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Profenofos induced biochemical, hormonal and histomorphological changes in female rat reproductive system

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Abstract
Profenofos is widely used, persistent and toxic organophosphorus insecticide. The present study was conducted to investigate toxic effects of profenofos on reproductive system of female albino rats. Two subchronic doses of 1/10th and 1/50th of LD_{50} of profenofos were administered orally for six weeks. Its exposure was associated with the alterations in the serum levels of hormones and various biochemical parameters viz: the concentration of total proteins, lipids, phospholipids and cholesterol in ovary of treated rats as compared to control rats. Activity of phosphatases (ACP and AKP) was significantly reduced in treated rats. Histomorphological studies of ovary and uterus further revealed a number of abnormalities with increase in atretic follicles, enhanced percent atresia, degeneration of uterine epithelium and enlarged intracellular spaces in endometrial glands in treated rats. The results infer that the profenofos exposure leads to pathophysiological conditions in the ovary and uterus at dose dependent manner.

Keywords: Profenofos, reproduction, histomorphology, biochemistry, pesticides

1. Introduction
Pesticides are the most effective means of pest eradication all over the world, but their use causes a number of adverse effects on non-target species including human beings by causing the toxicity [1, 2]. Organophosphorus pesticide (OP) exposure is a major public health issue in terms of death, morbidity, health care and general safety from toxicity, as OP residue levels have been detected in the soil, water bodies and vegetables [3]. These toxic compounds impair the cellular function, enzyme activity and produce cytotoxic changes through the generation of reactive oxygen species (ROS).
Profenofos is an organo-thiophosphate pesticide with broad insecticidal and acaricidal activity. It is a powerful stomach poison, widely used to control various flies and mites on crops like maize, soya bean, potato, sugar beet, paddy, tobacco, cotton and vegetables [4, 5]. Treatment of agricultural crops by profenofos leaves residues on crops and also contaminates surface water. The toxicity by profenofos appeared fatal even at relatively low plasma concentrations. Exposure to low levels of profenofos is known to produce a variety of biochemical changes in testis and liver of rats and also results in the accumulation of Ach (acetylcholine) and increase lymphocyte mobility and cytotoxicity in rats [6, 7].
Toxicities of organophosphorus insecticides cause adverse effects on many organs. Systems that could be affected by OPs are the immune system, liver, muscles, urinary system, reproductive system, pancreas and haematological system [8]. Profenofos cause various deformities like biochemical alterations and histomorphological changes in the male reproductive system [7, 9]. But the information related to its effects on health with particular reference to female reproductive toxicity are scarce, so our present study was designed to investigate the toxic effects of profenofos subchronic doses on biochemical constituents, hormones as well as induced histopathological features in ovary and uterus of female albino rats.

2. Material and Methods
2.1 Chemicals
Profenofos as Curacron 50 EC was obtained from Insecticides (India) Limited, Bharuch, Gujarat. Standard rat feed was purchased from Ashirwad Industries, Mohali, India.

All chemicals used were either of analytical grade or the highest purity commercially available. Hormone assay was performed by using ELISA kits.

2.2 Animals
The female albino rats aging 4-5 months and weighing 130-200g were procured from the Department of Livestock Production and Management, GADVASU, Ludhiana. All methods and procedures of animal handling during research were conducted in accordance with the guidelines of Committee for the Purchase of Control and Supervision of Experiments on Animals (CPCSEA), India and were duly approved by Institutional Animal Ethics Committee (IAEC), GADVASU, Ludhiana.

2.3 Experimental design
Female albino rats were divided into three groups each consisting of eight rats and was fed on standard feed. The first group of rats serving as control were given water and to the remaining two groups, profenofos dissolved in water was given at a dose level of 1/10th and 1/50th of LD_{50} i.e. 430.89 mg/kg of body weight (b.w.) by oral intubation for six weeks. The body weight of all the rats was recorded weekly. Vaginal smear of the rats was observed daily to know the stage of estrous cycle.

2.4 Sample preparation
After six weeks of treatment, the rats were anaesthetized using chloroform. After dissection, blood sample was collected directly from heart and used for the hormonal analysis. Ovaries, oviduct and uterus were excised immediately, cleared off the adhering tissue and weighted. One ovary of each animal used for the biochemical parameters which were assayed by standard methods viz., total proteins by Lowry *et al* [10], total lipids by Folch *et al* [11], phospholipids by Ames [12], cholesterol by Chiamori and Henry [13] and phosphatases (ACP and AKP) by Bessey *et al* [14].

2.5 Histological studies
Ovary and uterus tissues were also placed in alcoholic Bouin's fixative for 24 h and then dehydrated in graded series of alcohols, cleared in benzene and embedded in paraffin wax. The 5 μm thick sections were cut serially using microtome and then stained. The slides were observed under OLYMPUS CH20i microscope and photographed.

2.6 Statistical analysis
All data were expressed as mean ± standard error. Data was statistically analysed by one-way ANOVA test using CPCS and the differences were considered statistically significant at $p<0.05$.

3. Results and Discussion
3.1 Body, ovary, oviduct and uterus weight
After six weeks of treatment, it was observed that there was no effect on the body weight gain in profenofos treated rats. Growth rate (g/day/100g b.w.) was decreased significantly ($P<0.05$) at both doses of profenofos (1/10th and 1/50th of LD_{50}) as compared to control. The relative weight of ovary, oviduct and uterus was observed to be low in profenofos treated rats (Table 1).

Significant decrease in the weight of ovary was in corroboration with the finding where organophosphates like methyl parathion, dimethoate and monocrotophos given to female albino rats showed significant decrease in the weights of ovary [15]. Similar decrease in the weight of ovaries and uterus was also observed with methomyl (1/10 LD_{50}) treatment [16]. Similar results have also been reported [17].

Table 1: Effect of profenofos on body weight (g), relative ovary weight (g/100g b.w), relative oviduct weight (g/100g b.w) and relative uterus weight (g/100g b.w) of female albino rats as compared to control rats.

Treatments	Body weight		Growth rate (g/day/100g b.w.)	Relative ovary weight (g/100g b.w)	Relative oviduct weight (g/100g b.w)	Relative uterus weight (g/100g b.w)
	Intial (g)	Final (g)				
Control	168.33 ±2.78	183.33 ± 3.57	0.236± 0.06	0.015±0.002	0.007±0.00	0.12±0.008
1/50th	149.16 ± 4.90	169.33 ± 3.33	0.199± 0.08	0.013±0.00	0.004±0.00*	0.10±0.00
1/10th	145 ± 5.62	168.33 ± 6.00	0.132± 0.08	0.01±0.001*	0.004±0.00*	0.09±0.01*

Values are expressed as mean ± SE (n=8)
*Significant difference ($p<0.05$) as compared to control

3.2 Estrous cycle
The cyclicity becomes irregular in the animals of treatment groups after first week. It was observed that there was significant increase in diestrus phase in profenofos treated rats. Diestrus index was also increased dose dependently in all the treated groups as compared to the control rats (Table 2).

Table 2: Effect of profenofos on estrous cycle.

Groups	No. of cycles	Duration in days				Diestrus index
		Proestrus	Estrus	Metestrus	Diestrus	
Control	6.83±0.61	6.41±0.57	7.08±0.46	7.08±0.52	7.33±0.33	16.65
1/50th	6.17±0.55	6.16±0.40	7.25±0.57	6.83±0.67	8.5±0.83	19.31
1/10th	6.51±0.53	6.08±0.45	6.66±0.63	6.75±0.62	10.25±0.55*	23.29

Values are expressed as mean ± SE (n=8)
*Significant difference ($p<0.05$) as compared to control

Similar increased diestrus index was also observed with carbofuran treatment [18]. The number of regular estrous cycle was also reduced and significant increase in the duration of diestrus was observed in rats treated with monocroptophos [17]. Early study of [19] has also reported significantly altered estrous cycle in triazophos treated rats.

3.3 Biochemical observations
Profenofos treatments have been shown to induce significant changes in the biochemical constituents of the cell in ovary. Total soluble proteins, lipids and phospholipids in ovary were observed to be lower in profenofos treated rats as compared to control rats (Table 3, Fig 1). The activity of acid phosphatase

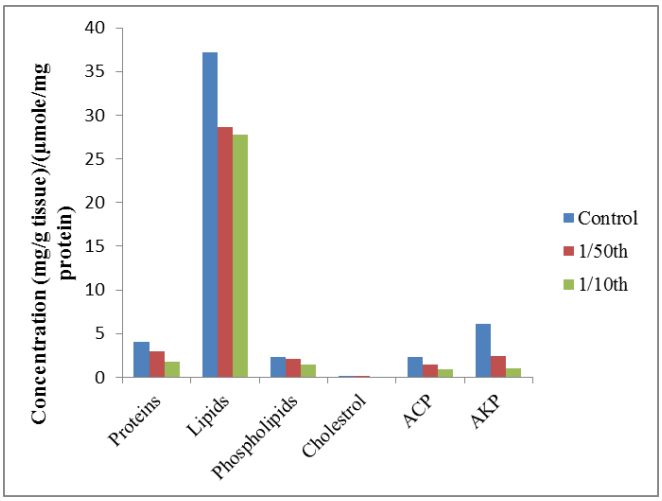
(ACP) and alkaline phosphatase (AKP) significantly ($P<0.05$) decreased in the ovaries of profenofos treated rats, while the level of cytoplasmic cholesterol in ovary was comparable in control and profenofos treated rats (Table 3, Fig 1). The present study is similar to the study conducted by [20] showed that the profenofos given at dose of 1/20th of LD_{50} to male albino rats showed a significant decrease in total lipids and phospholipids in the organ tissues of treated rats as compared to control.

Table 3: Effect of profenofos on ovary biochemical parameters as compared to control.

Parameters	Control	1/50th	1/10th
Protein	4.04±0.65	3.02±0.70*	1.74±0.42*
Lipids	37.17±0.21	28.69±0.12*	27.73±0.53*
Phospholipids	2.31±0.35	2.08±0.54	1.45±0.15*
Cholesterol	0.13±0.05	0.12±0.03	0.08±0.03
ACP	2.38±0.23	1.42±0.20*	0.94±0.04*
AKP	6.09±1.71	2.49±0.45*	0.98±0.20*

Values are expressed as mean ± SE (n=8)
Units: Proteins (mg/100 mg tissue), lipids, phosoholipids, cholestrol (mg/g tissue) and ACP, AKP (µmole/mg protein)
*Significant difference ($p<0.05$) as compared to control

Organophosphates like methyl parathion, dimethoate, monocrotophos and mancozeb given to female albino rats had resulted in significant decrease in total soluble proteins, total lipids, phosoholipids and cholesterol in ovaries of treated rats [21, 22]. Low levels of lipids, phospholipids and cholesterol in these rats indicated the decreased steroidogenic activity in atretic follicles and degenerating corpora lutea and are due to loss of intracellular membranes in degenerating tissues [23]. Profenofos induced significant decrease in the activity of phosphatases (ACP and AKP). The change in the activity of ACP and AKP is generally related to the intensity of cellular damage [24].



*Significantly different from control group ($p<0.05$).

Fig 1: Dose dependent effect of profenofos oral intubation on the level of total soluble proteins, lipids, phospholipids, cholesterol, ACP and AKP in the ovary of female albino rats (mean ± SE for n=8 rats in each group).

3.4 Hormone analysis

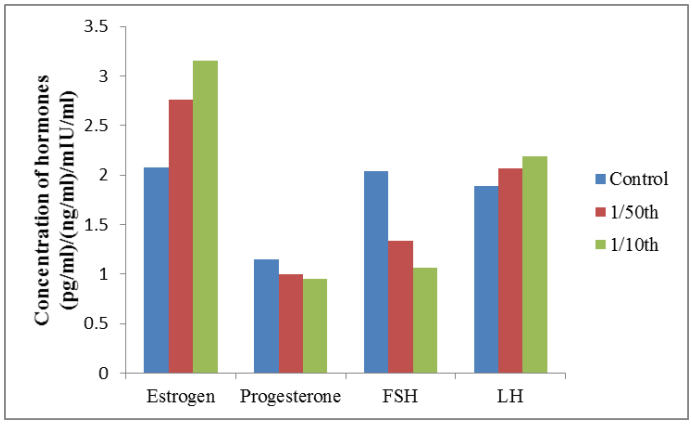
The present study has indicated the manner of profenofos poisoning by causing hormonal imbalance. The level of estrogen and follicle stimulating hormone (FSH) in plasma showed significant ($P<0.05$) increase while the level of progesterone and luteinising hormone (LH) in plasma showed significant decrease at both doses of Profenofos and in treated rats in comparison to control rats (Table 4, Fig 2).

The insecticides may act directly on the gonadotropins to alter the gonadotropin synthesis and secretion or indirectly by altering the pituitary cell responsiveness to GnRH or gonadal steroids which result in the alterations in the levels of FSH and LH affecting the feed-back mechanisms [25]. Pesticide intoxication can lead to disruption in balance of estrogen/progesterone at all stages of hormonal regulation [26]. Similarly, [27] showed that the mixture of cypermethrin and methyl parathion have resulted in alterations in reproductive hormones and enhanced levels of estradiol.

Table 4: Effect of profenofos on hormones in the plasma of treated rats as compared to control rats.

Hormones \ Treatments	Control	1/50th	1/10th
Estrogen	2.08±0.23	2.76±0.42*	3.15±0.23*
Progesterone	1.15±0.11	1.00±0.01*	0.95±0.04*
Follicle stimulating hormone	2.04±0.49	1.34±0.33*	1.06±0.23*
Luteinising hormone	1.89±0.04	2.07±0.14	2.19±0.23*

Values are expressed as mean ± SE (n=8)
Units: Estrogen (pg/ml), progesterone (ng/ml), FSH (mIU/ml) and LH (mIU/ml).
*Significant difference ($p<0.05$) as compared to control



*Significantly different from control group ($p<0.05$).

Fig 2: Dose dependent effect of profenofos oral intubation on hormones in plasma of female albino rats (mean ± SE for n=8 rats in each group).

3.5 Histological observations

All the phases of the follicular development viz. primary, secondary, tertiary, preantral and antral were observed in control and profenofos treated rats under light microscope. The total number of primary and preantral follicles was significantly more in 1/10th of LD_{50} profenofos treated rats (Table 5). Secondary, tertiary and antral follicles were significantly ($P<0.05$) reduced in number and more atretic follicles were observed in all the stages of follicles in profenofos treated rats. Overall follicular atresia was high in all the profenofos treated groups as compared to control (Table 5). Furthermore, 1/10th of LD_{50} profenofos treated rats ovary sections revealed degenerating oocyte and interstitial glands as a result of atresia attributed to profenofos dose level (Plate 1).

Table 5: Effect of profenofos treatment on follicular kinetics in the ovaries of treated rats as compared to control rats.

Follicles \ Treatments	Category of follicles	Control	1/50th	1/10th
Number of primary follicles	Normal	12.5±0.5	8.50±0.50	5.00±1.00*
	Atretic	6.5±1.50	6.50±0.50	8.50±0.50
	% atresia	34.41	43.33	62.96
Number of secondary follicles	Normal	9.50±0.50	6.00±0.00	4.50±0.5*
	Atretic	5.00±1.00	7.50±1.50*	7.50±1.50*
	% atresia	34.48	55.55	62.5
Number of tertiary follicles	Normal	5.50±1.50	3.50±1.50	3.50±0.50
	Atretic	1.50±0.50	5.50±0.50*	4.00±0.00
	% atresia	21.42	61.11	61.11
Number of preantral follicles	Normal	6.00±1.00	4.50±0.50	6.50±0.50
	Atretic	6.5±0.50	5.00±0.00	10.5±0.5*
	% atresia	52.00	50.00	61.76
Number of antral follicles	Normal	7.50±0.50	3.50±0.5	3.00±1.00*
	Atretic	3.00±1.00	6.50±1.50*	4.50±0.50
	% atresia	28.57	65.00	60.00

Values are expressed as mean ± SE of four animals in each group
*Significant difference (p<0.05) as compared to control

The present study is in the agreement with the study conducted by [18] where treatment with 1 and 1.3 mg/kg/d carbofuran caused a significant decrease in the number of healthy follicles with concomitant significant increase in the number of atretic follicles. A significant increase in the follicular atresia in profenofos treated rats is similar with the earlier studies of [16] that the increased atresia of ovarian follicles can be attributed to the deleterious effects of pesticides through the altered endogenous hormone levels. Overall follicular atresia was high in all the triazophos treated groups as compared to control [19] which is similar to present study. Similar follicular atresia pattern have also been reported by other workers [28].

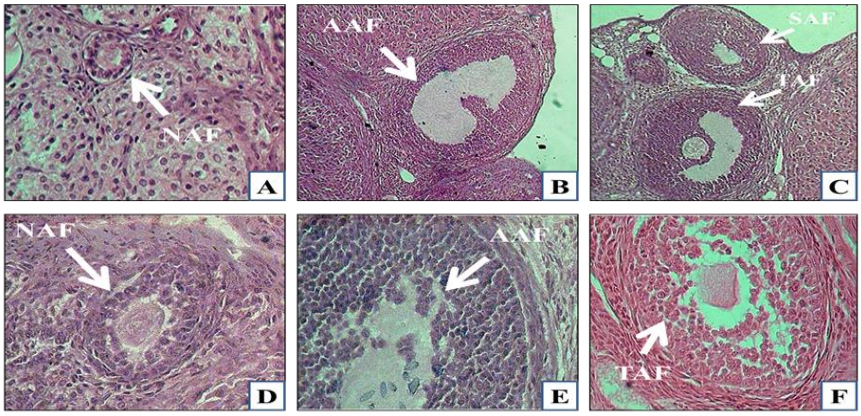


Plate 1: Fig. A, D T.S of ovary of control female showing normal antral follicle (NAF). Fig. A (10x), Fig. D (40x). **Fig. B, E** T.S of ovary of females treated with profenofos at a dose of 1/50th of LD_{50} showing atretic antral follicles (AAF). **Fig. B** (10x), Fig. E (40x). **Fig. C, F** T.S of ovary of females treated with profenofos at a dose of 1/10th of LD_{50} showing secondary atretic follicles (SAF), tertiary atretic follicles (TAF) Fig. C (10x), tertiary atretic follicles (TAF). Fig. F (40x).

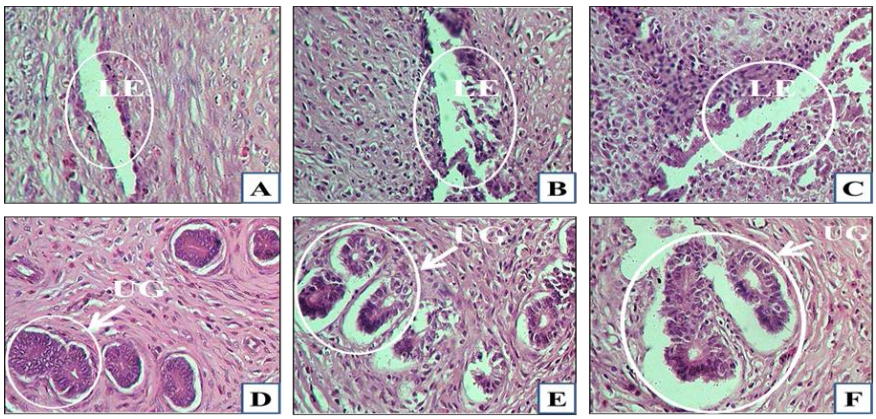


Plate 2: Fig A, D , T.S of uterus of control female showing intact columnar epithelium of lumen (LE) Fig. A (40x) and uterine glands (UG) Fig. D (40x). **Fig. B, E** T.S of uterus of female treated with profenofos at a dose of 1/50th of LD_{50} showing distorted epithelium of lumen (LE) Fig. B (40x) and decreased uterine glands with (UG) Fig. D (40x). **Fig C, F** T.S of uterus of female treated with profenofos at a dose of 1/10th of LD_{50} showing distorted and disappearance of epithelium of lumen (LE) Fig. B (40x) and decreased uterine glands with (UG) Fig. D (40x).

The uterus of control rats showed normal histological architecture of luminal epithelium and endometrial glands. Whereas, the luminal epithelium showed degeneration and its height was also decreased significantly in 1/10th and 1/50th of rofenofos treated rats as compared to control rats (Table 6, Fig 3). Also, less number of endometrial glands with distorted histoarchitecture was observed in profenofos treated rats (Plate 2). Myometrium was normal and intact in control rats whereas in profenofos treated rats myometrium was vacuolated (Plate 2) and there was an increase in thickening of myometrium of treated rats (Table 6, Fig 3).

Table 6: Effect of profenofos on uterine components of treated rats as compared to control rats**.**

Treatments / Uterine components	Control	1/50th	1/10th
Type of Luminal epithelium	CL	CL	CL
Epithelial height(µm)	22.08±1.76 (16.50-28.50)	14.56±1.23* (11.50-17.50)	10.45±0.31* (9.40-14.50)
Endometrial Glands	++	+	+
Myometrium: Thickness(µm)	90.09±2.47 (75.50-105.50)	110.49±1.97* (100.50-112.75)	120.07±3.32* (105.60-121.75)

Values are expressed as mean ± SE (n=8)
*Significant difference (p<0.05) as compared to control

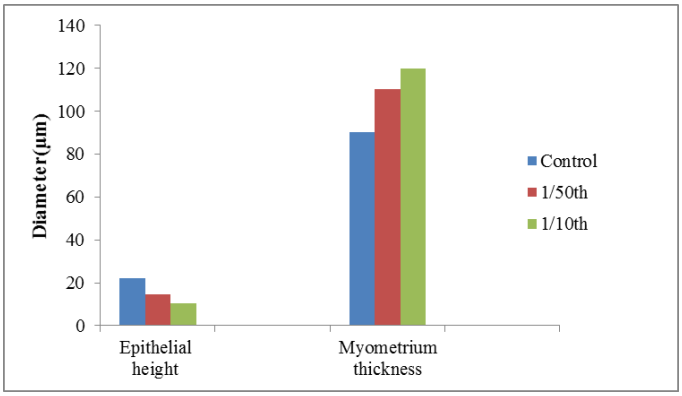


Fig 3: Effect of profenofos on uterine components in treated rats as compared to control rats.

4. Conclusion

Significant changes were seen in biochemical constituents of ovarian cells of treated rats. Histopathological changes were seen in ovary and uterus in all profenofos treated rats. Prominent follicular atresia in corroboration with increased atretic follicles, suggests the impact of profenofos as endocrine disrupter causing hormonal imbalance in ovaries of treated rats. Degenerated luminal epithelium and endometrial glands also reflects a number of pathophysiological conditions with pesticide exposure.

5. Acknowledgement

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