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Impact assessment of benomyl on cauda epididymis and vas deferens of male wistar rats

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Abstract : The aim of the study was to examine any potential effects of benomyl on cauda epididymis and vas deferens of male wistar rats. The wistar rats received the test dose orally for 30 days at a dose level of 2.5 mg, 5 mg. 7.5 and 10 mg/kg body weight each day. There was noticeably reduced sperm motility. Additionally, a substantial drop in sperm density was noted. Epididymis and auxiliary sex organs' protein and sialic acid contents have significantly decreased, according to a biochemical analysis. Histopathological research served as additional confirmation of this. Therefore, it may be concluded from the facts mentioned above that benomyl acts as a reproductive toxin.

Introduction:

Benomyl is an effective broad spectrum, systemic, benzimidazole fungicide widely used throughout the world against a wide range of fungal diseases of field crops, fruits nuts, ornamentals, mushrooms, nuts and turf (WHO, 1996). This locally prevalent systemic fungicide is frequently used to safeguard crops against infections. Research on benomyl histopathological effects has been published by Linder et al. in 1988, Hess et al. in 1991, and Lim and Miller in 1997.

Fungicide residues in crop fields have a number of fundamental causes, including indiscriminate and careless use, lack of safe management, inadequate spraying equipment, illiteracy, and a lack of scientific knowledge. These factors ultimately produce environmental pollution. The harmful effects of benomyl on biochemical components, hormonal profiles, and the histopathology of reproductive organs are currently poorly understood. As a result, this study was created to shed light on any potential harmful effects of benomyl at various doses and exposure times on the cauda epididymis and accessory sex organs of male rats.

Material and methods:

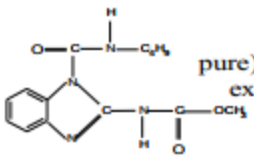
Chemical:

Benomyl (Chemical name-Methyle 1-(butyl carbamoyl)-2- benzimidazole carbamate

Trade name- Agrodit, Benex, Benlate, Benosan, Fundazole fungicide and Tersan

Molecular formula- $C_{14}H_{18}N_4O_3$

Technical grade benomyl (95% pure) procured from Agro Chemicals Pvt. Ltd., Jaipur was used for experiments as test fungicide.



Test animal:

24 Wistar strain adult male rats weighing between 150 and 200 g were used for the experiment. They were kept in polypropylene cages at room temperature with 12-hour cycles of natural light and dark and a relative humidity of 55±5%. They were given water at will along with typical commercial pallet feed that was purchased from Ashirvad Food Industries Ltd. in Chandigarh, India.

Testing dose and experiment design:

Four groups of six each were created from male rats that had been determined to be healthy. The animals in groups, and received Benomyl dissolved in olive oil administered orally at the dose level of 2.5, 5, 7.5, and 10 mg/kg b.wt./day, respectively, for 30 days. The control group I acted as the control and merely got the vehicle (olive oil). The animals were weighed and autopsied with light ether anaesthesia after 30 days.

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ISSN NO.-2347-2944
Vol.-7, No.-I, Issues-VIII YEAR- June-2017

Parameter 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). 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. 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 and sialic acid contents in the cauda epididymis and vas deferens.

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 the embedded in paraffin and bee wax (3:1 ratio; M.P. 55- 620 C). Sections were cut at 5 □m 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.

Result and discussion:

Significant reduction in the body weight of benomyl exposed rats was noticed at higher doses. Benomyl exposed sat revealed significant decrease in cauda epididymal weight at all dose levels except in group II, where non- significant change was observed. Exposure of rats to benomyl shows reduced significant decrease in the motility of sperm in cauda epididymis 35.37%, 35.49%, 25.84%, 15.49%.

The density of sperm in cauda epididymis was reduced significantly ($p < 0.01$ and $p < 0.001$) in benomyl treated rats at all the dose levels. The decline was 25.06%, 39.57%, 48.16% and 47.89% as compared to control group. Mating exposure test revealed 100% positive fertility in control rats whereas the rats exposed to different dose level showed 35%, 45%, 75% and 85% negative fertility respectively.

Protein content of cauda epididymis after oral administration of benomyl elevated significantly in rats when compared with control group. A significant decrease was observed in the sialic acid content of cauda epididymis of animals treated with various doses of benomyl. Sialic acid content of vas deferens after oral administration of benomyl in rats showed reduction in dose dependent manner.

Table 1: Body and organ weight in control and treated groups

Treatment	Body weight		Cauda Epididymis	Vas deferens
	Initial	Final		
	g		mg/ 100 g b wt.	
Group I (Control Vehicle)	168.93 ±7.17	183.10 ±6.96	515.72 ±7.38	145.32 ±6.67
Group II 2.5 mg for 30 Days	180.37 ± 5.96	195.92^{ns} ±7.23	435.55* ±18.32	115.35^{ns} ±5.28
Group III 5 mg for 30 Days	180.42 ±4.84	165.64^{ns} ±4.42	450.24* ±14.02	115.48^{ns} ±5.37
Group IV 7.5 mg for 30 Days	181.46 ±2.96	155.75* ±5.46	410.43** ±10.92	118.56^{ns} ±8.21
Group V 10 mg for 30 Days	175.58 ±3.31	220.44* ±10.12	385.16** ±22.91	130.41* ±8.92

Mean ± of 6 animals
ns = $P \leq 0.05$ (Non significant)
* = $P \leq 0.01$ (Significant)
** = $P \leq 0.001$ (Highly significant)

Table 2: Tissue Biochemistry (Group II, III, IV and V compared with Group I)

Treatment	Protein (mg/g)		Sialic acid (mg/g)	
	Cauda epididymis	Vas deferens	Epididymis	Vas deferens
Group I Control (Vehicle only)	215.38 ±7.34	265.13 ±6.24	5.08 ±0.18	5.58 ±0.13
Group II 2.5 mg for 30 Days	245.62* ±4.97	275.91^{ns} ±7.32	3.26* ±0.13	3.21* ±0.16
Group III 5 mg for 30 Days	250.36* ±7.72	280.92^{ns} ±6.86	3.27* ±0.28	3.42* ±0.19
Group IV 7.5 mg for 30 Days	255.63** ±4.12	294.89* ±2.81	3.80** ±0.29	3.78** ±0.47
Group V 10 mg for 30 Days	262.69** ±7.67	295.08* ±0.28	3.81** ±0.19	3.80** ±0.41

Mean ± of 6 animals
ns = $P \leq 0.05$ (Non significant)
* = $P \leq 0.01$ (Significant)
** = $P \leq 0.001$ (Highly significant)

Histological changes:

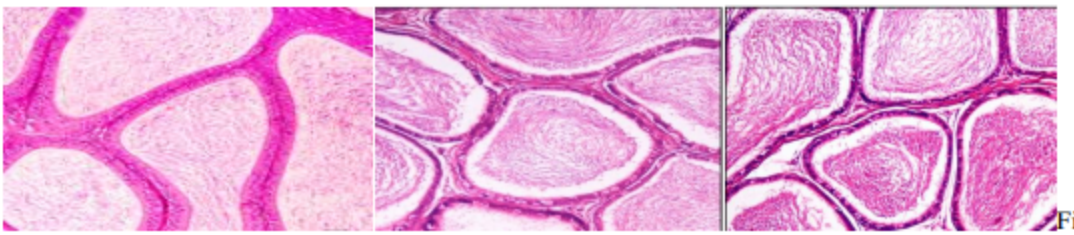
Many degenerative changes are seen in benomyl treated rats in comparison to control rats in dose dependent manner. Cauda epididymis of control rat showing large and compact tubules lined with pseudo stratified epithelial cells and stereo cilia are present. Inter tubular stroma contains connective tissues and blood vessels. The lumen is filled with mature spermatozoa (Fig: i).

Rats with 2.5 mg /kg. b.wt./day dose show shrunken tubule fused at certain points. Pseudo stratified cells are loosened. Stereo cilia are less than normal (Fig: ii).

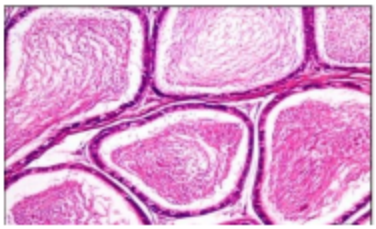
Rats treated with 5 mg/kg. b.wt./day dose show Degenerated cauda epididymis showing lumen filled with cellular debris and lesser spermatozoa (Fig: iii).

Rats with higher doses show cauda epididymis showing distorted tubules with cellular debris in the lumen (Fig: iv & v).

ISSN NO.-2347-2944
Vol.-7, No.-I, Issues-VIII YEAR- June-2017



(i):Cauda epididymis control Fig (ii): 2.5 mg for 30 days Fig (iii): 5 mg for 30 days



(iv): 7.5 mg for 30 days

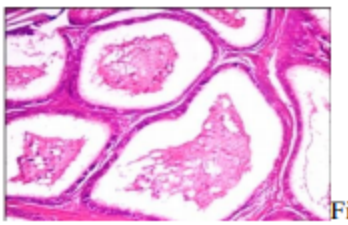


Fig (v): 10 mg for 30 days

Vas deferens:

Vas deferens of control rat showing thick muscular layer of outer longitudinal and inner circular muscle fibre. Central lumen is lined with columnar epithelium containing long stereo cilia (Fig: a).

Degenerative changes are seen in treated rats. Vas deferens treated with 2.5 mg/kg. b.wt./day dose exhibiting degenerated epithelium with reduced size longitudinal muscle layer is damaged to some extent (Fig: b).

Vas deference with 5 mg/kg. b.wt./day dose showing circular muscle layer detached at several points. Epithelial folds are also reduced (Fig: c).

Animals treated with higher **doses** showing diminished number of stereo cilia. Lumen contains cellular debris, damaged and degenerated vas deferens is visible (Fig: d & e).

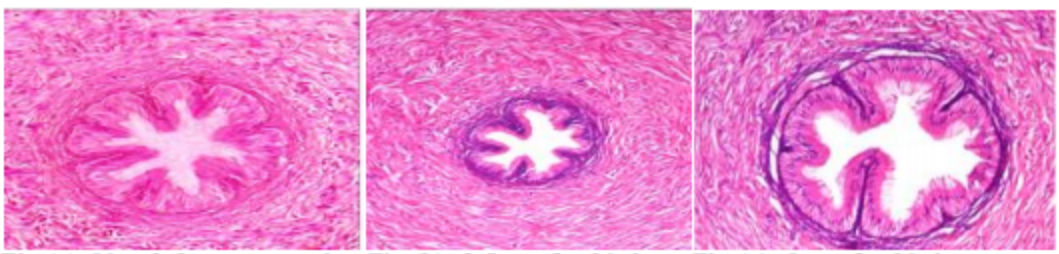


Fig (a): Vas deferens control Fig (b): 2.5 mg for 30 days Fig (c): 5 mg for 30 days

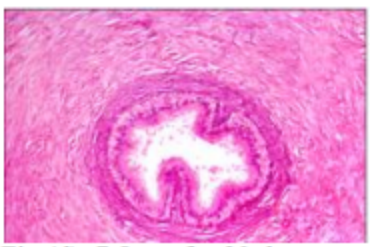


Fig (d): 7.5 mg for 30 days

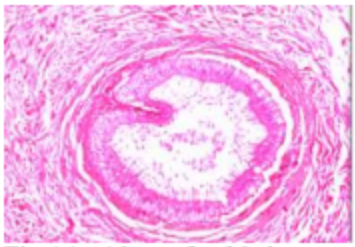


Fig (e) : 10 mg for 30 days

ISSN NO.-2347-2944
Vol.-7, No.-I, Issues-VIII YEAR- June-2017

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