The Effect Of Carnosine On Venom Induced Acute Kidney Injury In Experimental Rat Model

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Introduction

- Carnosine is a naturally occurring multifunctional dipeptide made up of the amino acids beta-alanine and L-histidine. hypothesized to have a number of antioxidant properties and has been shown to be an antiglycating agent. Snake venom causes ischemic AKI with occlusion of left renal artery. L-carnosine prevents the development of ischemia/reperfusion-induced renal injury and the effect is accompanied by the suppression of enhanced norepinephrine release in the kidney immediately after reperfusion. Thus the preventive effect of L-carnosine on ischemic AKI is probably through the suppression of enhanced renal sympathetic nerve activity induced by ischemia/reperfusion (Matsumura *et. al.*, 2006).

Aims & Objectives

- Development of Russell's Viper venom induced Acute Kidney Injury model.
- Evaluation of the role of carnosine in experimental venom induced Kidney Injury model.
- Establishing the mechanism of action of carnosine in ameliorating Acute Kidney Injury by snake venom.

Methods:

Venom Preparation : Russell's Viper venom procured as a gift from Dr. Debanik Mukherjee, Herpetologist, New Delhi, India, was prepared at the dose of 10µg/100g of animal. The dose was prepared by dissolving the venom in saline.

Preparation of Carnosine : Carnosine was prepared at the dose of 10mg/ml of saline. For injection the carnosine was prepared by incubating it with the venom solution for 10mins at 37˚C. For intraperitoneal (i.p) dose carnosine was kept the same barring the venom.

Collection and Conditioning of the Animals : 28-30 weeks old male albino rats(weight 130±10gm) were collected and was housed under control condition(room temp 23±2˚C, relative humidity 60±5%, 12 hour light dark cycle).

Grouping of animals : 18 rats were taken and they were divided into 3 groups, each group containing 6 animals. Groups are as follows

Group 1 : Control

Group 2 : Venom Injected

Group 3 : Venom Injected and treated with Carnosine.

- **Development of Snake Venom induced AKI**
- On the 0th day the animals of the venom injected group(group 2) were administered injection through the i.v route in the tail vein at volume of 200µl.
- The tail turn blackish after a while morphologically showing the venom indeed passed through i.v route since the venom induces hemorrhage. Confirmation of AKI is done by noting the time when this group animals first passes blood in their urine.

Treatment Schedule

Animals of each group was given an equal volume load so as they urinate. The schedule of volume load given is as under :

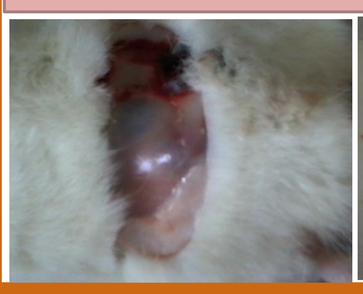
1. In control animals of group 1, in order to provide equal stress, 2ml saline was administered intraperitoneally(i.p) at a time 30mins from the time of i.v administration in animals of group 2 & 3. Then 100µl of saline was administered as i.p at 1hr interval upto 6 times. Then a final volume of 0.3ml was injected i.p as a load in order to obtain overnight urine.
2. In venom animals of group 2, similarly a volume of 2ml saline was injected through i.p at the time 30mins from their venom administration. Then 100µl of saline was continued to be administered through i.p till the 6th time after which a final volume load of 0.3ml was administered through i.p so as to obtain the overnight urine.
3. In the animals of group 3 in which carnosine incubated with venom was injected, a volume of 2ml carnosine that was prepared for i.p administration(dose 2mg/2ml saline) was given through i.p at the time 30mins from the time of i.v administration. . Then 100µl of carnosine was continued to be administered through i.p till the 6th time after which a final volume load of 0.3ml was administered through i.p so as to obtain the overnight urine.

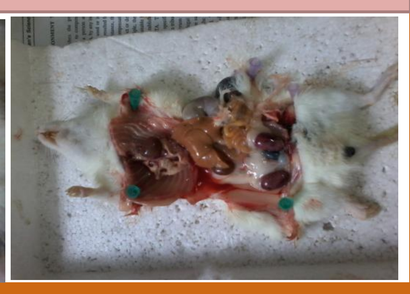
Next day, i.e 24hours later, after urine collection, all the animals were allowed to feed. Then again continued to the same treatment schedule starting from the time when i.p volume load was administered the previous day. Again urine was collected the next day, i.e 48 hours later. Eventually the animals were then sacrificed.

Results with Photographs

- Venom inflicted AKI model was established which was manifested by reduced urinary function and shows signs of proteinuria, hematuria/haemoglobinuria, and urinary crystals.
- Elevation of plasma creatinine, MG, AOPP were observed in venom injected group of rats as compared to sham control.
- Histopathological alterations including glomerular injury, increased Bowman's space, tubular necrosis, etc. were also observed in venom injected group of rats.
- Per oral treatment of carnosine showed to prevent all the above signs of renal inefficiencies and damage significantly ($p<0.05$).

Photograph showing Systemic Inflammation in group 2 venom induced animals shows hemorrhage & systemic inflammation

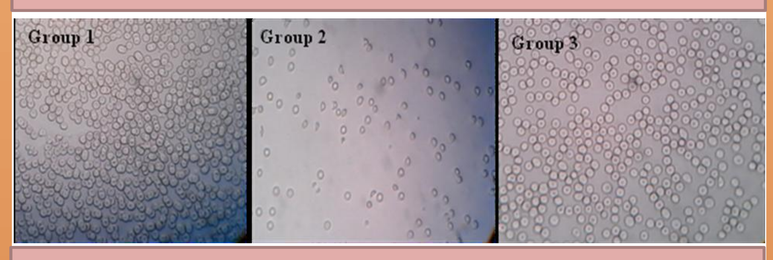




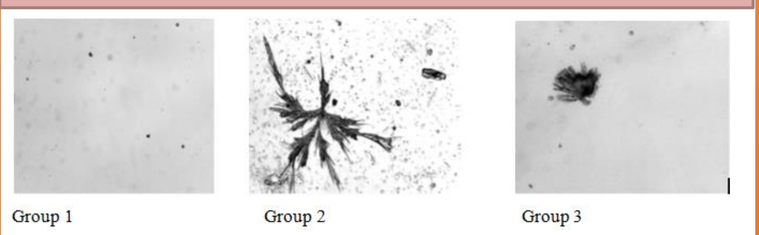
Plasma Biochemical Changes

Parameters	Control (n=6)	Venom Induced (n=6)	Carnosine Treated (n=6)	One Way Anova (p value)
Plasma Creatinine (mg/dl)	0.63±0.023	2.47±0.193	0.38±0.022	<0.001
Total Protein (g/dl)	165.65±7.17	66.23±3.98	161.18±5.85	<0.001
Plasma MG (µmol)	27.02±0.847	59.45±2.99	36.03±1.376	<0.001
Plasma AOPP (µmol Chloramine T equivalent)	73.38±5.75	170.6±6.41	68.50±2.444	<0.001
Parameters	Control (n=6)	Venom Induced (n=6)	Carnosine Treated (n=6)	One Way Anova (p value)
Plasma AGE (AU/mg protein)	0.13±0.005	0.44±0.089	0.14±0.006	<0.001
Plasma DT (Au/mg protein)	0.2±0.007	1.2±0.173	0.19±0.008	<0.001
Plasma Pentosidine (AU/mg protein)	0.25±0.011	0.83±0.118	0.41±0.033	<0.001
Plasma Trptophan Fluorescence (AU/mg Protein)	4.52±0.221	5.34±0.439	4.92±0.256	0.230 N.S*

Photomicrograph showing spherocytes in blood slide of group2 animals having AKI while only few numbers of spherocytes in group 3 animals upon comparison with control group1 animals.



Photomicrograph showing urine smear of animals of venom induced group 2 having different types of crystals as well as RBCs. In group 3 animals treated with carnosine very few RBCs and crystals are seen. Thus showing venom induction causes kidney injury while carnosine acts to prevent it, upon comparison with normal group 1 animals which has no RBC or crystals in the field of the slide.



Group 1 Group 2 Group 3

Parameters	Control n=6	Venom Induced n=6	Carnosine Treated n=6	One Way Anova(p value)
Urinary Creatinine (mg/dl)	145.21±11.668	57.47±4.963	164.57±5.415	<0.001
Urinary Microprotein (mg/l)	538.88±30.814	1589.6±148.802	1046.6±52.688	<0.001
Urine Volume (ml)	2.13±0.091	0.77±0.177	1.77±0.216	<0.001
Creatinine Clearance(dl)	495.56±47.958	19.61±4.842	784.04±109.97	<0.001
Urinary Microprotein : Urinary Creatinine Ratio	0.39±0.058	2.91±0.445	0.64±0.027	<0.001

Conclusions

These findings suggests that carnosine have renoprotective potential against venom induced AKI. More studies are needed to establish underlying mechanism.

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