ANTIHEMORRHAGIC ACTIVITY OF QUERCETIN AGAINST *Macrovia* erina obtusa VENOM

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ABSTRACT

Effect of quercetin’s antihemorrhagic activity against *Macrovia* erina obtusa venom was assessed by histochemical analysis and aspartate transaminase (AST), alanine transaminase (ALT), creatinin and coagulation homeostasis parameters of blood plasma of Wistar rats whit intraperitoneal administration of viperian venom, quercetin+venom and saline buffer. Histochemical analysis of study revealed that animal group treated by quercetin + venom was showing less hemorrhagic area (49.7%) as compared to venom group. Significant improvement in creatinin quantity, ALT and AST activity was reported from the venom-treated animals group when compared to the control group. The group treated by quercetin + venom showed significantly lower concentration of AST, ALT and creatinin level in blood plasma (24.9%, 28.5% and 30.8%, respectively), as compared to venom treated group. However, there were no significant differences was reported in the coagulation homeostasis parameters in venom and quercetin + venom groups. Present investigation testify the antihemorrhagic effect of quercetin against *M. lebetina obtusa* venom.

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1 Introduction

Macrovipera betina obtusa venom has mainly hemorrhagic and hemolytic properties (Sanz et al., 2008), with this snake venom’s metalloproteinases degrade most important components of the basement membrane, such as laminin and type IV collagen (Baramova et al., 1990, Mashiko et al., 1998). Phospholipase A2 is a constant component of any venom and is responsible for the highest damaging effect (Chen, 1979). It was also reported that injection of E. carinatus venom caused a significant rise in serum aspartate transaminase (AST) and alanine transaminase (ALT) in rats accompanied with disturbances in the hepatic and renal functions of the animals through severe hepatocellular injuries, necrosis of hepatocytes and kidney tubules as well as nephrotoxic action (Abdel-Nabi and Rahmy, 1992). The level of plasma AST and ALT are the indicators of liver injury, and the concentration of creatinine is an indicator of kidney injury and its functional disorders (Thomas et al., 2011).

In recent years, investigations of non-specific antitoxic substances became extremely urgent problem in context of anti-snake bite treatment. This is due to the fact that the specific immune serum treatments are very expensive and need specific storage conditions, these two conditions making it impossible to use them in the field conditions, where the most of bites take place.

The limitations of antivenom therapy have prompted to the scientists to explore new drugs derived from plants sources for the treatment of snakebite (Premendran et al. 2011). There is a wide range of products, among them number of possible non-specific antitoxic substances, which are based on herbal extracts, may explored. Recently, more attention has been paid to pharmacological screening of medical plants traditionally used for the treatment of snakebite as well as isolation and characterization of their active compounds (Mukherjee, 2012).

The application of herbal extracts for medical purposes is determined by the presence and complex action of various secondary metabolites. Quercetin is a plant derivative polyphenolic antioxidant, which has anti-inflammatory (McAnlis et al., 1999) and angioprotective action (Atalık et al., 2010; Jung et al., 2012.). Quercetin also prevents the autoimmune reactions, repressing the formation of prostaglandins and leukotrienes (Lee et al., 2008). It also has a properties of stabilizes the cell membrane (Margina et al., 2012), interact with membrane-associated Ca-calmodulin, which transports Ca²⁺ ions (Nishino et al., 1984), and changing the membrane (Denisa et al., 2010).

The present study aims to verify the M. lebetina obtusa venom neutralization potential of quercetin and explore some biochemical mechanism of it action.

2 Materials and Methods

2.1 Source of Venom

Lyophilized snake venom produced by "geb & ss" (Armenia) of Gyurza (Macrovipera lebetina obtusa) inhabiting in Armenia was used.

2.2 Experimental Animal

12 Male albino Wistar rats with weight between 200-220 grams were used for this study. Animals were kept under standardized conditions of temperature (22 ± 1 °C), humidity (55% ± 5%), and 12-12-h light-dark cycles with free access to food and water. The experimental protocol has been approved according to the local animal protection act. The animals were divided into three groups as the following viz Control group (4 rats were injected intra peritoneally (i.p.) with 100 µL 0.9 % NaCl only), Quercetin + venom group (4 animals were injected (i.p.) with 50 µL crude venom in 0.9 % NaCl (5mg/kg) and 50 µL of saline buffer whit quercetin 30µM/kg), Venom group ( 4 animals were injected (i.p.) with 100 µL crude venom in 0.9 % NaCl 5 mg/kg).

One hour after injections animals were decapitated under carbon dioxide narcosis. Blood sample was collected on 0.109M citrate anticoagulant, plasma supernatant was decanted following a 15 min. centrifugation at 2,500 g.

2.3 Alanine transaminase determination

The quantitative determination of ALT in rats blood plasma was provided on Cobas Integra 400 plus (Roche) according to the International Federation of Clinical Chemistry (IFCC), with pyridoxal-5’-phosphate (Bergmeyer et al., 1986). ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed in reaction was reduced by NADH in a reaction catalyzed by lactate dehydrogenase to form L-lactate and NAD+. The speed of the NADH oxidation is proportional to the ALT activity and was determined by spectrometric evaluation at 340 nm.

2.4 Aspartate transaminase determination

The AST quantitative measurement in rats plasma was provided on Cobas Integra 400 plus (Roche) by method according to the IFCC, but without pyridoxal-5’-phosphate (Bergmeyer et al., 1986).

AST catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate with formation of oxaloacetate and L-glutamate. Then the oxaloacetate reacts with NADH to form NAD+. The speed of the NADH oxidation is proportional to the AST activity and was determined by spectrometric evaluation at 340 nm.
2.5 Creatinine concentration determination

The creatinine determination in rats plasma was provided on Cobas Integra 400 plus by colorimetric method (Junge et al., 2004) by measuring the increase in absorbance at 552 nm. The color intensity of the quinine-imine chromogen formed is directly proportional to the creatinine concentration.

2.6 Thrombin time and fibrinogen quantity

Measurement of the fibrinogen quantity was in accordance with the Clauss method (Clauss, 1957) and the thrombin time was evaluated by the automatic coagulation analyzer (Maroch MG 1410).

2.7 Histochemical assay

Hemorrhagic status was assessed by digital microscope (Intel Play QX3 Computer Microscope) and image analyzer program pocket (ImageRepair3.19.) as described by Asatryan et al., (2007). Hemorrhagic status extent the damage of rats brain capillaries by histochemical method using fresh frozen sections, fixed 5-10% formaldehyde solution and incubated in Ca-adenosine triphosphate environment (Chilingarian, 1986).

Extent of hemorrhagic tissue damage was evaluated by measuring of “dark” area on the micrographs. The software tool devoted to hierarchical segmentation of an image and analyzing of the segments features. Each pixel of the binary image has the intensity of “0” (black) or “255” (white) and binarization grade was 128 of pixel intensity. The extent of damage in the venom group was taken as 100% and 0% in control group.

Table 1 The effect of intraperitoneal administered viperian venom and quercetin on AST, thrombin time, creatinin , ALT and fibrinogen of Wistar rats blood plasma (Mean ± SD, p<0.05).

<table>
<thead>
<tr>
<th>Sample</th>
<th>AST (u/dl)</th>
<th>Thrombin time (sec.)</th>
<th>Creatinine</th>
<th>ALT (u/dl)</th>
<th>Fib (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.7±0.2</td>
<td>12.1±0.4</td>
<td>4±0.1</td>
<td>2.4±0.2</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>Quercetin+venom</td>
<td>14.8±0.5</td>
<td>17.9±0.6</td>
<td>9±0.4</td>
<td>10.3±0.5</td>
<td>1.1±0.05</td>
</tr>
<tr>
<td>Venom</td>
<td>19.7±0.4</td>
<td>17.7±0.6</td>
<td>13±0.3</td>
<td>14.4±0.4</td>
<td>1.2±0.03</td>
</tr>
</tbody>
</table>

Figure 1 Effect of treatment on Wistar rats blood plasma AST, thrombin time, creatinin, ALT and fibrinogen.
2.8 Statistical analysis

All experiments were repeated 3-4 times and experimental results were expressed as means ± SD. All measurements were replicated three times. The data were analyzed by a one-way analysis of variance (ANOVA) and the values of p < 0.05 were considered as significant.

3 Results and Discussion

Results of the effect of intraperitoneal administered of viperian venom and quercetin on AST, ALT, creatinin and coagulation homeostasis parameters of Wistar rat blood plasma are presented in table 1 and Figure 1. Envenomations often produce persistent hemorrhage due to considerable degradation of fibrinogen and other coagulation factors, thus preventing clot formation (Ho et al., 1993). In present study any difference in thrombin time and fibrinogen concentration in rats plasma was not reported in the group treated by quercetin + venom and venom, it was testified that quercetin does not prevent fibrinolytic activity of M.lebetina obtuse venom.

However, the results of the study indicated that there was a significant increase in creatinin quantity, ALT and AST activity in the venom-treated animals group compared to the control group. The elevation in AST and ALT, gives evidence about the destruction of the liver and heart tissues as a result of venom injection.

The increase in ALT and AST activities recorded in the present study one hour after venom envenomation may be ascribed to brutal injuries and necrosis of hepatocytes as well as to nephrotoxic activities of the venom. With this high level of creatinine in plasma indicates sever disturbances in the kidney and similar observations were reported in rats following administration of various viper venoms (Abdel-Nabi , 1993; Abdel-Nabi et al., 1997).

In case of animals treated with quercetin + venom was showing significant reduction (p<0.05) in AST, ALT and creatinin level of blood plasma (24.9%, 28.5% and 30.8% respectively) compared to venom treated group.

Temorrhagic toxins such as metalloproteinases play an important role in pathogenesis of venom-induced hemorrhage (Bjarnason & Fox, 1994). Hemorrhage from venom induced vascular damage of vital organs can be fatal (Warrell, 1989).

Extent of hemorrhagic tissue damage was evaluated by measuring “dark” area by special program “Image repair”. The software devoted to hierarchical segmentation of an image and analyzing of the segments features. Each pixel of the binary image has the intensity of “0” (black) or “255” (white) and binarization grade was 128 of pixel intensity. The extent of damage in the venom group was taken as 100% and 0% in control group. The histochemical analysis data have shown (figure.2) that animal group treated by quercetin + venom had decreased hemorrhages area (49.7%) compared to venom group.

Considering the fact that quercetin did not show any anti-fibrinolytic activity it may be concluded that probable there is no direct inhibition mechanisms of proteolytic enzymes activity of M.lebetina venom by quercetin and its hemorrhages neutralizing properties may be mediated, particullary through its beneficial effects on endothelial cell functions (Shen et al., 2012).

Recent studies have reported that quercetin reduces the activity of viper venom phospholipase A2 (Cotrim et al., 2011), inhibiting the process of venom and cell membrane binding. However, the mechanisms of action of PLA2 are quite different from those of metalloproteinases.

Conclusion

Results of the study suggest that quercetin have been able to reverse venom induced enzymatic changes in blood plasma to a large extent and have significant venom neutralization capacity against hemorrhagic activity and can be used for the treatment of snakebites as nonspecific antitoxic compound against M. lebetina obtusa venom envenomination.
Antihemorrhagic activity of quercetin against *Macrovipera lebetina obtusa* venom.

Reference


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