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Effect of carbendazim on the liver of male Swiss albino mice

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Abstract : *The effect of a fungicide,carbendazim was examined on the liver of Swiss albino mice by using histological technique and by measuring serum glutamic pyruvic transminase (SGPT) level in the blood. A sublethal dose of carbendazim caused pathological changes in the liver. Simultaneous administration of ascorbic acid with carbendazim showed positive changes in the liver histology.*

Keywords : *Carbendazim , Olive oil, Ascorbic acid , Liver, SGPT.*

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Introduction :

Carbendazim is a widely used broad spectrum fungicide. It is widely used in China and India to control fungal pathogen on cereals, fruits, cotton, tobacco, turf, ornamentals, vegetables etc. In India, its trade name is Ruston-50, Benfil and Bavistin. Its molecular formula is $C_9H_9N_3O_3$.

Carbendazim has been reported to cause endocrine and developmental toxicity in rats and mice (Lu et al 2004; Farag et al 2011). Its repeated exposure leads to adverse effects on the testes in rats (Nakai et al 1992; Lim and Miller 1997; Moffit et al 2007) and causes hepatic tumor in mice (Beems et al 1976; Carter et al 1987). Surprisingly, carbendazim was classified by the World Health Organisation (WHO) as 'unlikely to present hazard in normal use' in 1993. But, now, it is considered one of the twelve most commonly detected pesticides in EU monitoring programmes, most often in apple samples, followed by grapes and strawberries (EC 2001). Carbendazim is one of the

23 active substances that are currently approved (or close to be approved) for preserving plant protection products, but some may be withdrawn from the European market, due to their particularly serious properties such as being a carcinogen, toxic for germ cell, and endocrine disruptors (ED) (SCA 2009).

Carbendazim is sparingly soluble in water (8mg/L at pH 7 and 25°C), but is soluble in olive oil.

Ascorbic acid is a water soluble antioxidant. It plays an important role in combating the effect of free radicals for vital cells (Sinisa et al 2008). Ascorbate can both chelate and reduce transition metal ions and in turn can reduce O_2 or H_2O_2 to superoxide and hydroxyl radical respectively (Carr and Frei 1999). Pesticides induce oxidative stress, leading to generation of free radicals and alteration in antioxidants, oxygen free radicals, the scavenging enzyme system, and lipid peroxidation (Banerjee et al 1999, Etemadi et al 2002).The protective role of ascorbic acid on carbendazim toxicity has not been undertaken by toxicologists and this is called for an examination of its efficacy in the present study.

Methodology :

Forty eight male Swiss albino mice with average weight (32±0.75g) were procured from the animal house of Patna Women's College. They were housed in poly propylene cages in air conditioned room at 17ºC in a 12hrs light and 12 hrs day cycle. They were fed on bengal gram, soya bean and tap water.

The animals were divided into six groups (n=8 per group). The mice of first group were treated with 0.5ml olive oil and served as control group. The second and third group were administered with single high but sub lethal dose of carbendazim (400mg/kg BW) suspended in 0.5ml olive oil. The fourth group was treated with a single high dose of 50mg /kg BW of L-ascorbic acid. According to Sahu and Das (1994), 10 mg/kg BW of ascorbic acid is quantitatively equivalent to the human therapeutic dose (500 mg/day;) in terms of body weight.The fifth and sixth group were pre-treated with 50mg/ kg BW of ascorbic acid followed by 400mg/kg of carbendazim .

The mice of second and fifth group were sacrificed on the second day of dose after giving light anaesthesia.The mice of all other groups were sacrificed on the fifth day of dose after giving light anaesthesia. Blood was collected through cardiac puncture for SGPT (Serum Glutamic Pyruvic Transaminase) analysis.The body weight, and that of liver was taken of all samples.

The relative weights of different organs of control and treated mice, the relative weights of the organs of the control and treated mice were compared with Students t-test. P value less than 0.05 was considered statistically significant.

Results and Discussion :

There was no perceptible change in the general and feeding behaviour of mice treated with carbendazim. There was no significant change in the initial and final body weights of the treated mice, but there was a slight fall in the final weight of the control group of mice treated with ascorbic acid (Table 1). There was no significant change in the weights of liver between carbendazim treated group and carbendazim + ascorbic acid treated group of

mice (Table 2). A rise in SGPT level was seen between the control (Group I) and treated mice (Groups II and III), but the difference was not significant statistically. However, there was significant fall in the SGPT level in the mice pre–treated with ascorbic acid as compared to the mice treated with carbendazim (t=1.84, P<0.05) (Table 3).

The liver of control mice or those given ascorbic acid showed normal histological profile (Fig. 1 and 2) whereas, the liver of mice treated with carbendazim showed enlargement of sinusoids, vacuolation in hepatocytes, pycnotic nuclei, degeneration of blood vessel endothelium and rupture of some hepatocytes (Fig 3 and 5). The histological profile of the mice treated both with carbendazim and ascorbic acid was indicative of some kind of recovery towards a normal histological pattern (Fig. 4 and 6).

Table 1. Comparison of initial and final body weights of mice. Values are Mean ± S.E.

	Initial weight	Final weight	t value	Level of significance
Control with olive oil	27.57±0.71	28.49±0.85	0.84	NS
Treated with Carbendazim (dissected after two days)	27.92±0.89	27.90±0.92	0.01	NS
Treated with Carbendazim (dissected after five days)	31.03±0.61	28.98±1.17	1.55	NS
Control with ascorbic acid	32.14±0.61	30.24±0.67	2.09	P < 0.05
Treated with Carbendazim + ascorbic acid (dissected after two days)	28.91±0.86	28.91±0.84	0.01	NS
Treated with Carbendazim + ascorbic acid (dissected after five days)	28.07±0.87	29.97±0.92	1.49	NS

Table 2. Comparison of weights of liver between carbendazim treated and Carbendazim + ascorbic acid treated mice. Values are Mean ± S.E.

	Dose with Olive oil	Dose with Vit C	t value	Level of significance
Control	1.58±0.04	1.61±0.05	0.39	NS
Treated with Carbendazim (dissected after two days)	1.57±0.05	1.54±0.04	0.53	NS
Treated with Carbendazim (dissected after five days)	1.53±0.02	1.52±0.06	0.14	NS

Table 3. Comparison of SGPT level between carbendazim treated and Carbendazim + ascorbic acid treated mice. Values are Mean ± S.E.

	Dose with Olive oil	Dose with ascorbic acid	t value	Level of significance
Control	32.13±2.47	35.36±3.97	0.68	NS
Treated with Carbendazim (dissected after two days)	38.68±3.66	36.48±3.26	0.48	NS
Treated with Carbendazim (dissected after five days)	42.14±6.69	28.77±2.76	1.84	P < 0.05

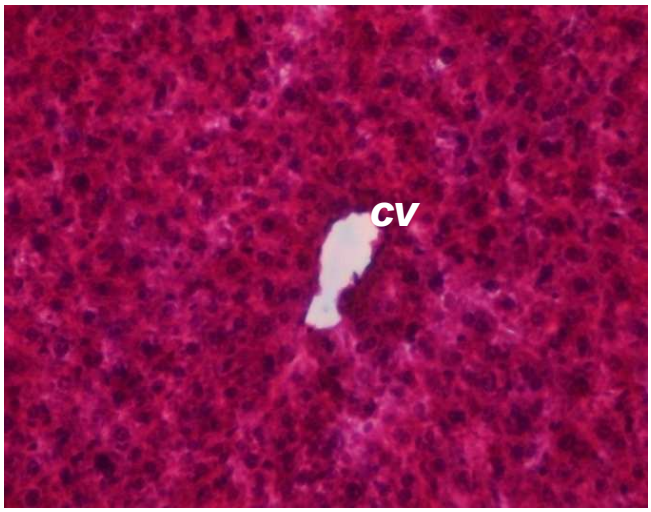

Fig 1. Liver microphotograph of control group fed with olive oil. Hepatocytes closely packed. Central vein (CV) normal occupying limited space. Magnification = X 400

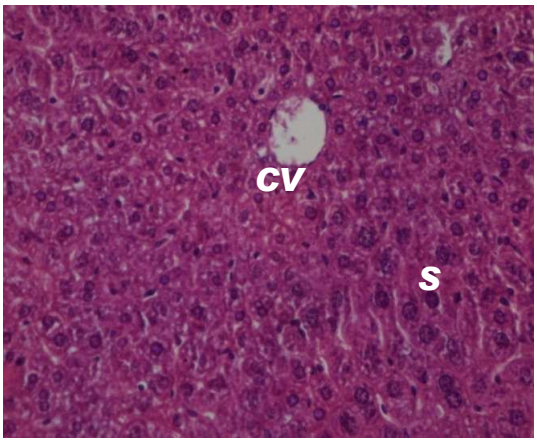

Fig.2. Liver of control group fed with ascorbic acid. Hepatocytes appear normal with intact cytoplasm and prominent nucleus. Magnification = X 400

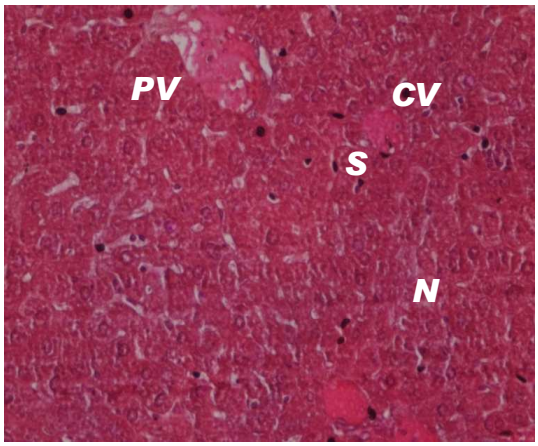

Fig 5. Liver of carbendazim (400 mg/kg BW) treated mice and dissected after five days.Congestion in portal (PV) and central vein (CV). Dilatation of sinusoids (S). Nuclear loss (N), nuclei not prominent. Magnification = X 400

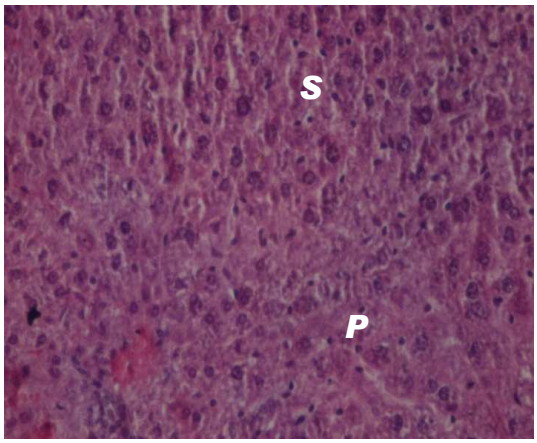

Fig 3. Liver of carbendazim (400 mg/kg BW) treated group and dissected after two days. Enlarged sinusoids (S), pycnotic nuclei (P). Vacuoles in hepatocytes (V). Magnification = X 400

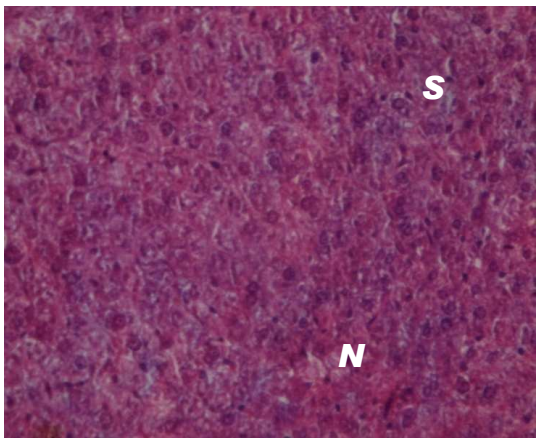

Fig 6. Liver of carbendazim (400mg/kg BW) + ascorbic acid (50mg/kg BW) treated group and dissected after 5 days. Nuclei visible. Sinusoids comparatively reduced. Magnification = X 400

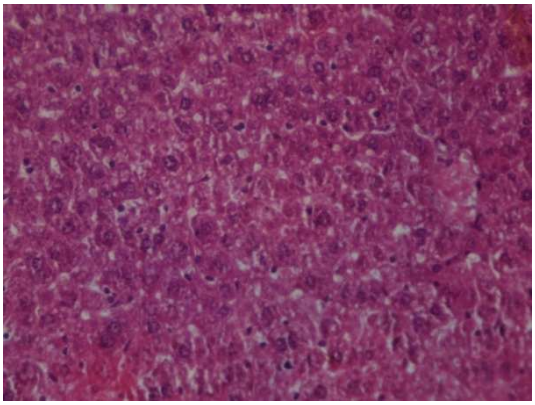

Fig 4. Liver of Carbendazim (400 mg/kg BW) + ascorbic acid (50mg/kgBW) treated group and dissected after two fays. Nuclei (N) appear normal. Magnification= X 400

Similar changes in the histology of liver caused by carbamates and benomyl was reported by Abdu Rabou (1996) and Balkan and Aktac (2005) respectively. Muthuviveganandavel et al (2008) administered 50 mM (single dose) of carbendazim intradermally to male rats and found similar histopathological changes in the liver (dissected six hours after dosing).

Conclusion :

It was found in the present study that carbendazim could have toxic effect on liver even when it is consumed in minimum quantity (400mg/

bw) for a short period of time that as a single oral dose. The present study also indicated that a high dose of L-ascorbic acid would exert significant effect to counteract carbendazim toxicity.

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