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Embryotoxic and gonadotoxic effect of carbendazim on female Swiss albino mice

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Abstract : *An experiment was conducted to investigate the effect of Carbendazim on the structure and function of liver, ovarian histology and development of embryo in the uterus of female Swiss albino mice. The study revealed significant embryotoxic and gonadotoxic effect in the group of mice treated with carbendazim (400 mg/kg BW). Histopathological changes were also found in the liver of treated group.*

Keywords : *Carbendazim, ovary, liver, embryo.*

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Introduction :

Carbendazim is used to control fungal pathogen on cereals, vegetables, fruits and is widely used as broad spectrum fungicide. Its trade name in India is Ruston-50, Benfil and Bavistin.

Carbendazim has been reported to cause endocrine and developmental toxicity in rats and mice (Sitarek 2001; Lu et al 2004; Farag et al 2011). 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substances that are currently approved (or close to be approved) for use in plant protection products, but may be withdrawn from the European market, due to their particularly serious properties such as being a carcinogen, toxic for reproduction, and Endocrine disruptors (ED) (SCA 2009).

Animals are exposed to Carbendazim through food. Liver is the most important site of detoxification. Information on the effect of a single oral dose of carbendazim on the liver of swiss albino mice is lacking.

Further, there are no reports on the effect of single sublethal dose of carbendazim on the gonadal and embryo toxicity in female mice. Therefore, the present study was undertaken to elucidate the effect of carbendazim on the ovarian follicular development and embryonic development in the uterus of Swiss albino mice. This paper also reports on the effect of exposure period (pre-organogenesis versus organogenesis) on the above mentioned responses.

Methodology :

Twenty one healthy virgin inbred Swiss albino mice weighing (23.45±1.22gm) were used for the experiment. The mice were procured from animal house of Patna Women's College. They were housed in groups in polypropylene cage in air conditioned room maintained at 25±2°C, in 12 hr light and 12 hr dark cycles .They were fed on Bengal gram, soyabean crunches, homemade breads, carrot and tap water.

The mice were divided into three groups (n=7 per group). The female mice were housed with male mice in the ratio of 3 female: 1 male. First day of gestation was identified by the presence of vaginal plug in female mice.

Mice of group –I were given normal food (and drinking water) with olive oil (0.5ml) and were considered as control group. Mice of group-II were administered a single sublethal oral dose of Carbendazim (400mg/Kg BW) suspended in 0.5ml of olive oil through gavages during the pre-organogenesis phase (1-5 days) of the gestation period. Mice of group-III were administered with the same single oral dose of carbendazim during the organogenesis phase (6-15 days) of the gestation period. On the 16th day of pregnancy mice were weighed and sacrificed after giving light anaesthesia. Weights of liver and kidneys were taken. Uterine horns were studied for number of live and dead foetus, litter number, litter weight, resorption site, embryonic death and external malformation. Blood was collected through cardiac puncture for SGPT (Serum Glutamic Pyruvic Transaminase) analysis.

Initial and final weights of mice were compared with Students t- test. Body weight gain, weight of liver, SGPT level and weight of embryos between control and treated groups of mice were compared with one-way ANOVA. $P<0.05$ was considered as statistically significant.

Results and Discussion :

There was no change in the general and feeding behaviour of mice. There was an increase in the final body weights of female mice as compared to the initial weights (Table 1), but there was no significant difference in the initial and final body weights of treated mice as compared to the control mice (Table 2). There was no significant difference in the weights of liver and SGPT level between the control and treated mice (Table 2). The ovary of control mice showed normal development of follicles, with intact granulosa cells in the Graafian follicle (Fig 1 and 2). The numbers of atretic follicles were less in this group (Table 3). In the mice treated with carbendazim during the pre-organogenesis period, the ovarian stroma contained large number of atretic follicles of different sizes and congested blood vessels at the centre of the ovary (Fig 3). Graafian follicles appeared with degenerated zona pellucida and cumulus oophorus. The theca externa cells also appeared to be damaged (Fig 4). In the third group of mice treated with carbendazim during the organogenesis period, the follicles appeared normal (Fig 5), but the Graafian follicle appeared with degenerated zona pellucida and pycnotic granulosa cells (Fig 6). The embryos of control group of mice appeared normal and were of equal size (Fig 7). Resorption of embryos was more in the group of mice treated with carbendazim in during the pre-organogenesis period (Fig 8), whereas embryonic death, teratogenic (club foot) changes and retarded growth of embryos were found comparatively more in the group of mice treated with carbendazim during the organogenesis period (Table 4), (Fig 9).

In the present study follicular atresia was found to be more in the treated group of mice as compared to the control. Primary and pre-antral follicles did not seem to be affected. These may help to recover the loss and replace the atretic follicles. This suggests that a single sub lethal dose may not cause irreparable damage to the ovary. Borgeest et al (2002) also found increase in follicular atresia in response to Methoxychlor. Gupta et al (2007) showed that primary follicles of primates may be more sensitive to Methoxychlor and its metabolites than of rodents. Koc et al (2009) also found less number of healthy follicles and more number of atretic follicles and corpus luteums in high dose of endosulfan and malathion (33 mg/kg.) treated rats. Ahmad et al (2011) found that diazinon is similarly toxic to the ovary of pregnant female mice.

The liver of control mice had normal hepatocytes around the central vein (Fig 10). Histological examination of the liver of pregnant females exposed to 400 mg/kg BW during the pre-organogenesis period and dissected on the 16th day of gestation period showed pycnosis of nuclei of hepatocytes (Fig 11). The liver of pregnant females exposed to 400 mg/kg BW during the organogenesis period and dissected on the 16th day of gestation showed degeneration in hepatocytes, dilation of the sinusoids between the hepatocytes and vacuolization of hepatocytes. However, significant changes were not seen in the SGPT level of the three groups of mice (Table 2).

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

	Initial Weight	Final Weight	t value	Level of signi-ficance
Control	24.61 ± 0.99	28.09 ± 2.10	1.49	NS
Carbendazim treated (on 4th day of pregnancy)	24.65 ± 0.59	30.84 ± 1.73	3.38	P<0.01
Carbendazim treated (on 12th day of pregnancy)	24.59 ± 0.52	28.13 ± 1.60	2.1	P<0.05

Table 2. Comparison of body weights, weights of liver and SGPT level between carbendazim treated and control mice. (Values are Mean ± S.E).

	Groups		
	Control	Carbendazim treated (on 4th day of pregnancy)	Carbendazim treated (on 12th day of pregnancy)
Initial weight (g)	24.61 ± 0.99	24.64 ± 0.59	24.59 ± 0.52
Final weight (g)	28.09 ± 2.10	30.84 ± 1.73	28.13 ± 1.60
Body weight gain (g)	3.47 ± 2.09	6.19 ± 1.62	3.86 ± 1.41
Weight of liver (g)	1.65 ± 0.17	1.82 ± 0.11	1.68 ± 0.13
SGPT (ALT) U/L	32.17 ± 4.47	31.99 ± 2.16	24.36 ± 4.19

Table 3. Effect of Carbendazim treatments on the number of ovarian follicles and corpora lutea.

	Number of follicles				
	Primary follicle	Secondary follicle	Graafian follicle	Atretic follicle	Corpus luteum
Control	21	12	4	13	11
Carbendazim treated (on 4th day of pregnancy)	23	13	3	22	9
Carbendazim treated (on 12th day of pregnancy)	15	10	8	21	9

Table 4. Embryotoxic effects of Carbendazim on Swiss albino mice

	Groups		
	Control	Carbendazim treated (on 4th day of pregnancy)	Carbendazim treated (on 12th day of pregnancy)
No. of Live fetus (per female) mean ± S.E	4 ± 0.67	5 ± 1.69	4 ± 1.2
Average weight of embryos (mean ± S.E.)	0.48 ± 0.02	0.54 ± 0.03	0.27 ± 0.01*
Resorption of embryo (no.)	1	7	0
Embryonic death (no.)	1	0	3
No. of embryos with Club foot	0	0	10
No. of embryos with retarded growth	0	0	7

* p<.0001

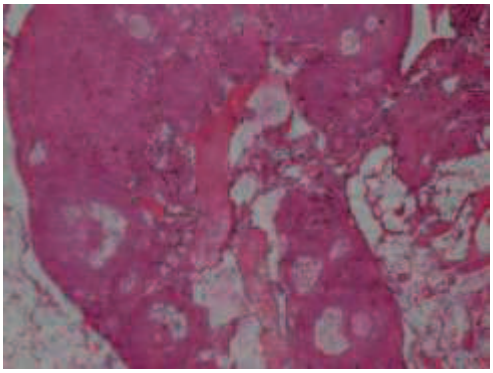


Fig 1. The ovarian stroma of control group of mice fed with 0.5 ml olive oil on 4th day of gestation (pre-organogenesis period) and dissected on 16th day of gestation showing developing follicles of different sizes. (Magnification = X 100)

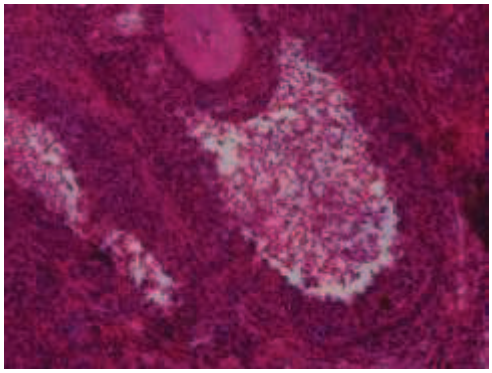


Fig 2. Graafian follicle of control group of mice fed with 0.5 ml olive oil on the 4th day of gestation (pre-organogenesis period) and dissected on 16th day of gestation. The granulosa and corona radiata cells appear normal and intact. (Magnification = X 400)

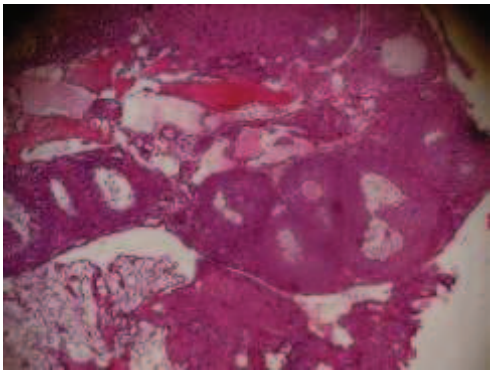


Fig 3. The ovary of mice treated with carbendazim (400 mg/kg BW) on the 4th day of gestation (the pre-organogenesis period) and dissected on 16th day of gestation. Showing a number of atretic follicles (Magnification = X 100).

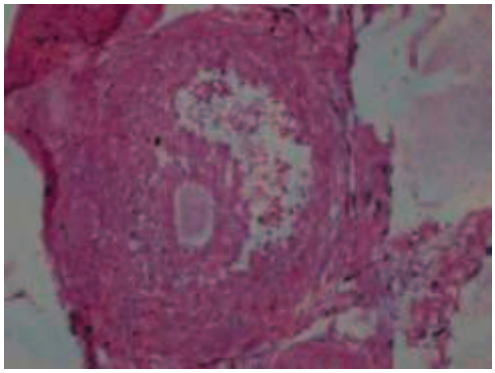


Fig 4. Graafian follicle of mice treated with carbendazim (400 mg/kg BW) on the 4th day of gestation (the pre-organogenesis period) and dissected on 16th day of gestation. Showing degenerated cumulus oophorus and corona radiata cells. (Magnification = X 400)

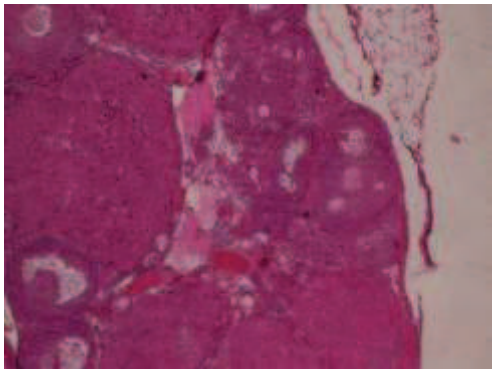


Fig 5. The ovarian stroma of mice treated with carbendazim (400 mg/kg BW) on the 12th day of gestation (the organogenesis period) and dissected on 16th day of gestation.(Magnification = X 100)

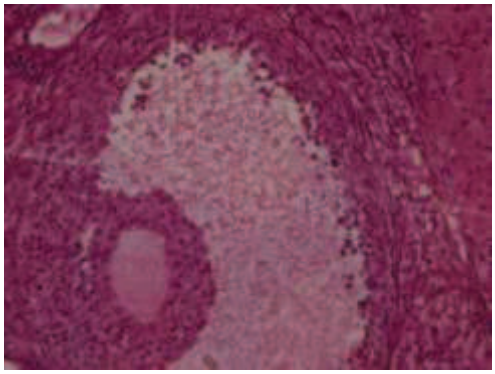


Fig 6. Graafian follicle of carbendazim (400 mg/kg BW) treated group on the 12th day (the organogenesis period) and dissected on 16th day of gestation. Pycnotic granulosa cells seen. (Magnification = X 400)

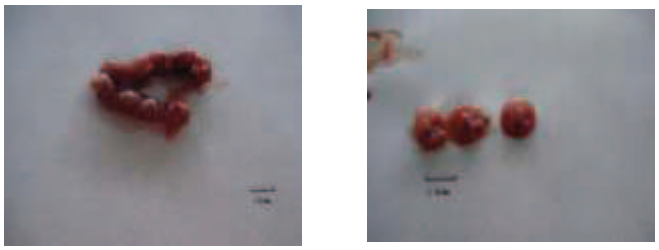
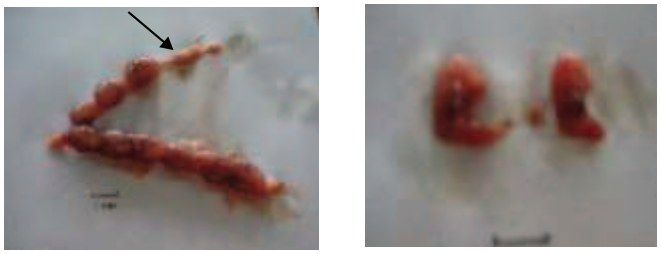


Fig 7. Uteri of control mice showing normal embryos of equal size.



(a) **(b)**

Fig 8. Uteri of mice treated with carbendazim (400 mg/kg BW) during the pre-organogenesis period and dissected after 12 days, showing resorption of embryo in (a) and relative size of embryo of treated group, in (b).

(a) **(b)**

Fig 9. Uteri of mice treated with carbendazim (400 mg/kg BW) on the 12th day (the organogenesis period) and dissected on 16th day of gestation, showing embryonic death in (a) and retarded growth of embryo in (b)

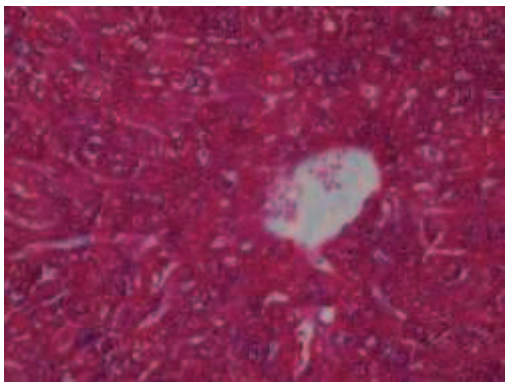


Fig 10. Liver of mice of control group. (Magnification = X 400.)

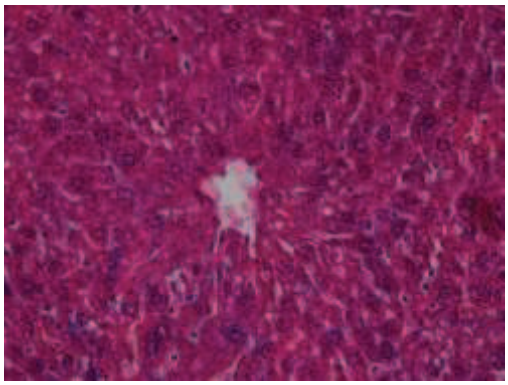


Fig 11. Liver of mice treated with carbendazim (400 mg/kg BW) on the 4th day of gestation (the pre-organogenesis period) and dissected on the 16th day of gestation. (Magnification = X 400.)

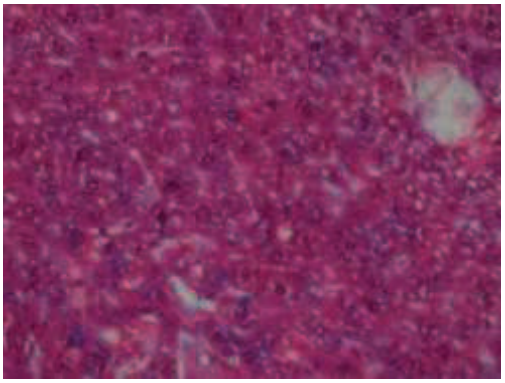


Fig 12. Liver of mice treated with carbendazim (400 mg/kg BW) on the 12th day of gestation (the organogenesis period) and dissected on 16th day of the gestation. (Magnification = X 400.)

Conclusion :

The study concluded that carbendazim could have embryotoxic and gonadotoxic effect even when consumed in minimum quantity (400 mg/kg BW) i.e. as a single sublethal dose. No pronounced effect was seen on liver.

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