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Original Article

Endosulfan and N-nitroso-N-methylurea could cause hepatomegaly and kidney tumor in rats*

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Abstract

Aim / Background: Pesticides and nitrites which could be available in foods are generally investigated individually, although they could be found in foods together and exhibit combined health effects. In this study we aimed to determine the combined effects of endosulfan which is a commonly used pesticide and N-nitroso-N-methylurea (NMU) which is a prototype for mammary tumor development in Sprague Dawley rats and could be formed in brine foods or also in body from nitrites which is commonly used in meat.

Method: Ninety female Sprague Dawley rats had been used. Animals were divided three groups which consisted 30 animals. The first group took a single dose endosulfan intraperitoneal (ip) on 19th day and a single dose NMU ip on 21 day, the second group took endosulfan by their drinking waters for the first 60 days of their life and a single dose NMU ip on 21 day, and the third group took only a single dose NMU ip on 21 day. Animals were controlled for their general health status, mammary mass development, daily water consumption and weekly weight gain. At the end of the study rats were examined carefully under anesthesia, and tissue samples were prepared from their mammary glands and intraabdominal organs. Tissue samples taken from mammary glands, liver and kidneys were histopathologically examined.

Results: It was determined a statistically significant hepatomegaly in the second group when we compared to other groups ($p < 0.05$). No animals developed mammary tumors. On the macroscopic examination and manual palpation of intraabdominal region, it was showed that solid tumors in kidneys as a ratio of 76.7% of the animals in the second group (bilateral in 1,3% of them, the others are unilaterally and 70% of the unilateral tumors are in the left kidneys while the rest of them are right localized). Also it was found that in the first group, 13.4% of the animals developed unilateral kidney tumors. The third group which had been applied only NMU developed no kidney or liver pathology macroscopically.

Conclusions: As a result it was considered that a diversity which is named as "genetic shift" could be developed in our animal research center and because of this shift we could not developed NMU induced mammary tumors in our Sprague Dawley rats. But it has been showed that long term endosulfan expose has resulted in kidney tumor development for the first time. Because of the genetic shifting mentioned above, it should be tested whether this experimental animal cancer model is limited for our research center or not by obtaining sprague dawley animals from different centers. Consumers and appliers should be educated about using pesticides, and healthy and safety food production and consumption should be considered as one of the primary subjects in public health studies.

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INTRODUCTION

Pesticides are toxic substances commonly used in order to eliminate harmful effects of animals or plants

in agriculture or daily public life. Pesticides are generally toxic to more than one species and could be detrimental to humans as well as to wildlife [1-5]. Although they are used widely, there are efforts to limit

their use. Pesticide residues on the crops could be considered as external toxic chemicals contaminants. Endosulfan is one of the commonly used pesticides in the agriculture, and also it was found in water sources, fish products, meat products, milk, air and falling as well as vegetables and fruits because of contamination [4-12].

Beside pesticides, there are a lot of chemicals which we add to foods through food processing and manufacturing. They have to be healthy for human and there are some regulations about these food additives. Nitrates and nitrites are the food additives which have special interest [13]. Because they are known as toxic for human, but they are superior to the other preservatives for meat and meat products [6]. There are some studies about the production of cancerogenic nitroso compounds in meat products which have contain nitrate/nitrite [14-16].

Pesticides and nitrites which could be available in foods are generally investigated individually, although they could be found in foods together and exhibit combined health effects. In this study we aimed to determine the combined effects of endosulfan which is a commonly used pesticide and *N*-nitroso-*N*-methylurea (NMU) which is a prototype for mammary tumor development in Sprague Dawley rats and could be formed in brine foods or also in body from nitrites which is commonly used in meat [17, 18].

MATERIAL AND METHODS

Ninety female Sprague Dawley rats had been used. All studies on rats conformed to the principles of the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources) [19] and study and methods were approved by local ethical committee. The laboratory animals used in the study were provided by the Medical Faculty. Sprague Dawley rats were divided three groups which consisted 30 animals. The first group took a single dose (2 mg/kg) endosulfan (Dr. Ehrenstorfer GmbH, Germany, Catalog No: 13120000) intraperitoneal (ip) on 19th day and a single dose (75 mg/kg) *N*-Nitroso-*N*-methylurea (NMU) (Sigma No: N1517, Sigma, Germany) ip on 21 day, the second group took endosulfan by their drinking waters for the first 60 days of their life (3 mg/kg/day) and a single dose (75 mg/kg) NMU ip on 21 day, and the third group took only a single dose NMU ip on 21 day.

Animals were controlled for their general health

status, mammary mass development, daily water consumption and weekly weight gain for 90 days. At the end of the study rats were examined carefully under anesthesia (ip ketamine (50 mg/kg) and dehydrobenzoperidol (2 mg/kg)), and tissue samples were prepared from their mammary glands and intraabdominal organs. Tissue samples taken from mammary glands, liver and kidneys were histopathologically examined. Sections taken from the samples fixed in formalin were stained with Hematoxylin and Eosin for examination.

All the obtained data were transferred to the computer and analyzed statistically with the SPSS for Windows 11.0 software. The data have been presented as mean \pm standard deviation (SD). By using frequency distribution graphs, and skewness and kurtosis statistics which describe the shape and symmetry of the distribution, it was decided that our data were parametric. So, parametric tests (ANOVA and chi-square tests) were used to compare the groups.

RESULTS

During the study no animal was died for any reason and the study was ended with 90 female Sprague Dawley rats. Anesthesia was applied before obtaining tissue specimens and the abdomen was examined with detail. Among the animals in second group exposed to NMU plus endosulfan in top water during 60 days, 76.7% of female SD rats were encountered renal tumor (1.3% bilaterally, others single side, among the single side ones %70 on left, 30% on right) when examined via palpation and macroscopically.

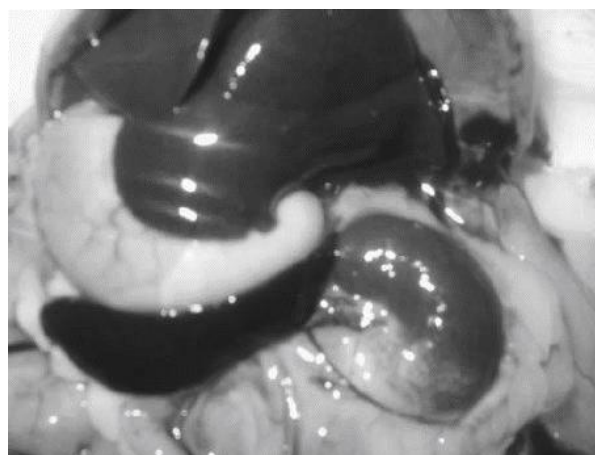


Fig. 1 Kidney with tumor from intra abdominal view

When examined macroscopically and via palpation, the first group those took single dose endosulfan together with single dose NMU, single side renal tumor was encountered 13.4% of the animals. Although second group animals had severe hepatomegaly, animals in first group have also been observed to have relatively bigger liver. Group 3, exposed NMU only, had normal liver and kidney when examined macroscopically and via palpation.

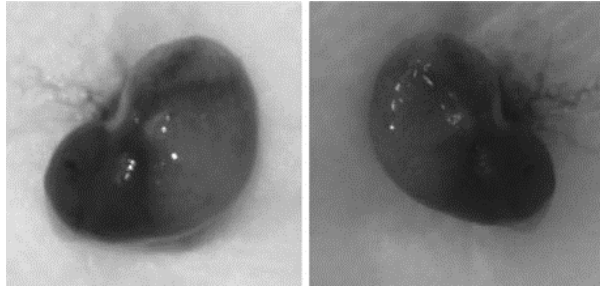


Fig. 2 Kidney with tumor after excision from abdominal wall

Daily water consumption of rats were followed and changes in daily water consumption depending on the endosulfan in top water or chemicals exposed to animals were examined closely. Although, water consumption according to groups varied between 42.7 ± 4.8 and 46.3 ± 5.6 ml/day there was no statistical significance for water consumption among groups ($p > 0.05$). Gaining weight ratio among groups was statistically insignificant ($p > 0.05$) although the group taken endosulfan with top water has less weight gain (Figs. 1 and 2). All rats' livers were weighted after removal and liver/body weight ratios were obtained. Diffuse hepatomegaly was encountered the groups first and second those exposed to endosulfan. Animals in groups those took endosulfan during 60 days had higher liver/body weight ratios than others and statistically significant difference was observed ($p < 0.05$) (Table 1).

Table 1 Female SD rats liver/body weight ratios according to groups

	Group 1	Group 2	Group 3
Liver/body weight ratio	0.0165 ± 0.0017	$0.0191 \pm 0.0023^*$	0.0125 ± 0.0031

* statistically significant difference had been encountered in 2nd group as compared with others ($p < 0.05$)

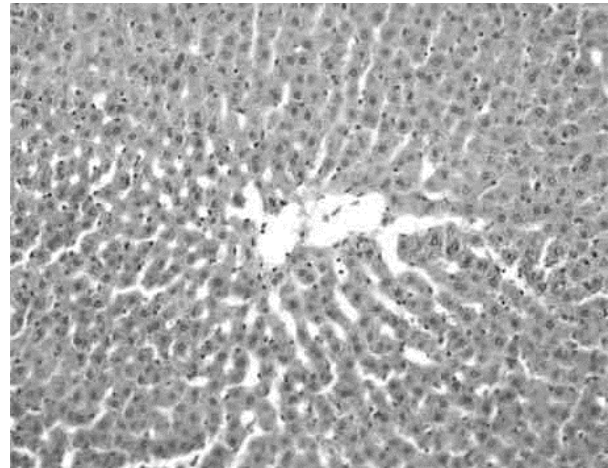


Fig. 3 A microscopic liver specimen view belonged to animals those took endosulfan during 60 days and single dose NMU at day 21st (2nd group). Portal vein at center and sinusoids widening were more pronounced than those first group liver exposed to single dose i.p. endosulfan at day 19th plus single dose NMU at day 21st (H&E 100x)

In group 1 and group 2 liver specimens, observation of sinusoidal widening was supported the hepatomegaly when examined histologically. Among animals those took endosulfan with top water and single dose NMU, sinusoidal widening of 2nd group animals were larger than those group 1st animals and hepatomegaly of this group was more severe (Table 1) (Fig. 3).

Infiltrative tumor had been established after histopathologic examination of the specimens of animals those had renal tumor. The tumors had been observed to have relatively higher mitotic frequency and infiltrated the normal glomerulus and renal tubuli. No pathology had been observed in animals those took single dose NMU when examined both macroscopically and microscopically (Fig. 4).

No tumor or growing breast had been encountered when breasts of female Sprague Dawley rats had been examined weekly and end of the study. However, breast tissue specimens were taken and examined microscopically. Breast tissues taken from animals had been accepted normal after specimens examined histopathologically, no fibrocystic changes or atypical characteristic related to carcinogenic improvement had been encountered. No calcification had been encountered in any specimens. Because of the possibility of histologic differences between human and Sprague Dawley rats breast tissues, related references were used for making true histopathologic examinations during evaluation [20, 18, 21].

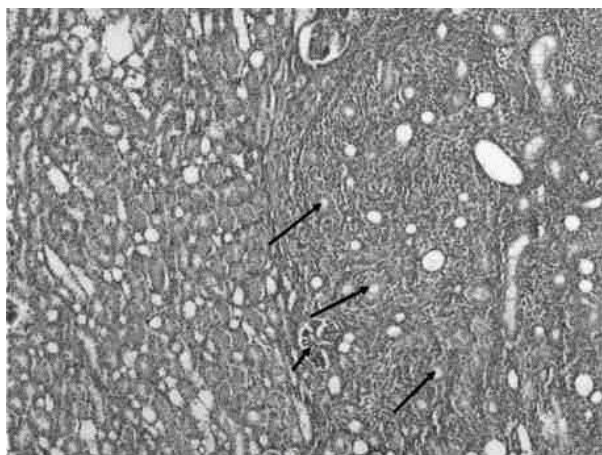


Fig. 4 Infiltrative tumors (right side of picture with arrows) together with normal renal tubuli. Renal tumors infiltrating normal glomerulus (short arrow) and normal renal tubuli (long arrows) could be seen (H&E, 50x)

DISCUSSION

Although developing of NMU induced mammary tumors would be expected in Sprague Dawley rats, we could not detect any mammary tumors [22, 23, 24, 18]. It was considered that a diversity named as “genetic shift” could be developed in our animal research center and because of this shift, we could not develop NMU induced mammary tumors in our Sprague Dawley rats [25, 26].

In this study, it has been showed that long term endosulfan exposure has resulted in kidney tumor development for the first time. It was known that NMU could be harmful for liver and kidney, but there is no study that shows tumor development resulting from NMU exposure [28]. Similarly, we did not find any tumor development in the liver or kidney of the animals which belong to the group that was exposed to NMU only. Also there was no histopathologic changes in these animals. But there are definite changes in the other animals that were exposed to endosulfan, and the hepatomegaly and diffuse hyperplasia in the second group that takes endosulfan chronically, are more prominent; and these changes are compatible with previous studies [29]. So, we could conclude that these changes in animals are resulted from endosulfan exposure. Also, it could be declared that we found a new tumor development method: the female SD rats that are exposed to oral administration of endosulfan (3 mg/kg/day) for 60 days and applied i.p. NMU (75 mg/kg) at the 21th day of their life could produce kidney tumor(s) at a rate of 75%.

Because there are no scientific documentation about kidney tumor development in rats resulting from endosulfan, new studies should be planned to explain

mechanism of kidney tumor development. And also it should be well documented whether there is an interaction between endosulfan and NMU.

Interactions of living style, environmental factors and genetic properties in the development of cancer and its necessary to control all of three factors in order to reduce cancer frequency in public showed that the importance of interdisciplinary collaboration and public education [8, 27]. Consumers and appliers should be educated about using pesticides, and healthy and safety food production and consumption should be considered as one of the primary subjects in public health studies.

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