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Original Article

Carbendazim induced Histopathological changes in Adrenal, Thyroid Glands and some Enzyme Activities in Adrenal Gland of *Rattus rattus*

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Abstract

The purpose of this study is to determine the effects of carbendazim on the level of certain enzymes and endocrine glands (Adrenal and Thyroid glands) of male rats. Carbendazim is a systemic fungicide with enzymatic activity against a number of plant pathogens. In this study, daily dose of 300mg/100g per day carbendazim were applied to rats by gavages for 15 and 30 days. At the end of the experiment ACP and ALP levels in adrenal glands were analyzed. A significant increase was observed up to 15 day as compared to control group. This shows that carbendazim caused histopathological alteration in thyroid and adrenal glands. These results suggest that carbendazim exposure for 15 days increase ACP, ALP enzyme level in adrenal glands and slightly decreased in 30 days exposure of carbendazim and 15 and 30 days carbendazim exposure effect the thyroid and adrenal glands. Alteration in histopathological changes in adrenal and thyroid suggested that the androgen deprivation after carbendazim exposures. These results conclude that carbendazim may effect directly on the glands or through hypothalamo-hypophysial axis

Key words**:** *Carbendazim, glands, enzymes, toxicity, ACP, ALP*

Introduction

Carbendazim (methyl-2-benzimidazole carbamate, MBC), a metabolite of benomyl is one of the most widespread environmental contaminant of major concern to human and animal reproductive health. Carbendazim is a systemic benzimidazole fungicide that plays a very important role in plant disease control.

Carbendazim is classified by the World Health Organization as 'Toxic Substance' (WHO, 1993). Short term exposure with carbendazim has been reported to induce the morphological changes in the duodenum, bone marrow and liver (Sherman, 1965; Sherman and Krauss, 1966). However, long term exposure with carbendazim resulted in the decreased survival rate, body weight, hematological, biochemical and histopathological alterations in adrenal, thyroid, liver and testis (Sherman, 1972; Barlas *et al.,* 2002; Selmanoglu *et al.,* 2003). Carbendazim has been reported to induce carcinogenic effects in liver of both male and female animals (Beems *et. al.,* 1976; Muthuviveganandavel *et al.,* 2008). Carbendazim is a suspected endocrine disruptor (Friends of the Earth, 2001; Lu *et al.,* 2004). It can disrupt the development of sperm and damage testicular development in rats (Du Pont, 1991; Lim *et al.,* 1997; Moffit, 2007; Gawande *et al.,* 2009). Long term exposure of male animals with carbendazim revealed the decreased testicular, epididymal weights, altered sperm morphology, testicular atrophy and thus infertility (Nakai *et. al.,* 1992; Lim and Miller, 1997; Moffit, 2007; Gawande *et al.,* 2009). Chronic low daose treatment of MBC is capable of inducing reproductive and endocrine toxicity through increased oxidative stress (Barlas *et al.,* 2002; Rajeshwary *et al.,* 2007)

In connection to these studies present experimental investigation was an attempt to evaluate the endocrine disrupting effects of carbendazim in male *Rattus rattus* by adopting different parameters via, histopathological changes in adrenal, thyroid glands and some enzymatic activities (ACP, ALP) in adrenal glands.

Materials and Methods

In the present experimental investigation twenty adult male rats, *Rattus rattus* weighing 70±5gms were used. The animals were divided into two groups of ten each. Group I, served as control, were fed with

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standard rats feed and corn oil (0.5ml) and water *ad libitum*, while group II received a daily dose i.e. 300mg/0.5ml/100g b.wt. (dissolved in corn oil) with standard laboratory diet for 15 and 30 days respectively. After the termination of experiments i.e., on 16th and 31st days all the animals were sacrificed and their adrenal and thyroid glands, were dissected out. Adrenal and Thyroid were weighed first and fixed (one side of adrenal and thyroid) in bouin's fluid for normal histopathological studies using hematoxylin and eosin staining (Ehrlich, 1886) and another side of the adrenal where homogenized in sucrose solution for the enzyme activities (ACP and ALP) estimation by adopting Bergmeyer, (1963) methodology.

Results

Carbendazim induced alterations in body weight, adrenosomatic indices (ASI) and Adrenal enzyme activities (ACP, ALP) after 15 and 30 days of Carbendazim exposures, Body weight and ASI levels were increased initially after Carbendazim exposure while these values were decreased significantly in later part of the experiment as compared to control group (figs 1 and 2). Apart from this, it has been also noticed that the adrenatic ACP and ALP levels were also increased after 15 days of Carbendazim exposure, while it lowered in the later part of the experiments i.e., after 30 days (figs 3 and 4). In histopathological studies we observed that thyroid treated up to 15 days with carbendazim reveled the degenerative and atrophied changes in epithelial cells when compared with control cells (fig. 5 & 6) however these degenerative changes were very much conspicuous with declined epithelium and large lumen after 30 days of exposure (fig. 7). Besides this, Adrenal exposed with carbendazim for 15 days also showed degenerative and pycnotic changes in zona glomerulosa (ZG) and zona fasciculate (ZF), few hypertrophied and vacuolated cells are also seen in medullary region, intercellular spaces are also formed by degenerative cells as compared control (fig. 8 & 9). However, these degenerative changes were more conspicuous showing involuntary changes in zona reticulate (ZR) region and hypertrophied medullary cells with accentric nucleic were noticed in later part of the experiment (fig.10).

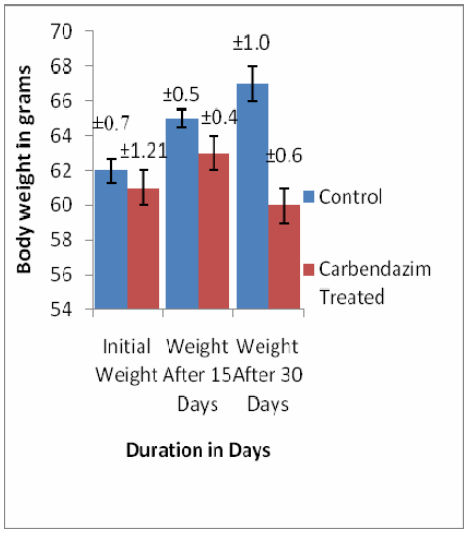

Fig.1: Body weight (gms) of Control and Carbendazim treated *Rattus rattus* after 15 and 30 days.

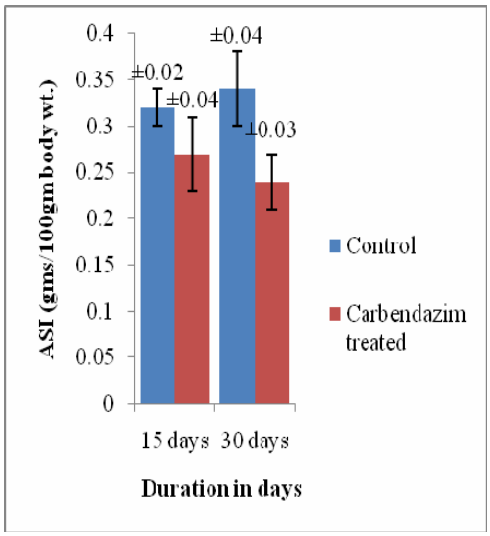

Fig. 2: Adrenosomatic indices (ASI; gms/100gm body weight) of Control and Carbendazim treated *Rattus rattus* after 15 and 30 days.

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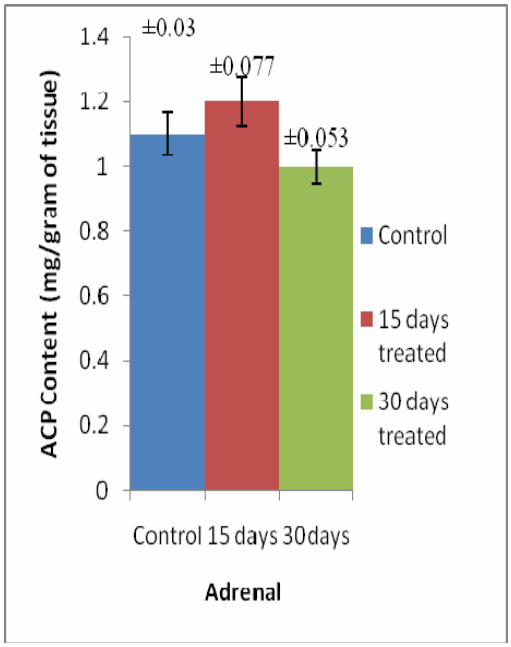

Fig.3: Adernal Acid phosphatase (ACP; mg/gm) activities in Carbendazim treated and control *Rattus rattus* after 15 and 30 days.

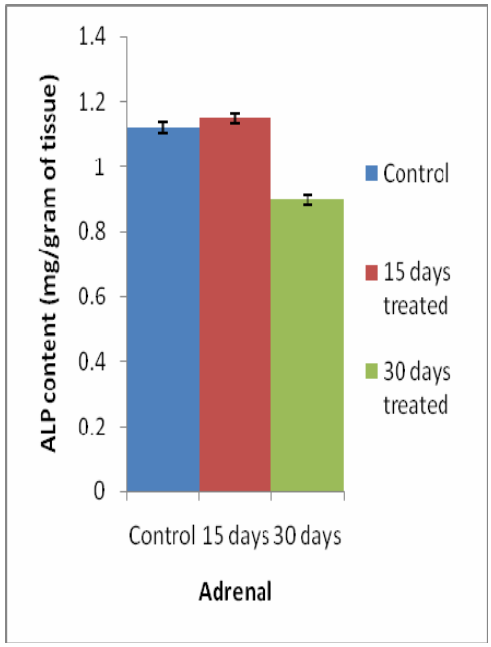

Fig. 4: Adrenal Alkaline phosphate (ALP; mg/gm) activity in Carbendazim treated and control *Rattus rattus* after 15 and 30 days.

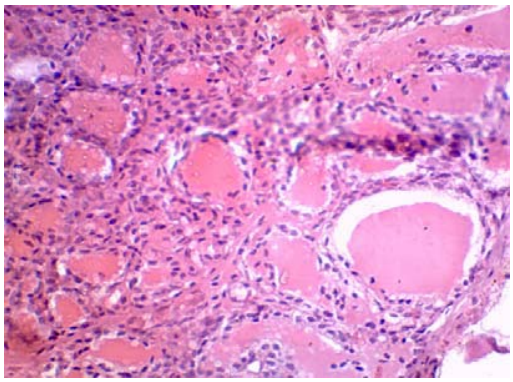

Fig.5: Showing normal histoarchitecture of control thyroid gland of *Rattus rattus* with organized epithelial cells in spherical or short, blind ending cylindrical masses called follicles. H&E X 400.

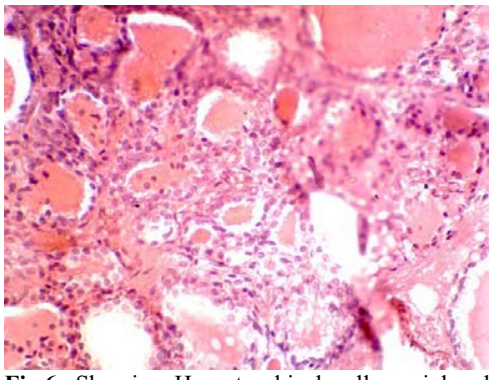

Fig.6: Showing Hypertrophical cells peripheral reabsorption in the follicles is seen. Intercellular spaces also seen in 15 days carbendazim treated thyroid sections. H&E X 400.

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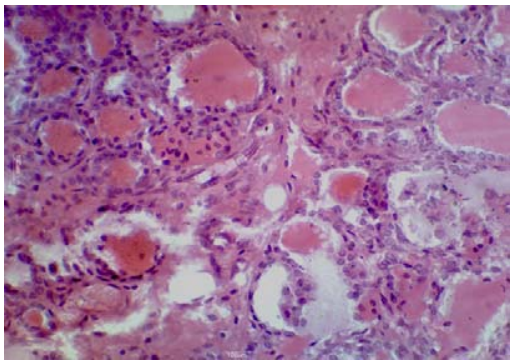

Fig.7: Showing the more severe and conspicuous changes in Follicles. Fibronoid degeneration in epithelial cells reflecting apoptic morphology. H&E X 400.

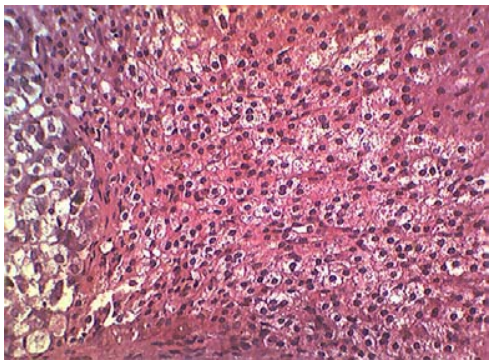

Fig.9: Showing damaged layers with intercellular spaces in medullary region formed by degenerative cells. Degeneration in ZG and ZF also noticed. H&E X 400.

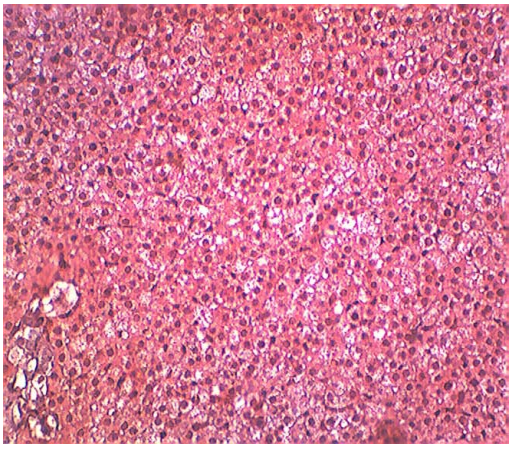

Fig.8: Showing normal adrenal cortex and medulla of control *Rattus rattus* with well defined \zona glomerulosa (ZG), \zona fasiculata (ZF), zona reticularis (ZR) and medullary (M) regions having active cells with prominent nuclei. H&E X 400.

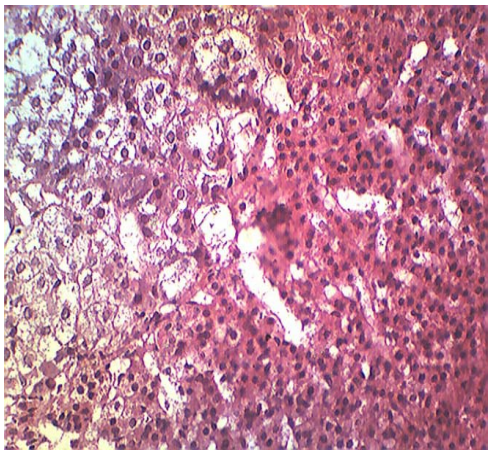

Fig.10: Showing degenerating cells in adrenal cortex and medullary regions. Hypertrophied cells with accentric nuclei showed in medullary region. ZG, ZF and ZR also showed involuntary changes with damaged cells. H&E X 400.

Discussion

Carbendazim is a systemic fungicide used to control the wide range of plant pathogens in fruits, vegetables, field crops and ornamental plants. Carbendazim is been reported to cause endocrine and developmental toxicity in rats (Lu *et al.,* 2004). At high dose level repeated exposure cause spermatogenic effect in rats and hepatic tumor in mice (Carter *et al.,* 1987). Torchinskly et al (1976) reported the testicular atrophy and decresed fertility after carbendazim exposure. Disruption of sertoli cell morphology has been also reported by carbendazim exposure in rats (Nakai and Hess, 1991; Nakai *et al.,* 1992; Moffit, 2007; Gawande *et al.,* 2009). Earlier results also suggested a decreased in body weight, adrenal weight and testicular weight after carbendazim exposure (Sherman, 1972). Our results reveled that carbendazim decrease body weight and ASI levels after 30 days. Acid and alkaline phosphatases are involved in protein synthesis, gonadal maturation and steriodenesis (Shaffi *et al.,* 1974: Guraya and Sidhu, 1975). Alternation in these enzyme activities in our result suggest that the MBC modulate enzyme activity and effects were more prominent in later part of the experiment

which may impair steroidogenesis in adrenal of *Rattus rattus*. Resent repot have reveled that carbendazim exposures induce histopathological changes in the reproductive organs and endocrine glands of rats and birds (Barlas *et al.,* 2002; Aire, 2005; Muthuviveganandavel *et al.,* 2008). Alternation in histopathological changes in the adrenal and thyroid in our results may suggest that the androgen deprivation and cellular degeneration of carbendazim exposures. These results also conclude that the carbendazim has deleterious effect on the adrenal and thyroid of male *Rattus rattus.* Carbendazim may effect directly on the glands or through hypothalamo-hypophysial axis.

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