazinon were measured in the samples' plasma. In this method, first, 5 ml of the plasma sample was placed in a falcon tube. The analysis was done in two stages. In the first stage, 15 ml of acetonitric and acetic acid 1.0% were added to the sample, and the sample was shaken for 2 minutes. Then, the extracted powder with 5.1 g of sodium acetate and 4 g of magnesium sulfate was added to the mixture. The mixture was again shaken for 2 minutes. After this stage, the sample was centrifuged at 4000 rpm (model universal 320 from Hettich Company) for 5 minutes. Then, 8 ml of the above mixture was poured into the clean-up kit for cleanup. The kit included 200 mg of PSA and 1200 mg of magnesium sulfate. After the transfer of the mixture into the kit, it was vortexed for 30 seconds and centrifuged at 5000 rpm for 5 minutes. Afterwards, 5mg of the sample supernatant was transferred into the vial. The solution was condensed using nitrogen, and 2 μl of it was injected to the GC-MS device for analysis.

2.4. Statistical analysis

Independent t-test was used for comparing the means in the healthy and afflicted groups. The significance level was considered as $P<0.05$, and SPSS version 20 was employed for statistical analyses.

3. RESULTS AND DISCUSSION

The demographic information of the healthy and afflicted groups is shown in Table 2.

Table 2. The demographic characteristics of the healthy and afflicted cow

Demographic Data	Afflicted cows	**Healthy cows**
Number	11	11
Breed	Holstein	Holstein
Age mean (months + SD)	52 ± 7.22	51.56 ± 7.95
Age (months)	26-101	25-101
Weight mean (Kg ± SD)	568.64 ± 16.64	568.18 ± 12.2
Weight (Kg)	500-600	500-600
Mean of days in milk (Days ± SD)	75.18 ± 12.21	74.36 ± 12.28
Days in milk (days)	36-162	39-160
Calving number mean (number ± SD)	2.73 ± 2.1	2.73 ± 2.1
Calving number record (number)	1-7	1-7
Milk production mean (Kg ± SD)	48.36 ± 2.36	48.27 ± 3.45
Milk production (L)	37-62	35-63

SD: standard deviation. In all of the above statistics P>0.05, and there is no significant statistical difference

The plasma levels of diazinon and malathion were measured in the cows with follicular cystic ovary and healthy cows using GC-MS method, and then they were compared with eachothetr (Table 3).

Table 3. Comparison of plasma levels of diazinon and malathion pesticides in both healthy and case groups

Pesticides' plasma level	milk cows with (n=11) follicular cystic ovary disease	healthy milk (n=11) cows	p-value
Diazinon	< 30 ppb	< 30 ppb	0.999
Malathion	< 30 ppb	< 30 ppb	0.999

Ppb: $1ngml^{-1}$

The plasma levels of both diazinon and malathion were very low, lower than the diagnostic threshold of GC-MS method in this study (30ppb). Therefore, the pesticides were not detected in the healthy and afflicted groups; consequently, no significant statistical difference was seen between the two groups (P>0.05). As diazinon and malathion are widely-used pesticides in Iran and can lead to cystic ovary disease through disrupting the endocrine system, the present study aimed to explore the level of these pesticides in the plasma of dairy cows with cystic ovary disease and healthy cows to find out whether the difference in the pesticides' plasma concentration. Balali-Mood and Abdollahi indicated that organophosphorus pesticides are not just neurotoxins that lead to neurotoxicity symptoms by inhibiting acetylcholinesterase from neuromuscular endings (12). One of the main proposed mechanisms is the activation of oxidative stress paths due to the consumption of these pesticides that leads to disruption in neurohormonal system and especially reproductive hormones (12). Mickinlay et al. and Kojima et al. indicated that pesticides have agonistic and antagonistic activity against aryl hydrocarbon receptors and nuclear membrane receptors (such as retinoic acid receptor, pregnane x, and PPAR), and they result in muscular atrophy through anti-androgenic effect. In addition, some organophosphorus materials have estrogenic function and are attached to estrogen receptors (agonist) and result in the increase of the estrogen expression (13, 14). Kang et al. indicated that some organophosphorus materials result in the increase of the weight of female reproductive organ through anti-androgenic activity and do not have anti-estrogenic and estrogenic activity (15). Fattahi et al. indicated that diazinon also affects sex hormones, increases FSH and LH levels, and significantly decreases testosterone level (10). The overall results of the above studies suggest that organophosphorus pesticides can contribute to the formation of ovarian cysts. A significant point is that the impact of diazinon and malathion in the possible incidence of cystic ovary disease in dairy cattle has not been explored, hence, the current study launched an investigation on this topic. As diazinon and malathion were not detected in the plasma of dairy cattle follicular cyst of ovary and healthy dairy cattle, there was no significant statistical difference between the healthy and afflicted groups; it is safe to say that there is no link between the impact of diazinon and malathion and follicular cystic ovary disease in dairy cattle. It is recommended to explore these pesticides in a bigger sample and population. Diazinon and malathion were not detected in the plasma of the samples explored in this study, so these pesticides were not present in the environment of these samples, or their amount was insignificant and below the detection threshold. Therefore, diazinon and malathion did not result in cystic ovarian disease as these pesticides did not exist in the environment and consequently in the plasma of the samples. Other possible causes of this disease can be explored in future studies.

4. CONCLUSION

The comparison of the plasma levels of diazinon and malathion as two widely-used organophosphates in Iran in the group of dairy cows with follicular cystic ovary disease and the group of healthy dairy cows was conducted. As none of the pesticides were detected in the plasma of the healthy and afflicted groups, no significant statistical difference was observed between the two groups; it can be said that there is possibly no relation between diazinon and malathion, and affliction with follicular cystic ovary disease, and probably the affliction with this disease is related to other factors.

ACKNOWLEDGMENT

The researchers would like to appreciate the cooperation of the owners of Abbasi Dairy Farm in Varamin who helped in the selection of samples.

FUNDING/SUPPORT

All stages of this study were supported by personal charge of Aida Sayad.

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

REFERENCES

1. Peter AT. An update on cystic ovarian degeneration in cattle. Reproduction in domestic animals = Zuchthygiene. 2004;39(1):1-7.
2. Arthur GH, al-Rahim AT, al-Hindi AS. Reproduction and genital diseases of the camel. The British veterinary journal. 1985;141(6):650-9.
3. Youngquist R. Cystic follicular degeneration in the cow. Current therapy in theriogenology. 1986;2:243-6.
4. Blowey R. Bovine Medicine, diseases and husbandry of cattle. Black Well Scientific Publications, Great Britain; 2004.
5. Peter A. An update on cystic ovarian degeneration in cattle. Reproduction in Domestic Animals. 2004;39(1):1-7.
6. Mokarizadeh A, Faryabi MR, Rezvanfar MA, Abdollahi M. A comprehensive review of pesticides and the immune dysregulation: mechanisms, evidence and consequences. Toxicology mechanisms and methods. 2015;25(4):258-78.
7. Guo Z, Qiu H, Wang L, Wang L, Wang C, Chen M, et al. Association of serum organochlorine pesticides concentrations with reproductive hormone levels and polycystic ovary syndrome in a Chinese population. Chemosphere. 2017;171:595-600.
8. Yang Q, Zhao Y, Qiu X, Zhang C, Li R, Qiao J. Association of serum levels of typical organic pollutants with polycystic ovary syndrome (PCOS): a case–control study. Human Reproduction. 2015;30(8):1964-73.
9. Garfitt S, Jones K, Mason H, Cocker J. Exposure to the organophosphate diazinon: data from a human volunteer study with oral and dermal doses. Toxicology letters. 2002;134(1):105-13.

10. Fattahi E, Parivar K, Jorsaraei SGA. The effects of diazinon on testosterone, FSH and LH levels and testicular tissue in mice. International Journal of Reproductive BioMedicine. 2009;7(2):59-64.
11. Wexler P. Encyclopedia of toxicology: Academic Press; 2005.
12. Balali-Mood M, Abdollahi M. Basic and clinical toxicology of organophosphorus compounds: Springer; 2014.
13. Kojima H, Sata F, Takeuchi S, Sueyoshi T, Nagai T. Comparative study of human and mouse pregnane X receptor agonistic activity in 200 pesticides using in vitro reporter gene assays. Toxicology. 2011;280(3):77-87.
14. McKinlay R, Plant J, Bell J, Voulvoulis N. Endocrine disrupting pesticides: implications for risk assessment. Environment international. 2008;34(2):168-83.
15. Kang HG, Jeong SH, Cho JH, Kim DG, Park JM, Cho MH. Chlropyrifos-methyl shows anti-androgenic activity without estrogenic activity in rats. Toxicology. 2004;199(2):219-30.