THE EFFECTS OF FENVALERATE ON PRODUCTION OF PHEROMONE, HORMONE AND HISTOPATHOLOGICAL CHANGES IN ENDOCRINE GLANDS OF FEMALE ALBINO RATS

G. Jayachitra[1], P. Ponmanickam[2], J. Vijaya Kumar[1], S.P. Sevarkodiyone[3] and T. Rajagopal[1,4*]

[1]Post Graduate Department of Biotechnology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi-626 124.
[2]Post Graduate Department of Microbiology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi-626 124.
[3]Post Graduate Department of Zoology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi-626 124.
[4]Post Graduate and Research Department of Zoology, Thiagarajar College (Autonomous), Madurai-625 009.

Abstract: In the present study, the average body weight, organ weight, LH, FSH, estradiol and progesterone significantly decreased in fenvalerate treated groups as compared to that of control. The gas chromatography analysis clearly showed the major 72 volatile compounds were identified in the control and fenvalerate treated groups. More number of volatile compounds was disappeared long time Fenvalerate treatment groups as compared to control rat urine. The SDS-PAGE analysis of liver, kidney, clitoral and ovary revealed that the significant changes in protein profiles in the fenvalerate administrated rats. The protein bands like 14 to 85 kDa were disappeared in the fenvalerate treated rats as compared to control rats. The result indicating the fenvalerate caused severely effect on protein secretion in the endocrine organs. Histopahological degeneration was observed in the liver, kidney, clitoral and ovaries of long time fenvalerate treated groups. The results clearly showed that the fenvalerate alters the organ cell architecture, pheromone production, sex hormone and fertility in the female rats which is does and duration dependent.

Key words: Fenvalerate, SDS-PAGEanalysis, Histopahological degeneration.

1. INTRODUCTION

The modern man is constantly exposed to a variety of toxic chemicals primarily due to change in life style. The food we eat, the water we drink, the air we breathe and the environment we live in are contaminated with toxic xenobiotics. Humans are exposed to such chemicals while still in the womb of the mother (*i.e*. earth). In the last hundred years or so, human activities have been destroying the natural system upon which life depends [1]. Pesticides including all xenobiotics whose specific purposes is to kill another form of life, including insects (insecticides), small rodents (rodenticides), or even vegetation (herbicides). Globally, the use of synthetic pesticides has increased rapidly in the last fifty years due to intensification of farming in order to obtain higher yields. However, over dependence on chemicals not only resulted in a high cost of production but also irreparable damage to the environment and long term health problems to humans and other forms of life including marine and terrestrial organisms [2].

Pesticides are agricultural chemicals used for controlling pests on the plant or animals. Problems associated with pesticide hazards to man and environment are not confined to the developing countries, but extended to developed nations and still facing some problems in certain location [3]. A number of long persistent organo-chlorides (O'Ch), which have been banned or severely restricted are

*****Corresponding author: *deer_raj@yahoo.co.in*

still marketed and used in many developing countries [4]. The ideal pesticide is pesticide which be effectively only against the pests and be harmless to people, animals and environment. However, they have some side / a non-target effect that may show undesired actions appears latter [5]. A large number of chemicals occurring in our environment may have potential to interfere with endocrine system of animals. Vinggaard *et al*. [6] reported that the many of the pesticide chemicals have been considered endocrine disruption because of their capacity to block or active hormone or pheromone receptors and/or to affect sex hormone or pheromone production.

Further, sexual-pheromonal communication system of insect and mammals which includes the production and emission of volatile chemical compounds by sexually receptive females, and a sequence of behaviors by males in response to these chemicals that culminates in mating. Use of insecticides, rodenticide, herbicide, termiticide etc. can result in pleiotropic effects to the pheromone communication [7]. According to Rajagopal [8] wild Blackbuck population is likely to continue to decline as a consequence of poaching and has also been considered lack of communication between their partner which is affected by environmental air pollution (*i.e*. use of pesticides). For instance, a reduction in the production and emission of pheromone, a delay in the onset and reduction in incidence of female calling, a reduction in female attractiveness, and a reduction in the ability of males to locate sources of pheromone have been observed in resistant moths [9, 10].

Fenvalerate is a synthetic pyrethroid insecticide commonly used in agriculture and other domestic applications due to its high insecticidal activity and low mammalian, avian and phytotoxicities [11]. Although fenvalerate is considered to have low acute toxicity to mammals, it is having severe neurotoxic effects and estrogenic acitivity casuing endocrine disorders. Imidacloprid (IM) is a neonictinoid compound, which is a class of neuro- active insectidies and manufactured after synthetic nicotine. It is widely used in pest control, seed treatment, termite control, flea control, as an insecticide spray and a systemic insecticide. IM is rapidly and almost completely absorbed from the gastrointestinal tract, an eliminated via urine and feces [12].

The possible ecological effects of fenvalerate which has still been commonly used in our country for various aims are not known well. However, few literatures in this respect long-term exposure specifically in mammals are available [13,14]. For the above mentioned, the aim of the present study was to check the endocrine-disrupting effects and reproductive toxicity of daily exposure to fenvalerate female rats *via* evaluation of effect on sex hormone level, emission of the pheromone production and biochemical parameters were studied.

2. MATERIALS AND METHODS

Study Animal: The female albino rats in adult stage weighing 150-200 g were used for the present study. In the animal house of Animal aquarium, the animals were maintained hygienically and they were separately housed under controlled temperature and sanitized conditions in polypropylene cages with sterile paddy husk. The rats were maintained in normal day and night schedule (12 hour light: 12 hour dark) and the pelleted food stuff containing 22 % crude protein, 4.12 % crude oil, 2.79 % crude fiber, 7.81 % ash and 1.34 % sand silica were use for feeding the animal that has been purchased from TANUVAS, Chennai, India before carrying out the experiment and during experimental work too. And in short and long duration of the experimental periods the animal were orally administrated with pesticide and pesticide plus vitamin-C, pesticide plus male urine and pesticide plus vitamin and male urine.

Experimental design: Thirty six female rats reared from this litter were divided into six groups of six each: group I, control rat (normal); group II, treated with fenvalerate (short time duration-30 days, 30 mg/ kg b.wt); group III, treated with fenvalerate (long time duration-60 days); group IV, treated with fenvalerate plus vitamin C (long time duration); group V, treated with fenvalerate plus male urine and group VI, treated with fenvalerate plus vitamin and male urine.

Body and reproductive organ weight measurement: The weight of animals was recorded at the starting time and in the end of the experiment [15]. The organ weight of treated animals was recorded the end of the experiment.

Collection of blood, organ and tissue sample: The urine samples were obtained from the six rats in each group during the experimental period. Albino rats (130-180 g, female) overnight (12) in metabolism cages at ambient temperature. After centrifugation at 400 X g for 10 min to remove residues, urine samples were mixed and stored in aliquot at -80^0c until use. Blood was collected by cardiac puncture and allowed to clot at room temperature. Serum was collected from the clotted blood by centrifugation (Cooling centrifuge – REMI) at 1500g for 15 min at 4°C and stored at 80°C until analyses. Serum was used for hormone assay. At the end of the experimental period, animals were overnight fasted and were euthanized by decapitation under deep anesthesia with diethyl ether inhalation and dissected via spargue. Organs such as ovary and clitoral gland were recovered and their weights were recorded. Ovary and clitoral gland gland was processed for further histological analysis.

Steroid hormonal assay: Serum plasma LH, FSH and Estradiol were measured by chemiluminescence immunoassay method (CLIA) [16]. Serum progesterone was determined by the carbonylmetallo immunoassay method (CMIA)/ micro particle enzyme immunoassay (MEIA). The inter and intra-assay coefficients of variation are <15 % and <10 %, respectively [17].

Gas chromatography and mass spectrometry analysis

Identification of volatile compounds by GC-MS:

The sample (*i.e.* fenvalerate treated and control urine sample) were fractionated and chemical compounds were identified by Gas Chromatography-linked Mass Spectrometry (GC-MS; QP-5050, Schimadzu, Japan). The 2 µl of the extract was injected into the GC-MS system on a 30 m glass capillary column with a film thickness of 0.25 µm (30 mx0.2 mm i.d. coated with UCON HB 2000) using the following temperature programme: initial oven temperature of 40ºC for 4 min increasing to 250ºC at 15ºC/ min; and then held at 250 ºC for 10 min. The gas chromatography (Schimadzu GC 15A) was equipped with FID detector connected to an integrator. The relative amount of each component was reported as the percent of the ion current. The GC-MS was under the computer control at 70 eV using ammonia as reagent gas at 95 eV performed chemical ionization. Identification of unknown compounds was made by probability-based matching using the computer Library built within the NICT 12 system.

SDS-PAGE analyses

Preparation of extract: Portions (1 g) of the ovary and clitoral gland were homogenized with PBS (pH 7.2) separately for each treated group. The homogenate was clarified by centrifugation at 12,500 x g for 15 min and the supernatant was removed. Further, the samples (proteins) were desalted from the supernatant by size exclusion chromatography (SEC) on spun columns of Sephadex G25. SDS-PAGE (one-dimensional) was performed as described by Laemmli [18]. All samples were run under reducing condition in 12% SDS-PAGE. The

protein (25 µg) was loaded on the gel for determination of molecular mass. Protein standard (protein molecular weight marker- medium range, Genei, Bangalore), 4 µl, was applied on the gel separately. The gels were run for 3 hrs at 50 V, and the proteins were stained with silver.

Once the gel was completed it was rinsed with distilled water for 2 min and stained with 0.5% CBB-R-250 stain for 2 hrs at room temperature. The stained gel was destained until the appropriate background was obtained. The gel was washed with distilled water and stored in the refrigerator for image analysis.

Histomorphological analysis: Histology of ovary and clitoral gland gland was studied by fixing the tissues in Bouin's fluid and adopting the routine paraffin method for light microscopic studies. 33–5 µm thick transverse or longitudinal paraffin sections, as may be applicable of testis and preputial glands were obtained using a rotary microtome (Lieica, jena, Germany) and stained with Harris haematoxylin and eosin, mounted in DPX (Dibutyl phthalate in xylene) mountant and observed in a hund wetzlar microscope (Germany). Images were captured through a charge-coupled device (CCD) camera (Canon (PC1356), Canon Inc., made in Japan)

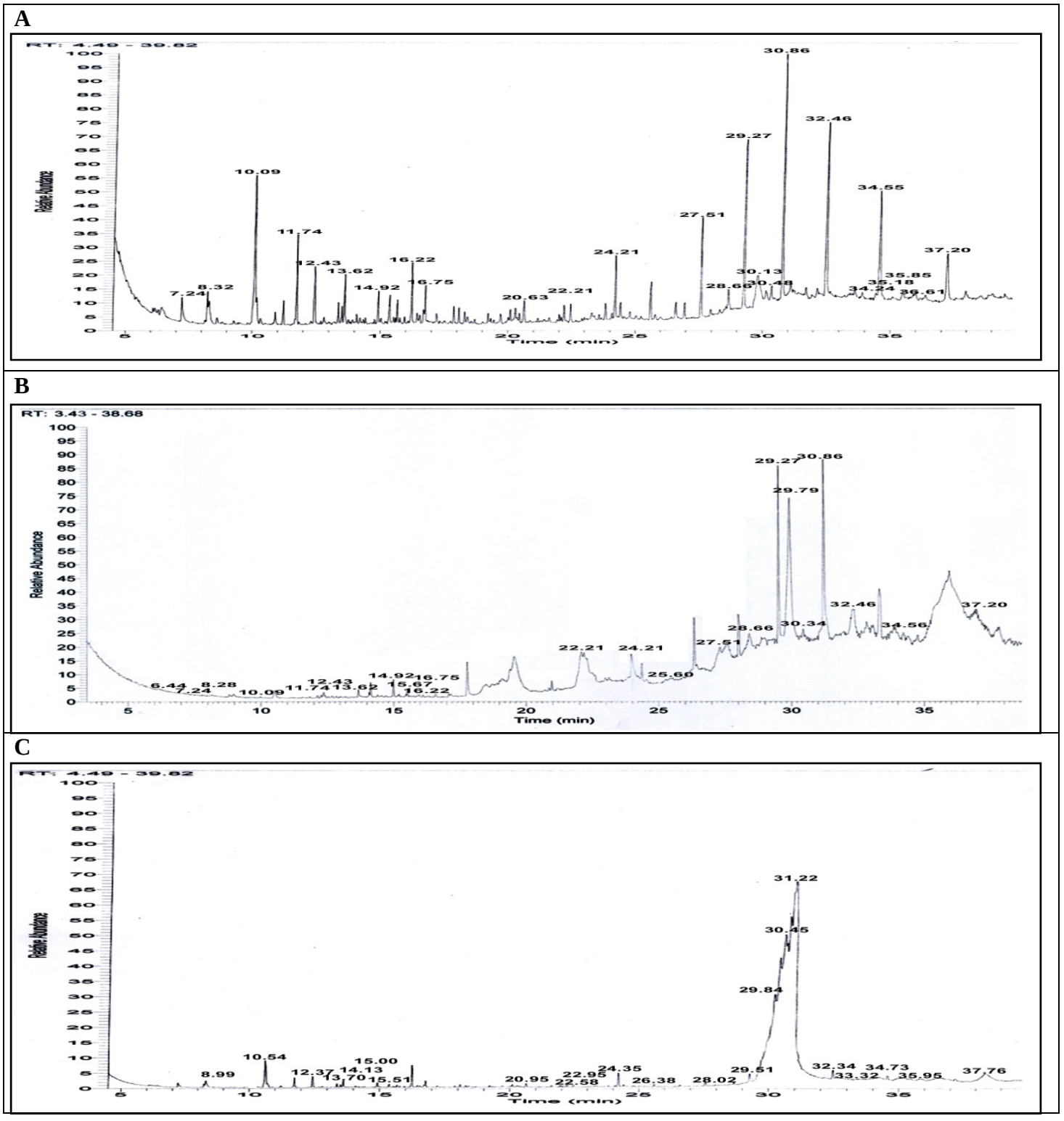
Statistical Analysis: All data were expressed as mean±SE of number of experiments. The individual comparisons of experimental groups were obtained by Duncan's Multiple Range Test (DMRT). A value of $p<0.05$ was considered to indicate a significant difference between groups. Valued with identical letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level.

3. RESULTS AND DISCUSSION

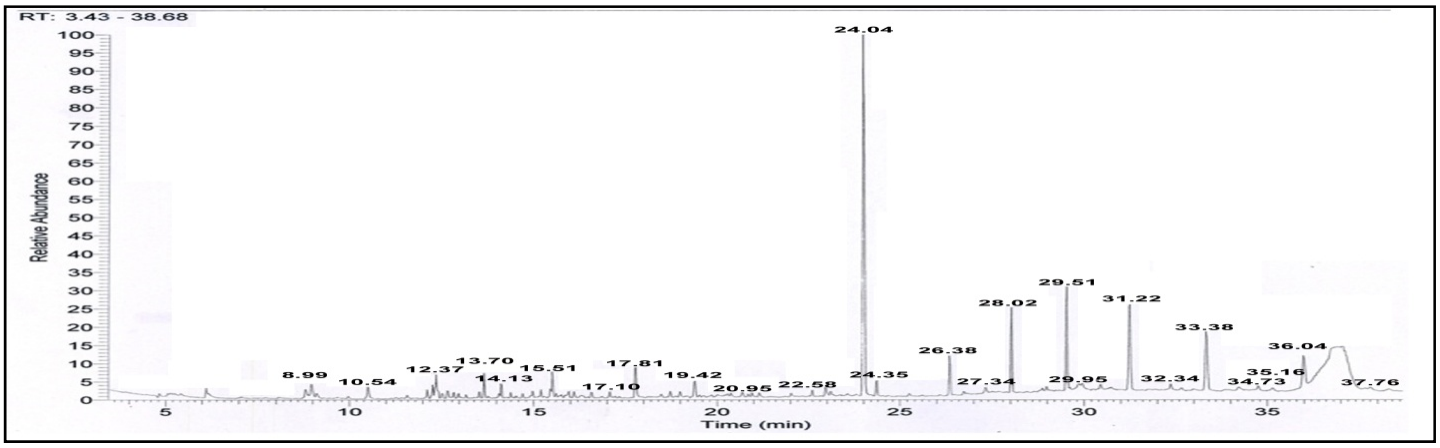
Effect of fenvalerate on pheromone production: The gas chromatography analysis clearly showed the major 71 volatile compounds were identified in the female urine of control group and treated groups (Fig. 1). The identified compounds belonged to alkanes (23volatiles), ketones (13 volatiles), aldehydes (10 volatiles), esters (8 volatiles), alcohols (6 volatiles), amines (4 volatiles), phenols (3 volatiles) and each in alkenes (2 volatiles) and carboxylioc acids (2 volatiles) (Table 1). Among the 71 volatile compounds, 25 compounds were identified in the each control (group I), treated group IV and treated groups V, 24 compounds in treated group II, 22 compounds in treated group III and 23 compounds in treated group VI.Urine is a highly complex biological materials; the nature of its chemical constituents dependent on endocrine status and body temperature as well as genetic background [19]. It is remarkable to note that the compounds such as 2-methoxypent-4-ynal, hexadecanoic acid,-1-methylethyl ester, n-cetyl thiocyanate, hexanedioic acid-bis(6-methylheptyl)-ester and 2-methyl-2-(3-pentanoyl)-5-isopropenyl-hexanone were present only in the long time fenvalerate treated groups (i.e. III, IV, V and VI treated groups). After fenvalerate treatment more number of volatile compounds was disappeared and appeared unique volatile compounds from the urine sample as compared to control rat urine. It is reported that the pesticides in mammals could be of direct or indirect effects on the immune systeminhibition of non-target serine hydrolases [20], inhibition of esterases in the lymphocytes membrane oxidative damage to the immune organ [21. 22] and interferes with immune cells proliferation and differentiation. Therefore, the present study demonstrated that the fenvalerate insecticide may inhibit pheromone production in the fenvalerate treated female rats.

Effect of fenvalerate on body and organ weight: The body weight taken on initial and final days of the experimental animals did not show much variation among the fenvalerate treated groups as compare to the control. The average body and organ weight gain was significantly reduced in treated group III as compared to control and other treated groups (Table

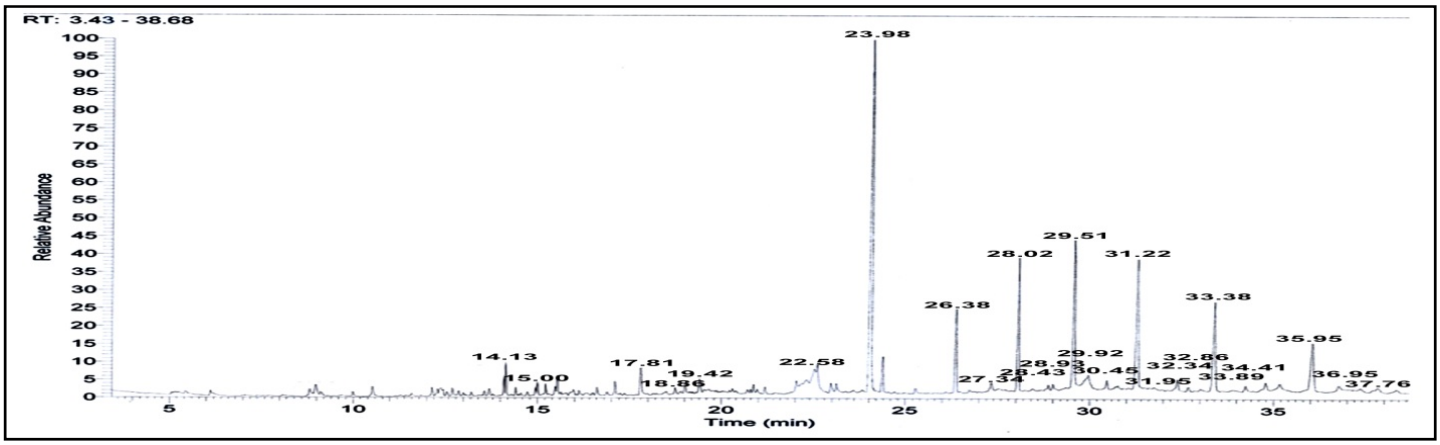
2). Previous studies reported that exposure of organophosphate has on adverse effect on body and organs weight. According to Suresh *et al.,* (2003) the exposure of methyl parathion at the dose level of 30 mg/kg was not showed significant changes in body and organ weights. Likewise, in the present study exposure of 30 mg/kg of fenvalerate was



D



E



F

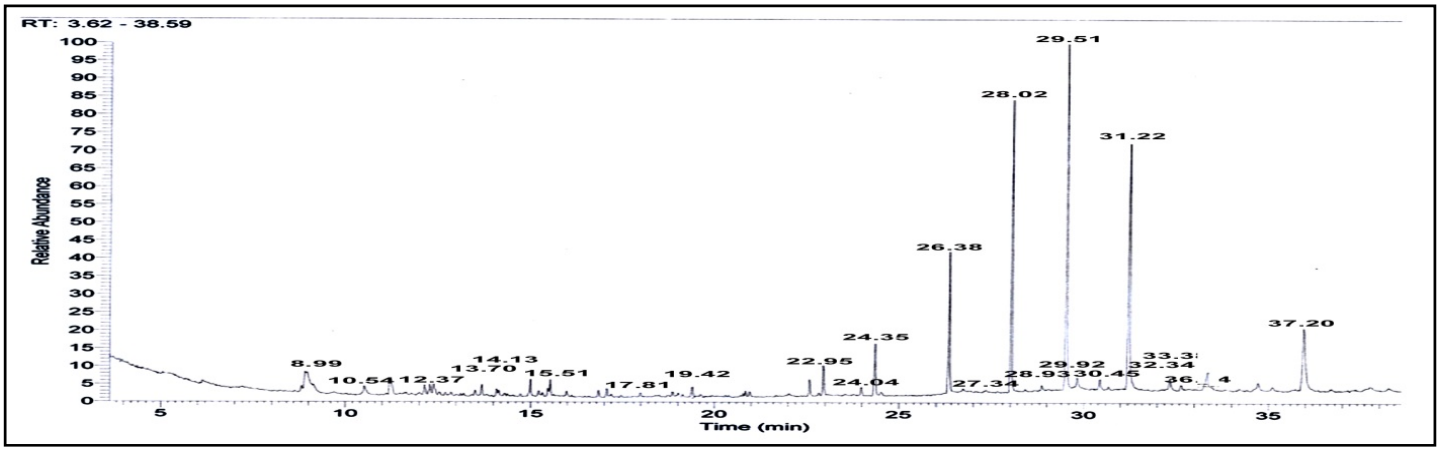


Figure 1. GC-MS profiles of volatile compounds from (A) control (B) short time fenvalerate treated group, (C) long time fenvalerate group, (D) the long time fenvalerate plus vitamin-C treated group, (E) long time fenvalerate plus male urine (*i.e*. pheromone) treated group and (F) long time fenvalerate plus vitamin-C and male urine treated group (Refer to Table 1)

Table 1: List of volatile compounds identified in the control and fenvalerate treated groups

S. No.	RT	Compound name	Experimental group					
			G1	G2	G3	G4	G5	G6
1	6.44	Benzoic acid	-	+	-	-	-	-
2	7.24	Methanesulphinic acid methyl ester	+	+	-	-	-	-
3	8.32	4-methyl-phenol	+	-	-	-	-	-
4	8.99	2-methyl-1-pentanol	-	-	+	+	-	+
5	10.09	2-ethyl-phenol	+	+	-	-	-	-
6	10.54	2-methyl-heptadecane	-	-	+	+	-	+
7	11.74	Dodecane	+	+	-	-	-	-
8	12.37	3-methyl-undecane	-	-	+	+	-	+
9	12.43	4- propyl-benzaldehyde	+	+	-	-	-	-
10	13.62	Pent-4-enal	+	+	-	-	-	-
11	13.70	1,1-difluoro-1,5-hexadiene	-	-	+	+	-	+
12	14.13	2-methoxypent-4-ynal	-	-	+	+	+	+
13	14.92	2-methyl-dodecane	+	+	-	-	-	-
14	15.00	4,4-dimethyl-1-penten-3-one	-	-	+	-	+	-
15	15.51	2-fluorobenzoic acid-triethyl-othoester	-	-	+	+	-	+
16	15.67	1,2-bis(c-trimethylsilypropoxy)-ethane	-	+	-	-	-	-
17	16.22	Dodecanoic acid-1-methylethyl ester	+	+	-	-	-	-
18	16.75	Pentadecane	+	+	-	-	-	-
19	17.10	1-acetyl-2-propylcyclohexane	-	-	-	+	-	-
20	17.81	N,N,N-tetramethyl-1,3-propanediamine	-	-	-	+	+	+
21	18.86	(S)-1-cyano-2-methylpropyl acetate	-	-	-	-	+	-
22	19.42	1-(2-hydroxyethoxy)tridecane	-	-	-	+	+	+
23	20.63	2-methyl-hexadecane	+	-	-	-	-	-
24	20.95	Tetradecanoic acid, methyl ester	-	+	+	-	-	-
25	22.21	Nonanoic acid, methyl ester	+	+	-	-	-	-
26	22.58	1-Iodo-3-methyl-2-pentanone	-	-	+	-	-	-
27	22.95	2-diazo-5-(2-methoxyphenyl)-6-methylheptan-3-one	-	+	+	-	-	-
28	23.98	2-(p-methoxyphenyl)-2-methyl-1-phenylpropan-1-one	-	-	-	-	+	-
29	24.04	4,4-dimethyloxazolo[2,3-d]triazaine-2,2-dioxide	-	-	-	+	-	+
30	24.21	2,6-bis(1,1-dimethylethyl)-4-methyl phenol	+	+	-	-	-	-
31	24.35	(R,R)-3,8-dimethyldecane	-	-	+	+	-	+
32	25.60	7,9-dimethyl-hexadecane	-	+	-	-	-	-
33	26.38	Hexadecanoic acid,-1-methylethyl ester	-	-	+	+	+	+
34	27.34	Hexadecamethylcyclooctasiloxane	-	-	-	+	+	+
35	27.51	Undecane	+	+	-	-	-	-
36	28.02	n-cetyl thiocyanate	-	-	+	+	+	+
37	28.43	a-fluoro-chalcone	-	-	-	-	+	-
38	28.66	1-[(1,1-dimethylethyl)dimethylsilyl]oxy]-1-phenyl-2-propanone	+	+	-	-	-	-
39	28.93	5-methyl-5-nitro-2-hexanone	-	-	-	-	+	+
40	29.27	Heptadecane	+	+	-	-	-	-
41	29.51	Hexanedioic acid-bis(6-methylheptyl)-ester	-	-	+	+	+	+
42	29.79	1,3-dimethyl-2(1H)-pyridinone	-	+	-	-	-	-
43	29.84	Heptacosane	-	-	+	-	-	-
44	29.92	5,10-bis(iso-butyl)porphyrin	-	-	-	-	+	+
45	29.95	2,3,4-5tetradydro-4-methyl-1,5-dioxo-1H-benz(c)azepine-3-carboxylate	-	-	-	+	-	-
46	30.13	1-chloro-1methoxyl-1-phenylacetone	+	-	-	-	-	-
47	30.34	Hexamethyl-disiloxane	-	+	-	-	-	-
48	30.45	3(tert-butylazo)-3-cyclopropylbutanoic acid	-	-	+	-	+	+
49	30.48	2-chloro-4,6-bis (2,4-dimethyl phenyl 1) sulfanyl] pyrimidine	+	-	-	-	-	-
50	30.86	[1,2-(2)H(2)]-5,7-cholestadlen-3a-ol	+	+	-	-	-	-

51	31.22	2-methyl-2-(3-pentanoyl)-5-isopropenyl-hexanone	-	-	+	+	+	+
52	31.95	2-hydroxy-2-cyclopenten-1-one	-	-	-	-	+	-
53	32.34	Dotriacontane	-	-	+	+	+	+
54	32.46	Docosane	+	+	-	-	-	-
55	32.86	1-(dodecycloxy)-4-methoxybutan-2-one	-	-	-	-	+	-
56	33.32	Pentatriacontane	-	-	+	-	-	-
57	33.38	5-pentylporphyrin	-	-	-	+	+	+
58	33.89	3-hydroxy-2-ethyl-5-methyl-4-pyrone	-	-	-	-	+	-
59	34.24	t-butyl(R)-3-(benzyloxy)-butanoate	+	-	-	-	-	-
60	34.41	N-(4-chlorobenzyl)pyrrole	-	-	-	-	+	-
61	34.55	2-methyl- 4-propylthiazole	+	-	-	-	-	-
62	34.56	Tricosane	-	+	-	-	-	-
63	34.73	Hexatriacontane	-	-	+	+	-	-
64	35.16	(5-hydroxyhexyl)-t-butyl carbonate	-	-	-	+	-	-
65	35.18	Phenyl-2-heptanonate	+	-	-	-	-	-
66	35.85	2-acetyl-5,6-dimethylpyridine-3-ol	+	-	-	-	-	-
67	35.95	Hexacosane	-	-	+	-	+	-
68	36.04	Nanacosane	-	-	-	+	-	+
69	36.61	1,3- Diethoxycarbonylbenzo(c) tellurophene	+	-	-	-	-	-
70	37.20	(E)-3,4,4-trimethyl-2-pentenol	+	+	-	-	-	+
71	37.76	Nonahexacontanoic, methyl ester	-	-	+	+	+	-
72	36.95	1-(Ethoxy)-3-methylbut-3-enyl benzene	-	-	-	-	+	-
		Total	25	24	22	25	24	23

Table 2: Relative body and endocrine organ weight of control and fenvalerate treated groups

Exptl. group	Body weight (gm)	Liver	Kidney (gm)		Clitorial gland (gm)		Ovaries (gm)	
			Right	Left	Right	Left	Right	Light
Group I	205.75±4.12^{a}	5.16±0.03^{a}	0.69±0.03^{a}	0.61±0.01^{a}	0.09±0.032^{a}	0.08±0.074^{a}	0.10±0.01^{a}	0.09±0.005^{a}
Group II	187.43±2.67^{b}	4.92±0.71^{ab}	0.52±0.01^{ab}	0.56±0.10a^{b}	0.07±0.025^{b}	0.07±0.05^{ab}	0.08±0.10^{b}	0.07±0.015^{b}
Group III	154.15±1.95^{de}	3.64±0.05^{b}	0.46±0.05^{b}	0.47±0.017^{b}	0.04±0.015^{c}	0.03±0.05^{b}	0.06±0.02^{c}	0.04±0.002^{c}
Group IV	163.85±0.77^{d}	3.41±0.14^{b}	0.43±0.014^{b}	0.44±0.025^{b}	0.04±0.015^{c}	0.03±0.025^{b}	0.04±0.012^{cd}	0.04±0.002^{c}
Group V	169.46±0.15^{cd}	3.53±0.10^{b}	0.40±0.037^{b}	0.41±0.025^{b}	0.03±0.014^{d}	0.02±0.02^{bc}	0.04±0.015^{cd}	0.03±0.017^{c}
Group VI	170.15±1.09^{c}	3.72±0.55^{b}	0.42±0.025^{b}	0.43±0.15^{b}	0.04±0.015^{c}	0.03±0.021^{b}	0.06±0.02^{c}	0.04±0.002^{c}

Values are mean ±SE of six rats in each group.
Means with same superscripts are not significant ($p<0.05$) to DMRT.

revealed the statistically significant difference in body weight of control and fenvalerate groups. However, the weight of organs such as liver, kidney, clitoral and ovary exhibited considerable changes in control and fenvalerate treated groups. In the present study, the body and organ weight of the long time fenvalerate treated groups (group III, IV, V and V) there were no significant changes among the fenvalerate treated groups. Authors have also observed similar effects after treating of Methyl parathion possible due to decrease the body and organ weights [23]. Brown and Casida [24] and Jadaramkunti and Kaliwal [25] reported that reduction of kidney, liver, testes, seminal vesicle and epididymis weights in rats treated with the highest does of dicofol. By contrast, some synthetic pyrethroids had no effect on body as well as organ weights [26, 27].

Effect of fenvalerate on liver function (SGOT, SGPT and ALP): The function of liver and kidney was predicted by the biochemical assays. The SGOT, SGPT and ALP are key enzyme for the testing the liver damage. The SGOT, SGPT and ALP level was significantly increased in fenvalerate treated groups as compared to control. Likewise, the SGPT level was considerably raised in short time treated group. However, there were no significant variations observed in fenvalerate treated groups (group III, IV, V and V) (Table 3). These results are in agreement with different previous researchers which indicated that the exposure to fenvalerate and other pesticides led to induce severe physiological and biochemical disturbances in experimental animals, buffalo calves [28], goats [29], cockerels [30], poultry [31], rabbits [32] and rats [33]. It is reported that the ALP is a membrane bound enzyme which has found application in diagnosis of liver damage. Abnormal levels of this enzyme is known, abnormally high level which usually suggests leakage from tissues and abnormally low level which may imply enzyme repression or inhibition [34].

Effect of fenvalerate on kidney function (creatinine and uric acid): Many pesticides can cause some toxic and adverse effects on the kidney tissues. In the present study to monitoring the kidney function based on the level of uric acid and creatinine in fenvalerate treated and control rats (Table 3). Kidney is one of the targets organs of experimental animals attacked by OP compounds [35]. The uric acid and creatinine levels was found higher in fenvalerate administered rats as compared to control. In this study uric acid and creatinine increase may be related to either increase in protein degradation, which is involved in uric acid formation, or the toxic effect of fenvalerate on the kidneys [36]. The creatinine excretion is dependent almost on the process of glomerular filtration. Previous study reported that significant rise in the serum creatinine level may due to the impairment of the glomerular function and tubular damage in the kidneys [35]. Increased creatinine level shows that damage of the glomerular function and tubular damage in the kidneys [37, 38].

Effect of fenvalerate on steroid hormone (LH, FSH, Estradiol and Progesterone): The main hormone which gives rise to female characteristics is estrogen, and the hormone mainly responsible for predominantly masculine characteristics is androgen. In the present study, LH, FSH, estradiol and progesterone significantly decreased in fenvalerate treated groups as compared to that of control. The highest levels LH, FSH, estradiol and progesterone have been noted in the control group. All the values are significantly difference according to Duncan post-hoc analysis (Table 4). Numerous pesticides have been reported to affect hormone synthesis and/or metabolism. These include the imidazole pesticides (propi- propiconazole, epoziconazoleand ketoconazole), fenarimol [39], TBT [40], and several organo-chlorine pesticides [41]. The results of a study by Cooper *et al*. [42] indicate that lindane may effectively block the response of estrogen-dependent tissues and that this apparent anti-estrogenic effect is responsible for the disturbances observed in the neuro-endocrine control of ovarian function in rats [43]. Other studies also suggest that lindaneis anti-estrogenic a disable to disrupt the estrus cycle [44]. Atrazine, simazine, and diamino-chlorotriazine expressed anti-estrogenic activity in uteri of female rats without expressing intrinsic estrogenic activity, but the precise mechanism is not known [45].

Table 3: Changes in values of creatinine, uric acid, SGPT, SGOT and ALP in fenvalerate treated and control group

Parameters	Experimental Group					
	Group-I	**Group-II**	**Group-III**	**Group-IV**	**Group-V**	**Group-VI**
Kidney enzymes Creatinine (mg/dL)	0.91±0.11^{bc}	1.02±0.38^{b}	1.56±0.23^{a}	1.51±0.24^{a}	1.43±0.07^{ab}	1.47±0.044^{ab}
Uric acid (mg/dL)	3.90±0.20^{cd}	5.34±0.30^{c}	17.00±1.92^{a}	16.73±1.26^{ab}	16.92±1.08^{ab}	15.14±1.52^{b}
Liver enzymes SGPT (mg/dL)	9.70±0.47^{c}	17.85±1.77^{bc}	21.64±1.92^{a}	20.58±2.52^{ab}	21.37±2.05^{a}	19.71±1.97^{b}
SGOT (mg/dL)	15.62±1.27^{d}	24.80±1.55^{b}	28.67±2.28^{a}	28.32±1.10^{a}	27.05±1.28^{ab}	18.10±1.92^{c}
ALP (mg/dL)	43.00±3.17^{e}	51.72±6.17^{d}	89.75±5.52^{a}	86.80±1.47^{b}	87.24±2.04^{ab}	60.45±6.52^{c}

Values are mean ±SE of six rats in each group.
Means with same superscripts are not significant ($p<0.05$) to DMRT.

Table 4: Effect fenvalerate on plasma levels of FSH, LH, estradiol and progesterone in rats

Parameters	Experimental Group					
	Group-I	**Group-II**	**Group-III**	**Group-IV**	**Group-V**	**Group-VI**
LH (ng/ml)	4.53±0.2^{a}	3.85±0.07^{b}	2.54±0.12^{c}	2.36±0.28^{c}	2.65±0.18^{c}	2.93±0.11^{bc}
FSH (ng/ml)	2.15±0.13^{a}	1.64±0.14^{ab}	1.05±0.07^{b}	0.98±0.14^{bc}	1.12±0.06^{b}	1.32±0.32^{b}
Estradiol (pg/ml)	8.24±1.30^{d}	7.62±0.30^{c}	5.40±0.16^{b}	5.21±1.92^{ab}	5.92±0.72^{b}	5.84±0.60^{a}
Progesterone (ng/ml)	12.29±1.52^{d}	9.33±1.07^{c}	7.20±0.87^{a}	7.14±1.35^{a}	7.68±0.95^{b}	8.05±1.03^{ab}

Values are mean ±SE of six rats in each group.
Means with same superscripts are not significant ($p<0.05$) to DMRT.

Decline in LH and FSH levels in the present study confirm the findings of Desaulniers *et al.* [46] and Lafuente *et al.* [47] who investigated the toxicological influences of PCB (126 and 153) and methoxychlorat different concentrations on the male rats. On the other hand, the results of the present study disagree with the findings of Tag El-Din *et al*. [48] who mentioned that the FSH level increased significantly after treatment with dicofol at lower and higher doses. The present results suggest that the significantly decrease of progesterone and estradiol level may be as a result of direct damage of fenvalerate on granulosa cells of the ovarian follicles and corpora lutea, which are the main site of ovarian estrogen biosynthesis.

Effect of fenvalerate on protein profiles in the different endocrine organs: The SDS-PAGE analysis of liver, kidney, clitoral and ovary revealed that the significant changes in protein profiles in the control and fenvalerate administrated rats (**Fig. 2**). The protein bands of the liver, kidney, clitoral and ovary showed different protein band fractions ranging from 14 to 85 kDa and some bands were missed in the fenvalerate treated rats as compared to control rats. The result indicating the fenvalerate caused adverse effect on protein secretion. Similar elevation in protein content caused by other organochlorine has also been reported [49, 50]. Our results tend to agree with Rajendran *et al.* [51] who reported that the production of novel proteins or the increased production of already existing proteins, which are only produced under stress conditions due to exposure of pesticides.

Histopathological effect of fenvalerate on different endocrine organs: Reproductive abnormalities caused by organo-phosphorate (OP) have been

observed in many vertebrates [52]. Histopahological degeneration was observed liver, kidney, clitoral and ovaries in the fenvalerate control and treated groups (Figures 3-6).

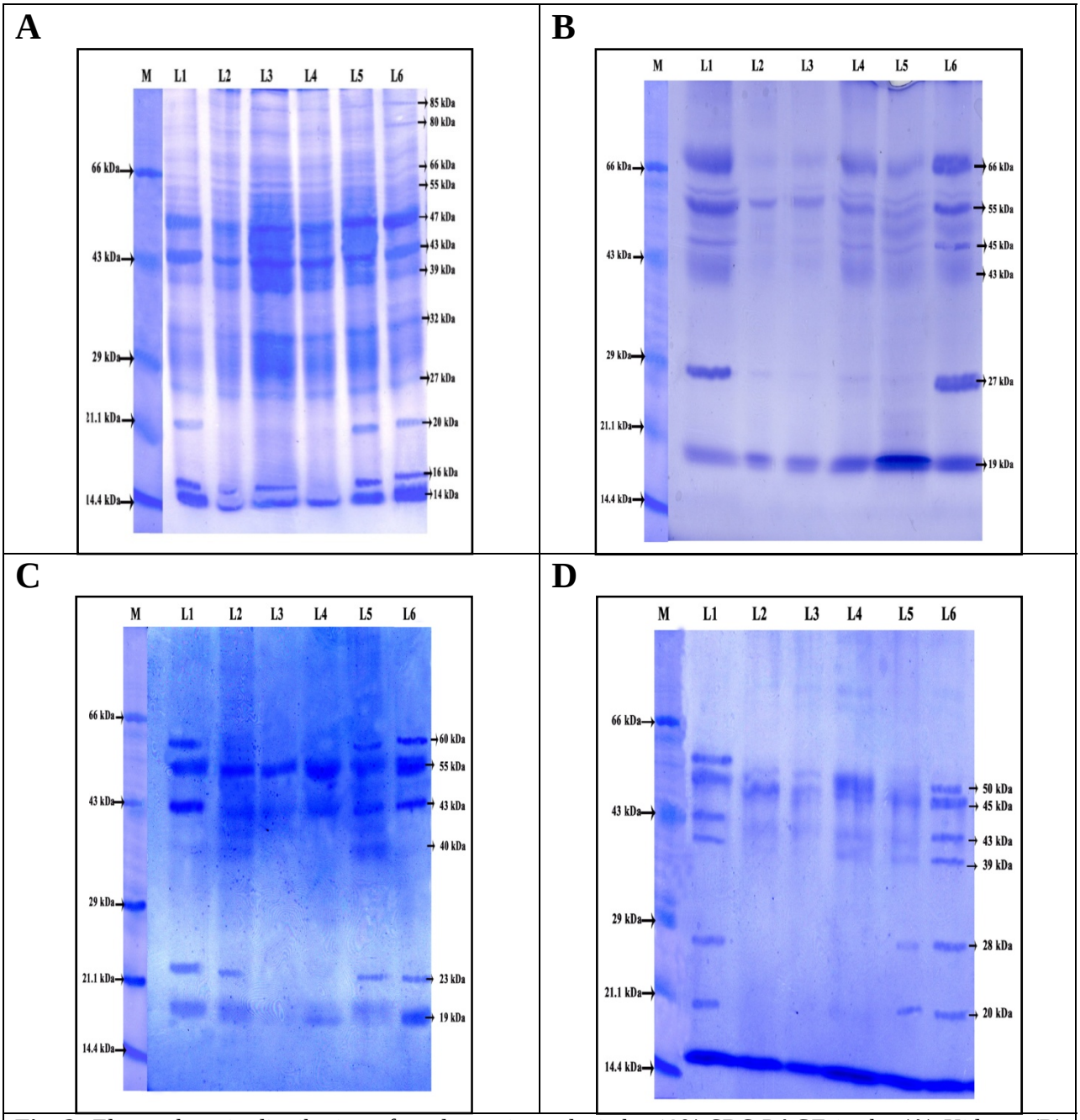


Fig. 2: Electrophoretic distribution of total protein analysis by 12% SDS-PAGE in the (A) Kidney, (B) Liver, (C) Ovaries and (D) Clitorial gland of fenvalerate treated and control groups.
(M) Marker, (L1) control (L2) short time fenvalerate treated group, (L3) long time fenvalerate group, (L4) the long time fenvalerate plus vitamin-C treated group, (L5) long time fenvalerate plus male urine (*i.e.* pheromone) treated group and (L6) long time fenvalerate plus vitamin-C and male urine treated group

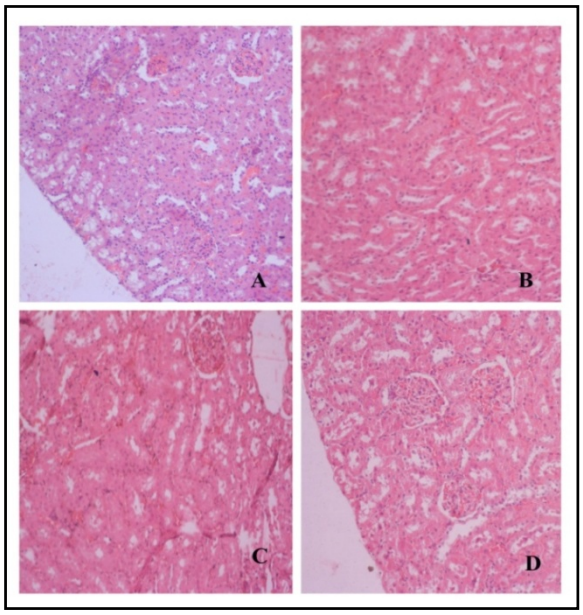


Fig. 3: Photomicrographs of kidney of control and fenvalerate treated rat. Photomicrographs control kidney showing normal architecture of tubular epithelium (A), mild swelling of tubular epithelium in the group II (B), severely clouding swelling of tubular epithelium group III (C) and groups IV, V, and VI (D).

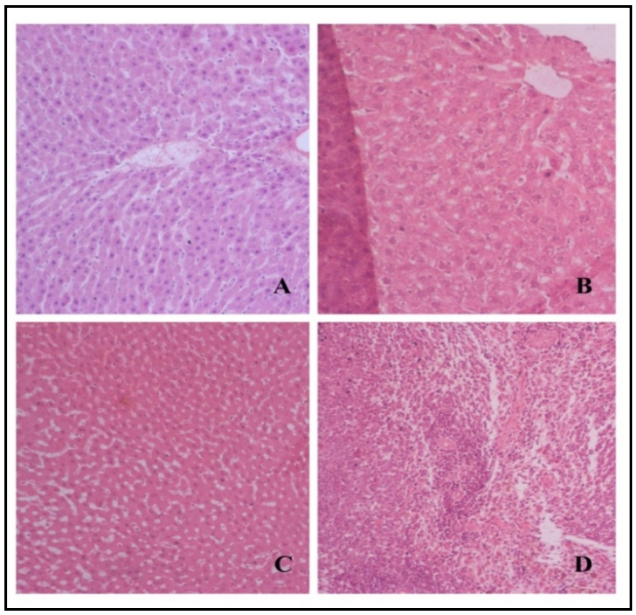


Fig. 4: Photomicrographs of liver from rats in control and fenvalerate treated rat. Photomicrographs of liver showing normal architecture of tubular epithelium cells in the control (A) and experimental group I (B), severely damaged the tubular epithelium cells in the group III (C) and groups IV, V and VI (D).

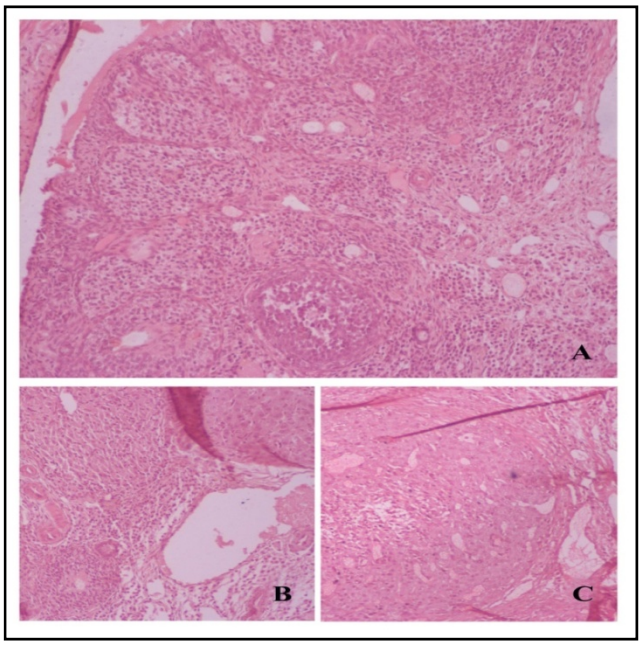


Fig. 5: Photomicrographs of ovaries from rats in control and fenvalerate treated groups. Photomicrographs of ovaries showing normal architecture of Graafian follicles and corpus luteum (A), Sloughing and disorganization of Graafian follicles and corpus luteum in the short time fenvalerate treated group (B) and long time fenvalerate treated group (C).

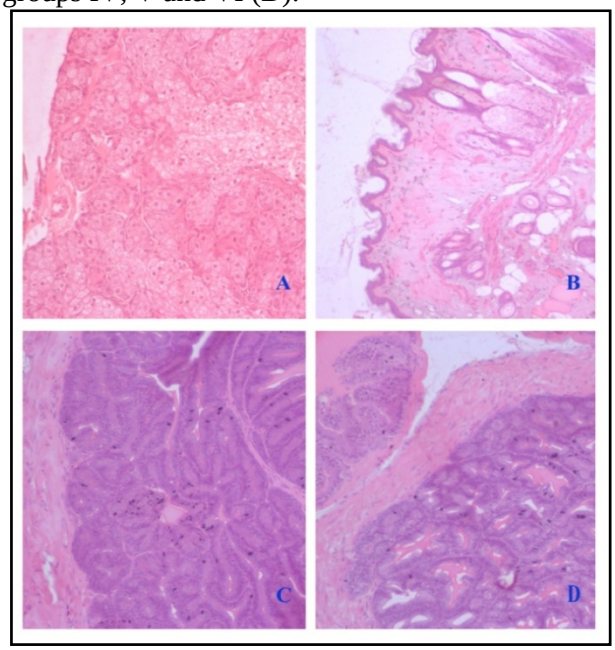


Fig. 6: Photomicrographs of clitorial gland from rats in control and fenvalerate treated groups. Photomicrographs control clitorial gland showing normal sebaceous secretary cells (A), slightly damaged the sebaceous secretary cells in the groups II (B), severely damaged the sebaceous secretary cells in the group III (C) and groups IV, V and VI (D).

In present study, the long time fenvalerate treated group liver tissues showed much severe histopathological alteration. Several cells also show histological features of necrosis. Similar histopathological changes such as mononuclear cell infiltration and parenchymatous degenerations of hepatocyte in liver were observed in rats exposed 250 mg/ kg orally alpha-cypermethrin [53].

Present investigation much histological variation observed in the fenvalerate treated groups as compared to control group. However, widened capsular space, degeneration and necrosis of renal tubular epithelia were noticed in fenvalerate exposed groups. These damaging changes reflected action of toxic metabolites of pesticides under study. It is remarkable change in the ovaries was widespread ovarian follicle atresia accompanied by a decreased number of normal antral and growing follicles were recorded in the long time fenvalerate treated groups (III, IV, V and VI) as compare to that of control. The severe damage to oocytes would lead to follicular atresia and cessation of development of antral follicles. Kamijima *et al*. [54] evaluated the 2-bromopropane exposure showed distorted shape of ovaries and uterus in female rats.

4. CONCLUSION

The present study concluded that the fenvalerate is a highly toxic chemical when applied through the oral route based on toxicological effects on the body and organ weight, haematological and biochemical indices and histopathological studies. If this toxin affects laboratory animals, may be having same effects on other mammals too. The toxic effect of fenvalerate on female rat endocrine organs is dependent on number of day exposure. However, further study is required for determining the mode of action of the insecticide fenvalerate on female endocrine system.

ACKNOWLEDGEMENT

The authors would like to acknowledge The Management and The Principal, Ayya Nadar Janaki Ammal College, (Autonomous) Sivakasi for providing the facilities to carry out this work successfully.

REFERENCES

1. R.A. Hess. *J. Reprod. Fertil. (Suppl).* 1998, 53**,** 247.
2. R. Xavier, K. Rekha and K.L. Bairy. *Mal. J. Nutr*. 2004, 10**,** 39.
3. J.R. Nuckols, R.B. Gunier and P. Riggs. *Environ. Health Perspect.* 2007, 115**,** 684.
4. R.M. Hajjo, F.U. Afifi and A.H. Bathah. *Food Addit. Contam.* 2007, 24**,** 274.
5. A.A. El-Kashoury, O.M. Mohamed and N.A. Said. *Egypt. J. Appl. Sci.* 2005, 20, 32.
6. A.M. Vinggaard, C. Hnida and V. Breinholt. *Toxicol. In-vitro* 2000, 14**,** 227.
7. A.M. El-Sayed and R.M. Trimble. *Can. Entomol.* 2002, 134**,** 331.
8. T. Rajagopal.. A study on the reproductive behaviour and pheromones of an endangered Indian Blackbuck (*Antelope cervicapra* L.) to enhance captive breeding and conservation. Ph.D. Thesis. Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. 2009.
9. C. Campanhola, B.F. McCutchen, E.H. Baehrecke and F.W. Plapp. *J. Econ. Entomol.* 1991, 84, 1404.
10. J. Delisle and C. Vincent. *Chem. Ecol*. 2002, 12**,** 47.
11. S. Giri, G.D. Sharma, A. Giri and S.B. Prasad. *Mutat. Res.* 2002, 520, 125.
12. N. Golamreza, D.V.R. Mazdakrazi, A. Hoshyar, S. Shahmomadloo and S. Feyzi. *Int. J. Fertil. Steril*. 2010, 4, 9.
13. P.C. Wsyolek, N.A. LaFaunce, T. Wachs and D.J. Link. *Bull. Environ. Contam. Toxicol.* 1981, 26, 262.
14. R. Frank, H.E. Braun, L.A. Miller and G.W. Allan. *Pestic. Sci.* 1984, 15, 600.
15. J.A. Cuthbert and S.M.A. Carr. *Inveresk research international report*, 1986, 3473.

16. M.K. Ausmanas, D.A. Tan, U. Jaisamrarn, X.W. Tian and C.F. Holinka. Climacteric, 2007, 10, 427.
17. A. Vessieres, M. Salmain, P. Brossier and G. Jaouen. J. Pharm. Biomed. Anal. 1999, 21, 625.
18. U.K. Laemmli. *Nature* 1970, 227, 680.
19. W.R. Klemm. *Chem. Senses*. 1977, 1, 77.
20. H.N. Nigg and J.B. Knaak. *Rev. Environ. Contam. Toxico*. 2000, 163, 29.
21. R.A. Handy, B.J. Moore and R.E. Linder. *Vet. Hum. Toxicol*. 2002, 34, 408.
22. K. Narayana, P. Narayan and U.J.D. Souza. *Indian J. Med. Sci*. 2004, 58**,** 528.
23. S.K. Maitra and R. Sarkar. *Arch. Environ. Contam. Toxicol*. 1996, 30, 384.
24. M.A. Brown and J.E. Casida. *Xenobiotica*, 1987, 17, 1169.
25. U.C. Jadaramkunti and B.B. Kaliwal. *Bull. Environ. Contam. Toxicol*. 2002, 69, 741.
26. L. Institoris, U. Undeger, O.Siroki, M. Nehez and I. Desi. *Toxicology,* 1999, 137, 47.
27. M.H. Aziz, A.K. Agrawal, V.M. Adhami, Y. Shukla and P.K. Seth. *Neurosci. Lett*. 2001, 300, 161.
28. G. Singh, L.D. Sharma, A.H. Ahmad and S.P. Singh. *J. Appl. Anim. Res.* 1999, 16, 205.
29. H. Kaur, K. Srivastava, S.K. Garg and D. Prakash. *Indian J. Toxicol.* 2000, 7**,** 212.
30. S.P. Singh, L.D. Sharma, S.P. Singh and A.H. Ahmad. *Indian J. Agronomy*. 2003, 8, 141.
31. U. Jayasree, A.G. Reddy, K.S. Reddy and B. Kalakumar. *Indian J.Toxicol.* 2003, 10**,** 111.
32. M.I. Yousef, F.M. El-demerdash, K.I. Kamel and K.S. Al-sahen. *Toxicology* 2003,189, 223.
33. S. Jabbar, A. Khawaja, A. Iqbal and S.A. Malik. *J. Pak. Med. Asso.* 1990, 40, 266.
34. O.Y. Adeniran, M.A. Fafunso, O.A. Adeyemi, A.O. Lawal, A. Ologundudu and A.A. Omonchua. *J. Appl. Sci*. 2006, **6,** 668.
35. S.A. Mansour and A.H. Mossa. *Pest. Biochem. Physiol*. 2010, 96, 14.
36. G. Eraslan, M. Kanburn and S. Silici. *Pestic. Biochem. Phys.* 2007, 88**,** 273.
37. M. Mohnssen. *Environ Res*. 2001, 87, 31.
38. L.O. Mora, L.M.G. Antunes, H.D.C. Francescato and M.L.P. Bianchi. *Pharmacol. Res.* 2003, 47**,** 517.
39. E.L. Gray and J. Ostby. *Toxicol. Indust. Health*. 1998, 14, 1.
40. J. Oehlmann, U. Schulte-Oehlmann, E. Stroben, B. Bauer, C. Bettin, P. Fiorni and B. Markert. *Experimentia* 1995, 40**,** 217.
41. J. Haake, M. Kelley, B. Keys and S. Safe. *Gen. Pharmac*. 1987, 18, 165.
42. G.S. Cooper, M.A. Klebanoff, J. Promislow, J.W. Brock and M.P. Longnecker. *Epidemiology,* 2005, 16**,** 191.
43. R.L. Cooper, R.W. Chadwick, G.L. Rehnberg, J.M. Goldman and K.C.Booth. *Toxicol Appl Pharmacol.* 1989, 99**,** 384.
44. N. Pages, M.P. Sauviat, S. Bouvet and F. Goudey. *J. Soc. Biol*. 2002, 196**,** 325.
45. M.K. Tennant, D.S. Hill, J.C. Eldridge, L.T. Wetzel, C.B. Breckenridge and J.T. Stevens. *J. Toxicol. Environ. Health*. 1994, 43**,** 183.
46. D. Desaulniers, K. Leingartner, M. Wade, K. Fintelman, E. Yagminas and W.G. Foster. *Toxicol. Sci.* 1999, 47**,** 158.
47. A. Lafuente, N. Marquez, Y. Pousada, Y. Pazo and A.L. Esquifino. *Arch. Toxicol.* 2000, 74**,** 270.
48. H.A. Tag El-Din, H.E. Abbas and A.A. El-kashoury. *Bull. Fac. Pharm.* 2003, 41**,** 179.
49. T. Shivanandappa and M.K Krishnakumari. *Indian. J. Exp.* 1981, 19**,** 1163.
50. V.K. Bhatnagar and A.N. Malviya. *Indian. J. Biochem. Biophys.* 1986, 15**,** 78.
51. U.M. Rajendran, K. Elango and N. Anand. *Pest. Biochem. Physiol.* 2007, 87**,** 164.
52. H.M. Dutta and D. Meijer. *Environ. Res*. 2003, 91**,** 157.
53. M. Luty, J. Belden, C. Wheelock, B. Hammock and D. Denton. *Ecol. Soc*. 2000, 9, 1.
54. M. Kamijima, G. Ichihara and X.Z. Yu. *J. Occup Health*, 1997, 39**,** 3.