Morphological Changes in Various Tissues After Russell'S Viper (Vipera Russelli) Envenomation

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Background: The effects of Russell 's viper Venom (RVV) on tissues were studied in 24 Rabbits injected with 1 MLD, ½ MLD and 1:20 MLD RVV.
Materials and Methods: Lungs, Livers and Kidneys of these animals were examined by light microscope. Lungs and Liver of all animals of all groups showed intravascular clotting with RBC sludging & formation of platelet thrombi.
Results: Kidneys of 1 MLD and ½ MLD group showed congestion of glomerular capillaries whereas interstitial blood vessels showed RBC sludging and platelet thrombi. Kidneys of 1:20 MLD group showed proliferation of endothelial and mesangial cells with degeneration of both proximal and distal tubules indicating both direct nephrotoxic action of RVV as well as damage produced by DIC like action of the venom.
Conclusion: The results of present work show that RVV produces DIC and haemodynamic changes in blood vessels of various organs in acute poisoning. In chronic poisoning in addition to DIC the Nephrotoxic effects are more prominent. Nephropathy seems to be due to both DIC and direct Neprotoxic effects of the venom.
Key words: Russell's viper venom, rabbits, liver, kidney and lungs.

Introduction

Snake bite is a public health problem in many countries of the world including Pakistan. It is estimated that about 30,000 to 50,000 deaths occur annually, around the world, due to snake bites. In Pakistan important poisonous snakes are Cobra (Naja naja), Russell's Viper (Vipera Russelli), Saw-Scaled Viper (Echis carinatus) and Kraits (Bungarus caeruleus). Almost every species of these snakes can cause renal failure but Russell's Viper is well known for its effects on coagulation and effects on various tissues.1,5,6 Russell's Viper is also known as one of the deadliest snakes. Lot of work has been done on Russell's Viper Venom (RVV), both on its coagulation action and tissue toxicity. Reports on nephrotoxicity of RVV are conflicting and on other organs are a few.1,2,3 Various experimental models have been produced on animals and both in vivo and in vitro studies have been done to see morphological effects in various tissues particularly kidney.1-3,6,7,13 In most of the experiments high doses of RVV i.e., 2 MLD, 1 MLD, ½ MLD were used.

Present study was designed to observe tissue changes in livers, lungs and kidneys of Rabbits using both high doses of RVV i.e., 1 MLD and ½ MLD as well as prolonged poisoning i.e., 1:20 MLD of RVV.

Materials and Methods

Snake Venom: Lyophilized Russell's Viper Venom was obtained from National Institute of Health, Islamabad. The venom was reconstituted in Phosphate bufferd saline (pH7) in such a way that one ml of diluted fluid contained 2.6 mg of crude RVV. The dilutions of venom were made with normal saline just before injection.

Animals: A total of 32 local domestic rabbits were used for this study. The average weight of the animals at the commencement of experiment was 1.5 kg.

Injection Schedule

Group I: Eight male animals were included in this group. Each of these animals received 1.0 ml of the RVV using intramuscular route. All these animals died 24 hours after envenomation. Autopsies were performed on these animals. Kidneys, Lungs and Livers of these animals were preserved in 10% formal saline.

Group II: Eight male animals were included in this group. Each of these animals received ½ ml of RVV diluted with ½ ml of normal saline using the intramuscular route. All these animals died thirty hours after envenomation. Autopsies were performed on these animals. Kidneys, Lungs and Livers of these animals were preserved in 10% formal saline.

Group III: Eight male animals were included in this group. Each of these animals received 10-18 doses of 1:20 dilution of RVV at the interval of three days. Six animals died after receiving 10-16 doses of venom;

whereas the remaining two animals were killed at the end of the experiment i.e., when they had received 18 doses of RVV. Autopsies were preformed on these animals. Kidneys, Lungs and Livers of these animals were preserved in 10% formal saline.

Group IV: Eight male animals were included in this group. Each of these animals received 1.0 ml of Physiological Saline through intramuscular route at the time of envenomation of each experimental animal. All these animals were sacrificed at the end of experiment. Autopsies were preformed on these animals. Kidneys, Lungs and Livers of these animals were preserved in 10% formal saline.

Gross Morphological Examination: A thorough macroscopic search was done for gross lesions in these organs.

Histological Processing and Microscopic Study: Representative blocks were taken and sections were stained with Haemotoxylin and Eosin stain after standard histological processing using acetone, xylene and alcohol. In a few selected section of kidneys methenamine-silver stain was also carried out.

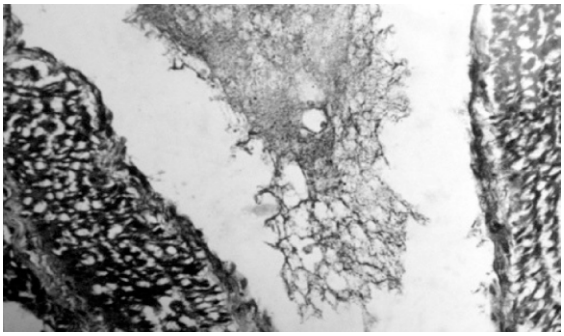
Results

Morphological changes were same in 1 MLD and ½ MLD groups and the changes were different in 1:20 MLD group particularly in kidneys. All these organs were normal in control animals.

Lungs: On gross examination lungs were congested, odematous and were having sub pleural petechial haemorrhages in almost all animals of 1 MLD, ½ MLD and 1:20 MLD groups.

Histological examination showed RBC sludging in arteries and veins of lungs with fine fibrin network **(Fig. 1).**

Fig-1: Photomicrograph of pulmonary blood vessel showing fibrin and platelet thrombus.



Early thrombus formation was also seen in lung Blood vessels. Some alveoli in 1:20 MLD group showed proliferation of their lining endothelium.

Liver: On cut section, liver was congested in all animals of all the groups.

Microscopic examination showed exaggeration of its lobules, congestion with RBC sludging in its blood vessels. Platelet and fibrin thrombi were seen in its veins and arteries in almost all animals of all groups.

Interstitial blood vessels showed RBC sludging & platelet thrombi **(Fig. 2).**

Fig-2: Photomicrograph of a glomerulous showing mesengial Cell proliferation and capsular adhesion. The changes were more prominent on Methenamine Silver stain.

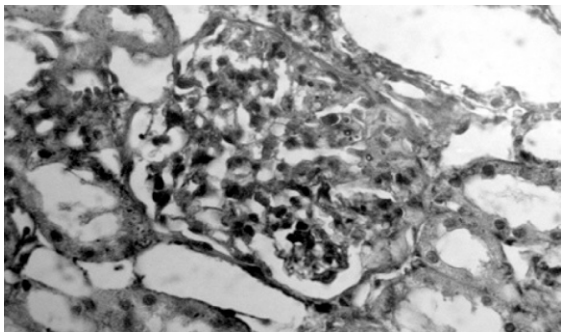


Fig-3: Photomicrograph of a glomerulous showing increased mesengial matrix (methenamine silver)

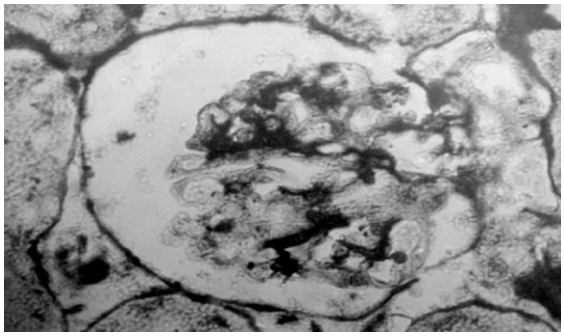
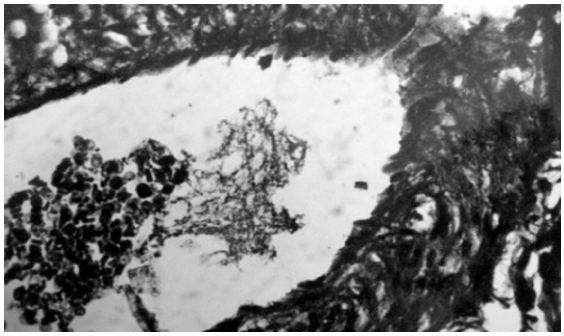


Fig-4: Photomicrograph of a renal vessel showing fibrin RBC thrombus.

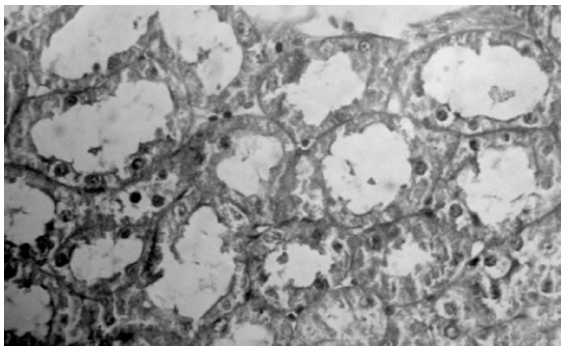


Kidneys: On gross examination, kidneys did not show any significant change.
Microscopic examination in 1 MLD and ½ MLD group showed congestion of glomerular capillaries.
Proximal and distal tubules showed necrosis, clear cell change with protein casts.
Kidneys of 1:20 MD group animals showed following changes.

Glomerular Changes: Glomeruli showed congestion with focal proliferation of both endothelial and mesangial cells. Mesangial matrix was also increased. Capsular adhesions were seen in most of glomeruli.

Tubules: Both proximal and distal tubules showed degeneration and necrosis with retention protein casts **(Fig. 5)**

Fig-5: Photomicrograph of proximal convoluted tubules showing epithelial cell degeneration.



Blood Vessels: Blood vessels were congested and were having platelet thrombi.

Discussion

In the present study, the main morphological abnormality was intravascular clotting in the blood vessels of lungs, livers and Kidneys in all groups of animals after RVV envenomation. It has been observed by many other workers[13-15] that RVV has got coagulant action both in vivo and in vitro. Hypo fibrinogenemia and thrombocytopenia are the main features of RVV and even Cobra Venom[16] poisoning. This change develops due to consumption Coagulopathy and ultimate deposition of fibrin threads and platelet aggregates in blood vessels of various organs.

The prominent morphological changes after RVV bites are observed in the Kidneys.[1,6, 7,8,9] In our experiment, intravascular clotting and congestion of glomeruli was seen in 1 MLD and ½ MLD group indicating DIC like effect of the RVV. The tubular necrosis was also seen in the animals of these groups indicating Nephrotoxic effect of RVV. The direct toxic effects of RVV are directly capable to produce renal tubular necrosis.

Chugh etal[8] and Sarangi etal[9] have also reported acute renal failure in patients of RVV bite. They also agree that its pathogenesis involves many factors including DIC, intravascular haemolysis, hypotension and direct nephrotoxicity.

In the present study more prominent changes have been observed in kidneys in relatively chronic poisoning by RVV i.e., using 0.05 MLD. These changes include proliferation of endothelial and mesangial cells with degenerative changes in tubules. Similar observations have been made by other workers[7,8,9] particularly in persons who do snake charming. Similar type of chronic poisoning by the various venoms can be observed in persons who do milking of snake venom. Even in snake bites, sometimes depot of venom stays at the site of bite and small amounts are released slowly and hence this type of chronic nephrotoxicity can be seen in these individuals.

The results of present work show that RVV produces DIC and haemodynamic changes in blood vessels of various organs in acute poisoning.

In chronic poisoning in addition to DIC the Nephrotoxic effects are more prominent. Nephropathy seems to be due to both DIC and direct Neprotoxic effects of the venom.

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