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Effects of Acute Grayanotoxin-I Administration on Hepatic and Renal Functions in Rats

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Abstract: The effects of acute Grayanotoxin-I (GTX-I) administration on hepatic and renal functions in rats were investigated. GTX-I was administrated to the animals of groups 1, 2 and 3 at a single i.p. dose of 1 mg/kg, 0.5 mg/kg and 0.25 mg/kg respectively, and group 4 (control) received i.p. saline (0.9 %) solution only. One hour following the administration of GTX-I or saline, urine analysis (leukocytes, urobilinogen, protein, pH, blood, ketone, glucose, nitrites) was performed and serum was evaluated for activities of glutamic pyruvic transaminase (GPT), γ-glutamyl transferase (γ-GT) and isoenzymes of lactate dehydrogenase (LDH) (as a percentage of total LDH activity), transferrin, ceruloplasmin and total protein concentrations and histopathologic changes in the liver and kidney. A single dose of GTX-I produced proteinuria and hematuria and decreased GPT, LDH_3, LDH_4, and γ-GT. But the loss of GPT, LDH_3 and LDH4 partially disguised by hepatic enzyme leakage which was the result of hepatic damage occured with increasing doses of GTX-I. Hepatic damage was also detected by light microscopy.

Key Words: Grayanotokxin, glutamic pyruvic transaminase (GPT), γ-glutamyl transferase (γ-GT), lactate deyhdrgenase $(LDH)_3$ serum total protein

Introduction

Grayanotoxins (GTXs) are toxic constituents isolated from the leaves or flowers of various *Rhododendron* species belonging to the family Ericaceae. These toxins are contained in the toxic honey produced by bees from the collected nectar of these plants (1). Toxic honey has been known since ancient times. In 400 BC, Xenophon in the Anabasis reported the poisoining of his troop by the honey of *Rhododendron ponticum* flowers. GTXs (GTX-I, GTX-II, GTX-III), mainly GTX-I, occurring in Ericaeae plants are the compounds responsible for poisoining. Pharmacological and physiological studies have demonstrated that GTXs have a wide range of acute systemic effects including hypotension, arrythmias, respiratory depression, nausea vomiting, dizziness and induced amystotic postures indicating a central nervous system effect (2), and they have been shown to depolarize the sodium dependent excitable membranes (3-5). On the other hand, it has reported that subchronic and chronic exposure to GTX-I cause hepatotoxicity and nephrotoxicity (6, 7). However, the acute effects of GTXs on hepatic and renal functions have seldom been studied. Nishikawa et al studied the effects of a single dose of GTX-III on liver and renal functions (8). But poisonings from honey orginating from *Rhododendron* and other Ericaceae plants have generally been assumed to be due to GTX-I (9). In addition, GTX-I has been positively identified in various toxic honey samples (1, 9). Therefore, we investigated the effects of acute GTX-I administration on hepatic and renal functions in rats, because these tissues are most susceptible to organotoxicity when exposed to any drug.

Materials and Methods

GTX-I, kindly provided by Dr. Tadamasa Terai (Department of Applied Chemistry, Osaka Institute of Technology, Osaka - JAPAN) was dissolved in physiological saline solution at a concentration of 0.15 mg/0.3 ml (to administer 0.3 ml GTX-I solution to an animal weighing 300 g in the highest dose group.

Fifty-12-week-old male Swiss albino rats weighing 160-250 g were divided into 4 groups. Animals in group 1, 2 and 3 received single i.p. doses that would cause acute effects of GTX-I (1, 0.5 or 0.25 mg/kg respectively), and group 4 (control) received saline

solution i.p. 0.9%. The groups contained 10, 15, 10 and 15 animals respectively. One hour after the administration of GTX-I or saline solution, urine analysis for leukocytes, urobilinogen, protein, pH, ketone, blood, glucose and nitrites was performed with Multistix 10-SG (Bayer Diagnostic, Ames Co., Division of Miles laboratories, USA) according to the manifacturer's instructions (10). Then the animals were sacrified and blood was collected into tubes. Serum was obtained for determination of the following: activities of glutamic pyruvic transaminase (GPT), γ-glutamyl transferase (γ-GT) and isoenzymes of lactate dehydrogenase (LDH) as a percentage of total LDH activity and transferrin, ceruloplasmin, and total protein concentrations. Determinations of these parameters were carried out with colorimetric in vitro diagnostic test reagents of Randox (GPT), Stanbio (γ-GT), Helena (LDH isoenzymes electrophoresis procedure on cellulose acetate), Bayer-ames-Serapak (transferrin) and p-phenylendiamine oxidase procedure (ceruloplasmin) (11), and Peter's procedure (total protein) (12). Some parameters could not be obtained for all rats because of insufficient sera and urine.

In order to determine histopathologic changes, the liver and kidney were excised and immediately fixed in 3% formalin, stained with hematoxylin and eosin, and examined by light microscopy.

One-way analysis of variance and Kruskall Wallis variance analysis was done for significance. Individual comparison within the groups was carried out by Scheffe's procedure. In all cases a difference was considered significant when $p<0.05$.

Results

The results showed that the administration of GTX-I at a single low dose caused a decrease in serum GPT, LDH_3, LDH_4 and γ-GT activities in rats. However, the decrease in GPT activity and LDH_3, LDH_4 activities as a percentage of total LDH activity disappeared at a high dose of GTX-I. In addition, serum total protein level was markedly reduced and this effect was proportional to the dose of GTX-I. No significant changes were observed in serum LDH_1, LDH_2 and LDH_5 activities as a percentage of total LDH activity or in ceruloplasmin and transferrin concentrations in serum. The data are presented in Table 1.

Urine analysis findings are presented in Table 2. Urine analysis showed that the acute GTX-I administration produced proteinuria and hematuria. In addition, urine keton bodies expressed a decrease in the rats of groups 1 and 2.

The results of histopathologic examinations on the excised livers were as follows: livers of rats that received GTX-I at a dose of 0.25 or 0.5 mg/kg had no significant histopathologic differences from control livers (Figure 1), while the livers of rats that received GTX-I at a dose of 1 mg/kg had significant changes in central vein dilatation, congestion, focal necrosis, inflammatory cell infiltration in portal tract and parenchyma (Figure 2). Histopathological examinations of the kidney revealed no significant histopathologic alterations.

Table 1. Effects of Acute GTX-I Administration on Biochemical Serum Data of Rats

I.p. dose of GTX-I	GPT (U/Liter)	g-GT (U/Liter)	LDH1	LDH2	LDH3	LDH4	LDH5	Transferrin (mg/100 ml)	Ceruloplasmin (g/Liter)	Total protein (g/Liter)
Group 1 1 mg/kg	21.4±7.0 (8)✶✚✣	3.1±1.0(3)★	6.8±4.8(8)	7.2±2.7(8)	8.3±3.2(8)✣	14.3±5.0(8)✣	63.0±13.0(8)	98.1±53.3(9)	0.5±0.2(7)	7.8±0.4(8)✶
Group 2 0.5 mg/kg	13.2±6.5(15)✶†✣	8.0±1.6(13)	7.0±4.0(8)	7.0±2.0(8)	10.3±4.0(8)✣	13.3±5.7(8)✣	62.2±9.5(8)	120.2±37.3(15)	0.5±0.1(11)	8.0±0.4(13)✶†
Group 3 0.25 mg/kg	10.7±3.7(8)✶✚†	6.7±2.7(10)	8.5±7.3(7)	8.0±4.3(7)	5.3±2.0(7)✶✚†	5.6±3.0(7)✶✚†	72.3±13.0(7)	126.8±31.4(10)	0.5±0.1(10)	8.8±0.2(10)✶†
Group 4 Control	34.8±4(15)	11.1±4.2(13)	6.5±5(11)	7.7±4(11)	9.7±3.8(11)	12.8±5(11)	63.0±12.5(11)	117.4±26(13)	0.5±0.3(14)	8.9±0.5(13)

Data are expressed as the mean±S.D. One-way analysis of variance analysis of Kruskall Wallis was performed for significance, then Scheffe's procedure was used for individual comparison within the groups (Number of animals given in parantheses).

✶: $p<0.05$ when compared with control

†: $p<0.05$ when compared with group 1

✚: $p<0.05$ when compared with group 2

✣: $p<0.05$ when compared with group 3

Table 2. Effects of Acute GTX-I Administration on Urine Data of Rats

I.p. dose of GTX-I	Leucocytes (counts/µl)	Urobilinogen (mg/100 ml)	Protein pH	(g/liter)	Ketone (Erythroytes/µl)	Blood (g/liter)	Glucose (mg/100 ml)	Nitrities
Group 1 (n=6) 1 mg/kg	200±132	0.20±0.00	300±36*	7.60±0.68	0*†	78±29*†	Negative	Negative
Group 2 (n=8) 0.5 mg/kg	167±120	0.20±0.00	253±45*	7.80±0.64	0*†	36±12.2	Negative	Negative
Group 3 (n=8) 0.25 mg/kg	128±81	0.20±0.00	220±32*†	7.15±0.51	0.16±0.09	22.6±9*††	Negative	Negative
Group 4 Control	100±56	0.20±0.00	61±9	7.20±0.61	0.20±0.11	Negative	Negative	Negative

Data are given as the mean±S.D. Kruskall Wallis procedure was performed to compare the values, then Scheffe's procedure was used in order to compare the groups with one another.
*: p<0.05 when compared with control
†: p<0.05 when compared with group 1
†: p<0.05 when compared with group 2
†: p<0.05 when compared with group 3

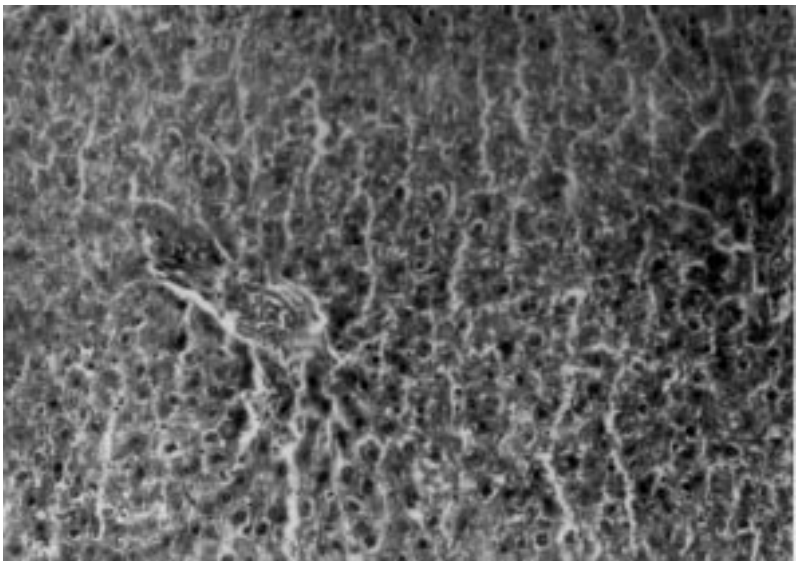

Figure 1. Representetive light micrograph of liver of a rat from control group, showing no significant lesions (H & E. x 40)

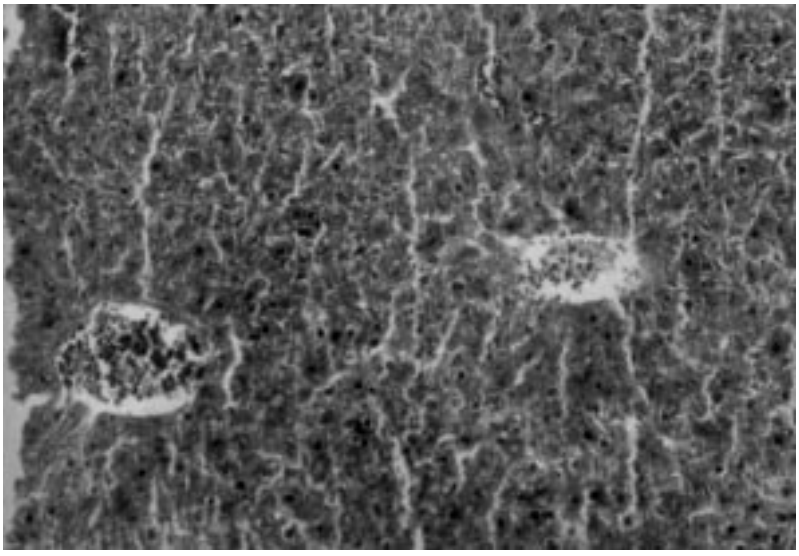

Figure 2. Representetive light micrograph of liver of a rat from group 1, which received GTX-I at a dose of 1 mg/kg, showing central vein dilatation, congestion, focal necrosis (H & E. x 40)

Discussion

Recent studies have shown that nephrotoxicity is caracterized by proteinuria, hematuria, glucosuria, etc. (13-16). Therefore, the proteinuria and hematuria observed in our study may be ascribed to a nephrotoxic effect of acute GTX-I administration. On the other hand, it is well known that intermediate-sized proteins such as albumin, erythropoietin, IgG and hormone binding proteins, and low molecular weight inhibitors of the clotting cascade, are lost in the urine by proteinuria and their concentrations in serum are reduced in nephrotoxicity (17). Therefore, our findings of proteinuria, reduced serum total protein level and some enzyme activities (GPT, LDH_3, LDH_4, γ-GT) were consistent with the findings mentioned above. However, in consideration of the fact that there were no significant histopathologic changes in the tissue detectable by light microscopy, it was suggested that this nephrotoxicity may have caused ultrastructural changes in cells.

It is known that the hepatic damage is characterized by decreases in the enzyme levels in hepatocytes and increases in serum because of hepatic enzyme leakage (18-20), and numerous studies undertaken to examine hepatotoxicity induced by drugs and chemical substances in rats have shown that hepatotoxicity, resulting oxidative stress and hepatic damage led to an increase in the serum GPT levels (18, 21-24). On the other hand, Kalapos et al. (1993) have reported increased LDH release in relation to hepatotoxicity in their study (25). In addition, it is well known that the decrease in liver functions leads to impaired fatty acid oxidation and a decrease in levels of plasma and urine ketone bodies (26). In our study, the administration of GTX-I at a single low dose caused a decrease in serum GPT, LDH_3, LDH_4 and γ-GT activities in rats. The decrease in serum GPT, γ-GT, LDH_3, and LDH_4 activities, observed at low-dose levels of GTX-I, may be ascribed to a nephrotoxic effect of acute GTX-I administration. However, the decrease in GPT activity and LDH_3, LDH_4 activities as a percentage of total LDH activity disappeared at a high dose of GTX-I. These results indicate that exposure to low doses level of GTX-I does not cause a hepatic damage, but exposure to high doses produce hepatotoxicity. That is why the decrease of serum GPT, LDH_3, LDH_4 activities observed at low doses of GTX-I was disguised by hepatic enzyme leakage, which was the result of hepatic damage caused by increasing doses of GTX-I. This was supported by significant histopathologic changes, including central vein dilatation, congestion, focal necrosis, inflammatory cell infiltration in portal tract, and parenchyma in the liver only in rats that received high doses of GTX-I.

When the results of this study were evaluated in the light of the previous studies mentioned above (13-16, 18-25), it was concluded that exposure to a single dose of GTX-I led to nephrotoxicity characterized by hematuria and proteinuria together with reduced serum total protein level and hepatotoxicity, which occurred at only high doses.

In conclusion, it should be remembered that the poisoinings from honey orginating from *Rhododendron* and other Ericaceae plants may affect the functions of the kidney and liver and cause nephrotoxicity and hepatotoxicity.

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