Turkish Journal of Biology

Volume 26 | Number 2 Article 4

1-1-2002

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Turk J Biol
26 (2002) 73-76

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Received: 18.10.2001

Abstract: The effects of a sublethal concentration of three plant growth regulators (PRGs) on serum enzymes in rats were investigated under laboratory conditions. 100-ppm of PRGs, indoleacetic acid (IAA), indolebutiric acid (IBA) and kinetin were administered orally to 8 rats *ad libitum* for 3 weeks. The hormon treatments caused different effects on the level of serum alanin aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), amylase and creatine phosphokinase (CPK) in comparison to those of control rats. According to the results, while the levels of LDH and CPK were increased significantly by IBA, the levels of AST, LDH and CPK were increased significantly by IAA. In addition, the levels of AST, LDH and CPK were increased significantly by kinetin. In conclusion, these chemicals have toxicological effects on the animals in subchronic treatment.

Key Words: Plant Growth Regulators, Serum Enzymes, Rat

Bazı Bitki Büyüme Düzenleyicilerinin Subkronik Uygulanmasının Sıçanların Serum Enzim Düzeyleri Üzerine Etkisi

Özet: Bazı bitki büyüme düzenleyicilerinin subletal konsantrasyonda laboratuar şartlarında sıçanların serum enzim düzeyleri üzerine etkileri araştırıldı. Sekiz adet dişi sıçandan oluşan gruplar deneme boyunca içebildiğince 100 ppm'lik IAA, IBA ve kinetin uygulamasına üç hafta maruz bırakıldı. Sonuçlar gösterdi ki; bu büyüme düzenleyicileri serumun alanin aminotransferaz (ALT), aspartate aminotransferaz (AST), lactate dehidrogenaz (LDH), amilaz ve kreatine fosfokinaz (CPK) enzim seviyeleri üzerine farklı etkilere sahip oldukları gözlendi. Sonuçlara göre; LDH ve CPK'nın düzeyleri IBA tarafından önemli derecede arttırlırken, AST, LDH and CPK düzeyleri IAA tarafından önemli derecede arttırıldı. Keza, AST, LDH ve CPK seviyeleri kinetin tarfından önemli derecede arttırıldı. Sonuç olarak; bu kimyasallar subkronik uygulamalarda toksik etkilere sahip oldukları görüldü.

Anahtar Sözcükler: Bitki büyüme düzenleyicileri, Serum enzimleri, Sıçan

Introduction

Today, because the effects of plant growth regulators (PGRs) on plants are well known, they are used widely in agriculture. The toxic effects of these chemicals on animal are limited, therefore, this subject has attracted the interest of many researchers recently.

Many chemicals are currently used in agriculture, and PGRs are among those widely used. On the other hand, some of these are endogenous hormones of plants that they are most likely included in the diet of all herbivorous and omnivorous animals. The amounts of these substances in the environment may soon exceed those of insecticides (1).

The effects of different PGRs on insects have been investigated (2-5), but reports concerning vertebrates are very limited (6,7). In the literature, it is reported that PGRs cause increases in the number of splenic plaque forming cells and circulating white blood cells, hematocrit values, and thymus weight in young deer mice (8). El-Mofty and Sakr (9) found that Gibberellin A_3 (GA_3) induced liver neoplasm in Egyptian toads, and they suggested that the tumors could be diagnosed as hepatocellular carcinomas. GA_3 also induces microabsceses and hydropic degeneration in the liver and mononuclear inflammatory infiltration in the kidneys of laboratory mice, but not tumors (10). On the other hand, it is reported that fecundity, longevity and egg viability have been changed in different insects by PGRs treatment (11,12). Some PGRs, the effects of which we investigated in another study, affected the carbonic anhydrase izoenzymes of erythrocytes in humans and bovines. While

indoleacetic acid increased the activation of both bovine carbonic anhydrase and human carbonic anhydrase-II, kinetin was found to have no effect on bovine or human carbonic anhydrase-I or human carbonic anhydrase-II isozymes *in vitro* (13). The effects of IAA and kinetin were also investigated on human serum enzymes in vitro. IAA was found to inhibit AST and activate amylase, CPK and LDH. Kinetin inhibited muscle creatine kinase (CK-MB), while it activated AST and ALT (14).

In order to achieve a more rational design of PRGs, it is necessary to clarify the mechanism of PRGs' toxicity effect comprehensively and its structure toxicity. For this aim, the treatment of PRGs was done orally because the effect of chemicals represents a well characterized in vivo toxicity model system.

Materials and Methods

Materials

Plant growth regulators (PRGs) of technical grade were supplied by the Sigma Chemical Co. (St. Louis, MO, USA).

Animals: Rats (Sprague-Dawley albino) weighing 150-200 g were provided by the animal house of the Medical School of Yüzüncü Yıl University, and were housed in 4 groups, each group containing 8 rats. The animals were fed a standard laboratory diet purchased from Van Animal Feed Factory (Van, Turkey), and they had access to food *ad libitum* during the experiments. The animals were housed at 20±2°C in a daily light/dark cycle.

Rat Treatment: This investigation was performed on female rats. A dosage of IAA, IBA and kinetin was used. The rats were exposed to 100 ppm of IAA, IBA and kinetin *ad libitum* for 3 weeks. One hundred milligrams of PRGs were dissolved in 1 ml of 1 N NaOH, and then were diluted with tap water to obtain a 100 ppm dose. For the control rats, only 1 ml of NaOH was added to 1000 ml of tap water. The control rats were given only this drinking tap water.

At the end of the treatment, the rats were anesthetized by inhalation of diethyl ether, and after blood samples were obtained, they were sacrificed. The samples were obtained from a cardiac puncture using a syringe for the determination of enzyme levels. Blood samples were put immediately into ice-chilled siliconized disposable glass tubes. The serum samples were obtained by centrifuging blood samples at 3000 rpm for 15 min at 4°C, and enzyme levels were measured in these serum samples.

Measurement of Enzyme Levels: Serum enzyme levels were measured by autoanalyzer (BM/HITACHI-911), using the kit.

Analysis of Data: All data were expressed as mean ± standard error (SE). For statistical analysis the SPSS/PC+ package (SPSS/PC+, Chicago, IL, USA) was used. For all parameters, means and SE were calculated according to the standard methods. The Mann-Whitney U test was employed differences between means of the treatments and the control rats. The significance level p= 0.05 was used for all tests (15).

Results

The results showed that the treatment of rats with IAA, IBA and kinetin hormones produced changes in the levels of serum ALT, AST, LDH, amylase and CPK. According to the results, the exposure to sub-chronic doses of IAA induced significant increases in the levels of AST, LDH and CPK, but did not change significantly the other enzymes. To find out the significance of the increases in different serum enzymes on exposure to IAA for three weeks, the data obtained were subjected to the Mann-Whitney U test. The observed value of p for AST was 0.003; for LDH it was 0.004 and for CPK it was 0.005. The treatment with IBA caused a significant increase in the levels of LDH and CPK. As for the other enzymes, no significant differences existed between the levels of the treatment groups compared to those of the control group. To find out the significance of the increases different blood parameters on exposure to IBA for three weeks, the data obtained were subjected to the Mann-Whitney U test. The observed value of p for LDH was 0.05 and for CPK it was 0.05. On the other hand, kinetin induced significant increases in the level of AST, LDH and CPK, but did not change significantly the other enzymes. To find out the significance of the increases in different blood parameters on exposure to kinetin for three weeks, the data obtained were subjected to the Mann-Whitney U test. The observed value of p for AST was 0.003; for LDH it was 0.001 and for CPK it was 0.001. The Table shows means and standard deviations of serum enzyme levels of the treated and the control rats.

Table. Serum enzyme levels of rats (Mean ± SE).

ENZYMES	CONTROL	IAA	IBA	KINETIN
AST(U/L)	109.9±8.9	164.9±21.1[a]	143.5±13.3	163.4±8.7[f]
ALT (U/L)	62.4±4.9	70.9±4.1	58.8±3.5	66.5±6.1
LDH (U/L)	458.9±97.8	1373±212.5[b]	811.9±91.1[d]	1870±200[g]
AMYLASE (U/L)	1952±169	1887±87.5	1919±87.5	1765±141
CPK (U/L)	250±50.3	917±193.6[c]	592±59[e]	939±103.6[h]

[a]: p<0.003, [b]: p<0.004, [c]: p=0.005, [d]: p<0.05,
[e]: p<0.05, [f]: p<0.003, [g]: p<0.001, [h]: p<0.001

Discussion

The effects of pollutants on nature became a subject of interest for scientists from the beginning of the second half of the 20th century, and subsequently, investigations on the effects of these pollutants on human beings, plants, and animals were initiated. PRGs are commonly used agricultural chemicals that regulate plant development with their inhibitory or activatory effects. In our study, indoleacetic acid, indolebutiric acid and kinetin were chosen because information on their toxicological biological effects in higher animals is very limited.

Research shows that the toxicological or biological effects of PGRs are divergent, and the dose-effect relationship changes in living organisms. For example, kinetin increases DNA in the nuclei of a fibroblastic cell culture at low doses, but at high doses kinetin causes foamy and vacuolized cytoplasm in these cells (16). So far, no study examining the effect of PGRs in vivo has been carried out on rat serum enzymes. Therefore, we were unable to compare our results with previous ones. In addition, because of the high variability in analyzing enzyme-chemicals interaction in vitro and in vivo due to inconsistent factors like treatment time and manner, purity and species tissue differences, it is difficult to compare data from different laboratories regarding the toxicological effect.

The present study indicates that PGRs possess toxicological properties. This is evidenced by our observation that, upon PGR treatment in vivo, the level of hepatic and muscle enzymes increased in rats. In addition, the PGRs exerted different effects on rat liver and muscle function, causing AST, ALT, LDH and CPK release. The reasons for such effects of PGRs are not understood at present, but one may be the effect of the ranking of test chemicals on tissues. However, it is conceivable that the PGRs, being toxic like other pesticides, might be interacting primarily with the liver and muscle tissue cells' membrane, resulting in structual damage and changes in enzyme leakage. Further studies are required to correlate the in vivo damage to liver and skeletal and heart muscle isoform of LDH and CPK.

Conclusion

It is postulated that liver and muscle damage indicator enzymes might offer a marker of choice for monitoring the biotoxicity of direct acting compounds such as PGRs. It is impossible to forbid the utilization of these kinds of chemical, which are used against harmful insects and cause product losses under these conditions today. However, the necessity of using regulators should be decreased by improving the resistantce of plant species to diseases and unfavorable conditions. This kind of plant species can be developed with the aid of biotechnological and plant improving procedures.

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