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# Toxic effects of benomyl on seminal vesicle and ventral prostate of male wistar rats

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***Summary:*** ***The aim of the study was to examine any potential effects of benomyl on seminal vesicle and prostate gland 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. Seminal vesicle and prostate gland' 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.

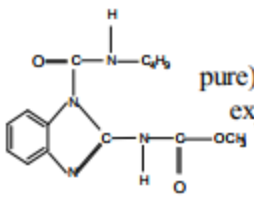
**Material and methods:**

**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.

**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 seminal vesicle and prostate gland.

**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:**

Pesticides, despite their known toxicity are used throughout the world (Salameh *et al.,* 2004) and have shown to exert various toxicological effects in human and animals (Gray *et al.,* 1994; Campbell *et al.,* 1998; Chia, 2000; Windham, 2001; Garry *et al.,* 2002; Rao *et al.,* 2005; Joshi *et al.,* 2005 and Joshi *et al.,* 2006). There are a number of possible ways in which humans can be exposed and toxic effects of these chemicals may have consequences to food consumers, formulators, production workers, farm workers and other home applicators.

The weight of Seminal vesicle showed remarkable reduction at various doses of benomyl for 30 days. A significant decrease in the ventral prostate weight was noticed in the group III, IV and V whereas non-significant reduction in II treated group (Table 1).

Androgens are the regulators of normal structure, functions and growth of accessory sex organs. In the present investigation significant reduction in the weight of seminal vesicle and prostate gland was recorded, indicates that the level of androgen was not enough to maintain the weight of the accessory organs (Moser *et al.,* 2001; Marty *et al.,* 2001, O'Conner *et al.,* 2002; Anas *et al.,* 2005).

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

| Treatment | Body weight | | Seminal vesicle | Ventral prostate |
|---|---|---|---|---|
| | Initial | Final | | |
| | g | | mg/ 100 g. b. wt. | |
| Group I (Control Vehicle) | 168.93 ±7.17 | 183.10 ±6.96 | 410.34 ±25.63 | 360.47 ±15.63 |
| Group II 2.5 mg for 30 Days | 180.37 ± 5.96 | 195.92$^{ns}$ ±7.23 | 330.27$^{ns}$ ±26.63 | 290.19$^{ns}$ ±15.32 |
| Group III 5 mg for 30 Days | 180.42 ±4.84 | 165.64$^{ns}$ ±4.42 | 310.28* ±10.91 | 281.52* ±11.21 |
| Group IV 7.5 mg for 30 Days | 181.46 ±2.96 | 155.75* ±5.46 | 305.42* ±15.82 | 254.53* ±21.18 |
| Group V 10 mg for 30 Days | 175.58 ±3.31 | 120.44* ±10.12 | 265.39** ±24.42 | 235.32** ±12.01 |

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

Non significant increase in the protein content of seminal vesicle in the group II, III and IV was noticed whereas rats of V group showed significant increase in comparison to control. Same results as seminal vesicle showed in protein content of ventral prostate. A non significant to significant decrease ($p < 0.01$) and ($p < 0.001$) in the protein content of ventral prostate was noticed in all the treated groups (Table 2).

Prostate gland does an important role in male reproduction by functioning of sperms (Kumar and Majumdar, 1995). Prostate development and functions depend upon testicular androgen (Reiter *et al.,* 1995). Lack of testicular androgen might be the cause of prostate gland weight loss (Singh and Joy, 2001; FAO/WHO, 2002).

Benomyl exposure to animals resulted in marked decline of sialic acid content of seminal vesicle in all the groups except II and III group. The reduced sialic acid content of seminal vesicles caused deteriorating effects on the structural integrity of sperm cells (Verma *et. al.,* 2005). Statistically significant decrease was observed in sialic acid content of prostate in group IV and V.

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

| Treatment | Protein (mg/g) | | Sialic acid (mg/g) | |
|---|---|---|---|---|
| | Seminal vesicle | Ventral prostate | Seminal vesicle | Ventral prostate |
| Group I Control | 230.46 ±15.21 | 230.87 ±7.78 | 5.08 ±0.18 | 5.31 ±0.19 |
| Group II 2.5 mg for 30 Days | 250.38$^{ns}$ ±15.78 | 245.69$^{ns}$ ±9.93 | 4.90$^{ns}$ ±0.12 | 4.81$^{ns}$ ±0.39 |
| Group III 5 mg for 30 Days | 258.98$^{ns}$ ±1.32 | 260.44$^{ns}$ ±10.12 | 4.75$^{ns}$ ±0.43 | 4.60$^{ns}$ ±0.28 |
| Group IV 7.5 mg for 30 Days | 265.48$^{ns}$ ±6.97 | 268.96$^{ns}$ ±3.55 | 4.62* ±0.20 | 4.20* ±0.15 |
| Group V 10 mg for 30 Days | 295.31* ±1.84 | 272.46* ±7.87 | 4.41** ±0.33 | 3.60** ±0.22 |

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

**Histopathological changes:**

Histopathological alterations observed in benomyl treated rats are discussed along with the microphotographs.

**Seminal Vesicle:**

The seminal vesicles are composed of tubular alveoli and the mucosa is thrown into an intricate system of folds with the epithelium overlaying the lamina propria (Gonzales, 1989). Seminal vesicles secrete substances which directly stimulate sperm motility and antigens that seem to prevent female immune response against spermatozoa (Bukovsky *et. al.,* 1991) and embryo (Thaler *et. al.,* 1989).

The sex differentiation and growth of seminal vesicles are highly dependent on androgens (Higgins and Burchell, 1978; Lieber *et. al.,* 1980; Curry and Atherton, 1990), thus reduced androgen level have adverse effects on histo architecture of seminal vesicles. Intoxicated rats in comparison to control rats showed adverse effects on histo architecture of seminal vesicle with disruptive changes of muscles and connective tissue along with highly reduced or almost no secretion in the lumen in Fig (i) to Fig (v).

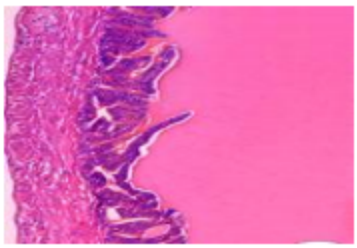


Fig (i): Seminal vesicle control

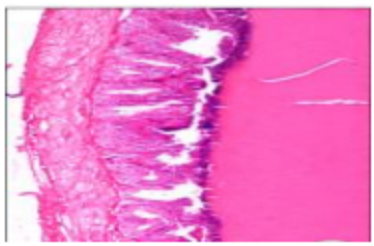


Fig (ii): 2.5 mg for 30 days

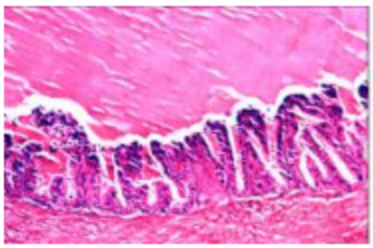


Fig (iii): 5 mg for 30 day

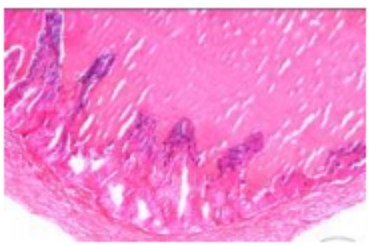


Fig (iv): 7.5 mg for 30 days

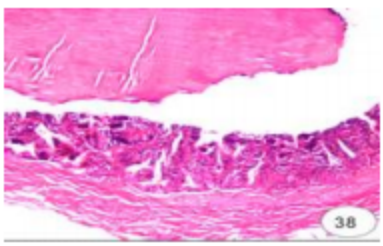
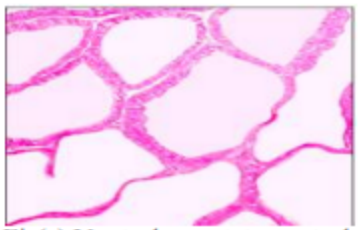



Fig (v): 10 mg for 30 days

**Ventral Prostate:**

Prostate plays an important role in male reproduction and its secretion is essential for the normal function of spermatozoa (Kumar and Majumdar, 1995). Administration of benomyl caused histopathological alterations in the ventral prostate. The secretary material of prostate was reduced severely and proliferation of epithelium with the crypts invaded in lumen has been observed in Fig (a) to Fig (c).

Similar observations were noticed by Akbarsha *et al.,* (1990). Absence of secretions in prostate indicates changes in the functions of Leydig cells on target organs (Pakrashi and Pakrashi, 1977). Prins *et al.,* 1991 reported non-functional phase of the prostate due to decrease in the sialic acid content.


Fig(a):Ventral prostate control

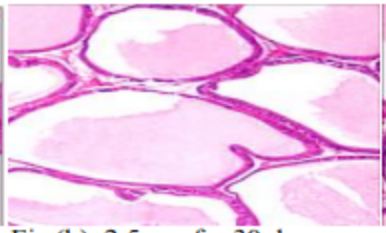


Fig (b): 2.5 mg for 30 days

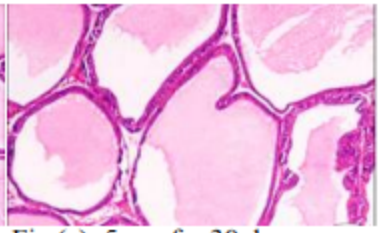


Fig (c): 5 mg for 30 days

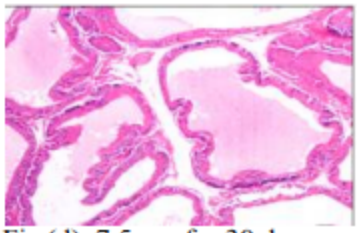


Fig (d): 7.5 mg for 30 days

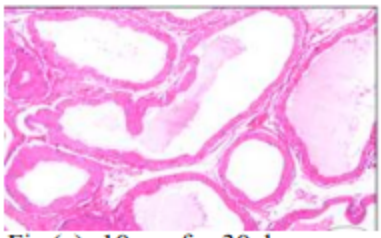


Fig (e): 10 mg for 30 days

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