African Journal of Biotechnology Vol. 10(12), pp. 2290-2294, 21 March, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2509
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Investigation of the ecotoxicologic effect of pesticide industry wastewater on the pancreas and liver of rats

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Accepted 15 March, 2011

In this study, when the raw wastewater, which resulted from the manufacturing of 2,4-dichlorophenoxyacetic acid (2,4-D) dimethylamine salt and 2,4-D acid isooctylester herbicides in the factory, was discharged to the ecosystem without treatment, its ecotoxic effect on the pancreas and liver of rats was investigated. In this research, 16 Wistar Albino race male rats were grouped into two (Group 1- control, n = 8; Group 2- wastewater, n = 8) and were then used. The rats in Group 1 were fed by standard feed, while rats in Group 2 were fed by a diet including 200 mg/kg/day factory composite raw wastewater for 16 weeks, and dissection was carried out for all of them. In the research, it was determined that the body, liver and pancreas weights of rats were decreased when compared to the control group; however, there was no significant statistical difference (P > 0.05) between the two groups. In the histopathological investigation, on the other hand, it was determined that atypical cell focuses (ACF) (neoplastic variations) were observed in the liver and pancreas of rats in Group 2 and the quantitative analysis of the ACF was performed. In the livers of rats in Group 2, dilation in sinusoids close to the vein centralis and hydropic degeneration in parenchyma were observed when compared to the control group. Since there is a possibility that the neoplastic variations caused by this wastewater could be transformed into adenoma or carcinoma during long-term treatment, it also seems possible that it could be carcinogenic.

Key words: 2,4-Dichlorophenoxyacetic acid (2,4-D), herbicide, wastewater, atypical cell focuses, pancreas and liver, ecotoxic effect.

INTRODUCTION

2,4-Dichlorophenoxyacetic acid (2,4-D) group herbicides have been used in agriculture and forestry since 1940 (Garabrant and Philbert, 2002). The common usage of these herbicides displaced the mechanical selection of weeds (Khalil, 2003). It was asserted that 2,4-D group pesticides are moderately stable chemicals, their half-lives vary between 20 and 200 days, they cause deceleration in the development of products to which they are applied and they can be determined in the areas even after they have been applied (Donald et al., 1999). Since 2,4-D group pesticides can be removed by rain and irrigation water, it is known that their residues can be determined in streams in rural areas and in great divides (Sierra Club of Canada, 2005). The recent studies showed that both pesticides used in agricultural areas and toxic chemical substances, as well as liquid and industrial wastes mix with streams, sea and lakes, in various ways affect small and big mammalians which are fed with this water, and even human beings (Yönten, 2006). Such pesticide pollution, in many countries, can be observed in increasing amounts in water and soil as a result of its uncontrolled and unaware usage in Turkey. In pesticide production plants, substantial amount of pesticides is given to the discharge systems after washing the boilers. It is assumed that every year, approximately 20,000 tonne of pesticides from pesticide industries and

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from the imported pesticides is discharged to water and land without any treatment all over the world. More or less, 20% of these pesticides, on the other hand, resulted from industrial usage (Göktürk, 2007). In order to increase the efficiency per unit area in agriculture, the herbicides which are offered to the market cause universal qualified environmental problems such as acute and chronic poisoning, biological unbalance, environmental pollution and food contamination, teratogenic, mutagenic and carcinogenic effects towards human beings and animals (Özdas et al., 2006). The number of scientific studies showing that the agricultural usage of pesticides have negative ecological effects and toxic effects on living things in the medium and environment and on the immune system and different tissues, respectively increases day by day (Sharma et al., 2005; Monteiro et al., 2006; Alexander et al., 2007; Sahinkaya et al., 2007; Kalipci et al., 2010a; 2010b; 2010c). In the consideration of studies performed on human beings with 2,4-D, it was determined that it causes two types of cancers. These are soft tissue sarcoma and non-Hodgkin lymphomas (NHL). The studies revealed that there was a relationship between the usage of 2,4-D analog herbicides and the occurrence of soft tissue sarcoma (Vineis et al., 1986; Woods and Polissar, 1989). It was shown that soft tissue sarcomas occurred in human beings who had an interaction with 2,4-D for long years and that the rate of becoming cancerous was high in the stomach, pancreas, lung, skin and urinary bladder (Sarma and Jacobs, 1982; Olsson and Brabdt, 1981; Axelson et al., 1980; Hogstedt and Westerilund, 1980). Zahm et al. (1990) concluded that the rate of non-Hodgkin lymphomas in workers who applied 2,4-D analog herbicides in agricultural areas was higher than the normal populations. Coggon et al. (1991) suggested that, the risk for the occurrence of various cancers in workers who were exposed to 2,4-D group herbicides partially increased. Lynge (1985) showed that the rate of malignant lymphoma was higher than normal populations in his researches on workers who worked more than ten years in two factories producing 2,4-D herbicides in Denmark.

In this study, when the composite raw wastewater of a factory producing pesticide during production of 2,4-D acid dimethylamine salt and 2,4-D acid isooctylester is given to the ecosystem without any treatment or is transported to the ecosystem indirectly, its ecotoxic and/or cancerogenic effect is investigated in the pancreas and liver of Wistar Albino race male rats.

MATERIALS AND METHODS

Experimental animals

In this research, 14-day old Wistar Albino race male rats, the weights of which varied between 22 and 30 g, were used (n = 16). The rats were grouped into two comprising 8 rats in each group. These animals were kept as groups with a maximum of 4 rats in special rat cages at approximately 24°C room temperature. Consequently, 12-h artificial light and 12-h darkness were applied in order to provide their regular biological rhythm. Before starting the experiments, an approval from the research ethics committee was given on the 25th of June, 2009, and 2009/39 numbered decision was taken from the "Experimental Animals Ethics Committee" of Selcuk University's Medical Faculty Experimental Medicine Research and Application Center. The experimental studies were performed by keeping to the instruction of the experimental animal ethics committee.

Application dosages

The rats in control group (Group 1) were fed with standard rat feed (n = 8), while those in wastewater with pesticide group (Group 2), on the other hand, were fed with a diet that has composite raw wastewater with a rate of 200 mg/kg/day (n = 8). The raw wastewater taken from pesticide factory was collected during 2,4-D acid dimethylamine salt and 2,4-D acid isooctylester production. Feed and water (*ad libitum*) suitable for each group were always present for rats and they were all fed for 16 weeks.

Taking tissue samples and their evaluation

Before starting the dissection of rats, the body weights of rats were recorded. After application of the anaesthetic agents [Ketamine (60 mg/kg) and ether], they were sacrificed by cervical dilation. The livers and pancreases of rats in all groups were taken out as a whole by abdominal distention. After dehydration of pancreases and livers which were spread on an absorbing paper, their body weights were measured and recorded. The pancreases and livers were determined by keeping them in 10% formal solution for 24 h. First of all, general tissue follow-up was applied in order to evaluate the quantitative amount and microscopic determination of atypical cell focuses (ACF) in the livers and pancreases of rats. After general tissue follow-up, the tissues were blocked in hard paraffin and hematoxylen and eosin dye were applied to the sections which were taken in 5 µm thickness from these prepared blocks by rotary microtome (Thermo Scientific, Shandon Finesse 325). These preparates were investigated under Olympus BX 51 Model Research Microscope and the pictures of the necessary regions were taken by Olympus DP12 Model camera. Olympus V.01.03 was used as application software.

Statistical analysis

In the evaluation of the data, SPSS 16.0 statistical package software was used. It was determined by One-Sample Kolmogorov-Smirnov test that the data showed a normal distribution. On the other hand, One Way ANOVA test was used for the determination of difference between groups. In this study, error performance was taken as 0.05. However, a mathematical formula was applied in order to determine the properties (ACF per mm^2, ACF per mm^3, average focus diameter, average focus volume, etc.) of atypical cell focuses (PTSDC; Planar to Spatial Data Converter by Anthony FLAKS).

RESULTS

Results of body, pancreas and liver weights

The body weights and weights of liver and pancreas of rats present in Groups 1 and 2 are given in Table 1. In the group, which was fed with a diet that comprised factory raw wastewater, it was determined that there was a

Table 1. Average body weights together with weights of pancreas and liver of rats in each experimental group (Average ± standard deviation) p< 0.05.

Group	Weight		
	Body weight (g)	Pancreas weight (g)	Liver weight (g)
1 (Control)	305.50 ± 47.138	1.524 ± 0.348	9.753 ± 1.292
2 (Factory raw wastewater)	276.0± 66.513	1.467 ± 0.668	8.320 ± 2.652

Table 2. Quantitative values of AACF occurrence in the pancreas of rats (average ± standard deviation) p < 0.05.

Group	Area of AACF per mm^2	Volume of AACF per mm^3	Rate of AACF size over the entire pancreas size (%)	Average focus diameter (mm)	Average focus volume (mm^3)
1 (Control)	0	0	0	0	0
2 (Factory raw wastewater)	0.149 ± 0.050	0.906 ± 0.142	0.137 ± 0.069	0.128 ± 0.004	0.0011 ± 0.0006

AACF, Atypical acinar cell focuses.

decrease in the body weight and weights of pancreas and liver when compared to the control group.

Histological results

Fundamentally, no atypical cell focuses (ACF), nor atypical acinar cell adenoma nor adenocarcinoma, were encountered in pancreases and livers of rats in the control group (Group 1). When pancreases of rats in factory raw wastewater group (Group 2) were investigated, it was determined that atypical acinar cell focuses (AACF) occurred. In the livers of rats in Group 2, on the other hand, it was determined that ACF were present. Moreover, when compared with the control group, dilation in sinusoids close to the vein centralis was observed in the livers of rats in Group 2. However, cell adenoma or adenocarcinoma was not encountered in the rats of Group 2.

Quantitative analysis

Quantitative values of ACF formed in the rats in each experimental group were determined and are given in Tables 2 and 3.

DISCUSSION

Herbicides and insecticides are the most commonly used formulations in the world's pesticide market. In Turkey, while insecticides are at the first rank, herbicides are at the second important part of the pesticides (Özdas et al., 2006). In 2001, 2,4-D active substance application in the entire agricultural sector was at the fifth rank among all pesticides in U.S.A. (Donaldson et al., 2004). When the average usage amounts of 2,4-D between 1999 and 2002 was calculated for Turkey, it was determined that the rate of herbicide consumption by years was 42.68%. Being cheap causes 2,4-D to be still used commonly (Delen et al., 2005).

In a research performed with 2,4-D, it was concluded that any carcinoma was not encountered in the livers of rats since the amount of 2,4-D was 45 mg/kg/body weight/day and less (McClintock and Gollopudi, 1990). In another experimental study, on the other hand, Jefries et al. (1995) fed the mice with 2,4-D diets including 15 and 45 mg/kg/body weight/day for two years. Although they observed that a part of the lesions increased in the kidney and liver of these mice, no carcinogenic structure was encountered.

In this study, pancreas and liver of rats were investigated qua histopathology at the level of light microscope in order to examine the ecotoxicity of wastewater, which resulted during 2,4-D production and thus, their quantitative analysis was performed. It was determined that the weights of body, pancreas and liver of the rats which were fed with feed including pesticide industry wastewater decreased when compared to the control group. It was also confirmed that this difference observed in the weights of body, pancreas and liver between groups was not significant between groups in terms of statistics (P>0.05). When composite factory raw wastewater group (Group 2) and control group (Group 1) were compared, it was observed that atypical acinar cell focuses (ACF) were not present in the pancreas of rats in control group; however, ACFs were observed in the group (Group 2) which was given composite factory raw wastewater in the diet. It was determined that average diameters of these formed focuses were 0.128 ±0.004 mm and their average volumes were 0.0011 ± 0.0006 mm^3 (Table 2). As a result of the histological investigation of rat livers in Group 2, hydropic degenerations in parenchyma of the liver were observed especially more prominently in localized hepatocyte of central vein periphery. In addition to this

Table 3. Quantitative values of ACF occurrence in the liver of rats (Average ± standard deviation) $p < 0.05$.

Group	Area of ACF per mm^2	Volume of ACF per mm^3	Rate of ACF size over the entire liver size (%)	Average focus diameter (mm)	Average focus volume (mm^3)
1 (Control)	0	0	0	0	0
2 (Factory raw wastewater)	0.255 ± 0.072	1.530 ± 0.053	0.210 ± 0.010	0.218 ± 0.009	0.031 ± 0.005

ACF, Atypical cell focuses.

dilation in sinusoids and kupffer cell proliferation was also observed. However, no finding was encountered in the livers of rats in the control group (Group 1) except the histological structures peculiar to this organ. In the livers of rats in Group 2, on the other hand, atypical cell focuses (ACF) were observed. It was determined that average diameters of these formed focuses in the liver were 0.218 ± 0.009 mm and their average volumes were 0.031 ± 0.005 mm^3 (Table 3).

As a result of this study, observation of neoplastically varied cell groups (ACF) in the rats which were fed with a diet that comprised 200 mg/kg/day factory raw wastewater indicates the possibility of these pesticides to initiate cancer. In the study of Kaioumova et al. (2001), it was determined that 2,4-D (DMA) had acute poisoning effect on rats and caused severe damage on lymphatic organs such as spleen and thymus. In the studies performed with 2,4-D, it was confirmed that anorexia, weight loss, vomiting, keratosis on the skin, depression, a general nervousness state and atonia were observed when 2,4-D was given at various dosages one after the other to mice, guinea pigs and rabbits (Hayes and Laws, 1991; Yalcinkaya, 2006). It was indicated that the presence of dioxin azaserin, even if it is in a low amount in the content of these pesticides, caused an increase in the neoplastic variation of pancreas in rats (Öztas, 2000). For this reason, it was possible to put forward that these pesticides might be effective in some way in the increase of pancreas and liver cancers for which an increase was observed depending on the environmental factors.

In this study, it was determined that several ecotoxic effects were observed in the pancreas and liver of rats when raw wastewater, taken from the pesticide industry as a result of 2,4-D acid dimethylamine salt and 2,4-D acid isooctylester production, was given to the ecosystem without any treatment. For this reason, the activity of such industries should be prevented without foundation of a wastewater treatment plant or without actuating the present one. As such, the number of inspections by relevant institutions should be increased. Özdemir et al. (2008) show inhibition of some pesticide that causes toxic effects with physicochemical and fenton processes treatment.

Effects such as industrial wastewater, sewage, direct pesticide application to water surface and mixing of residual chemicals with surface water by accident or purposely by discharging, cause significant increase in pesticide concentrations of reception waters. Pesticide residuals in water and determination of negative effects of decay or conversion products on living things made it necessary to apply and develop various technologies for the treatment of pesticides from wastewater which is discharged to water reception (Balkaya, 1998; Balkaya and Bayrakli, 2000). It is also necessary to perform more extensive researches related with 2,4-Ds to both encourage the need for the research of new technologies about pesticide treatment and minimize their ecotoxic effects on human beings.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Selcuk University Research Fund (BAP) for financially supporting this work (Project No: 09101055).

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