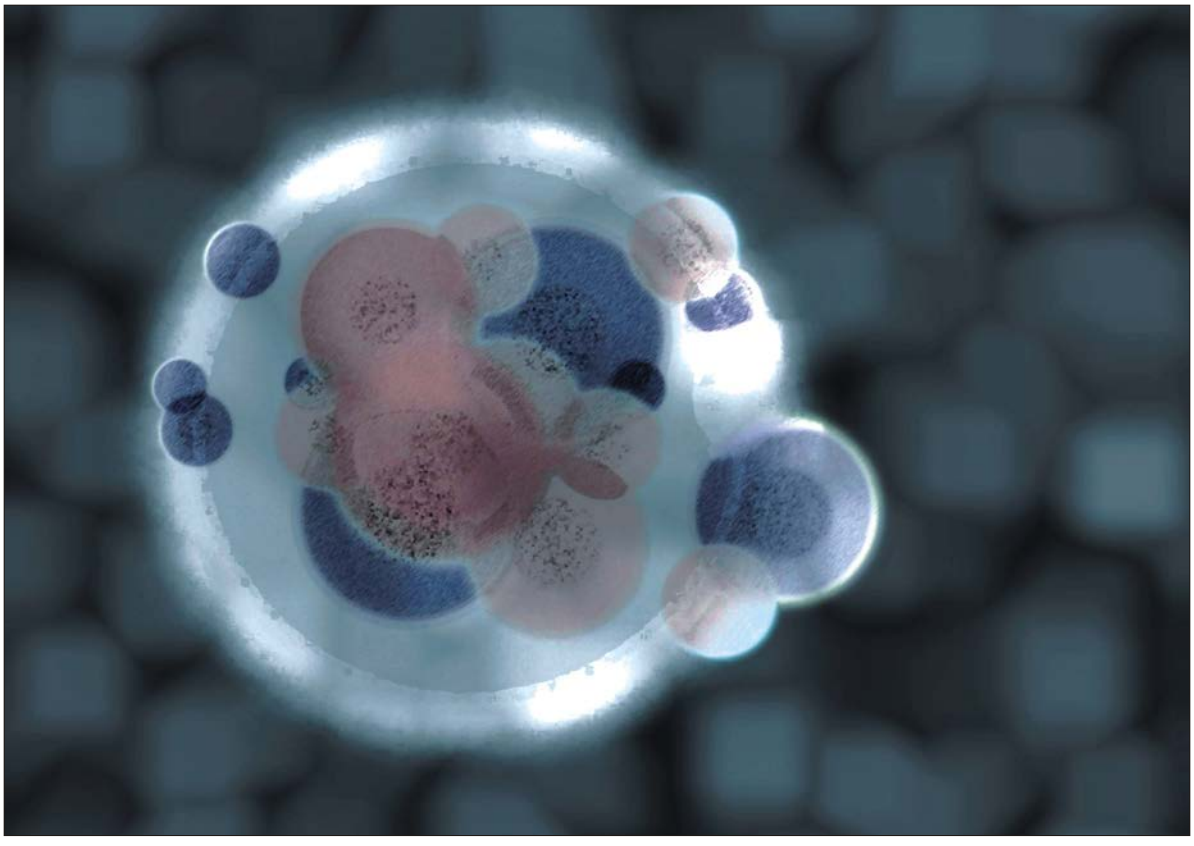

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Study on the Liver Functions in Rats Exposed to Benomyl

Seda Balkan and Tülin Aktaç
Department of Biology, Faculty of Science and Art, Trakya University, 22080 Edirne, Turkey

Abstract: The present study investigated the toxic effect of benomyl on the liver functions of the rats. Benomyl was administered at 200 mg kg^{-1} per day dose gavage to male and female rats (Spraque Dawley) for 5 days. Body weights, organ weights, biochemical and haemotological parameters and histological changes to the liver of rats were investigated. Body weights were not changed significantly but female and male liver weights, male spleen and liver weights were increased. Red blood cells (RBC), hemoglobin (HGB) and haematocrit (HCT) values were decreased in male and female rats which were treated with benomyl but white blood cells (WBC) and thrombocytes (PLT) values were not significantly different from controls. In the male rats, alanin aminotransferase (ALT), alcaline phosphatase (ALP), creatine phosphokinase izozyme (CK-MB) and amylase (AMYL) activities were changed. Also, in the female rats, alanin aminotransferase (ALT), aspartat aminotransferase (AST), creatine phosphokinase izozyme (CK-MB), amylase (AMYL) and lactat dehydrogenase (LDH) activities were changed. There was no significant change in serum gamma glutamyl transferase (GGT) levels of male and female rats. Therefore, it might indicate that benomyl has not carcinogenic effect in rats. Histopathological examinations revealed the cytoplasmic vacuolisation, enlargement of sinüsoids, picnotic nuclei, disintegrated membrans and degeneration of the blood vessel endothelium.

Key words: Liver functions, benomyl, rat, liver enzymes

INTRODUCTION

Methyl1-(Butylcarbamoyl)-2-benzimidazole carbamate (benomyl) is a widely used systemic funguside. Benomyl has low general toxicity and antimitotic activity in mammals[1,2]. It has been found that this compound disrupts microtubule formation by binding to tubulins[3-5]. Recently, because of the antimitotic effect of benomyl and its relatively low toxicity, it was reported that benomyl might be useful as an adjuvant in cancer chemotheropy[1].

The various effects of different doses of benomyl and its metabolite, carbendazim, have been demonstrated in previous reports[6-11]. However, there is limited information about the toxic effects of benomyl on the function of liver of mammals[12-14].

Benomyl is used widely to prevent and control plant disease caused by fungi in Turkey. Therefore, the present study was planned to investigate the benomyl-induced haematological, biochemical and histopathological changes to the liver of rats.

MATERIALS AND METHODS

Animals: Male and female Spraque-Dawley rats (200-250 g weighing) were obtained from the Laboratorium of Experimental Animals in Trakya University. Rats (10 male and 10 female) were divided into four groups, each containings animals. Two of them were experiment groups and the other two were control groups. Animals were fed with a standard laboratory diet and tap water during the experimental period.

Administration of benomyl: Benomyl was supplied by DuPont (Cenevre), purity >95%. Benomyl was dissolved in corn oil (1.5 mL/kg/rat/day)[15] and was prepared daily depending on the body weight of rats. Administration of benomyl (200 mg/kg/day) was made by gavage for 5 days.

Haematology and biochemistry: At the end of the fifth day, two blood samples were taken from the heart of each rat after cervical dislocation. Blood samples with anti-coagulant EDTA were analysed for haematological parameters [white blood cells (WBC), red blood cells (RBC), thrombocytes (PLT), hemoglobin (HGB) and haematocrit (HCT)] by Coulter STKS Counter.

After centrifugation of the other blood samples at 3500 rpm for 10 min, serum was seperated. Serum enzyme activities [alanin aminotransferase (ALT), aspartat aminotransferase (AST), alcaline phosphatase (ALP), amylase (AMYL), lactat dehydrogenase (LDH), creatin phosphokinase izozyme (CK-MB) and gamma glutamyl

Corresponding Author: Dr. Tülin Aktaç, Department of Biology, Faculty of Science and Art, Trakya University, 22080 Edirne, Turkey
Tel: 90 284 2352824 Fax: 90 284 2354010

transferase (GGT)] were measured using Synchron LX20 otoanalyzor and Becman Coulter kits.

The result of biochemical, hematological, body weights and organ weights were analyzed by student-t test.

Histopathology: At the end of the experiment period, the liver samples were fixed in formalin (10% solution) for 24 h and then embedded in parafin wax. Sections of 5 μm thickness were cut and stained with hematoxylin-eosine.

RESULTS AND DISCUSSION

Body weights were not changed significantly, female liver ($p<0.01$) male spleen ($p<0.01$) and liver ($p<0.001$) weights were increased significantly (Table 1).

An important reduction was observed in the amounts of RBC, HGB and HCT of male and female rats, the other biochemical parameters (WBC and PLT) were not significantly different from controls. In the male rats, ALT, ALP, CK-MB and AMYL activities were changed statistically. In the female rats, ALT, AST, CK-MB, AMYL and LDH activities were changed statistically. There was no significant change in GGT activities of male and female rats (Table 2 and 3).

The results of the light microscopic investigation showed that the liver of rats treated with benomyl has necrotic changes, compared to the control group (Fig. 1 and 2). These changes were cytoplasmic vacuolisation, picnotic nuclei, enlargement of sinusoids, disintegrated membrane. In addition, we also observed the degeneration of the blood vessel endothelium.

In the present study, the benomyl-induced (200 mg/kg/day) haematological and histopathological changes on the liver of male and female rats were investigated. No significant change in the body weight of rats with benomyl was determined. It was reported that the decrease of the body weight of pregnant rats was induced by 1000 mg/kg/day dosage of benomyl[16]. This reduction in body weight may be attributed to the dosage of benomyl and to the physiological states of the animals.

The male rats that received benomyl (125-1000 mg/kg/day) treatments during prepuberty, showed no significant effects in liver, kidney and testis weights[17]. Also, Dalvi[12] reported that 100 mg/kg/day benomyl given intraperitoneally did not cause a significant change in the liver weight of rats. In contrast, a significant increase in the liver weight was observed in female rats after exposure with benomyl (1000 ppm and 4000 ppm) for 15 days. Also, it was reported that the liver,

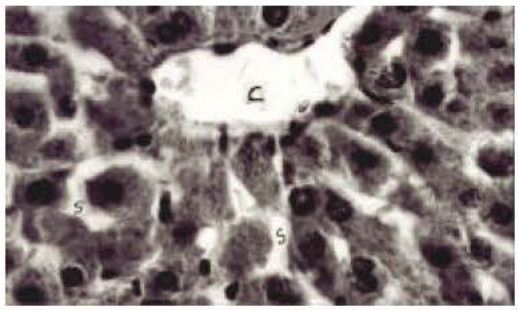

Fig. 1: The liver tissue of rats in control group. (×400) c: central ven, s: sinusoids

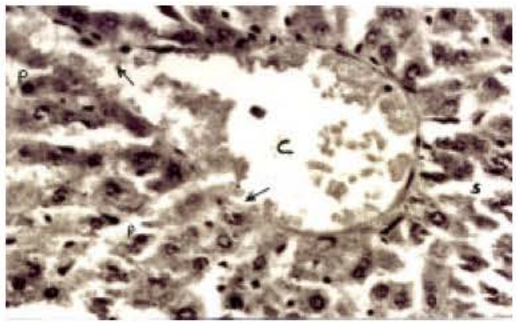

Fig. 2: The liver tissue of rats in treatment group. (×200) c: central ven, degeneration of the central ven endothelium (arrows), s: enlargement of sinusoid, p: picnotic nuclei

kidney and spleen were enlarged in rats that have been fed with different doses for 7 days between 40 and 600 mg/kg/day. The liver showed the highest enlargement[14]. These results are similar with our results. We found that the liver weights were increased in male and female rats, treated with 200 mg/kg/day benomyl for 5 days. In addition, there was an increase in the spleen weight of male rats. Gray *et al.*[18] reported that 400 mg/kg/day carbendazim (benomyl metabolite) did not cause any change in weight of liver and kidney. In another study, there was no effect on the tissues weights of rats, treated with 150, 300 and 600 mg/kg/day. carbendazim for 15 weeks[19]. The difference in the results may be due to exposure time and main fungicide used or its metabolite.

In the haematological analysis, results showed that benomyl decreases the RBC, HBG and HCT values in male and female rats. The decrease in red blood cells (together with HBG and HCT) may indicate a disruption of erythropoiesis or destruction of RBC[20]. Similarly, it was reported that carbendazim caused a dose-dependent decrease in RBC, WBC and lymphocyte values in rats[19].

The significant changes in the liver enzyme activities, markers of liver toxicity, in male rats (increase of ALT,

Table 1: Body weights and organ weights of rats in control and treatment groups

	Female		Male	
	Control (g)	Treated (g)	Control (g)	Treated (g)
Body weights at start	172.00±4.60	196.00±4.00	182.80±4.44	209.40±4.16
Final body weight	170.00±4.66	195.00±3.1	180.00±16.53	211.80±1.38
Liver	6.86±0.22	8.59±0.47*	6.28±0.25	10.56±0.34**
Kidney	0.84±0.03	0.92±0.04	1.09±0.12	0.86±0.03
Spleen	0.48±0.03	0.48±0.04	0.43±0.04	0.66±0.06*
Ovarium	0.09±0.02	0.07±0.01	-	-
Testes	-	-	1.22±0.03	1.35±0.06

*p<0.01, **p<0.001

Table 2: Results of biochemical analysis of rats in control and treated groups

	Female		Male	
	Control (U mL^{-1})	Treated (U mL^{-1})	Control (U mL^{-1})	Treated (U mL^{-1})
ALT	68.4±1.77	85.2±10.49 **	43.4±4.19	74.4±5.24*
AST	125.6±8.96	244.8±45.94***	152.4±10.06	143.0±4.66
ALP	289.0±29.17	257.8±39.4	182.2±17.4	392.4±30.4**
CK-MB	962.0±238.3	1643.6±102.9***	1419.8±134.1	1024.6±162**
AMYL	862.8±35.2	1647.2±314.4***	680.0±52.51	988.8±36.61***
LDH	617.3±230.7	2157.6±82.61**	1559.4±153.71	1683.2±329
GGT	1.4±0.25	1.8±0.37	1.4±0.46	2.0±0.32

*p<0.01, **p<0.001, ***p<0.05

Table 3: Results of haematological analysis of rats in control and treated groups

	Female		Male	
	Control	Treated	Control	Treated
WBC (×10^{3}µL)	6.14±0.72	5.82±0.78	7.36±1.12	6.16±1.24
RBC (×10^{6}µL)	7.79±0.08	6.81±1.78**	8.22±0.10	7.12±0.17**
PLT (×10^{3}µL)	1092.00±26.4	1313.00±112.5	868.00±61.47	973±62.64
HGB (g dL^{-1})	15.62±0.18	12.18±0.36*	14.18±0.32	12.96±0.36***
HCT (%)	39.88±0.56	34.66±0.9**	41.22±0.64	36.2±0.84*

*p<0.01, **p<0.001, ***p<0.05

ALP, LDH and AMYL) and female rats (increase of AST, ALT, LDH, AMYL and CK-MB) were determined. Similarly, Igbedioh and Akınyele[13] reported that AST, ALT and ALP enzyme activities increased in rats, treated with benomyl. In contrast, it was reported that the rats treated with carbendazim have no change in AST, ALT, ALP and AMYL activities. As a result, it can be indicated that benomyl is more effective than carbendazim.

GGT, which catalyses the transfer of gamma-glutamyl group to a wide variety of amino acid acceptors[21] is localized in the focal areas of hepatocytes[22]. GGT is used widely as a marker enzyme which is found to be raised in the preneoplastic lesions of the liver during chemical carcinogenesis[23]. Abnormally high levels of GGT were observed in tumors of a variety of tissues, including hepatocellular carcinomas[24,25]. Shukla *et al.*[10] reported that the increase in serum and liver GGT levels of rats was determined and that this result was indicative of a toxic or preneoplastic response of the liver to benomyl. In contrast it was shown that serum GGT levels in chickens, treated with benomyl, was remained unchanged. In the present study, there was no significant change in serum GGT levels of male and female rats, treated with benomyl. Therefore, it might be indicated that benomyl has no carcinogenic effect in rats.

The microscopic investigation showed that benomyl caused histopathological changes in the liver of rats. We observed enlargement of sinusoids, vacuolation of hepatocyte cytoplasm, nuclear membrane invaginations, picnotic nuclei and degeneration of blood vessel endothelium in the liver of treated rats. In previous studies, similar results were seen as an oral treatment of benomyl or its metabolite, carbendazim[13,14,20].

Finally, we can conclude that the dose of 200 mg/kg/day of benomyl is toxic, but not carcinogenic for the male and female rats and that the effect of benomyl differs from male to female. Recently, since, benomyl has an antimitotic effect and has relatively low toxicity, it was reported that benomyl might be useful as an adjuvant in cancer chemotherapy[1].

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