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Hypoglycemic Effect of Herbicide 2,4-Dichlorophenoxyacetic Acid (2,4-D)

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SUMMARY

Herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic auxin which promotes uncontrolled plant growth is widely used. The aim of our study was to investigate, using an experimental model, the effect of herbicide 2,4-D on liver function tests, enzyme a amylase and glucose blood level. BALB/C mice were treated i.p. with the herbicide (30 mg/kg 2,4-D) for four consecutive days. Twenty-four hours after the last injection, the treated and the control animals were weighed and sacrificed for biochemical analysis: haematocrit, glucose blood level, serum activities of enzymes alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, and a amylase, as well as liver reduced glutathione. Herbicide 2,4-D significantly decreased glucose blood level in mice. There were no changes in liver function tests or activity of enzyme a amylase. In this study on mice we confirmed the results obtained in the previous study, which showed a hypoglycemic effect of herbicide 2,4-D on agricultural workers. To elucidate the mechanism of this effect, a further research is needed.

Keywords: 2,4-Dichorophenoxyacetic acid; Herbicide; Glucose; Liver; Mice

INTRODUCTION

One of widely used herbicides is 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic auxin which promotes uncontrolled plant growth. The highest levels of exposure to 2,4-D occur in occupational settings, the amount of herbicide absorbed depending on the type of work and safety measures used. Toxic mechanism of 2,4-D is still poorly understood in mammals (Garabrant and Philbert, 2002). Herbicide 2,4-D increases lipid peroxidation in animal and human cells in vitro (Palmeira, 1995; Bukowska 2006).

Our previous study showed that concentrations of thioethers in urine were significantly higher among agricultural workers after occupational exposure to the herbicide containing 2,4-dichlorophenoxyacetic acid dimethylamine (2,4-DMA) and in experimental animals treated with the same herbicide (Mikov, 2000). It is possible that herbicide 2,4-D initiates its hepatotoxic effect via depletion of reduced glutathione (GSH) in hepatocytes (Palmeira 1994, 1995). Paulino et al. reported that 2,4-DMA had a hypoglycemic effect in rats (Paulino, 1996) and a hyperglycemic effect in cattle (Paulino, 1995). The International Programme on Chemical Safety (IPCS) Task Group for Environmental Health Criteria for 2,4-D reported that high doses of 2,4-D can affect glucose metabolism, and produce hypo- or hyperglycemia in humans (WHO, 1984). Our study of agricultural workers showed that glucose blood level was significantly decreased after occupational exposure for 2-4 days to herbicide containing 2,4-DMA (Mikov, 1999).

The aim of the present study was to investigate, using an animal model, the effect of herbicide containing 2,4-DMA on glucose blood level.

MATERIAL AND METHODS

Animals. Albino BALB/C male mice were used, 2-4 months old (Pasteur Institute Novi Sad, Serbia), which were kept in a natural dark-light cycle and fed with standard diet (Veterinarian Institute Zemun, Serbia) and water ad libitum. Herbicide containing 2,4-Dichlorophenoxyacetic acid dimethylamine as active compound (464 g/l 2,4-D and 200 g/l dimethylamine in water) was obtained from ICN Galenika Co (Zemun, Serbia) for use in experiment, since human exposure to herbicide involves not only active substance, but all chemicals present in a commercial formulation. 2,4-D in commercial formulation is of technical quality. Impurities in total are up to 4.8% and they are: 2-chlorophenoxyacetic acid, 4-chlorophenoxyacetic acid, 2,6-dichlorophenoxyacetic acid, 2,4,6-trichlorophenoxyacetic acid, bis-(2,4-dichlorophenoxyacetic acid), 2,4-dichlorophenol, 4-chlorophenol and perchloroetilen.

Experimental design. The animals were divided into experimental and control groups, with 5 mice in each. The experimental group of animals was treated i.p. during four consecutive days with herbicide containing 2,4-D dimethylamine as active compound (30 mg/kg 2,4-D in the form of dimethylamine salt, per body weight on each day), whereas the animals in control group received saline solution. The applied dose was comparable with occupational exposure of humans; in our previous study, it was found that agricultural workers were occupationally exposed to this herbicide for 2-4 days, 5-6 hours per day, in form of aerosol (Mikov, 1999). The mice received during 4 days of the treatment one i.p. LD_{lo} (lowest published lethal dose) of 2,4-D in the form of dimethylamine salt, which corresponds to 120 mg/kg (RTECS, 2000).

Twenty-four hours after the last injection, the treated and the control animals were weighed and sacrificed, and venous blood was used for determination of: haematocrit, glucose blood levels (Jovanovic, 1981) and serum activities of enzymes alanine aminotransferase (ALT) (Harder and Rej, 1983), aspartate aminotransferase (AST) (Rej and Harder, 1983), gamma glutamyl transferase (GGT) (IFCC, 1983) and a amylase (Kaufman and Tietz, 1980). The liver of each animal was removed, blotted by filter paper, weighed and then homogenized in an electric homogenizer in a glass vessel with Teflon pestle. The liver homogenate was used to measure reduced glutathione levels (GSH) (Kapetanovic and Mieyial, 1979). The Ethic Committee of Medical Faculty Novi Sad (Serbia) approved the study.

Statistical analysis. The results were expressed as mean ± standard deviation (SD). Statistical analysis was performed by Student t-test. A mean difference was significant, at the 0.05 level.

RESULTS

The results obtained for the measured haematocrit and glucose in blood, as well as, liver glutathione of the animals are presented in Table 1.

Table 1. Haematocrit, glucose and liver glutathione (GSH) levels in 2,4-D-exposed and control animals

Group	Haematocrit (%)	Glucose (mmol/l)	GSH	
			(umol/g l)	(nmol/g b.w.)
2,4-D-exposed	46.22±5.45	5.08±1.14*	1.40±0.37	77.81±18.80
Controls	44.84±3.39	7.36±0.99	1.13±0.18	60.25±11.48

* significant difference at $p<0.05$ level (n = 5)

The glucose blood level was significantly lower in the exposed group compared to the controls ($p<0.05$).

The results obtained for the measured activities of enzymes alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, and a amylase in the serum of the animals are presented in Table 2.

Table 2. The serum enzyme activity in 2,4-D-exposed and control anumals

Group	ALT (u/l)	AST (u/l)	GGT (u/l)	α amylase (u/l)
2,4-D-exposed	0.35±0.18	2.10±1.40	0.09±0.02	10.74±5.36
Controls	0.28±0.15	1.05±0.44	0.08±0.01	12.30±4.99

ALT = alanine aminotransferase
AST = aspartate aminotransferase
GGT = glutamyl transferase

The activities of enzymes ALT, AST, GGT, and a amylase showed no statistically significant differences.

DISCUSSION

Paulino CA et al, reported that in rats orally treated with very high single dose of 2,4-DMA (600 mg/kg), 8 h and 24 h after treatment, serum concentrations of glucose decreased and serum activities of enzymes ALT and AST increased. Also, serum activity of enzyme a amylase increased 8 h after 2,4-DMA dosing, but did not change after 24 h (Paulino, 1996).

In our present study herbicide 2,4-D significantly decreased glucose blood levels in mice, but there were no changes in liver function tests or activity of enzyme a amylase. The study of Aydin H.et al, showed 2,4-D accumulation in rat liver. However, no significant difference in activities of aminotransferases was found (Ayidin, 2006). Hypoglycemic effect could be explained by stimulatory effect of herbicide 2,4-D on b pancreatic cells or direct hypoglycemic herbicide effect. Since we used a commercial herbicide in this study, it cannot be excluded that some other constituents of the formulation might have influenced the results to some extent.

The results of the presented preliminary study using the animal model confirmed that herbicide 2,4-D exerts a hypoglycemic effect, which was also the conclusion of our previous study involving agricultural workers. To elucidate the mechanism of this effect, a further research is needed. The results of the study emphasize the importance of further investigations in this important domain of herbicide toxicity.

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Hipoglikemijski efekt herbicida 2,4-dihlorfenoksisirćetna kiselina (2,4-D)

REZIME

Primena herbicida 2,4-dihlorfenoksisirćetne kiseline (2,4-D), sintetskog auksina koji podstiče nekontrolisani rast biljke, u svetu je veoma rasprostranjena. Cilj ovog rada bio je da se, korišćenjem eksperimentalnog modela, ispita uticaj herbicida 2,4-D na funkcije jetre, aktivnost enzima a amilazu i nivo glukoze u krvi. BALB/C miševi tretirani su intraperitonealno četiri uzastopna dana herbicidom (30 mg/kg 2,4-D). Dvadeset četiri časa nakon završenog tretmana, tretirane i kontrolne životinje su izmerene i žrtvovane radi određivanja: hematokrita, nivoa glukoze u krvi, serumske aktivnosti enzima alanin-aminotransferaze, aspartat-aminotransferaze, gama-glutamil transferaze i a-amilaze, kao i redukovanog glutationa jetre. Herbicid 2,4-D značajno je smanjio nivo glukoze u krvi kod miševa. Nije nađena razlika u funkcijama jetre ili aktivnosti enzima a-amilaze. Ovi rezultati su u skladu sa rezultatima prethodnog istraživanja kod poljoprivrednih radnika o hipoglikemijskom delovanju herbicida 2,4-D. Radi razjašnjavanja mehanizma ovog delovanja neophodna su dalja istraživanja.

Ključne reči: 2,4-dihlorfenoksisirćetna kiselina; herbicid; glukoza; jetra; miš