

Toxicological Effects of Dimethoate (Rogor 30 %EC) on Ovary of Female Rat *Rattus Rattus Norvigicus*

Dr. Gabbar Singh Lodhi

Department of Zoology Government Ganesh Shankar Vidhyarthi College Mungaoli -473443, Madhya Pradesh India
Correspondence Author Email: [drgabbarlodhi1982\[at\]gmail.com](mailto:drgabbarlodhi1982[at]gmail.com)

Abstract: Dimethoate is a widely used organophosphorus (OP) insecticide applied to kill mites and insects systemically and on contact. The toxicological effect of dimethoate on ovary of female rat which total 90 animals were divided into 3 groups of 30 each; each group were further divided into three sub groups for 15, 30 and 60 days. The first group was served as a control administered with daily dose of vehicle olive oil orally by the help of cannula; the second and third group was exposed with daily dose of dimethoate (rogor 30 % EC) dissolved in olive oil by the help of cannula. In the result we find that in the histopathological changes ovary in the rats females were treated with dimethoate. The initial morphological changes in the ovary following decided dose of dimethoate include reduced size of graffian follicles. The stromal cells and ovarian follicles become degenerated condition after exposure of dimethoate upto 15 days as compared to control and supplemented groups. After 30 days dimethoate exposure showed reduced gross size of ovarian follicles and in the follicles were some atrophic and degenerative changes in ovarian follicles with reduced oocytes. The interstitial tissue is also in atrophied condition and corpus luteum is hypertrophied and as compared to the control 15 days and olive oil supplements treated groups. dimethoate acts by interfering with the activities of cholinesterase, an enzyme essential for the proper functioning of the nervous system of insects and humans.

Keywords: Dimethoate toxicity, ovarian damage olive oil, organophosphorus insecticide histopathological changes acetylcholine esterase

1. Introduction

Since the dawn of human civilization, man has been trying to improve the agriculture; modern agriculture employs a number of enhancing crop yield and protecting the same. Agricultural crop are destroyed by insects. Various types of fungi and bacteria cause disease in plants. According to estimate there is an annual loss of 30 percent in agricultural pests. The food problem of our country can be solved to a great extent.

Dimethoate is an insecticide used to kill mites and insects systemically and on contact. It is used against a wide range of insects, including aphids, thrips, plant hoppers and whiteflies on ornamental plants, alfalfa, apples, corn, cotton, grapefruit, grapes, lemons, melons, oranges, pears, pecans, safflower, sorghum, soybeans, tangerines, tobacco, tomatoes, watermelons, wheat and other vegetables. It is also used as a residual wall spray in farm buildings for house flies. Dimethoate has been administered to livestock for control of botflies. Dimethoate is available in aerosol spray; dust, emulsifiable concentrate, and ULV concentrate formulations (Hayes *et al.*, 1990; Meister, 1992). Dimethoate is one of a class of insecticides referred to as organophosphates. These chemicals act by interfering with the activities of cholinesterase, an enzyme that is essential for the proper working of the nervous systems of both humans and insects.

Organophosphorous insecticides dissolve in fats and do not dissolve easily or do not dissolve at all in water. They are easily absorbed from the alimentary tract, as well as through the respiratory tract and skin. These compounds reveal multidirectional effect on the organism. The mechanism of their neurotoxic effect consists in inhibiting the activity of

acetylcholinesterase, the accumulation of acetylcholine and excessive stimulation of the nervous system (Vale, 1998; Bajgar, 2004; Jintanna *et al.*, 2009) organophosphate (sometimes abbreviated OP) is the general name for esters of phosphoric acid. Phosphates are probably the most pervasive organophosphorus compounds. The EPA lists organophosphates as very highly acutely toxic to bees, wildlife and humans. Recent studies suggest a possible link to adverse effects in the neurobehavioral development of fetuses and children, even at very low levels of exposure. Organophosphates are widely used as solvents, plasticizers and EP additives.

2. Materials and Methods

Experimental Design:

Total 90 adult mature female rats, *Rattus rattus norvigicus* were used for present studies and were divided into three groups of thirty each. They were further divided into three sub groups for 15, 30 and 60 days respectively. The dose of dimethoate was finalized after observing various literatures and conformed through experimental investigation. In this investigation we have used 1mg/kg body weight of dimethoate.

Group- I: The animals (female rats) of this group served as control, received balanced diet and water *ad libitum* dose with vehicle olive oil through cannula for 15, 30 and 60 days.

Group-II: The animals of this group were introduced with dimethoate (1mg/kg body weight/day) dissolved in olive oil, per animal through cannula for 15, 30 and 60 days.

Group-III: The animals of this group received only vehicle olive oil through cannula for 15, 30 and 60 days.

After the completion of different duration's *i.e.* 15, 30 and 60 day of all animals of each group were sacrificed by cervical dislocations at different intervals *i.e.* 16th, 31st and 61st day of experiments.

3. Results

The normal ovary of female rats is roughly differentiated into an outer cortex and inner medulla. In mature ovary the cortex contains follicles and corpora lutea in various stages. The medulla consists only the large blood vessels of the organ. One cell of the mass of epithelial cells give rise to an immature ovum or oocyte. The remaining cells form a layer surrounding the ovum or oocyte as a sac or follicles called follicular epithelium or granulosa. Immature ovum or oocyte and surrounding follicular epithelium or granulosa constitute the primordial cells follicle. The stroma of the ovary surrounding the follicular epithelium or granulosa becomes organized into connective tissue layers, the theca externa and intema (Figs. 1 & 6). The histological observations of control group showed developing follicles (primordial, primary and secondary follicles), corpus luteum and graafian follicles were observed in the cortex of ovarian primordial follicles are composed of an oocyte surrounded by a small number of squamous granulosa cells. Structural morphology

of healthy oocytes in developing follicles appears similar regardless of the stage of development. (Figs. 1 & 6).

The initial morphological changes in the ovary following decided dose of dimethoate include reduced size of graafian follicles. The stromal cells and ovarian follicles becomes degenerated condition after exposure of dimethoate upto 15 days as compared to control and supplemented groups (Figs. 3 & 8). After 30 days dimethoate exposure showed reduced gross size of ovarian follicles and in the follicles were some atrophic and degenerative changes in ovarian follicles with reduced oocytes. The interstitial tissue is also in atrophied condition and corpus luteum is hypertrophied and as compared to the control 15 days and olive oil supplements treated groups (Figs. 2 & 7)

In later part of the experiments up to 60 days dimethoate exposure we found some atrophic changes in the oocyte in the ovary. The cells of the cumulus oophorus are deattached from oocyte. Oocyte shrinked and reduced in size and degeneration of corpus luteum as compared to control, 15, 30 days and olive oil supplemented groups (Figs. 3 & 8)

Beside the animals supplemented olive oil the 30 day and 60 days in ovary of female rats recovery showed normal histoarchitecture of ovary similar to control groups (Figs. 5 & 10).

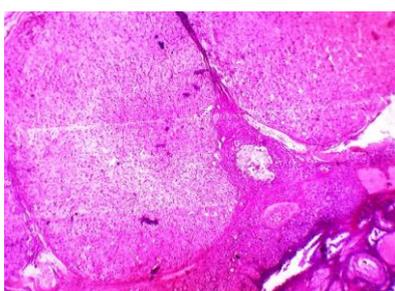


Figure 1: Control (H&E 100X)



Figure 2: Olive oil (H&E 100X)

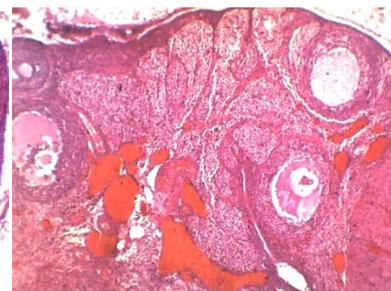


Figure 3: 15 Days (H&E 100X)



Figure 4: 30 Days (H&E 100X)

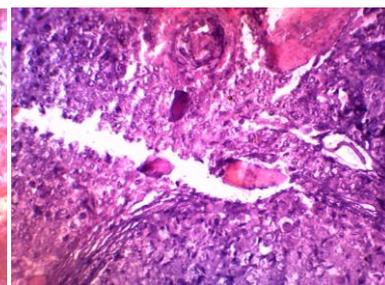


Figure 5: 60 Days (H&E 100X)

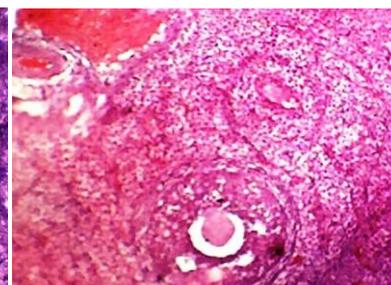


Figure 6: Control (H&E400X)

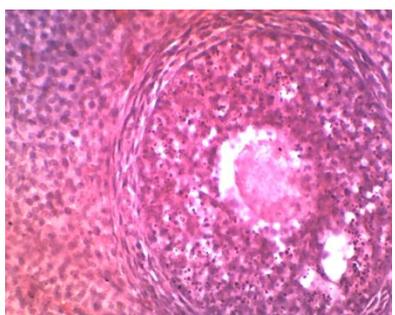


Figure 7: Olive oil (H&E400X)

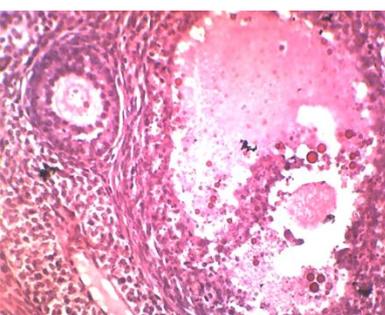


Figure 8: 15 Days (H&E400X)

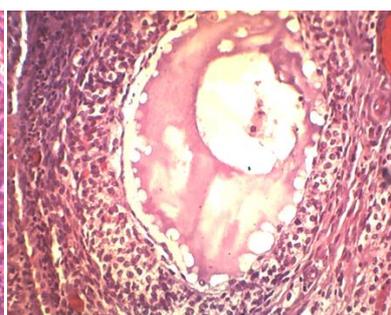


Figure 9: 30 Days (H&E400X)

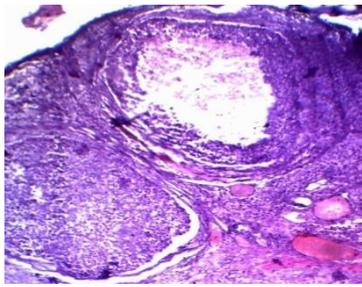


Figure 10: 60 Days (H&E400X)

4. Discussion

The ovary is the female reproductive organs that produce the ovum. The ovary consists of both epithelial and mesenchymal components. The mesenchymal tissue differentiates into interstitial tissue which will become closely associated with the germinal elements of ovary and addition to providing a nutritive environment for the oocytes, will also provide an important source of hormones during particular phases of female cycle.

Follicles start to grow at all times and as they develop, they produce more number of thecal and granulosa cells. The conversion of follicle to atretic stage is functional rather than a degenerative process and it is considered to be an integral part of ovarian function (Zuckerman and Weir, 1977). Follicular dynamics has revealed a significant decrease in the number of healthy follicles and increase in the number of atretic follicles in mancozebe treated rats (Swartz and Mall, 1987; Baligar *et al.*, 2001).

(Dhondup and Kaliwal 1997; Mahadevaswami and Kaliwal, 2002) showed significant increase in ovarian weight with a concomitant increase in compensatory ovarian hypertrophy and in the number of healthy follicles with a concomitant decrease in the number of atretic follicles and interrupted estrous cycle may be due to the direct effect on the ovary or may be due to hormonal imbalance in any stage of the hypothalamo - hypophysial ovarian axis in mice of dimethoate exposure. There is also the possibility that the decrease in healthy follicles with concomitant increase in atretic may be due to affecting gonadotropin secretion by central nervous system mechanism (Goldman *et al.*, 1997). Dimethoate may have catalyzed on oxidative stress which in turn raised the levels of lipoperoxides and activity of COX-2. As a consequence, a decreased expression of steroidogenic acute regulatory (StAR) gene stimulated cholesterol transport into the mitochondria (Walsh and Stocco, 1998). Also dimethoate could have blocked the activities of hydroxysteroid dehydrogenases and impaired the LH and FSH dependent signal for stimulation of androgen crucially on the lipoperoxidase levels (Astiz *et al.*, 2009); Ozcen Oruc, 2010). Histopathological examination revealed the ovary necrotic oocytes and atretia with dimethoate treatment (Eman *et al.*, 2011; Pugazhvendan *et al.*, 2009).

The presence of the highest atresia in the ovarian follicles and mesenchyme in the ovaries of neonates whose mothers were exposed to the highest dosages of the biological insecticide could be the result from the effects of oxidative stress produced by insecticide (Tsai *et al.*, 2006). According to Murdoch (1998) and Behrman *et al.*, (2001), the oxidative

stress induces apoptosis in ovarian cells and is associated with mechanisms involved in the regression of the corpus luteum and follicular atresia. Radhika and Kaliwal (2002) who showed that there was significant decrease in the weights of ovary and uterus after dimethoate exposure. Adiaxamma *et al.*, (1994) made similar observation in rats treated with monocrotophos and have stated that decrease in weight and size of ovaries is due to extensive fibrosis and atretic follicles. The basic functional unit of reproduction within the ovary is the follicle (Hsueh *et al.*, 1984). Follicles start to grow at all times and as they develop, they produce large number of thecal cells. The conversion of follicles to atretic state is functional rather than a degenerative process and is considered to be an integral part of ovarian function (Zuckerman *et al.*, 1977; Hirshfield, 1991). Most of the follicles undergo atresia and very few mature to ovulate among the new crop of recruited follicles during every cycle. After the early stage of gonadotropin independence, the entire process of follicle growth becomes dependent on the continuous presence of gonadotropins (Hogdon, 1989). In the present study there was a decrease in the number of small, medium, large and total number of healthy follicles with concomitant increase in the number of medium, large and total number of atretic follicles with dimethoate treatment were observed. Similar findings have been reported with different pesticide treatments in rats and mice, Swartz and Mall (1989). Swartz *et al.*, (1989) have reported that chlordecone induces follicular toxicity by reducing healthy, large and medium sized follicles with increase in atretic follicles. An organophosphate edifenphos is reported to decrease significantly some of the follicular stages and total number of healthy follicles and significantly increases the number of atretic follicles in a dose dependent manner (Jayadevi *et al.*, 1998). (Evans *et al.*, 1997) have shown that the ovarian androgen and inhibin secretion by follicles may play an important part in the regulation of FSH secretion and follicular dynamics. In the present study treatment with dimethoate might have interfered with ovarian function and indirectly acted at the level of hypothalamus or pituitary gland or also directly on the ovary by causing fibrosis as reported by (Adilaxmmama *et al.*, 1994). By doing so it must have interfered with the signals provided by ovary via hormonal feedback loops and stop timely release of gonadotropins FSH and LH, regulators of folliculogenesis, this may be one of the reasons for decrease in pool of healthy follicles and increase in the pool of atretic follicles. Treatment with carbamate fungicide sodium N methyl dithio carbamate is shown to block ovulation by inhibiting secretion of luteinizing hormone in rat (Goldman *et al.*, 1994).

Habibollah *et al.*, (2010) reported the histological effects of diazinon on the ovarian tissue showed significant changes in the mean numbers of primary and secondary graafian follicles but there was a significant decline in mean number of corpus luteum. Similarly, our result showed that dimethoate exposure adversely affected the histoarchitecture of ovary of rats in a dose of duration dependent manner.

In the present experimental investigation concluded that dimethoate organophosphate insecticide is adversely toxic on thyroid, adrenal gland, hepatic, renal and ovary of female. The findings demonstrate that some hematological,

biochemical and histopathological alterations induced by dimethoate. It also concluded that dimethoate has the potential toxic insecticide for living beings.

Finally, it may be suggested that we should least amount of dimethoate organophosphate insecticide uses in agriculture and other fields.

References

- [1] **Adilaxmamma K., Janardhan Redy A., and Redy K.S., (1994):** Monocrotophos reproductive toxicity in rats. *Indian J. of Pharmacology.* 26:126-9.
- [2] **Astiz M., Hurtado de catalfo G., De Alaniz M et al., (2009):** Involvement of lipids in dimethoate-induced inhibition of testosterone biosynthesis in rat interstitial cells. *Lipids.*, 44:703.
- [3] **Bajgar J., (2004):** Organophosphates/nerve agent poisoning: mechanism of action, diagnosis, prophylaxis, and treatment. *Adv. Clin. Chem.* 38:151-216.
- [4] **Baligar P.N., and Kaliwal B.B., (2001):** Induction of gonadal toxicity to female rats after chronic exposure to mancozeb. *Ind. Health.* 39:235-243.
- [5] **Behrman H.R., Kodaman P.H., Preston S.L., and Gao S., (2001):** Oxidative stress and the ovary. *J. Society Gynecology Investigation*, 8:40-2.
- [6] **Dhandup Pasang and Basappa Basavanneppa Kaliwal (1997):** Inhibition of ovarian compensatory hypertrophy by the administration of methyl parathion in hemicastrated albino rats. 11(1):77-84.
- [7] **Eman A. Abd- Gawad., Mohamed M.M. Kandiel., Amany A. Abbass., and Adel A. Shaheen., (2011):** Impact on some organophosphorus insecticides on growth performance fecundity and semen characteristics in Nile Tilapia (*Oreochromis Niloticus*). *Lucrari stintific Seria Medicina Veterinara* vol. 54.
- [8] **Evans A.C.O., Komar C.M., Wandji S.A., and Fortune J.E., (1997):** Changes in androgen secretion and lutenizing hormone pulse amplitude are associated with the recruitment and growth of ovarian follicles during the luteal phase of the bovine estrous cycle. *Biol Reprod.* 57:394-401.
- [9] **Goldman J.M., Parrish M.B., Cooper R.L., and McElory W.K., (1997):** Blocked of ovulation in the rat by systemic and ovarian intrabursal administration of the fungicide sodium N-methyl dithiocarbamate. *Reprod Toxicol.* 15:185-90.
- [10] **Goldman J.M., Stocker T.E., Cooper R.L., McEhroy K.W., and Hein J.F., (1994):** Blockade of Ovulation in the rat by the fungicide sodium N-methyl dithiocarbamate: Relationship between effects on the lutenizing hormone surge and alterations in hypothalamic catecholamines. *Neurotoxicol Teratol.* 16:257-68.
- [11] **Habibollah Johari Mehrdad Shariati Sharam Abbast Esfandyar Sharifi and Hamid Reza Askari (2010):** The effect of diazinon on pituitary gland axis and ovarian histological changes in rats. *Indian Journal of Reproductive Medicine* Vol. 8(3)125-130.
- [12] **Hayes, W.J., and E.R., Laws (ed.), (1990):** Handbook of Pesticide Toxicology, Vol. 3, classes of pesticides. Academic Press, Inc., NY.
- [13] **Hirshfield A.N., (1991):** Development of follicles in the mammalian ovary. *Int Rev Cytol* 124, 43-101.
- [14] **Hodgon G.D., (1989):** Neuroendocrinology of the normal menstrual cycle. *J Reprod Med* 34 (suppl 1) 68-75.
- [15] **Jayadevi Math R., Jadramakunti U.C., and Kaliwal B.B., (1998):** Effect of edifenphos on follicular dynamics in albino rats. *Indian J. Exp. Biol.* 36:39-42.
- [16] **Jintana S., Sming K., Krongtong Y., Thanyachai S., (2009):** Cholinesterase activity, pesticide exposure and health impact in a population exposed to organophosphates. *Int Arch Occup Environ Health.* 82: 833-842.
- [17] **Mahadevaswami M.P., and Kaliwal B.B., (2004):** Evaluation of dimethoate toxicity on pregnancy in albino mice. *J. Basic Clin. Physiol. Pharmacol.* (15) 211-221.
- [18] **Meister, R.T., (ed.). (1992):** Farm chemicals handbook 92. Meister publication 2008-08-13 <http://www.merriamwebster.com/dictionary/pollution> Retrieved 2010-08-26.
- [19] **Murdoch W.J., (1998):** Inhibition by oestradiol of oxidative stressinduced apoptosis in pig ovarian tissues. *J. Reproduction and Fertility.* 114:127-30.
- [20] **Ozcan Oruc E., (2010):** Oxidative stress, steroid hormone concentration and acetylcholinesterase activity in *Oreochromis niloticus* exposed to chlorpyrifos pesticide. *Biochemistry and Physiology.* 96:160-166.
- [21] **Pugazhvendan S.R., Narendiran N. J., Kumaran R.G., Kumaran S., and Alagappan K.M., (2009):** Effect of malathion toxicity in the freshwater fish ophiocephalus punctatus-a histological and histochemical study. *World Journal of Fish and Marine Sciences.* 1 (3):218-224.
- [22] **Radhika P. Rao., and Basappa B. Kaliwal., (2002):** Monocrotophos induced dysfunction on estrous cycle and follicular development in mice. *Industrial Health.* 40:237-244.
- [23] **Swartz W.J., and Mall G.M., (1989):** Chlordecone induced follicular toxicity in mouce ovaries. *Reproductive Toxicol.* 3:203-206.
- [24] **Tsai S.F., Yang C., Liu B. L., Hwang J.S., and Ho S.P., (2006):** Role of oxidative stress inthuringiensin-induced pulmonary toxicity. *Toxicologic Appl. Pharmacol.*, 216:347-53.
- [25] **Vale J.A., (1998):** Toxicokinetic and toxicodynamic aspects of organophosphorus (OP) insecticide poisoning. *Toxicol Lett.* 102-103:649-652.
- [26] **Walsh L.P., and Stocco D.M., (1998):** The effects of roundup and dimethoate on steroidogenesis acute regulatory (StAR) protein in mouse MA-10 Lydig cells. *Proceeding of the society for the study of reproduction society for the study of the reproduction.* *College Station, Texas Madison, WI.* 182:8-11.
- [27] **Zuckerman L., and Weir B.J., (1977):** The ovary-1 general aspects. 2nd eds. Academic press New York.
- [28] **Zuckerman L., and Weir B.J., (1977):** The ovary-1 general aspects. 2nd eds. Academic press New York.