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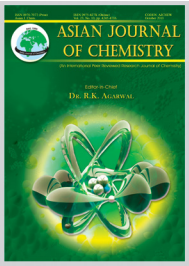

Investigation of Ecotoxic Effect of 2,4-Dichlorophenoxyacetic Acid Dimethylamine on Liver and Pancreas

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In this study, ecotoxic effects of 2,4-dichlorophenoxyacetic acid dimethylamine salt herbicide were investigated on the liver and pancreas of rats. Azaserine-rat model developed by Longnecker and Curphey was used in this research. As a result of this study, hydropic degenerations in parenchyma which were on the point of being more distinct in localized hepatocytes especially in the vena centralis periphery of the liver, dilatation in sinusoids and kupffer cell proliferation were observed. It was found that 200 mg/kg/day dose of 2,4-dichlorophenoxyacetic acid dimethylamine salt caused the formation of neoplastic variations in the pancreas and liver of rats. It was also indicated that the development of neoplastic variations which were experimentally formed by using azaserine was increased (diameter of focus, volume of focus). These results indicate the possibility of these herbicides to be cancer initiator during long exposures.

Key Words: 2,4-Dichlorophenoxyacetic acid dimethylamine salt, Herbicide, Atypical cell focuses, Pancreas, Liver, Ecotoxic effect, Quantitative analysis.

INTRODUCTION

2,4-Dichlorophenoxyacetic acid (2,4-D) herbicides are commonly used for both agriculture and the control of grass and weed all over the world[1-3]. Their esters, on the other hand, are generally used in the areas of pine plantation and cutting areas and in the regions of non-evergreen trees[4]. Uyanikgil[5] indicated that 2,4-dichlorophenoxyacetic acids remained on the soil for 2 to 4 weeks after application, while Öztürk[6] determined that they remained for 1 to 18 months. It was reported by U.S. EPA[7] that the half-lives of 2,4-dichlorophenoxyacetic acids on the soil were 6.2 days whereas Griffin[8] indicated that their half-lives were 10 days.

In the toxicological researches, it was informed that abnormalities in central nervous system, circulation/respiration, urogenital or muscle and skeleton of human beings in West Minnesota were observed more than normal depending on the usage of herbicides produced from 2,4-dichlorophenoxyacetic acid and phenoxy acetic acid[9]. Recently, the studies in Croatia indicated that there was an increase in chromosome abnormalities and brother chromatid variation frequencies in workers who were exposed to compounds of atrazine, malathion, cyanazine and 2,4-dichlorophenoxyacetic acid mixtures[10-12]. In another study[13], it was determined that the brain neoplasm of employees who worked for production, formulation or packaging stages of 2,4-dichlorophenoxyacetic acid was more than normal people. In addition to toxic effects of 2,4-dichlorophenoxyacetic acid group herbicides on birds and beneficial insects, it was determined that aquatic life, little invertebrates, fish, frogs, reptiles and algaes were affected negatively. Since water solubility of 2,4-dichlorophenoxyacetic acid and its absorbance by soil particles are not good, it can leak to underground water[14]. Unconscious usage of agricultural pesticides causes some environmental problems and the residuals of them threaten the lives of every kind of living things by causing environmental pollution[15].

In this study, it was investigated that whether 2,4-dichlorophenoxyacetic acid dimethylamine salt herbicide had ecotoxic or carcinogenic effect on pancreas and liver of rats or not. Moreover, possible effects of 2,4-dichlorophenoxyacetic acid dimethylamine salt herbicide on the development of neoplastic variations were searched by histological techniques after occurring experimental neoplastic variations on the exocrine pancreas and liver of rats with azaserine-rat model.

EXPERIMENTAL

In this research 14-day old Wistar albino race male rats, the weights of which varied between 22 and 30 g, were used (n = 32). The rats were grouped into four including 8 rats in each group. These animals were kept as groups with maximum

4 rats in special rat cages at *ca.* 24 °C room temperature and 12 h artificial light and 12 h darkness were applied in order to provide their regular biological rhythm. Before starting the experiments, research ethics committee approval was taken with 25.06.2009 dated and 2009/39 numbered decision from "Experimental Animals Ethics Committee" of Selcuk University Medical Faculty Experimental Medicine Research and Application Center. The experimental studies were performed by keeping to the instruction of experimental animal ethics committee.

Application dosages and azaserine-rat model applied on experimental animals: The rats in control group (group 1) were fed by standard rat feed. The rats in group 2 were fed by a diet including 200 mg/kg/day dose of 2,4-dichlorophenoxyacetic acid dimethylamine salt. Azaserine-rat model developed by Longnecker and Curphey[16] was used in order to investigate experimentally occurred neoplastic variations in pancreas and liver cells. 0.3 mL injectable water soluble azaserine (30 mg/kg body weight) was injected to 2 week rats in group 3 (azaserine control) and group 4 (azaserine + 200 mg/kg/day 2,4-dichlorophenoxyacetic acid dimethylamine salt) experimental groups for the formation of carcinogenesis in their pancreas and liver cells. Other groups were injected with only 0.3 mL injectable water. Injection was applied intraperitoneal once in a week throughout successive three weeks. During one month subsequent of injection, the formation of atypical cell focuses was possible in exocrine pancreas and liver and after adding 2,4-dichlorophenoxyacetic acid dimethylamine salt to their feed, the effect of this herbicide on occurred neoplastic variations was investigated. After one week from the last injection, the rats were fed with normal standard diet (Purina), a diet including 2,4-dichlorophenoxyacetic acid dimethylamine salt and as *ad libitum* with water for each group as mentioned before. The rats were always fed with feed and water that were suitable for their groups during 16 weeks.

Taking tissue samples and their evaluation: Before starting the dissection of rats, their body weights were recorded. After application of anaesthetic agents [ketamine (60 mg/kg) and ether], they were sacrificed by cervical dislocation. 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 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 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. 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 AND DISCUSSION

Results about body, pancreas and liver weights: The body weights and the weights of liver and pancreas of rats present in group 1, group 2, group 3 and group 4 are given in Table-1. It was determined in the group which was fed by a diet including 2,4-dichlorophenoxyacetic acid dimethylamine salt in the dose of 200 mg/kg/day (group 2) that there was a decrease in body weight and weights of pancreas and liver when compared to control group (group 1). In the group (group 4) to which azaserine was applied and a diet including 200 mg/kg/day dose of 2,4-dichlorophenoxyacetic acid dimethylamine salt was given, it was determined that there was a decrease in the body weights and the weights of pancreas and liver when compared with azaserine control group (group 3).

Histological results: In anyway, atypical cell focuses, atypical acinar cell adenoma or adenocarcinoma were not encountered in pancreases and livers of rats in control group (group 1). When pancreases of rats which were fed by a diet including 2,4-dichlorophenoxyacetic acid dimethylamine salt (group 2) were investigated, it was determined that atypical acinar cell focuses (AACF) occurred. It was observed that the zymogen granule content present in the cytoplasms of atypical acinar cell focuses which had hypertrophic property was denser and dyed dusky-coloured due to their increased acidophilic property. In the livers of rats in group 2, on the other hand, it was determined that atypical cell focuses were present. Moreover, when compared with control group, dilatation in sinusoids close to the vena centralis was observed in the livers of rats in group 2.

TABLE-1
AVERAGE BODY WEIGHTS TOGETHER WITH WEIGHTS OF PANCREAS AND LIVER OF RATS IN EACH EXPERIMENTAL GROUP (AVERAGE ± STANDARD DEVIATION) $p < 0.05$

Groups	Weights		
	Body weights (g)	Pancreas weights (g)	Liver weights (g)
Group 1	305.50 ± 47.138	1.524 ± 0.348	9.753 ± 1.292
Group 2	*294.14 ± 47.217	*1.393 ± 0.290	*8.714 ± 1.506
Group 3	*327.0 ± 32.357	*1.677 ± 0.369	*10.596 ± 1.743
Group 4	**267.0 ± 42.289	**1.440 ± 0.405	**8.298 ± 1.985

*Different value than the control group; ** Different value than the azaserin control group.

When pancreases of rats in azaserine group (group 3) were investigated, it was observed that atypical acinar cell focuses were formed as a result of azaserine application and they were separated distinctly from acinar cells phenotypically that formed normal parenchyma in their environment. In the livers of rats in this group, it was determined that atypical cell focuses occurred and atypical cell adenoma or adenocarcinoma were not encountered in anyway.

When pancreases of rats in azaserine + 2,4-dichlorophenoxyacetic acid dimethylamine salt group (group 4), it was observed that atypical acinar cell focuses occurred. In the livers of rats in this group, on the other hand, it was determined that atypical cell focuses were formed and atypical cell adenoma or adenocarcinoma were not encountered in anyway.

Quantitative analysis: The quantitative values of atypical cell focuses formed in rats of each group were determined and are given in Tables 2 and 3. When control group (group 1) and 2,4-dichlorophenoxyacetic acid dimethylamine salt group (group 2) were compared, atypical cell focuses were not present in the pancreas and liver of rats in control group whereas atypical cell focuses were observed in the pancreas and liver of rats in the group (group 2) to which 2,4-dichlorophenoxyacetic acid dimethylamine salt was given within their diet. As a result of comparison for azaserine + 2,4-dichlorophenoxyacetic acid dimethylamine salt group (group 4) and azaserine control group (group 3), it was observed that atypical cell focus load was significantly increased in all quantitative parameters belonging to the pancreas and liver of rats in azaserine + 2,4-dichlorophenoxyacetic acid dimethylamine salt group (Tables 2 and 3). From this point of view, it was concluded that 2,4-dichlorophenoxyacetic acid dimethylamine salt caused formation of atypical cell focuses in the pancreas and liver of rats and it seemed possible to increase the focus diameter, focus volume of experimentally formed atypical cell focuses and atypical cell focuses per mm^2 and per mm^3 by using azaserine.

When body weights, liver and pancreas weights of groups participated in the research were compared, it was determined that there was a difference in the weights of body, liver and pancreas between groups, however, there wasn't a significant difference between groups in terms of statistics ($p > 0.05$). In the research of Tayeb *et al.*[17], 2,4-dichlorophenoxyacetic acid was applied to the rats by oral gavage in dosages of 15, 75 and 150 mg/kg for 4 weeks. When 2,4-dichlorophenoxyacetic acid applied rats were compared with control group, it was determined that there was a decrease in the body weights and there was an increase in the weights of liver after 4 weeks. The data related with body weights obtained in present research showed parallelism with the study of Tayeb *et al.*[17]. In another study, Ates *et al.*[18] applied 2,4-dichlorophenoxyacetic acid to the rats by oral gavage in dosages of 20, 40 and 80 mg/kg/day for 28 days. As a result of the study, there was a decrease in the body weights of the groups to which 40 mg/kg/day and 80 mg/kg/day dosages were applied and when the liver weights of rats were compared, it was determined that there wasn't a significant difference in terms of statistics. The results of this study were in accordance with present study.

In the study of Sulik *et al.*[19], it was determined that subacute poisoning was observed in rats which were exposed to 2,4-dichlorophenoxyacetic acid, it caused hepatoxicity, histochemical and histological variations were observed in the livers and these variations were more especially in new-born rats. In another study, it was suggested that histopathological variations observed in the livers of rats which were fed with 2,4-dichlorophenoxyacetic acid on the basis of 100 and 150 mg/kg/day might depend on the carcinogenic property of this substance[20]. Longnecker and Webb[21] indicated that multiple focal nodular acinar cell dysplasias were more common in the pancreas of pancreatic cancer patients than control groups. These researchers reported some histological similarities between lesions occurred in rats by azaserine and these dysplastic acinar cell focuses observed in human beings. For this reason, neoplastic variations occurred in acinar cells are essential in terms of origin of adenocarcinomas observed in human beings[22]. In a research performed by Longnecker[23], it was informed that acinar cell focuses were changed into acinar cell nodules, adenoma and carcinoma from time to time when

TABLE-2
QUANTITATIVE VALUES OF ATYPICAL ACINAR CELL FOCUSES (AACF) OCCURRED IN THE PANCREAS OF RATS (AVERAGE ± STANDARD DEVIATION) $p < 0.05$

Groups	Area of AACF per mm^2	Volume of AACF per mm^3	% Rate of AACF size over the whole pancreas size	Average focus diameter (mm)	Average focus volume (mm^3)
Group 1	0	0	0	0	0
Group 2	*0.140 ± 0.021	*0.711 ± 0.127	*0.128 ± 0.032	*0.100 ± 0.003	*0.0009 ± 0.0001
Group 3	*0.158 ± 0.038	*0.961 ± 0.134	*0.147 ± 0.035	*0.132 ± 0.002	*0.0012 ± 0.0001
Group 4	**0.187 ± 0.040	**1.137 ± 0.162	**0.172 ± 0.014	**0.161 ± 0.004	**0.0014 ± 0.0002

*Different value than the control group; **Different values than in the azaserin control group as well as control group.

TABLE-3
QUANTITATIVE VALUES OF ATYPICAL CELL FOCUSES (ACF) OCCURRED IN THE LIVER OF RATS (AVERAGE ± STANDARD DEVIATION) $p < 0.05$

Groups	Area of ACF per mm^2	Volume of ACF per mm^3	% Rate of ACF size over the whole liver size	Average focus diameter (mm)	Average focus volume (mm^3)
Group 1	0	0	0	0	0
Group 2	*0.200 ± 0.017	*1.216 ± 0.052	*0.190 ± 0.005	*0.185 ± 0.006	*0.028 ± 0.006
Group 3	*0.250 ± 0.045	*1.520 ± 0.128	*0.210 ± 0.019	*0.236 ± 0.008	*0.034 ± 0.004
Group 4	**0.278 ± 0.058	**1.691 ± 0.130	**0.224 ± 0.012	**0.241 ± 0.004	**0.035 ± 0.008

*Different value than the control group; **Different values than in the azaserin control group as well as control group.

present acinar cells were changed into acinar cell focuses. Therefore, it was investigated in this study that whether 2,4-dichlorophenoxyacetic acids form atypical cell focuses or not and their quantitative loads were searched.

As a result of present study, it was concluded that 2,4-dichlorophenoxyacetic acid dimethylamine salt at a dosage of 200 mg/kg/day (group 2) caused the formation of cell groups (atypical cell focuses) which underwent neoplastic change in the liver and pancreas of rats. Moreover, hydropic degenerations in the liver of rats and dilatation in sinusoids were observed. The findings about dilatation in sinusoids were in accordance with the results of Ates *et al.*[18] with 2,4-dichlorophenoxyacetic acids. When pancreas and liver of azaserine control group and azaserine + 2,4-dichlorophenoxyacetic acid dimethylamine salt group (group 4) were compared in terms of atypical cell focus load, it was found that 2,4-dichlorophenoxyacetic acid dimethylamine salt at a dosage of 200 mg/kg/day increased the development of neoplastic structures (the focus volume, diameter, atypical cell focuses per mm^2 and per mm^3) formed by azaserine.

Conclusion

Since there is a possibility for neoplastic variations resulted from these pesticides to change into adenoma or carcinoma during long treatment, it has a probability to be a cancer initiator. According to these results, longer researches should be performed with 2,4-dichlorophenoxyacetic acid dimethylamine salt herbicide in different dosages and it should be determined whether it has carcinogenic effect on human beings or not.

Conflict of interest: The authors declare that there are no conflicts of interest.

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