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

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Received:26.6.2010; Revision: 4.8.2010; Accepted:8.8.2010; Published:15.8.2010

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 dose 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...
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 revealed 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 reticulata (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.
Fig. 3: Adrenal 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 Hypertrophic cells peripheral reabsorption in the follicles is seen. Intercellular spaces also seen in 15 days carbendazim treated thyroid sections. 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 has been reported to cause endocrine and developmental toxicity in rats (Lu et al., 2004). At high dose level repeated exposure cause spermatogonic 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 \textit{Rattus rattus}. Recent report have revealed that carbenzadim 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 carbenzadim exposures. These results also conclude that the carbenzadim has deleterious effect on the adrenal and thyroid of male \textit{Rattus rattus}. Carbenzadim may effect directly on the glands or through hypothalamo–hypophysial axis.

Acknowledgement:
The authors are grateful to Prof. Meenakshi Banerjee, Head, Department of Biosciences, for her elegant and ardent experimental support.

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