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Effect of Ethion Administration Schedules on Estrous Cycle and Follicular Dynamics in Albino Mice

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Abstract: **Ethion an organophosphate was orally administrated at 16mg/kg/day to Swiss albino mice for 5, 10, 20 and 30d days, control mice received similar. Quantities of olive oil. Daily body weight were recorded and the mice sacrificed by cervical dislocation after 24 hours the terminal exposure of ethion, in the number of follicles, and histological evaluation of the control mouse, ovary, revealed the presence of many developing follicles Graafian, corpora luteal and Atretic follicles the treatment with ethion foe 5 and 10 d showed no significant changes in the number of small ,medium and large healthy and atretic follicles how ever there was significant decrease in the Total number of healthy follicles with d ethion treatment the histological evaluation of the ovary of mouse following Ethion Treatement for 5d revealed the presence of many developing follicles, corpora luteal and atretic follicles treated and Treatment with Ethion for 20,30,d showed a significant decrease in the number of small, medium, large and total number of atretic follicles when compared with control. The histological evaluation of the ovary of the mice following Ethion treatment for 20, 30 d showed developing follicles, corpora luteal many atretic many atretic follicles but not Graafian follicles There was a decrease in the ovary uterus. Weight, protein, sialic and 3βHSD content, but there was increase in the cholesterol content in treated mice compared to control.**

Keywords: **Ethion. Histology, ovary, enzymes, Toxicity.**

1. INTRODUCTION

Ethion is an organophosphate pesticide used to kill aphids, mits, scales, thrips, leafhoppers, maggots and foliar feeding larvae.it may be used on wide variety of food, fiber and ornamental crops, including Greenhouse crops, lawns and turf. Ethion is often used on citrus and apples (USDA-soil conservation service, Syracuse, NY. Hartley, D and H.Kidd, et al., 1983).Ethion insecticide is block the ovulation by inhibiting the secretion of luteinizing hormone in rat (Hartley, D and H.Kidd, et al, .1983).Endocrinology homeostasis affected by pesticides is evident by the suppression of GnRH release, which may be acted directly on the Gonadotrophins to alter the gonadotrophins synthesis and secretion indirectly by altering the pituitary response to GnRH or gonadal steroids. Their by pesticides by affecting the levels of FSH and LH and the feed Back mechanisms (US EPA, 1989.sept.30) Washington, DC.1989 (sept29) U S EPA, Washington, DC, the pesticides primarily act on CNS either as nerve poisons or other acetyl cholinesterase inhibits. They may be affect the normal functioning of other organism. A3-generation Reproduction study with rats given dietary doses as highest as 1.25mg/kg/day did not show any Ethion related Reproductive effect (cheminova agro A/S, 1991) and DC: U.S.DOT. And Hollenbeck, W.H.and K.M Cunningham-Burns, 1985.Pesticides and human health, New York: Springer-Verlag.,

Once in the Bloodstream, ethion may cross placenta (U.S EPA. Washing, DC, when rats were given dose of 0.02,0.6 or 2.5mg/kg on days 6 to 15 of pregnancy ,development effects were seen only the highest dose tested. In fetus of the high

dose group, there was an increased incidence of delayed ossification of the pubic bones. The development NOEL in this study was 0.6mg/kg, when rabbits where given doses of0,0.6,2.4.or9.6mg/kg on days 6 to 18 of pregnancy. Fetuses from the highest dose tested exhibited.

2. MATERIALS AND METHODS

Chemical:

Technical grade Ethion(purity98%)was obtained from Rallies India Ltd., Mumbai(India)had been used for the experiments the effective i.e.,. 16mg/kg bodyweight dose was given orally in olive oil for 5,10,20 and 30d below their acuteLD_{50} level of intoxicant according to their Body weight The mouse oral LD_{50} for Ethion is 62mg/body weight o,o,o;o-tetraethyl S.S'-methylenebis(phosphorodithioate)S,S'-methylenebis(o,o-diethyl phosphorodithioate)$C_9H_{22}0_4P_2S_4$.

Animals and Treatment:

Laboratory bred adult virgin females Swiss albino mice were used in the experiments. The mice were maintained in the laboratory, P.G.Depatment of Studies in Zoology, Karnataka University, Dhār wad. Mice weighing 25- 30g of age 90-120 d were used .They were housed in separate polypropylene cage containing sterile paddy husk as bedding material. The animals were provided with standard pellet diet Gold mohar (Hindustan Lever Ltd., Mumbai) and water ad libitum throughout the study. The mice were maintained under normal days day/night schedule(12L:12D)at room temperature $26^0c\pm1^0$Animals showing normal regular estrous cycle(4 to 5D) for at least three consecutive cycles were taken for the experiment. The experiment was conducted with five groups, each carrying 10 animals; ethion was administered orally in olive oil vehicle at effect dose of 16mg/kg body weight/day for 5, 10, 20 and 30 days Ethion administered orally because the major available Ethion residue in the environment enters the non –target animals is by orally. All the animals were killed on 24 hours after the last dose treatment and ovary taken out for histology and biochemical studies. And Estrous cycles bye Zarrous et al., (1964)

Morphometric analysis of ovarian follicles serials section of ovary were evaluated for follicles stages as described by Moawed et al,.1989,Pederen and peters 1968,Bolon et al.,1997 and Swartz and Mall 1989.

Histological studies:

Ovary was removed, washed in saline fixed in bouin's fluid, dehydrated in ethanol and embedded in paraffin. Serial sections at 5µm were prepared and stained with haemotoxylin eosin.

Biochemical estimations:

Freshly removed ovary freed from adherent tissues weighed to nearest milligram was used for biochemical studies such as protein by Lowry et al.,(1951).cholesterol by Abell et al.,(1952)and sialic acid by Yao et al.,(1989),3βHSD by shivandappa and venkatesh(1997).

Statistical analysis:

Statistical significance between the control and experiment data were subjected to analysis of variance (ANOVA) together with Dunnett's test ($p<0.05$).

3. RESULTS AND DISCUSSION

Histological studies:

The ovary histological observation of the control mouse showed graafian follicles and developing follicles. Corpora luteal and atretic follicles at autopsy the animal was in diestrus, harris haematoxylin and eosin, (fig.1) the durational study in mice with 16mg/kg/day Ethion exposure for 5 d showing atretic follicles and developing follicles, corpora lutea at autopsy the animal was in diestrus. Harris haematoxylin and eosin, 30x.(fig.2) the histology observation of the ovary in the mice exposed ethion for 10,20 and 30days revealed the loss of Graafian follicles and increased many Atretic follicles and size of the ovary decreased at the autopsy the animal was diestrus, harris haematoxylin and eosin, 30 x. (figs3-5)

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Photographs:

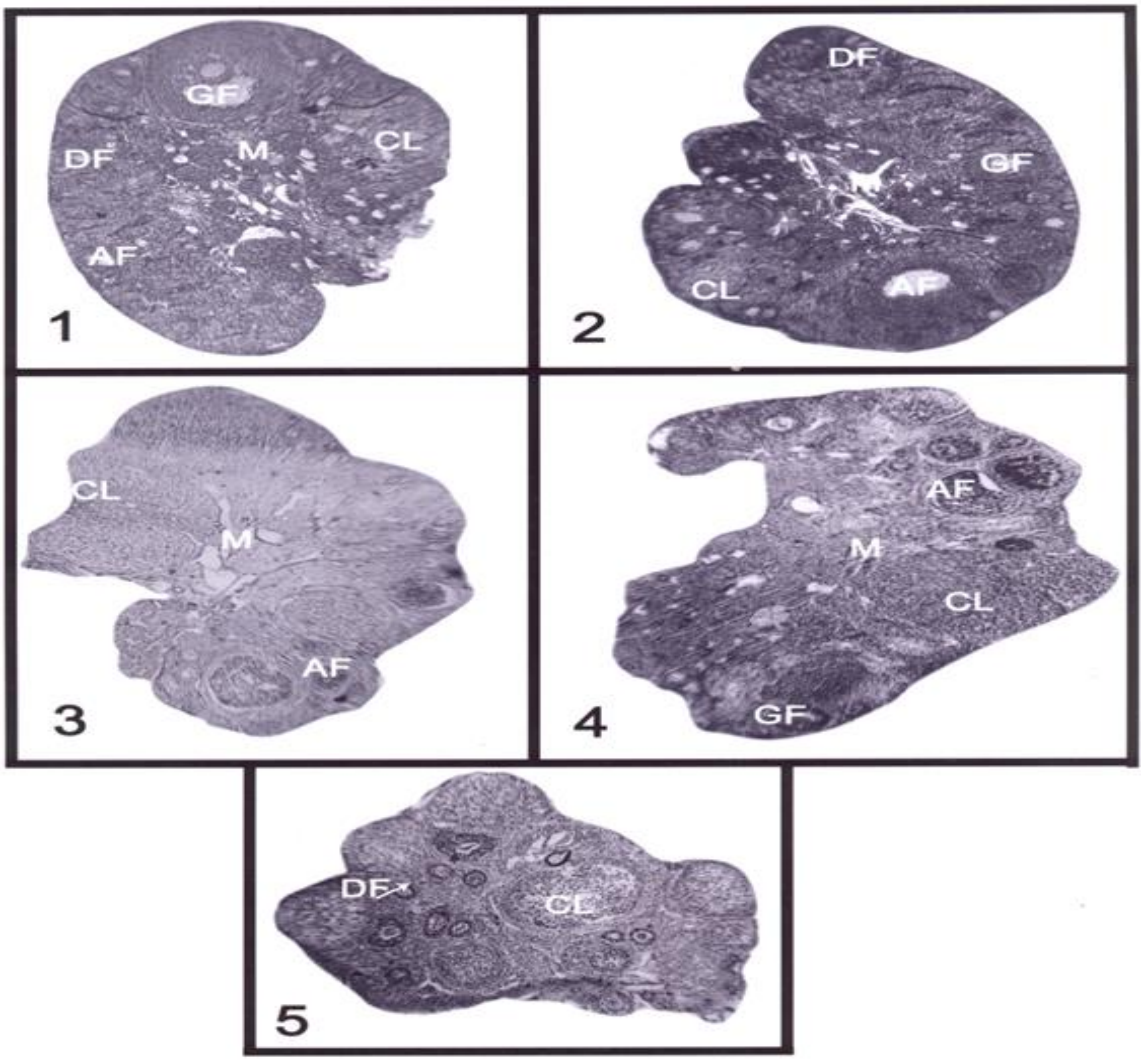

Fig .1 transverse section through the hilus of the control mouse taken at autopsy on 31st showing Graafian follicles, developing follicles , atretic and corpora lutea, atretic follicles At autopsy the animals was in diestrus.harris haematoxylin and eosin.

Fig.2transverse section through the hilus of the ovary of the mouse treated with 16mg/kgd ethion for 5 d showing atretic follicles and developing follicles, corpora lutea At autopsy the animals was in diestrus. Harris haematoxylin and eosin, 30x.

Fig.3 transverse section through the hilus of the ovary of the mouse treated with 16mg/kg d ethion for 10 d showing few developing Absence of graafian follicles, corporlutea and many Atretic follicles, At autopsy the animals was in diestrus. Harris haematoxylin and eosin, 30x.

Fig.4 transverse section through the hilus of the ovary of the mouse treated with 16mg/kg d ethion for 20 d showing few developing Absence of graafian follicles, corporlutea and many Atretic follicles, At autopsy the animals was in diestrus. Harris haematoxylin and eosin, 30x.

Fig.5 transverse section through the hilus of the ovary of the mouse treated with 16mg/kg d ethion for 30 d showing few developing Absence of graafian follicles, corporlutea and many Atretic follicles, size of the ovary decreased At autopsy the animals was in diestrus. Harris haematoxylin and eosin, 30x.

Photographs original exposure at×100

Abbreviation: GF-Graafian follicle DF-Developing follicle AF-Atretic follicles, CL-corpora lutea

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Table 1: Ethion induced temporal effect on the estrous cycle in albino mice.

Table2Treatment Duration (days)	Number Of Cycles	Duration in days				Diestrus Index
		Proestrus	Estrus	Metestrus	Diestrus	
control	5.61±0.50	7.41±0.76	7.71±0.79	7.51±0.47	7.41±0.46	24.66
5	4.70±0.42	7.32±0.39	4.10±0.91	7.91±0.82	10.71±1.09	35.66
10	4.32±0.30	7.11±0.48	5.91±0.45	5.82±0.29*	11.22±0.83*	37.33*
20	4.31±0.27	6.43±0.61	6.10±0.50*	4.71±0.58*	12.80±0.56*	42.66*
30	4.21±0.47*	5.70±0.52*	6.03±0.51*	3.90±0.45*	14.41±1.03*	48.00*

Data are mean±SEM of 10 animals, diestrus index=$\frac{dayes\ with\ diestrus}{total\ days\ of\ treatment}$×100

*Significant p≤0.05 compared to control Biochemical studies

Biochemical studies:

Biochemical contents±

Temporal study on ovary and uterus biochemical contents exhibited that treatment with 16mg/kg/day Ethion for 20 days in albino mice the of ovary and uterus weight, protein, sialic acid and 3βHSD contents are decreased but there was increased in the cholesterol content in treated mice compared to control.

Tables 2: Ethion induced temporal effect on the number of healthy follicles in ovaries of albino mice.

Treatment (mg/kg/days)	Healthy follicular size µm (diameter)			Total number Of healthy Follicles
	Small (<20µm)	Medium (20-70µm)	Large (<70µm)	
Control	234.00±2.37	75.40±4.30	8.00±0.13	317.40±0.30
5	223.21±4.50	72.50±0.22	6.01±0.22	301.72±0.35
10	212.91±0.22	69.26±0.36	5.68±0.16	287.85±0.16
20	202.58±6.24*	58.35±o.26*	5.04±0.15*	265.97±0.18*
30	186.56±8.10*	52.89±0.21*	5.00±0.13*	244.45±0.24*

Data are mean ±SEM of animals. *Significant p≤0.05 compared to control.

Table 3: Ethion induced temporal effect on the number of atretic follicles in ovaries

Treatment (mg/kg/day)	Atretic follicular size in µm (diameter)		Total number of Atretic follicle
	Medium (20-70µm)	Large (<70µm)	
Control	13.32±0.26	2.68±0.26	16.00±0.10
5	14.64±0.42	3.12±0.15	17.76±0.15
10	17.64±0.42	3.86±0.17	21.28±0.21*
20	19.20±0.09*	4.46±0.21*	23.66±0.16*
30	22.34±0.21*	5.02±0.90*	27.36±0.16*

Data are mean±SEM of 10 animals, * Significant P≤0.05 compared to control

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Table 4: Ethion induced temporal effect on biochemical contents of ovary in albino mice

Treatment Duration (mg/kg/d)	Ovary Weight (mg/100g body Weight)	Biochemical µg/mg wet weight of tissue			
		Protein	cholesterol	Sialic acid	3βHSD
Control	87.64±0.22	182.8±0.73	4.18±0.03	9.99±0.99	0.37±0.03
5	80.07±0.76	156.8±4.49	4.28±0.02	8.50±0.79	0.25±0.01
10	73.72±0.47	141.2±0.37	5.29±0.02*	9.36±0.01	0.21±0.00*
20	62.68±0.54*	134.6±10.3*	7.11±0.04*	9.24±0.03	0.22±0.03*
30	57.14±0.72*	120.6±1.74*	7.33±0.02*	8.00±0.01*	0.11±0.00*

Data are mean±SEM of 10 animals, * Significant P≤0.05 compared to control

Table 5: Ethion induced temporal effect on biochemical contents of uterus in albino mice

Treatment Duration (mg/kg/d)	Uterus Weight (mg/100g body Weight)	Biochemical µg/mg wet weight of tissue			
		Protein	cholesterol	Sialic acid	3βHSD
Control	0.456±3.28	186.6±0.24	3.31±0.09	12.57±0.13	0.42±0.05
5	0.424±4.24	155.4±1.60	3.08±0.02	12.23±0.09	0.41±0.01
10	0.376±2.68*	145.6±1.69*	4.68±0.06*	11.29±0.03*	0.35±0.04
20	0.354±1.34*	136.2±1.46*	5.00±0.04*	10.35±0.08*	0.33±0.01*
30	0.328±2.64*	130.8±0.37*	5.46±0.06*	09.82±0.02*	0.30±0.04*

Data are mean±SEM of 10 animals, * Significant P≤0.05 compared to control

4. DISCUSSUION

The present study ovary and uterus histological observation of the control mice showed treated with ethion for 5, 10.20and 30 days the total follicular dynamics significantly decrease this could be due to morphological and chemical induced injury that can manifest itself in different ways. The acute effect consists of accumulation of lipids and appearance of degenerative process leading to the death of cells. The necrosis process can effect small groups of isolated parenchymal cells the group of cells and the developmental NOEL in this study was 0.6 mg/kg, when rabbits were given dose of o0.06, 2,4 04 9.6 mg/kg on days 6 to 18 of pregnancy. Fetuses from the high dose tested exhibited, the pesticide and human health, New York: springer-verlag., once in the bloodstream ethion cross placenta (U.S EPA.Washing. DC, when rats were given of 0.02, 0.6 or 2.5 on days 15 of pregnancy. Development effects were seen only the hight dose tested in fetuses of hight dose group, there was an increased incidence of delayed ossification of the public bones, treatment with organophosphate insecticide , phosphomidon alone and congestion of sinusoids, ballooning of hepatocytes with pycnotic nuclei and focal necrosis was found (Dikshit et al., 1980)Choudhary et al., (2003) have revealed that the treatment with endosulfan, 10 mg/kg/day I rats causes liver damages histological study revealed hepatocytes and cell with nuclei. Acidophilic cytoplasm and cell with nuclear fragmentation induced by permethrin whereas DDT causes cytoplasmic vacuolization and hepatocytes necrosis (Kostka et al., 20000

Study on ovary and uterus folliculars dynomics and decrease the follicles and loss of Graafian follicles and biochemical changes occurs on effect of ethion dose 16mg/kg/d shows the absence of follicles and sizes of ovary decreases and chemical changes found .and also it has been reported that rapidly loss In protein of the brain during pesticide toxicity was reported (Richardson, 1981).ti has been suggested that acute treatment with monocrotophos showed tissue specific

inhibition of microsomal cyt-p-450 in hepatic and extrahepatic tissues resulting the loss of haemoprotien in rats (Siddiqui et al.,1987).swamy et al.,(1992)have reported that the decrease in total proteins and soluble proteins indicate their metabolic utilization. The increase in the activity of proteases correlated with decrease of soluble protein the increasing duration of the exposure of ethion causes decrease the proteins and sialic acid and 3βHSD of mice it has been reported there was decrease the level of proteins and sialic acid it has been reported that there was significantly decreases I the level of blood glucose and globulin I mancozeb treated rats due to low thyroxin level because of impaired thyroid function(Nebbia and Ferrero,1997),Ivanova-chemishanska (1982)has reported that the changes in the level of proteins and glycogen suggest either increased catabolism of biomolecule to meet the enhanced energy demand of animals under stress their reduced synthesis due to Impaired tissue function.

The present study of suggestion that prolonging exposure of ethion caused increase in the level of cholesterol in ovary and uterus it has been reported there was increased serum cholesterol level in rats exposed to BHC. the plasma cholesterol level were considered as valuable indicator of drug-induced disruption of lipid metabolism marked dose dependent increase of serum cholesterol in BHC fed rats suggested increased synthesis shivandappa and krishnakumari (1981) have also reported that in rats treated with BHC significant reduction is caused in hepatic DNA and RNA, with an indication of cells death due to focal necrosis the present study the exposure of ethion treatment the size of follicle were decreased and significantly decrease the level of protein and increase the level of cholesterol and accumulation of cholesterol in the ovary and uterus kidneys and testis or impaired biliary function(shivanadappa and krisnakumari1981). Similar results were also reported in rats treated with dimethoate (Siddiqui et al., 1991) Diethyl dithiocarbamate inhibits hepatic cyt-p-450 dependent activity in rats (Stott et al., 1997). The increase in cholesterol level indicates inhibitory action of pesticide on Cyt-p-450 enzymes (shivanadappa and krishnakumari, 1981: siddiqui et al., 1987: stott et al, .1997) might be due to high affinity binding (Zarh et al., 2002). In the present cholesterol increase in the ovary and uterus might be due to inhibition in the activity of enzymes involved in cholesterol break up results into deposition of cholesterol in the cell. Recently it has been reported that mancozeb and carbofuran treatments have altered the levels of protein, glycogen and total lipids in ovary uterus and liver in intact and hemicastrated rats and mice(Mahadevaswami et al., 2000: Baligar and kaliwal.2001) the results reveal that there was a decrease in the number of estrous cycles, the duration of proestrus, estrous and metestrus, with a concomitant significant increase in the duration of diestrous phase and there was a gradual increase in the diestrous index in treated mice compared to control, there was decrease in the total number of healthy follicles, but there was a increase in the total number of atretic follicles in treated mice compared to control, and there was decrease in the ovary weight, protein, sialic acid and 3βHSD content, but there was increase in the cholesterol content in treated mice compared to control. And significantly decrease in uterus weight, protein sialic acid and 3βHSD content, but there was increase in cholesterol content in treated mice compared to control.

By the results of present study it can be concluded that the ethion affects the estrous cycle and follicular dynamics of mice by suppressing the GnRH release, which may be acted directly on the Gonadotrophins to alter the pituitary response to GnRH or gonadal steroids. Therefore, the use of ethion as an insecticide or pesticide is harmful to the gerom cells of a living beings

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