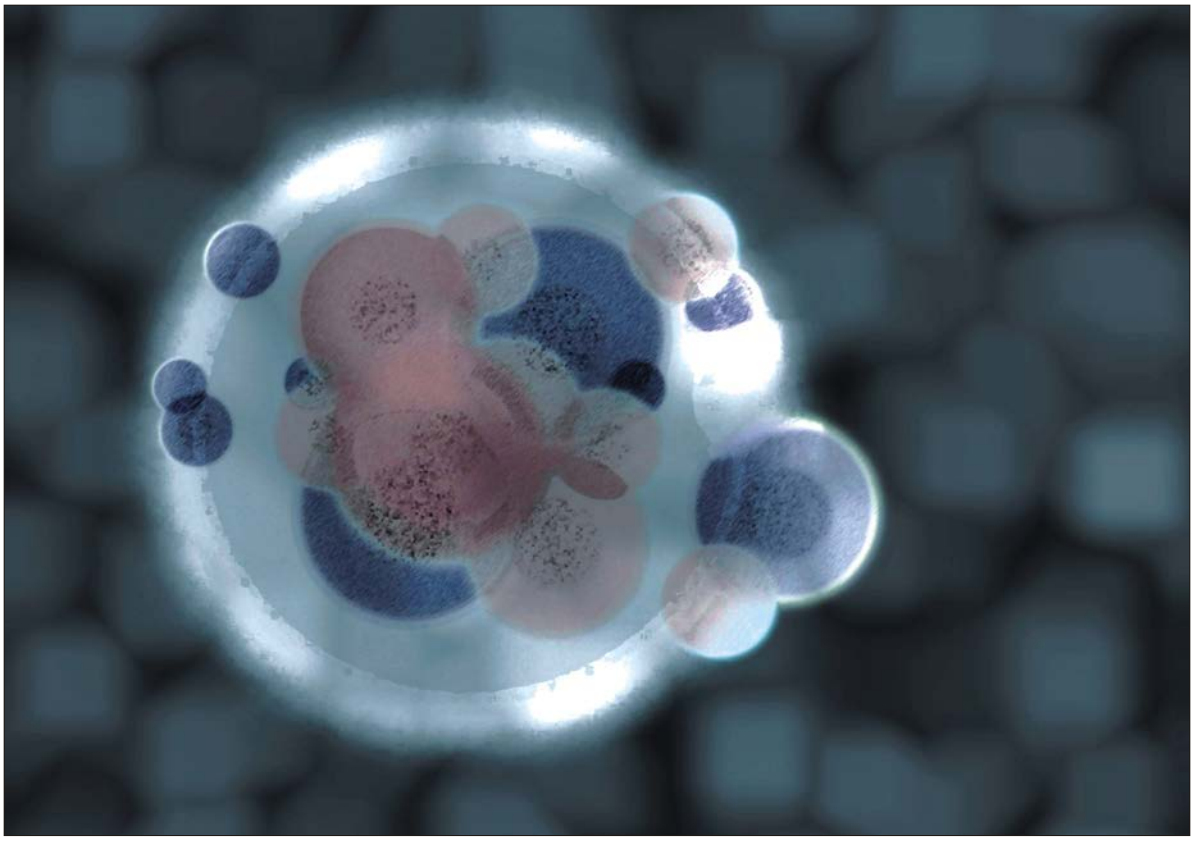

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Research Article
Beneficial Role of Quercetin on *Echis coloratus* Snake Venom Induced Hepato-renal Toxicity in Rats

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Abstract

Background: Scientists are exploring the application of new drugs derived from plant sources as an alternative to snakebite anti-venom therapy, which is endowed with several limitations. Naturally produced antioxidants like the flavonoids, which are widely found in the plants are largely consumed in daily diet, at present. **Methodology:** This study investigates and evaluates the potential effects of some flavonoids (quercetin) on envenoming of albino rats by sub-lethal venom (3.84 mg kg^{-1}, i.p.) doses of *Echis coloratus* (Ec) viper snake. Beneficial effects of quercetin (30 μM kg^{-1}, i.p.) doses on the induced hepato-renal toxicity are assessed by the measurement of selected biomarkers. **Results:** Sacrificed animals sera and tissues, which were collected for biochemical studies, showed significant increase in the levels of AST, ALT, ALP and creatinine. There was significant histopathological damage of the tissue's architecture and rise in the liver and kidney MDA levels. **Conclusion:** It is concluded that the reversal effects of modulation of the biochemical parameters and histological damage are attributed to the potential protective effects that ensued after administration of quercetin.

Key words: *Echis coloratus*, quercetin, MDA, rats, creatinine, AST

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hot weather places of the world are endowed with many snakes that belong to the carnivorous, poikilothermic reptiles. Vipers, like *E. coloratus* have vasculotoxic venom component that deteriorate the human vascular system. Those ones found in the central region of Saudi Arabia have not been studied thoroughly, especially their venoms that have received little attention[1]. Several factors have different physiological and biological effects on the bitten victims. Components of viper venoms have mainly enzymatic activities that break intracellular organelles, leading to necrosis, edema and hemorrhage[2] that culminate into loss of several body organs due to dysfunction tissue loss[3-5].

It was shown that several viper venoms constituents that are characterized by high molecular weights will show a slow absorption pattern through the lymphatic system[6,7]. Victims that have been envenomed usually show symptoms that vary according to type, size and sex of the snake and bitten victim, in addition to environmental, venom and treatment factors[8,9].

Several investigators have reflected the after snake envenoming signs and symptoms as several clinical abnormalities. The development of acute hepato-renal toxicity[10], organ dystrophy[11], cytotoxic cellular manifestations[12] and hypoglycemia[13] metabolic complications[14] all are induced acutely by envenoming bites.

Some studies have suggested that treatment by specific antivenins decrease the extent of the hemostatic failure, but still the management of *E. coloratus* victims remains uncertain. Victims subjected to such venoms and treated non-specifically reflected extremely urgent problems associated with antitoxic substances. This situation only stressed the queries and barriers associated with antivenin therapy that put researchers and scientists on the alert of finding alternative new drugs of plant and herbal origin to treat snakebiteand replace or limit the untoward effects brought about by antivenin use[15].

Recently, envenoming herbal treatment received more concentrated attention by subjecting traditionally used medical plants to pharmacological screening and isolating their active components[16]. Herbal extracts like quercetin has anti-inflammatory, polyphenolic antioxidant[17], angio protective activity[18,19] and appreciated beneficial effects on the immune system[20]. Further, extra benefits are associated with cell membrane stabilization[21], calcium ions transport[22] and membrane alteration[23].

In this present study, aim to investigate the biochemical activity mechanisms of quercetin and its beneficial neutralizing and ameliorating hepato-renal toxicity effects induced by *E. coloratus* viper venom in rats.

MATERIALS AND METHODS

Materials: Adult male albino Wistar rats (200-220 g), *E. coloratus* lyophilized snake venom, quercetin-3,3′,4′,5,6 pentahydroxyflavone′ 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (Sigma, USA) and other laboratory chematerials.

Preparation of venom solution: *Echis coloratus* lyophilized snake venom were obtained from Research Department of the Prince Sultan Riyadh Military Medical City, Riyadh, Saudi Arabia. The venom was dissolved in physiological saline (0.9% NaCl) (final concentration 10 mg mL^{-1}) under mild mixing for 10 min at 4°C and then, centrifuged at 10,000 rpm for 10 min at 4°C. The pellet was discarded and the supernatant was aliquoted and stored at -20°C at a maximum time of one week and administered intraperitoneally (i.p.) at a dose of 3.84 mg kg^{-1} b.wt. (LD_{50}) in a maximum volume of 0.2 mL (Fig. 1).

Preparation of quercetin: Quercetin -3,3′,4′,5,6 Pentahydroxyflavone′ 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (Sigma, USA) was dissolved in 1 M NaOH and administration by intraperitoneally (i.p.) at a dose of 30 μM kg^{-1} b.wt., in a maximum volume of 0.2 mL (Fig. 2).

Fig. 1: *Echis coloratus* snake venom

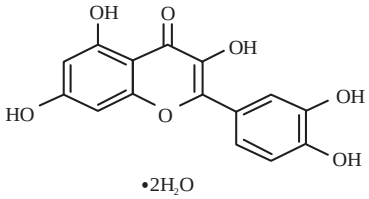

Fig. 2: Structure of quercetin

Animals: Adult male albino Wistar rats with weight between 200-220 g 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.

Methods: The animals were divided into four groups of six rats. Control group I were injected intraperitoneally (i.p.) with 200 µL normal saline (0.9% NaCl) only. Group II were injected (i.p.) with 200 µL crude venom (3.84 mg kg^{-1}) alone. Group III were injected (i.p.) with 200 µL venom (3.84 mg kg^{-1}) and quercetin (30 µM kg^{-1}). Group IV were injected (i.p.) with 200 µL quercetin alone.

One hour later, injected animals were decapitated and blood sample were collected for biochemical analysis and tissue organs were collected for histopathological analysis.

Ethics statement: Experiments were done according to the Saudi Arabian law of protection of animals. The study procedures involving animal experiments were approved by the institutional ethics committee.

Biochemical analysis: Blood sample were collected from each rat into plain centrifuge tubes, left for 1 h at room temperature (25°C±2) and serum were separated by centrifugation at 600×g for 15 min and analyzed, without delay for the concentration of creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined by using kits purchased from United Diagnostic Industry (UDI) Dammam, Saudi Arabia. A few pieces of liver and kidney were fixed with 10% neutral buffered formalin for histopathological investigations, whereas the majority of pieces were homogenized in ice-cold medium containing 50 mM tris-HCl and 300 mM sucrose, pH 7.4 and finally stored at -80°C until use in the various biochemical determinations.

Determination of TBARS (oxidative stress): The levels of TBARS, a marker of lipid peroxidation[24-26] were estimated in liver and kidney tissues using the method of Niehaus and Samuelsson[27]. Tissues were homogenized in tris-HCl buffer (pH 7.5) followed by centrifugation to remove the cellular debris. An aliquot (100 µL) of supernatant from the tissue homogenate was mixed with 2 mL of TBA-TCA-HCl (1:1:1) reagent (0.37% TBA, 15% TCA and 0.25 N HCl) and the tubes were placed in a water bath at 90°C for 10 min. The tubes were allowed to cool at room temperature and then the absorbance of the colored solution was measured against a reagent blank at 535 nm.

Histopathological studies: Tissue samples were fixed in 10% neutral formalin for 24 h and paraffin blocks were prepared and routinely processed for light microscopy. Slices of 4-5 µm were obtained from the prepared blocks and stained with hematoxylin-eosin. The preparations obtained were visualized using a Nikon microscopy at a magnification of 400×.

Statistical analysis: Results are presented as Mean±SEM. Comparisons were done by two-tail unpaired Student's t-test or one-way ANOVA as appropriate. The $p \leq 0.05$ were considered significant.

RESULTS

Hepato-renal function biomarkers: The administration of *Echis coloratus* venom to the rats caused significant increase in AST, ALT and ALP and blood serum creatinine levels, when it was compared with control group. Co-administration of quercetin decreased the rise in AST, ALT and ALP and blood serum creatinine levels in Fig. 3-6, respectively.

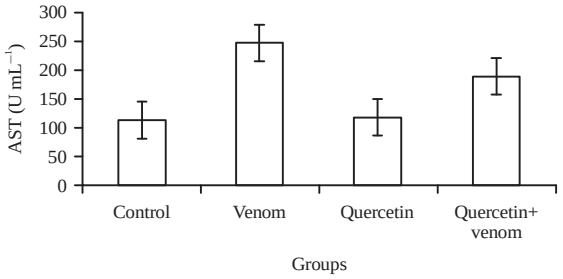

Fig. 3: Effect of the treatments of the groups in serum AST activity, values is expressed as Mean±SEM (n = 6). Results are represented in the form of Mean±SE, *Significant at p<0.05

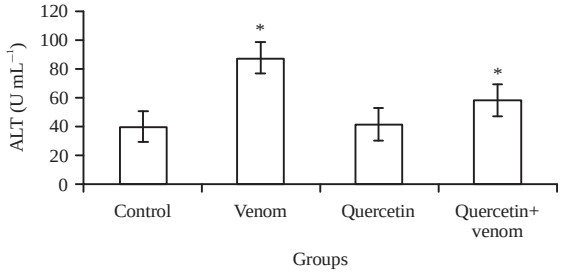

Fig. 4: Effect of the treatments of the groups in serum ALT activity, values is expressed as Mean±SEM (n = 6). Results are represented in the form of Mean±SE, *Significant at p<0.05

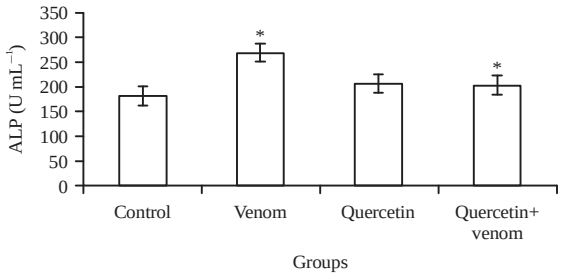

Fig. 5: Effect of the treatments of the groups in serum ALP activity, values is expressed as Mean±SEM (n = 6). Results are represented in the form of Mean±SE, *Significant at p<0.05

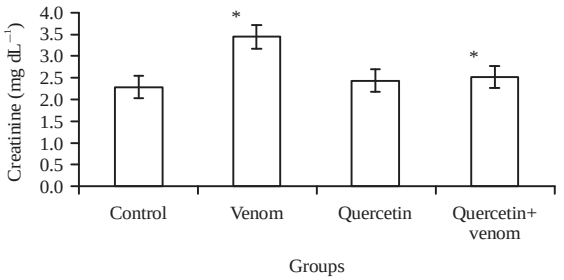

Fig. 6: Effect of the treatments of the groups in serum creatinine activity, values is expressed as Mean±SEM (n = 6). Results are represented in the form of Mean±S.E, *Significant at p<0.05

Oxidative stress: In the liver and kidney tissues, MDA was significantly increased in *Echis coloratus* venom treated group as compared to control group and this rise in MDA was decreased by quercetin (Table 1).

Histology: The histology of the liver tissue of the control and quercetin-treated animals showed normal histological structure of hepatocytes, central vein blood sinusoid and nuclei (Fig. 7), whereas in venom group showed cellular infiltrations, degenerative changes of hepatic cells with cell necrosis and disarrangement of normal hepatic cells were observed. The histology of liver tissues of venom+quercetin group showed less degeneration, necrosis and disarrangement of normal hepatic cells (Fig. 7).

The histology of the kidney tissue of control and quercetin-treated animals showed normal morphological appearances (Fig. 8), whereas the venom group showed congestion, necrosis and degeneration in the epithelial cells of renal tubules and swelling in the lining endothelium of the glomerulus. The histology of kidney tissues of venom+quercetin group showed less congestion, necrosis, degeneration in the epithelial cells of renal tubules and swelling in the lining endothelium of the glomerulus (Fig. 8).

Table 1: Effect of quercetin on *Echis coloratus* venom induced MDA levels in rat tissue

Tissues	Control	Venom	Quercetin	Venom+quercetin
Kidney	64.96±16.12	87.08±17.38*	63.25±29.61	35.55±3.83*
Liver	58.45±7.64	76.86±9.42*	62.93±6.19	49.12±2.49*

Results are represented in the form of Mean±SE, *Significant at p<0.05, Values expressed as Nm of MDA g^{-1} weight of tissue

DISCUSSION

Human hemostasis is severely deteriorated by *E. coloratus* envenoming and it was reported that anemia ensued due to excessive bleeding in about a third of the victims[28]. In this study, it was noticed that envenomed rats showed hemorrhagic signs and spots in different organs after sacrifice, similar to the reported observations by other investigators[9]. Such observations could be attributed to factors are coagulopathic and defibrinating in content[9]. Organs with hemorrhagic areas are formed due to their cells basal membrane alterations of the endothelial lining vascular permeability that allowed blood to ooze into tissues in the vicinity. Envenoming by *E. coloratus* usually allows for the formation of thrombotic hepatic portal vein leading to blood vessels congestion and damage of the cell membrane endothelial lining. On the other hand, phospholipase A_2 enzyme releases plasma lysolecithin and also directly destroys cell membranes[9]. Venoms of such vipers like *E. coloratus* and limited numbers of other snakes have the ability to form thrombin by precursors of prothrombin[29].

Liver function markers (ALT, AST and ALP) reflected hepatic dysfunction by showing significant increase of their activity levels in rat sera, in comparison with control groups. This situation is speculated and envisaged through free radicals production induced by *E. coloratus* viper envenoming that led to hepatotoxic oxidative stress and the ensuing extra hepatic obstruction and damage in liver function[30,31]. Rise in liver enzymes that followed viper venom administration is indicative of hepatocellular injury[32]. Hepatocytes mitochondria get congested with liver enzymes and the biochemical indices increased due to the altered transportation input/output of the mitochondrial membrane[33-36].

On the other hand the induced renal stress was viewed through elevation of the serum creatinine, which is a marker of nephritis[37-42]. As a protein metabolism waste product that is normally excreted by kidneys, creatinine excess levels in serum affirms some degree of deficient kidney function[43] as a specific kidney marker[44].

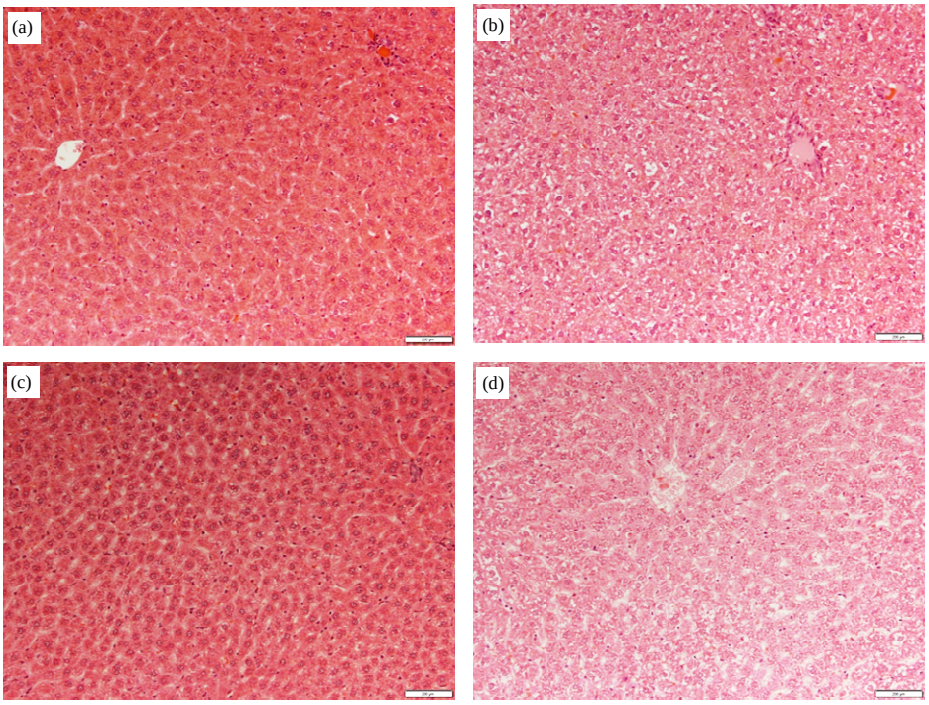

Fig. 7(a-d): Effect of quercetin on snake venom induced histological changes of liver tissue. Quercetin enhanced the protective mechanism of the liver and helped in the restoration of the damaged histology. Representative sections of the liver of (a) Normal rats, (b) Venom treated rats, (C) Quercetin treated rats and (d) Pretreated with quercetin+venom

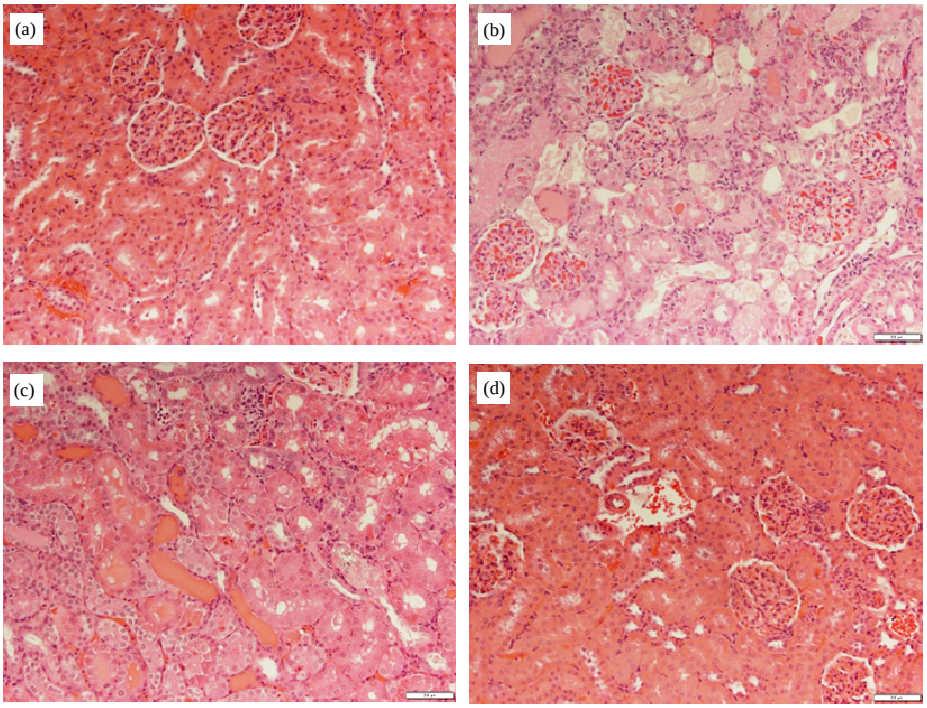

Fig. 8(a-d): Effect of quercetin on snake venom induced histological changes of liver tissue. Quercetin enhanced the protective mechanism of the kidney and helped in the restoration of the damaged histology. Representative sections of the kidney of (a) Normal rats, (b) Venom treated rats, (c) Quercetin treated rats and (d) Pretreated with quercetin+venom

The out coming results of the excess hepatic and nephrotic tissue MDA levels also confirm the oxidative stress and lipid peroxidation indication[45] and free radicals action[46]. Extensive explanation of the free radicals production and various mechanisms of actions and the countering antioxidant defense mechanisms were previously reported[47,48]. This has been modulated in several recent studies[49-55].

The scavenging ability of quercetin[56] and polyphenol compounds[57] convey protective effects against the actions of venoms and free radicals. Confirming similar studies on rats using *Vipera russelli* venom were previously done by Ali *et al*.[58].

The findings of the present study confirm the outcome of previous studies on mice MDA levels[53] mechanisms involved in lipid peroxidation, fatty acids, adipose tissue[59,60] and inhibition of PLA2[61] and membrane stabilization improvement[62,63]. Quercetin could have restored the balance between the venom-induced free radicals and the defensive antioxidant system mechanisms with the tissue redox state[64]. The massive cellular activity and mechanisms involved in the associated venom detoxification and nuclear interruption lead to appreciable karyorrhexis and pyknosis in the nuclei, in the outcome of this present study that reflected the cellular changes and necrosis, following administration of *E. coloratus* venom. Such findings were also concluded following the employment of other venoms (cobra snakes) done by Rahmy *et al*.[65].

Kidneys histological tests confirmed the renal stress evaluated by sera analysis, viewed in the tissue alterations. Further, cortical renal necrosis and related to intravascular coagulation added to vasospasm toxic effect action mechanisms were previously discussed[9,66].

CONCLUSION

The present experimental results indicate that quercetin was beneficial in countering the toxic effects of *E. coloratus* venom and or has an alternative or complementary treatment strategy of envenoming. Further experimental approaches could address quercetin, which could possibly lead to the development of pharmaceutical formulations for treating snake bite victims.

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