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Effect of Fytolan on Cauda Epididymis and Accessary Sex Organs of Male Rats.

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ABSTRACT

The purpose of the study was to investigate the possible effects of fytolan on cauda epididymis and accessory sex organs of male rat. The tested dose was given orally to the wister rats for 30 days at the dose level 5 mg, 7.5 mg and 10 mg/kg.b.wt./day. Marked reduction in the sperm motility was observed. A significant decrease in sperm density was also observed. Biochemical assay shows significant reduction in protein and sialic acid content of epididymes and accessory sex organs. This was further confirmed by by histopathological studies. Thus, from above results it can be inferenced that fytolan act as reproductive toxicant.
Keywords: Fytolan, cauda epididymis, sperm motility, sperm density, rat.

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INTRODUCTION

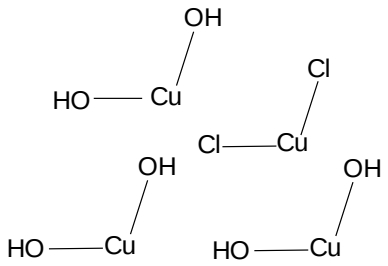
Fytolan is a broad spectrum copper based fungicide. It controls a wide range of fungal and bacterial disease on fruits, vegetables and ornamental plants so being used widely to protect crops from the diseases. Copper released from fytolan are found to be toxic for crop [1]. It affects protein and enzyme like lipase, intracellular diastase and some glucose metabolizing enzyme by denaturing them and thus inhibit the germination. Indiscriminate and non-judicious use, further lack of safe handling, improper spraying equipment, illiteracy and insufficient scientific knowledge are some of the root cause of fungicide residues in crop field, which ultimately leads to the environment pollution.

So far little information is available on toxic effect of fytolan on biochemical constituents, hormonal profile and histopathology of reproductive organs. Hence, this study was designed to bring into light the possible toxic effects of fytolan on cauda epididymis and accessory sex organs of male rats at different doses and duration.

MATERIALS AND METHODS

Chemical

Fytolan (Chemical name- copper oxychloride; Trade name- Blitox, Blue copper, Agrizan; Molecular formula- $CuCl_2.3\ Cu(OH)_2$). Technical grade fytolan (98% pure) obtained from Kisaan chemicals pvt. Ltd., Jaipur was used as test fungicide for experimentation.



Fytolan

Test animal

Twenty four healthy adult male rats of wistar strain weighing 150-200 gms were used for experimentation. They were housed in polypropylene cages at room temperature with natural light and dark cycles (12 h dark, 12 h light) and relative humidity 55±5 %. They were fed on standard commercial pallet feed procured from Ashirvad food industries ltd.,Chandigarh, India and water *ad libitum*.

Testing dose and experimental design

Proven healthy male rats were divided into four groups of six animals each. The control group I served as control and received only the vehicle (olive oil) whereas the animals of group II, III and Iv received fytolan dissolved in olive oil adminstrated orally at the dose level of 5, 7.5 and 10 mg/kg b.wt./day respectively for 30 days. After 30 days, the animals were weighed and autopsied using light ether anaesthesia.

Parameters studied

Reproductive organs were excised blotted free of blood and weighed and were used to perform by following parameter.

Sperm density

The sperm density was calculated in million per ml as per dilution by the method of Prasad *et al*., (1972) [2]. Total number of sperms were counted using haemocytometer after further diluting the sperm suspension from testis.

Sperm motility

Sperm motility was assayed by the method of Prasad *et al*., 1972 [2]. The epididymis removed immediately after anaesthesia and known weight of cauda epididymis was gently teased in a specific volume of physiological saline (0.9 % NaCl) to release the spermatozoa from the tubules. The sperm suspension was examined within five minutes after their isolation from epididymis. The results were determined by counting both motile and non-motile sperms in at least ten separate and randomly selected fields. The results were finally expressed as percent motility.

Tissue biochemistry

Tissue was analyzed to carry out protein [3] and sialic acid [4] contents in the testis.

Histopathological studies

The main reproductive organs testis was fixed in Bouin's fixative and cut into pieces and processed through ethanol-xylene series. The tissues were than embedded in paraffin and bee wax (3:1 ratio; M.P. 55-62^{0}C). Sections were cut at 5 µm thickness and stained with Harris haematoxylin and eosin (H&E).

Statistical analysis

The data obtained from the above experiments were subjected to statistical analysis. Student's t-test was performed for test of significance.

RESULTS AND DISCUSSION

In the present study fytolan was administered to rats at dose level of 5, 7.5 and 10 mg/kg. b. wt./day for 30 days, which brought mark alterations in cauda epididymal weight, function and histology. Further significant reduction in the weight of accessory sex organs was recorded, indicates that the level of androgen was not enough to maintain the weight of accessory sex organs [5, 6, 7, 8] as shown in table no. 1. Prostate gland plays an important role in male reproduction by functioning of sperms [9]. A marked reduction in the weight of prostate gland was recorded in fytolan treated rat as because development and functions of fytolan is depend upon testicular androgen [10]. Lack of testicular androgen might be the cause of prostate gland loss [11, 12].

In the present study, inadequate sperm count and motility were observed and resulted in complete sterility in exposed rats as shown in figure 2 & 3. Fytolan treatment also induced a significant decline in sperm reserves in the cauda epididymis, which indicates antispermatogenic effects of test compound. Many reports showed that most of fungicides are inhibitors of spermatogenesis at gonad level by affecting hormone production [13, 14, 15].

Marked inhibition of sperm motility after fytolan exposure may be due to low levels of ATP content [16] or may be androgen deprivation effect of fytolan [17]. The epididymal spermatozoa are highly dependent on testosterone and epididymal protein for their final maturation, further progressive motility and fertilizing capacity of the spermatozoa has been reported [18, 19]. Low fructose concentrations in seminal vesicle may be another cause of low sperm motility. Similar effects on sperm motility were also reported with other fungicides and this negative impact affects fertilizing ability of the sperm [20].

In the present study fytolan caused a significant reduction in sperm density in cauda epididymes. Testosterone and FSH are the main hormones required for maintaining normal spermatogenesis [21, 22]. Similar results of decreased sperm density by other pesticides intoxication were reported by other researchers [23, 24]. Reduction in sperm density in cauda epididymides is related to androgen metabolism alteration [25, 26, 27, 14].

Fytolan at various dose levels and durations produced many degenerative changes in the accessory sex organs as shown in figure no. 1, whereas control of cauda epididymis showing large and compact tubules lined with pseudostratified epithelial cells, lumen filled with mature spermatozoa. In fytolan treated rats

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lumen was filled with cellular debris and less amount of spermatozoa (Figure 1.1). Seminal vesicle of treatment groups showed degeneration in secretary epithelial cells, muscle layer also shows rupture at various points as compare to control (Figure 1.2). Ventral prostate showed disrupted epithelium and lumen with less secretion (Figure 1.3). Treatment groups showed increased lumen size with lack of epithelial folds and lumen with celluar debris in vas deference (Figure 1.4). Degeneration in the male reproductive system, referring to reduced organ weight and inhibition of spermatogenesis were also noted in earlier studies [28, 29, 30].

Therefore, it can be concluded that fytalon produces significant toxic changes in cauda epididymis and accessory organs.

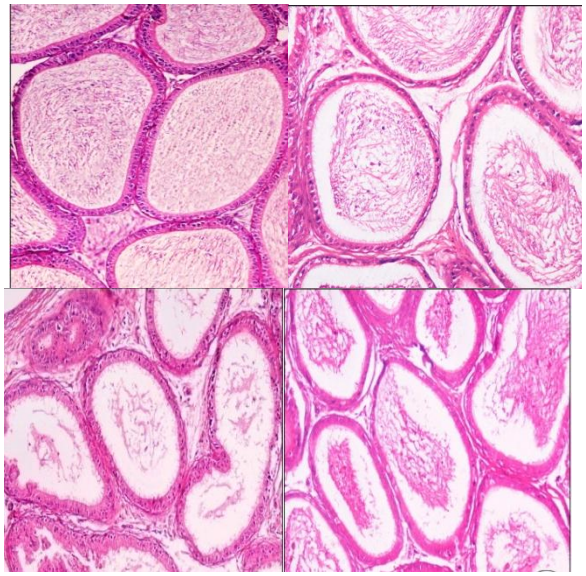

Fig. 1.1: Cauda epididymis

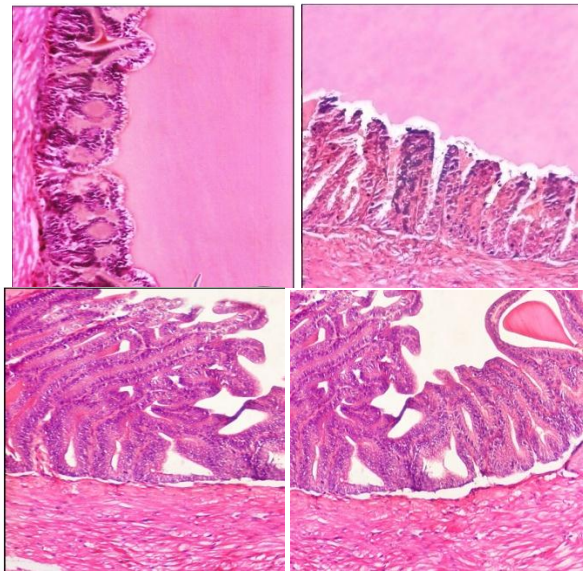

Fig.1.2: Seminal vesicle

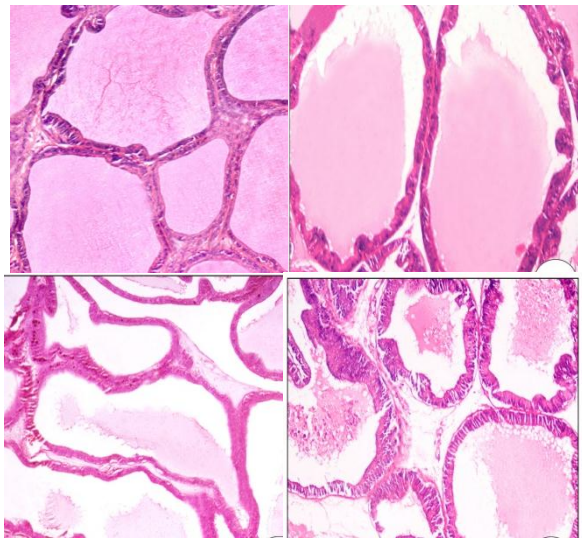

Fig. 1.3: Ventral prostate

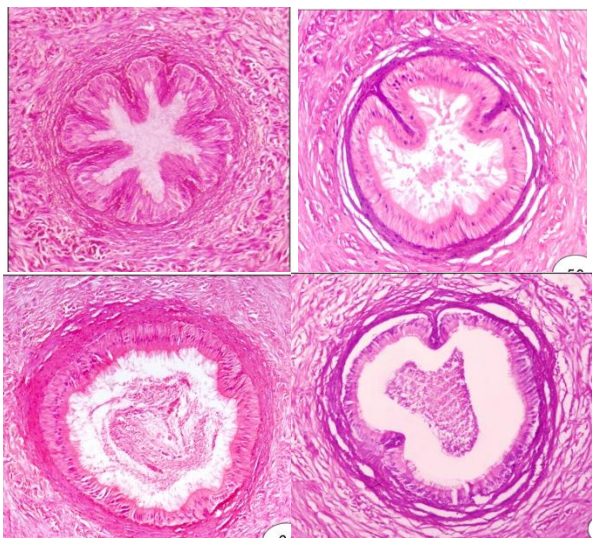

Fig. 1.4: Vas deferens

Figure 1: Microphotograph of control cauda epididymis, seminal vesicle, ventral prostate and vas deferens were compared with treatment groups shows many degenerative changes

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Fig. 2: Sperm Density (Cauda Epididymis)

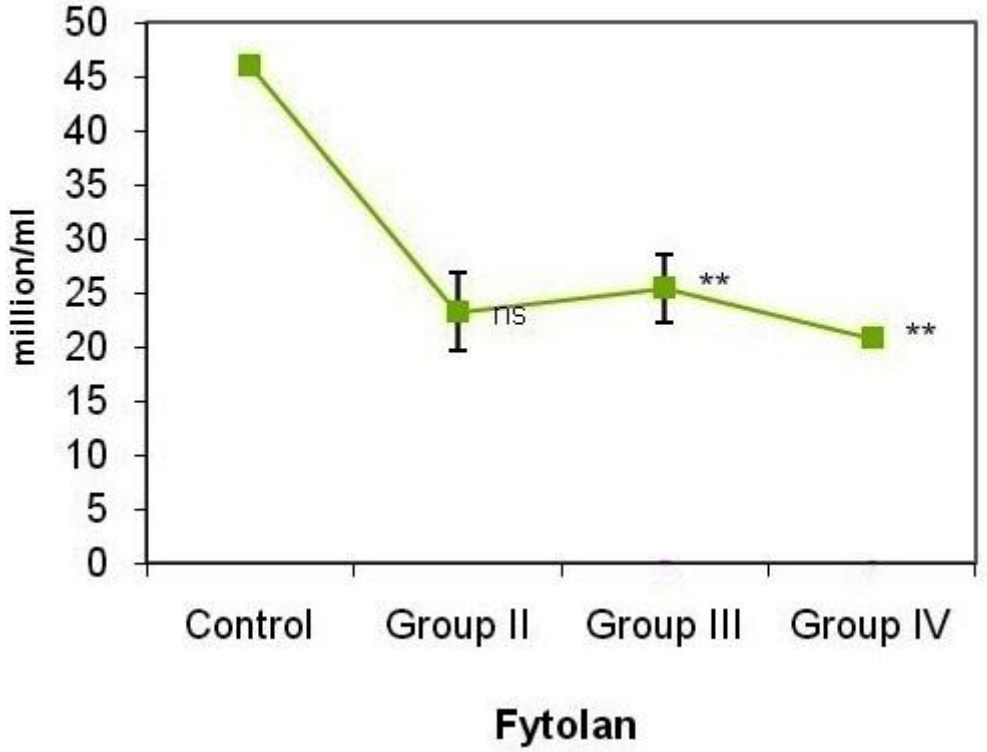

Fig. 3: Sperm Motility (Cauda Epididymis)

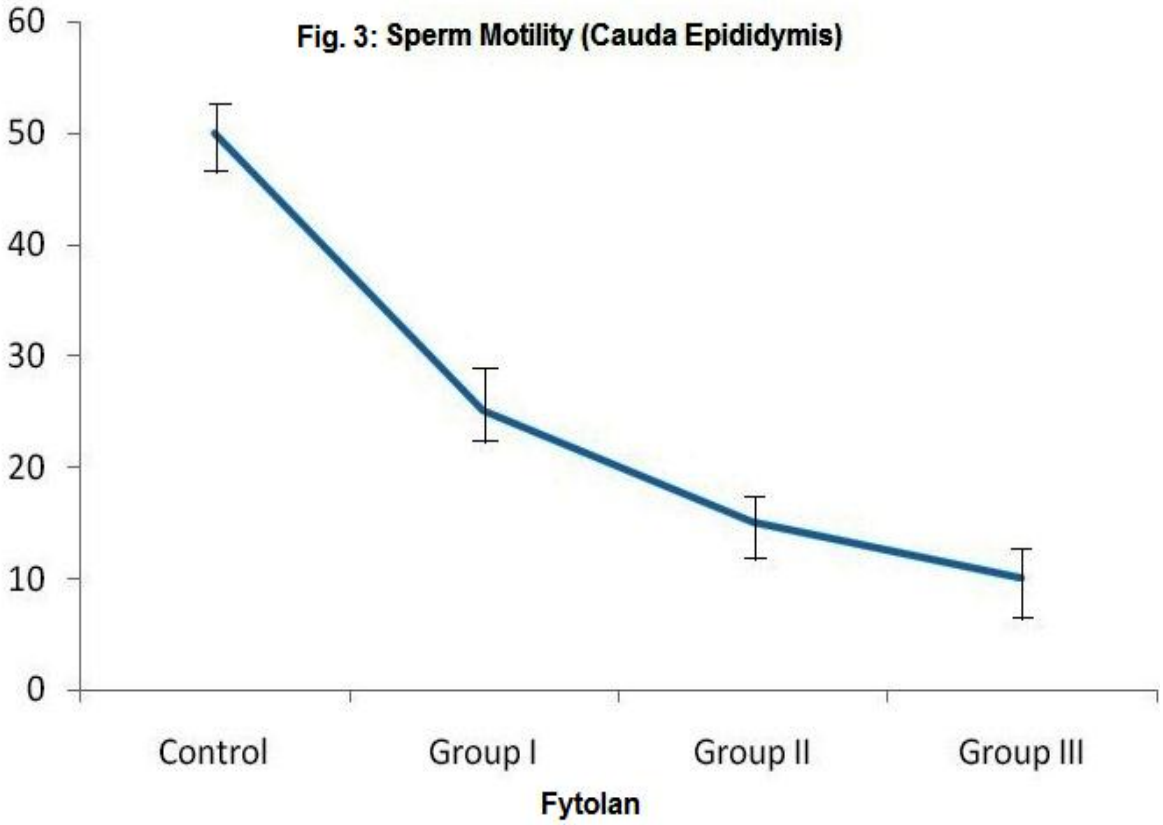

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Table 1: Body and organs weight in control and treatment groups

Treatment	Body weight		Epididymides	Vas Deferens	Seminal Vesicle	Ventral Prostate
	Initial	Final				
	gm		mg/100g body wt.			
Group I Control (vehicle)	177.65 ± 4.8	196.50 ± 9.54	516.20 ± 8.51	126.54 ± 4.21	412.66 ± 24.21	340.91 ± 26.01
Group II 5 mg for 30 days	167.45 ^{ns} ± 2.95	155.41 ^{ns} ± 7.21	320.24* ±14.34	100.67 * ±4.32	251.96 * ±23.25	230.93* ± 19.24
Group III 7.5 mg for 30 days	180.10 ^{ns} ± 2.79	165.94 ^{ns} ±5.41	260.01** ±9.42	81.53 * ±6.11	185.00* ±10.10	205.72* ± 15.50
Group IV 10 mg for 30 days	181 18^{ns} ±6.67	165.68 ^{ns} ± 10	180.43** ±20.01	73.41.±1.01	122.31* ±28.27	115.29** ± 17.38

** = highly significant (P≤0.001)
(Mean ± SEM of 6 Animals) Group II, III and IV Compared with group I
ns = non-significant
* = significant (P≤0.01)

Table 2: Tissue Biochemistry (Mean ± SEM of 6 Animals) Group II, III and IV Compared with group I

Treatment	Protein (mg/g)				Sialic acid (mg/g)			
	Cauda Epididymides	Seminal Vesicle	Ventral Prostate	Vas Deferens	Cauda Epididymides	Seminal Vesicle	Ventral Prostate	Vas Deferens
Group I Control	225.12 ±4.41	230.42 ±2.98	242.90 ± 3.32	267.56 ± 6.61	4.65 ± 0.06	5.10 ± 0.05	5.00 ± 0.21	5.75 ±0.12
Group II 5 mg	258.17* ± 6.65	240.14* ±0.34	255.17^{ns} ±4.86	269.06^{ns} ±8.37	4.24* ± 0.07	4.05* ± 0.24	4.11* ± 0.10	5.14* ±0.10
Group III 7.5 mg	245.44* ± 3.66	261.49* ±7.63	271.07* ±6.34	295.16* ±3.63	3.38* ± 0.36	3.50** ± 0.33	3.47* ± 0.29	4.12* ±0.47
Group IV 10 mg	256.38* ± 5.37	275.34* ±9.32	265.87** ±2.28	298.48* ±2.57	3.75** ± 0.12	4.47** ± 0.04	4.00** ± 0.01	4.51* ±0.11

ns = non-significant
* = significant (P≤0.01)
** = highly significant (P≤0.001)

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