The Effect of Indole-3-Acetic Acid on Some Metabolic Enzymes in Kidney of the Second Cross Maternal Mice and Their Offsprings*

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Objective: **Indole-3-acetic acid (IAA) generates reactive oxygen species (ROS) and excited oxygen intermediates. IAA causes renal dysfunction, hypoglycemia, and myotonia. The first aim of the study is to investigate the possible indirect effect of the plant growth hormone IAA on some renal enzyme activities such as hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH) of second cross offsprings of the second cross maternal mice (*Mus musculus*) that are given IAA. The second aim was to investigate the possible direct effect of IAA on the same renal enzymes in second cross maternal mice.**
Method: **Female mice was divided into two groups: IAA administered group and ethanol control group. Two chemicals used were applied intraperitoneally. IAA was administrated to maternal mice as a 1/40 dilution of 300 mg/kg body weights in 3-day intervals. Ethanol was administrated in the controls. Spectrophotometric methods were used to determine the activities of enzymes in the kidney of the second cross-maternal mice and their offsprings.**
Results: **In the second cross-maternal mice, the activities of LDH, MDH, and G6PD were higher in IAA administrated animals than in controls. 6PGD and HK showed decreases in IAA study group compared to control group. In the offsprings of the second cross maternal mice, the activities of G6PD, HK, and MDH enzymes were decreased, and 6PGD and LDH enzymes were increased in IAA group compared to control group. Even so differences were not statistically significant.**
Conclusion: **The results obtained suggested that metabolic enzymes studied were not affected from IAA toxicity. This may be due to low dose of IAA.**

Key words: *IAA, metabolic enzymes,* mus musculus, *kidney.*

The indole-3-acetic acid (IAA) is a major metabolite of tryptophan metabolism (1). It is found in blood, cerebrospinal fluid, and in several organs such as liver, kidney, lungs and brain (2-4). A number of investigation have shown that plasma levels of IAA and its metabolites elevated in some human diseases such as renal dysfunction and phenylketonuria (5-7). There are reports that administration of IAA causes hypothermia and myotonia in mice (4,8) and hypoglycemia in rats and humans (9,10), and also exhibits an anti-inflammatory effect in mice (11). In several studies, it has been shown that IAA generates reactive oxygen species, and oxygen intermediates were excited by peroxidase in phosphate buffer and rat neutrophil homogenate (12-14). However, we could not find any investigations concerning the direct effects of IAA on some renal enzyme activities such as hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH) of the second cross maternal mice and indirect effects on their offsprings

The first aim of the study was to investigate the possible indirect effect of the plant growth hormone IAA on some renal enzyme activities such as HK, G6PD, 6PGD, LDH, and MDH of second cross offsprings of the second cross maternal mice (*Mus musculus*) that are given IAA. The second aim was to investigate the possible direct effect of IAA on the same renal enzymes in second cross maternal mice.

Material and Method

Animals: Swiss Albino mouse (*Mus musculus*) was routinely produced in our laboratories. They were given free access to food and water. Adult females weighing 18-22 g were used. For mating, two females and one male were held in an elevated stainless steel cage for two days. Then, the male was taken from the cage. Female mice were divided into two groups

IAA Application: IAA was dissolved in 70% ethanol before the application. All the females were injected (just after the separation of males) intraperitoneally with a 1/40 dilution of 300 mg/kg body weight in 3 day intervals until their sacrifice (4). This dose was experienced as non-lethal dose which could be used in long term in the study.When their pregnancies were apparent, females were taken into a separate cage. Following birth, the offsprings were kept together until the 35th day. At this time the males

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Table I. The effect of indole-3-acetic acid on HK, G6PD, 6PGD, LDH and MDH enzymes in kidneys of the second cross maternal mice.

		HK	G6PD	6PGD	LDH	MDH
Treatment	N	(mU/mg prot.)	(mU/mg prot.)	(mU/mg prot.)	(mU/mg prot.)	(mU/mg prot.)
Control	5	66.2 ± 8.6	39.8 ± 5.1	27.5 ± 2.9	6188 ± 734	14208 ± 774
IAA	7	65.7 ± 10.5	46.0 ± 9.7	27.3 ± 3.4	6665 ± 719	15984 ± 1439

N: Animal number.

Table II. The effect of indole-3-acetic acid on HK, G6PD, 6PGD, LDH and MDH enzymes in kidneys of the offsprings of the second cross maternal.

		HK	G6PD	6PGD	LDH	MDH
Treatment	N	(mU/mg prot.)	(mU/mg prot.)	(mU/mg prot.)	(mU/mg prot.)	(mU/mg prot.)
Control	5	33.9 ± 6.7	28.3 ± 5.1	19.5 ± 7.5	5429 ± 821	15411 ± 2035
IAA	7	27.1 ± 5.8	25.3 ± 6.0	19.9 ± 5.1	5843 ± 667	13841 ± 1736

N: Animal number.

and females were separated from each other. IAA applications to female parents continued until the 55th day of age. These applications continued for two generations.

The Control: The control group was given 70% ethanol.

In each generation, at least 6 mating pairs were allocated to IAA experimental group. On the other hand, minimum 5 mating pairs formed the control group.

Enzyme Studies: For biochemical analysis, after weighing kidney tissues, they were homogenized in 0.15 M ice-cold KCl. The homogenate was centrifuged at 48,000 x *g* for 30 minutes. The supernatant was stored at –40 º C for a maximum period of 15 days if not used immediately. Activities of all enzymes were measured in NAD(P)/NAD(P)H-dependent reactions at 340 nm using a molar extinction coefficient of 6,220 M^{-1} cm^{-1}. The activities of enzymes HK, G6PD, MDH, and LDH were determined according to manufacturer's handbook (15). MDH and LDH activities were measured from the oxidation of NADH, and HK and G6PD activities were measured from the reduction of $NADP^+$ at A_{340} nm at 25 ºC for a 5-minute period. Enzymatic assay for 6PGD was done according to Rudack *et al.,* (16). 6PGD activity was measured from the reduction of $NADP^+$ at A_{340} nm at 30 ºC for a 5-minute period.

Protein assay: Protein assay was done according to Lowry *et al*. (17), bovine serum albumine serving as the standard.

Statistical analysis: Data were analyzed by using SPSS for Windows computing program. Non-parametric statistical methods were used to analyze the data. Mann-Whitney U Tests were used for comparisons of pairs. The results were expressed as mean ± SE. P values (<0.05) were regarded as statistically significant.

Results

Kidney tissue HK, G6PD, 6PGD, LDH, and MDH activities were presented in Table 1 and 2.There were no statistically significant differences in HK, G6PD, 6PGD, LDH, and MDH activities between the groups in both the second cross maternal mice and their offsprings.

Discussion

Since there are many metabolic pathways interacting one with another, the flux of metabolism responds to physiological effectors. To ensure effectiveness of interaction and response, the level of activity of the various pathways must be controlled. So, the enzymes studied here are all important in a sense that they represent three main metabolic pathways (glycolysis, citric acid cycle and hexose monophosphate pathway), and are primarily responsible for generating NADH and NADPH (and their oxidized forms), required for energy generation and reductive biosynthesis, respectively. For example, G6PD and 6PGD are two main enzymes in pentose phosphate shunt, the alternative pathway for glucose metabolism, and are the main providers of cells' NADPH reserves. MDH is the enzyme of Krebs cycle, generating both one of three NADHs and converting malate to oxaloacetate, the initiation precursor of this cycle. The enzymes HK and LDH catalyze the first and the last steps, respectively, in glycolytic pathway. While the former one is a glucose-phosphorylating enzyme for the degradation of glucose by respiration and fermentation, the latter establishes the balance of the coenzymes by utilizing NADH for regeneration of NAD^+.

In several studies, it has been shown that IAA generates reactive oxygen species (ROS) (3,12,14). In a study, it was shown that ROS including superoxide and hydroxyl radical, hydrogen peroxide, singlet oxygen and nitric oxide can cause cellular injury when they are generated

excessively or the enzymatic and non enzymatic antioxidant defense systems are impaired (18). IAA is cytotoxic on neutrophils, macrophages and lymphocytes (3). In a study carried by Pires de Melo *et al*. (3) IAA caused death and marked ultrastructural changes in cultured neutrophils and increased catalase and glutathione peroxidase. In addition, IAA has been shown to have a strong teratogenic effect in experimental animals (19).

In the present study, we have not obtained any statistically significant difference in studied enzymes activities. There are no studies examining the effect of IAA in vivo on HK, G6PD, 6PGD, LDH and MDH activities in mouse and their offsprings' kidneys. It was therefore impossible to compare our results. However, Çelik et al., (20) found that seventy-five ppm of IAA caused an increase in malondialdehyde levels in rat liver and kidney at subacute treatment for 25 days. In a study carried by Çelik and Kara (21), IAA was found to activate LDH, CPK and amylase, and to inhibit AST. In another study, Çelik et al. (22) found that the levels of LDH, CPK and AST were increased significantly by IAA at subchronic treatment in rat.

In conclusion, IAA, at the used dose did not have effects on HK, G6PD, 6PGD, LDH, and MDH activities in both second cross maternal mice and their offsprings. This may be due to low dose of IAA or the studied enzymes activities did not show any significant difference since ROS produced by IAA may have been scavenged by antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase.

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