Research Article

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EFFICACY OF JEEVARAKSHAKA GUTIKA IN THE MANAGEMENT OF COBRA VENOM INJECTED WISTAR ALBINO RATS

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ABSTRACT

Jeevarakshaka gutika (JRG) is a herbomineral compound used in snake bite cases in Kerala. The folklore practitioners were using this drug from many years and found good efficacy against cobra bite. In the present study an attempt has been made to explore the therapeutic value and underlying mechanism of action in wistar albino rats injected with cobra venom. The lyophilized snake venom of cobra (*Naja naja*) was dissolved in saline and required concentration was prepared. The lyophilized polyvalent snake venom antiserum was used as reference standard drug. The test drug JRG was evaluated for its efficacy to neutralize various actions of venom like lethality, haematological, biochemical and histological changes. The median lethal dose of cobra venom found to be 550 µg/kg body weight of rat. Orally administration of JRG at a dose of 540 mg/kg bodyweight effectively neutralised the lethal effect of LD 50 cobra venom and moderate neutralisation of 2 X LD 50 dose. The test drug didn't neutralise the toxic effect of cobra venom *in vitro*, all the animals died after intra muscular administration of incubated cobra venom and test drug. Haematological examination has shown significant increase in RDW-CV and non significant decrease in WBC, lymphocyte, monocytes and platelet count in comparison to normal control group. JRG has shown significant increase in WBC, platelet and MCHC and significant reduction in neutrophils, lymphocyte, mean corpuscular volume and red cells distribution width in comparison to venom control group in comparison to venom control group.

Keywords: Antiserum, Jeevarakshaka gutika, lyophilized snake venom, mean corpuscular volume.

INTRODUCTION

Snake bite is one of the major health problems at the global level. Considering the geographical pattern of whole world, it is estimated that the true incidence of snake bite/envenomation could exceed 5 million per year. About 1,00,000 of these develop severe sequel. Around 30 to 40 thousands of people die every day due to snake bite all over the world[1-3]. Anti Snake Venom (ASV) is a specific antidote to snake venom actions. In India polyvalent ASV is available which contains antibody against cobra, Russell's viper, common krait and saw-scaled viper. ASV neutralizes the circulating venom only and no amount of ASV will neutralize or combine with venom once the venom is attached or absorbed to target organs i.e. platelets, RBC's vascular endothelium, Renal tubules, muscles and neuromuscular receptors[4,5]. Snake venoms area cocktail of many components i.e. enzymes, polynucleotide's toxins, non toxic protein, carbohydrates, metals, lipids, free amino acids, nucleotides and biogenic amines. Venom acts quickly and causes death if it is not treated immediately. In most of the cases, there is likely to be a long distance to reach nearest hospital facilitated with anti venom therapy and enhances the mortality rate[6,7].

Folklore practitioners manage these emergency situations with wide range of herbomineral formulations, which are affordable to the majority of victims who belongs to very low socio-economic communities[8]. A thorough review of our literature and experience of the physicians of Kerala in India led to selection of research drug Jeevarakshaka Gutika (JRG) which is mentioned in Prayogasamucchyam by Kocchunni Tampuran - a Visha Chikitsa text belonging to the tradition of the physicians of the state of Kerala and no where found in literature of Ayurveda. The author of this authentic book of toxicology is known to be of Kochi dynasty[9,10]. The present knowledge in Visha (poison) and its Chikitsa (treatment) is based on a few books written exclusively on the subject like Uddesa, Ulpala and Lakshanamritha. Although references of JRG are available in many texts viz., Kriyakaumudi, Kodasserimargam, Prayogika Vishavaidya etc, the one which is mentioned in Prayogasamuccaya has been pharmacologically analysed for its toxicological effects[5]. Since long time JRG has been used successfully in the snake bite management by the physicians with the intension of extending the time required for spreading of

Visha to Uttarottara Dhatu. Therefore, it is felt that there is a necessity to evaluate the efficacy of JRG in the management of snake bite using experimental animals.

MATERIALS & METHODS

All the chemicals and reagents used in the experimental study were procured from standard and reputed firms and are of analytical grade regularly used in the laboratory. The biochemical and enzymatic kits for biochemical investigations were obtained from ERBA Diagnostic Mannheim, Transasia Biochemicals Ltd. Daman.

Chemicals

1. The lyophilized snake venom of cobra (*Naja naja*) was procured from Haffkines Institute, Mumbai- India.
2. Lyophilized polyvalent snake venom anti sera (ASV) was obtained from Koppa Taluq hospital Chikmagalore District Karnataka India.
3. Jeevarakshaka gutika (JRG) – Prepared according to classical method mentioned in the book Prayoga Sammuchaya written by Kochunni tampuran of Kochi dynasty and Purchased from Manmathan Vaidyar a folklore practitioner from Kerala.

Experimental animals

Wistar albino rats of either sex weighing between 180 to 220g were obtained from the animal house attached to the Pharmacology Laboratory, SDMCRA, Udupi and were used for present study. The animals were fed with rat pellet and water *ad libitum*. The animals were maintained at controlled condition i.e. temperature (23 ± 2ºC) and humidity (50 ± 5%) and animals were exposed to natural day and night cycles. The experiments were carried out in conformity with guidelines of the Institutional Animal Ethical Committee with its approval (IAEC approval no, 2012-13- HSN 06).

Test drug

The test drug Jeevarakshaka gutika (JRG) was made into fine suspension in vehicle with suitable concentration. All the animal were dosed constant volume i.e. 1ml/100g body weight. The test formulation was administered through oral rout to respective animal through oral feeding needle attached to disposable syringe. The intended human dose converted to rat dose on the basis of body surface area as per Paget and Barnes.

Therapeutic equivalent to human dose (TED) = 6g X 0.018X 5 i.e. = 0.54g/kg body weight.

Procedure
Acute oral toxicity study of JRG

The acute oral toxicity study was carried out as per OECD guidelines 425 using AOT software. The Jeevarakshaka Gutika tablet was made into a suspension in 0.5% gum acacia and dosed in the following order 175, 550, and 2000mg/kg body weight. After the dosing the animals were observed for 14 days for mortality. The LD 50 was determined by using AOT software.

Evaluation of LD 50 of the cobra venom

The median lethal dose LD 50 of Cobra venom was determined by acute toxicity study by following OECD guidelines 425. The acute toxicity of cobra venom was assessed by intramuscular administration of different concentrations of venom dissolved in physiological saline to groups of wistar albino rats weighing 180g-220g according to AOT software. The general behaviour and mortality were observed for 48 h after the administration of venom.

Neutralization of lethality

The study comprises 4 groups of 6 animals each with body weight ranging from 180- 220g. Group I rats received only normal diet and tap water and served as normal control group. Group II rats served as positive control group administered with only cobra venom. Group III rats considered the standard group, which received cobra venom plus polyvalent snake venom (ASV). The test groups were divided into two groups, the first one with JRG administered at therapeutic dose and the second one in which the double the therapeutic dose along with cobra venom.

In-vivo neutralization potency

The *in-vivo* neutralization potency of JRG was assessed by intra muscular administration of LD 50, 2×LD50 dose of cobra venom into different groups of rats. The test and standard drugs were administered after 5 minutes of venom administration. The following parameters were assessed for *in vivo* neutralization of venom[11-14].

1. Number of death at different time intervals
2. Percentage survival at different time interval

In- vitro Neutralization of potency

The *in vitro* neutralization test was carried out by mixing 540mg and 1.08g of JRG with LD-50, 2×LD-50 of the venom sample in a test tube and incubating the mixture at 37 ^{0}C for 30 minutes and then administered intra muscularly to the rat. The following parameters were assessed to determine *in vitro* neutralization[15].

1. Number of death at different time intervals
2. Percentage survival at different time interval

In vivo neutralisation of JRG on repeated dose

The test group rats were administered with JRG for 5 consecutive days; on 5th day one hour after the last JRG doses 1/4th LD50 cobra venom was injected and the animals were sacrificed under deep ether anaesthesia. Blood was collected and sent for biochemical investigation and the following organs liver, kidney and

stomach were excised out and cleaned carefully and histopathological examination was carried out.

Statistical analysis

The lethal dose (LD50) of the venom was expressed as µg/kg body weights of rats and was generated by AOT software (OECD 425 guidelines). The in vitro, in vivo neutralisation and biochemical data were expressed as Mean ± SEM of 6 animals per group. Parametric one way Analysis of Variance (ANOVA) test was performed using Graph pad prism 5.0. The minimum level of significance was identified at $p<0.05$- with Dunnet's multiple 't' test as post hoc test.

RESULTS

The antivenom potential of JRG against cobra venom in rats was assessed by in vivo and in vitro neutralisation methods. The median lethal dose (LD 50) for rats was assessed by following OECD 425 guidelines using AOT software and was found to be 550 µg/kg body weight of rat by im route. The median lethal dose of test drug JRG was found to be more than 2000 mg/kg body weight of rat by oral route.

***In vivo* neutralisation of lethality of cobra venom**

JRG administered group has shown complete detoxification of cobra venom administered at LD50 and all six rats were survived during 24 hours of observation and hence the survival rate is 100%. All the animals in the cobra venom group died after the venom administration. The standard administered with polyvalent antivenom has shown 100% survival rate (Table 1).

JRG administered group has shown mild detoxification of cobra venom administered at 2 X LD50 and five out of six rats died during 24 hours of observation. And hence the survival rate is 16.6 %. All the animals in the cobra venom control group died after the venom administration. The standard group administered with polyvalent antivenom showed 100% survival rate (Table 2).

***In vitro* neutralisation of lethality of cobra venom**

To assess *in vitro* neutralisation , the test drug were mixed with LD 50 and 2 X LD50 of cobra venom and incubated at 37 º C for 30 minutes and then injected i.m into the rat. The test drug was found to be not effective in neutralising the lethal activity of cobra venom. The entire animal in the cobra venom and test group died after venom administration (Table 3 & 4).

***In vivo* neutralisation of JRG on repeated dose**

Twelve haematological parameters were assessed viz- WBC count, differential count, RBC count, platelet count and derived blood indices like MCV, MCH, MCHC, and RDWCV. Administration of venom resulted in only one change that is significant increase in RDW-CV. Though moderate changes were observed in WBC count – (decrease), lymphocyte percentage (decrease), monocytes percentage (increase) and platelet count (decrease) in venom control in comparison to the normal control the observed effect was found to be statistically non-significant. The JRG administered along with venom group rats have shown significant elevation in WBC, platelet, mean corpuscular haemoglobin content and significant reduction in neutrophils, lymphocyte, mean corpuscular volume and red cells distribution width in comparison to venom control group (Table 5).

The analysis of data related to biochemical parameters has shown there is a significant reduction in alkaline phosphatase activity, serum total cholesterol and creatinine kinase levels in cobra venom control group in comparison to normal control group. Whereas the standard group administered with polyvalent antivenom and JRG has shown nearly normal values in comparison to venom control group. The bilirubin total significantly increased in venom control group in comparison to normal control group and the observed elevation was not affect by JRG and ant venom therapy (Table 6).

The histological examination of the sections of heart from different groups was carried out at different magnifications to ascertain the venom induced changes and influence of test drug and reference standard treatment on these changes. Examination of sections of heart from venom control group revealed presence of myocarditis, necrotic lesions and hemorrhagic streaks in two rats, mild myocarditis was observed in one rat and rest of the rats exhibited only minor changes in the profile. The heart sections from test drug administered and venom injected group revealed normal profile in all the six rats. In ASV treated group normal profile was observed in 4/6 rats in the remaining two mild oedema and myocarditis was observed (Figure 1).

Examination of kidney sections from venom injected group revealed almost normal profile in sections from 3/6 rats. Glomerular dilatation was observed in one rat and fatty changes in another. In test drug administered and venom injected group almost normal profile was observed in section from 4 rats. Mild cell infiltration and haemorrhage was observed in one rat and hyaline changes with fatty changes were observed in another rat, necrosis was also observed. Normal profile was observed in ASV treated group (Figure 2).

Examination of liver sections from venom injected group revealed the following changes in different degree- cell infiltration, micro and macro fatty changes, sinusoidal dilatation and haemorrhage. This was observed in sections from almost all the rats included in this group. In test drug administered and venom injected group almost normal profile was observe in 2 rats. Mild fatty changes with sinusoidal dilatation were observed in 3 rats. Sinusoidal dilatation and mild fatty changes were observed in 1 rat. In ASV treated group normal profile was observed in 2 rats and in rest of the rat's mild fatty changes in the parenchyma cells with sinusoidal dilatation was observed (Figure 3).

Examination of stomach sections from venom injected group revealed almost normal profile in sections from 3/6 rats. In others focal cell infiltration, fatty changes, erosion and necrosis of epithelial layer was observed. In test drug administered and venom injected group sections from all the rats exhibited normal cytoarchitecture. In ASV treated group 4/6 rats exhibited normal profile. Mild oedema in submucosal layer with mild cell infiltration was observed in the remaining rats (Figure 4).

Table 1: *In vivo* neutralising effect of Jeevarakshaka Gutika (JRG) in rats administered with LD50 (550 µg/kg body weight) of cobra venom

Group	Mortality*	% survival
Control	6/6	0
Venom + polyvalent anti venom	0/6	100
Venom + JRG (0.54g/kg)	0/6	100

* number of death/no. of rats used during 24 hours

Table 2: *In vivo* neutralising effect of Jeevarakshaka Gutika (JRG) in rats administered with LD50 (2 x 550µg/kg body weight) of cobra venom

Group	Mortality*	% survival
Control	6/6	0
Venom + polyvalent anti venom	0/6	100
Venom + JRG (0.54g/kg)	5/6	16.6

* number of death/no. of rats used during 24 hours

Table 3: *In vitro* neutralising effect of Jeevarakshaka Gutika (JRG) in rats administered with LD50 (550 µg/kg body weight) of cobra venom

Group	Mortality*	% survival
Control	6/6	0
Venom + polyvalent anti venom	0/6	100
Venom + JRG (0.54g/kg)	6/6	0

* number of death/no. of rats used during 24 hours

Table 4: ***In vitro* neutralising effect of Jeevarakshaka Gutika (JRG) in rats administered with LD50 (2 x 550µg/kg body weight) of cobra venom**

Group	Mortality*	% survival
Control	6/6	0
Venom + polyvalent anti venom	0/6	100
Venom + JRG (0.54g/kg)	6/6	0

* number of death/no. of rats used during 24 hours

Table 5: Effect of Jeevarakshaka Gutika (JRG) on haematological parameters

Group	WBC (10^3//µl)	RBC (10^6/µl)	N 10^3/µl	L 10^3/µl	M 10^3/µl	MCV (fl)	MCH (pg)	PCV (%)	MCHC (g/dl)	RDW-CV (%)	RDWSD (fl)	Platelet (10^3/µl)
Normal control	5416 ± 681	7.59 ± 0.57	41.9 ± 5.87	49.26± 6.4	8.84± 0.89	56.37 ±1.06	21.15± 0.189	42.62± 2.6	37.57± 0.56	14.25 ± 0.26	27.97± 1.1	5.94± 8.4
Venom control	3150 ± 434	7.37± 0.21	45.77 ± 2.26	41.75± 2.38	12.48± 0.52	58.2± 0.57	21.42± 0.18	38.55± 5.2	36.87± 0.15	24.11± 2.8**	28.18± 0.4	5.02± 0.53
Venom + Antivenom antisera	5566 ± 712	7.31 ±0.17	17.25 ±7.04	17.25 ± 7.04	11.41± 0.88	56.21± 1.22	20.95± 0.42	40.98± 0.6	37.38± 0.27	13.68± 0.4**	27.21± 1.2	7.08± 0.35
Venom + JRG	8714± 111***	7.49± 0.58	13.17 ± 4.9*	13.17± 4.9*	13.64± 1.9	54.67± 0.88*	20.70± 0.25	41.04± 3.5	37.98± 0.19*	12.88± 0.32	24.5± 1.0*	7.69± 0.95*

N – **Neutrophils, L – Lymphocytes, M - Monocytes**

Data expressed in Mean ± SEM, * P<0.05, in comparison to venom control group.

Table 6: Effect of Jeevarakshaka Gutika (JRG) on biochemical parameters

Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	U (mg/dl)	Cr (mg/dl)	TP (g/dl)	Ch (mg/dl)	CK (IU/L)	Ch (IU/L)	B (mg/dl)	BT (mg/dl)
Normal control	140.8 ±12.79	59.8± 4.07	898.6± 40.81	18.66± 2.5	0.65± 0.02	6.27± 0.2	69.1± 4.09	384.7 ±93	741.18± 80.4	0.108± 1.0	0.12± 0.019
Venom control	234.01± 38.14	66.57± 3.3	219.8± 19.7##	27.00± 1.2	1.31± 0.84	6.34± 0.2	48.42± 3.14##	91.9± 8.5##	1194.4± 64.8	0.10± 2.7	0.21± 0.014##
Venom+ Antivenom antisera	198.17± 31.43	59.50± 5.65	133.66± 19.65	25.00± 3.7	0.58± 0.01	5.76± 0.1	53.16± 4.13	276.4± 35**	881.05± 70.16	0.10± 0.0	0.20± 0.0
Venom+ JRG	326.68± 87.26	46.85± 11.9	164.88± 41.70	35.35± 8.9	0.65± 0.02	7.03± 0.3	49± 4.4	-	1022.9± 41.92	0.11± 0.02	0.21± 0.02

U – Urea, Cr – Creatinine, TP - Total Protein, Ch – Cholesterol, CK - Creatinine kinase, Ch – Cholinesterase, B – Bilirubin, BT - Bilirubin Total

Data expressed in Mean ± SEM, ##P<0.01, in comparison to normal control group. **p<0.01 in comparison to venom control group.

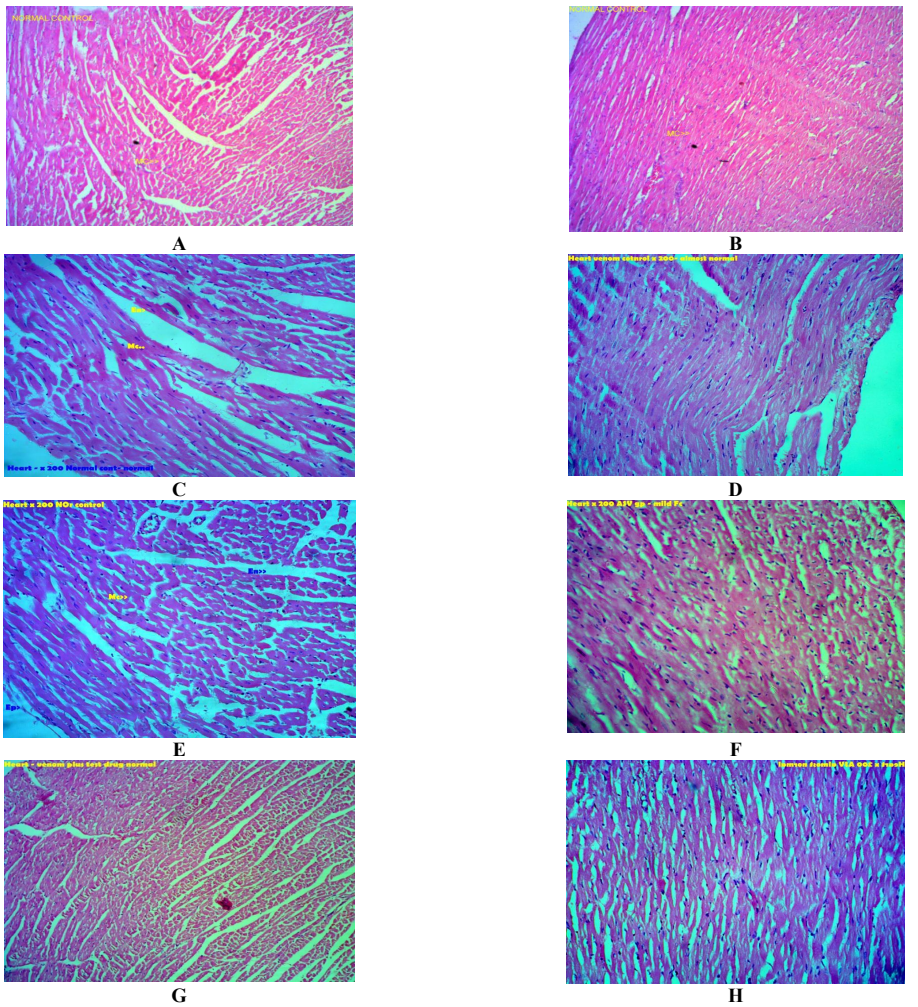
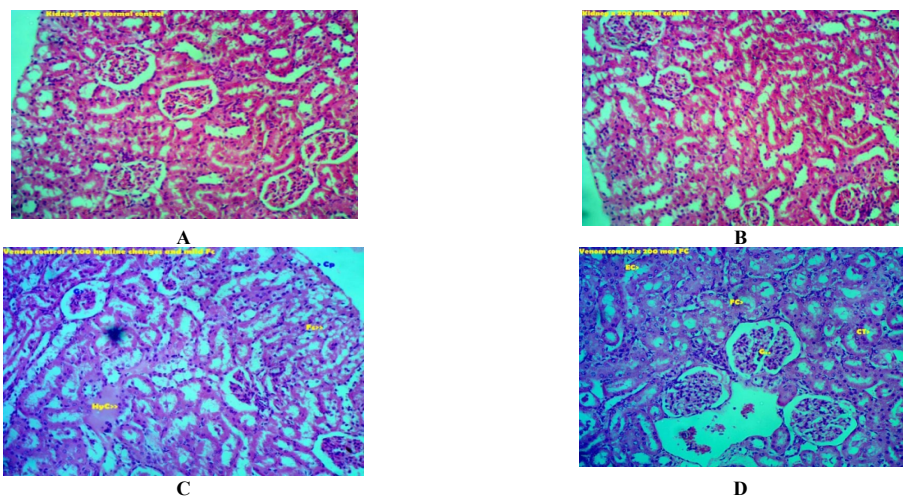


Figure 1: Histopathology of heart

A& B - control group shows normal cytoarchitecture, C & D – cobra venom only treated group, E & F – Venom plus ASV, G & H – Venom plus Test drug., MC- myocardium, NL- Necrotic lesion, HS- Haemorrhagic streak



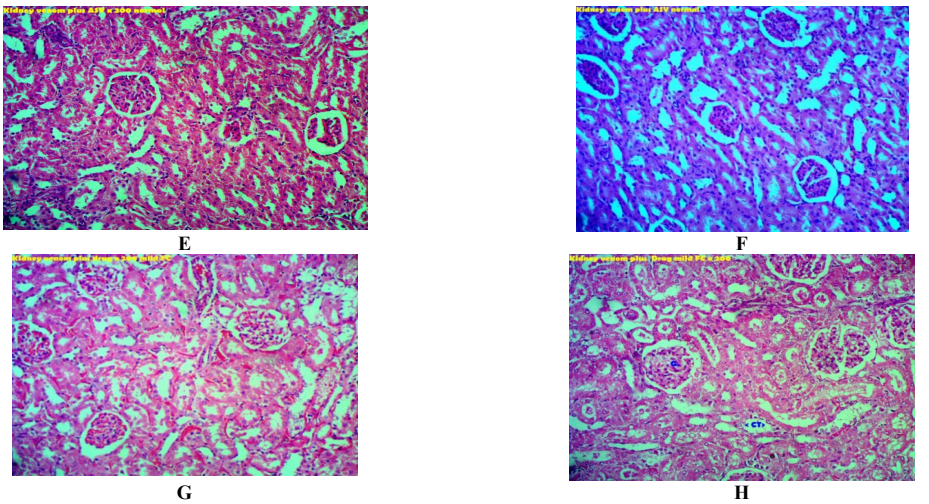


Figure 2. Histopathology of Kidney

A& B - control group shows normal cytoarchitecture, C & D – cobra venom only treated group, E & F – Venom plus ASV, G & H – Venom plus Test drug. HYC- Hyaline changes, MCI- Mild cell infiltaration, FC- fatty changes, GD- Glomerular dilatation.

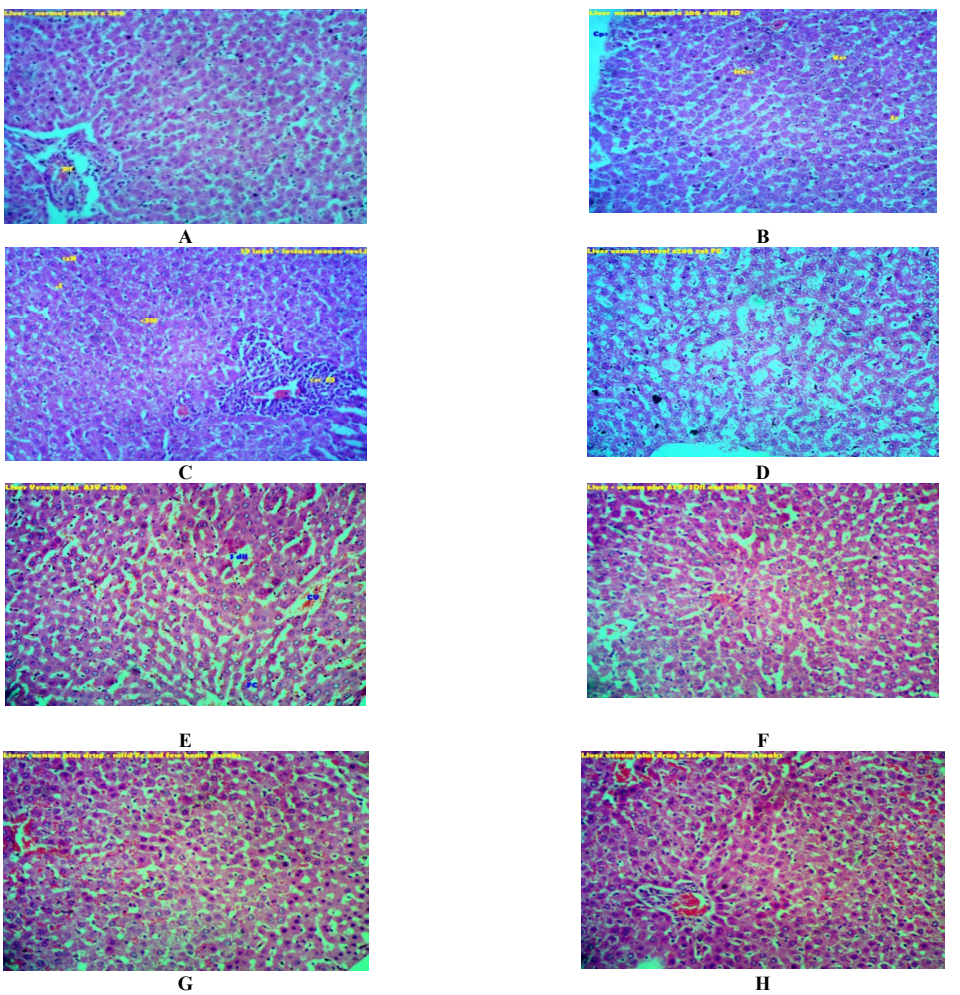


Figure 3. Histopathology of Liver

A& B - control group shows normal cytoarchitecture, C & D – cobra venom only treated group, E & F – Venom plus ASV, G & H – Venom plus Test drug. SD- Sinusoidal dilatation, FC- Fatty changes, HG- Haemorrhages

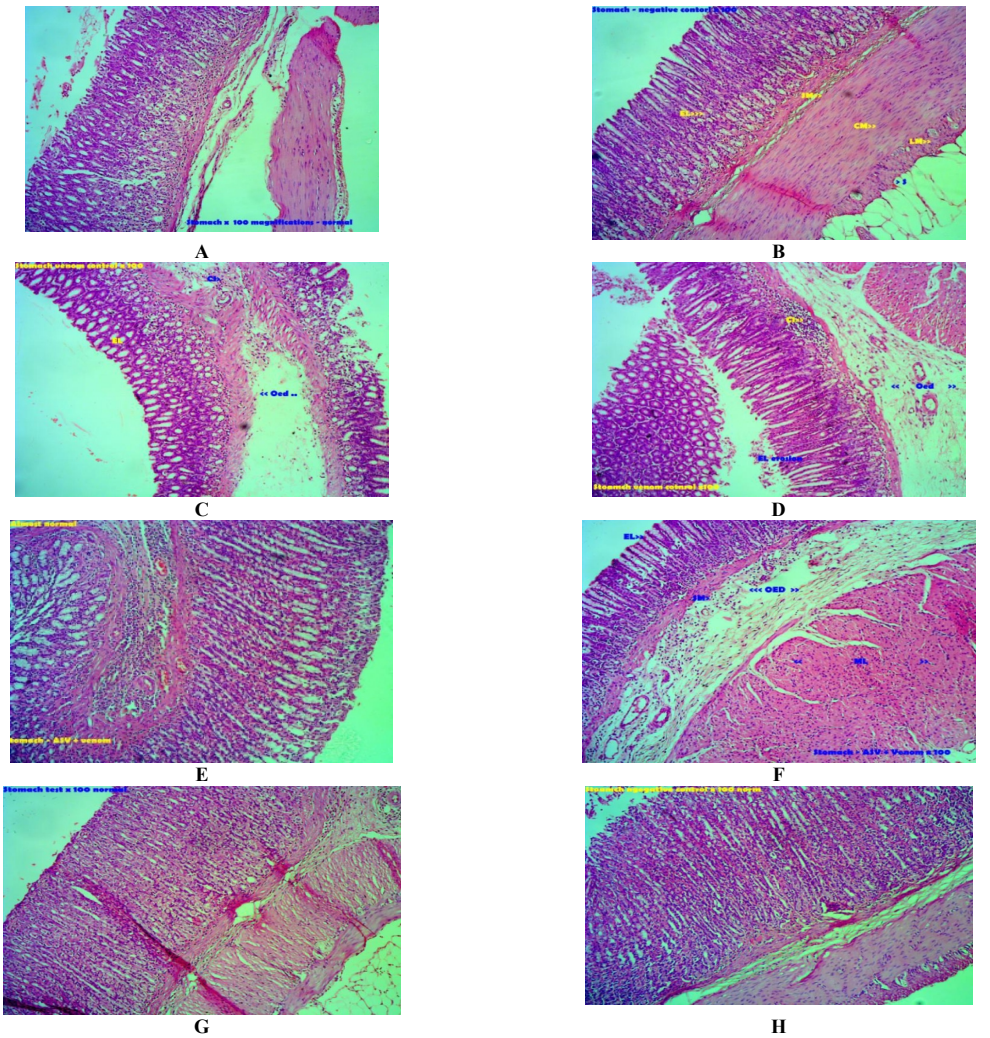


Figure 4. Histopathology of stomach

A& B - control group shows normal cytoarchitecture, C & D – cobra venom only treated group, E & F – Venom plus ASV, G & H – Venom plus Test drug. FCI- Focal cell Infiltration, FC- Fatty changes, Er- Errosion

DISCUSSION

The venom used in this study was found to have a LD50 of 555 µg/kg intra muscularly by AOT programme using OECD 425 protocol. This dose was used for the present study. In the first phase, the neutralizing effect of pretreatment of test drug (JRG) on the LD50 dose envenomation was assessed. Administration of test drug prior to venom injection provided 100 protections- all the 6/6 rats survived similar to anti venom group.

In the second part in which the test drug was tested against 2 x LD50 dose of venom- 1/6 animal survived providing 16.6% protection. Anti venom provided 100 % protection. Further the duration of survival in the test drug administered group was more than 2.5 fold in comparison to the control group. Based on this it can be inferred that test drug has the potential to protect the rats against sub-lethal dose. Even against high dose envenomation it provided moderate protection.

The TED and TED x 2 dose of test drug failed to neutralize the venom when incubated in vitro. This clearly shows that the test drugs do not contain components which can neutralize the toxic potential venom constituents. The protection observed against LD 50 dose seems to be due to antagonism of the effect of different venom components on body systems. Thus the data provides evidence for the anti venom efficacy of the test drug.

Next for the detailed study sub lethal dose of cobra venom was injected to rats to note down the ¼ LD 50 doses of venom induced changes in haematological, biochemical, and histopathological parameters. The effect of test drug on venom induced changes was assessed to note down its antivenom potential. The measurement of these parameters is of importance in the assessment of the pathophysiological state of snake bite victims. Venom induced changes in haematological parameters: injection of sub lethal dose venom resulted in only mild to moderate non-significant decrease in total RBC count, Hb concentration, MCHC, PCV, increase in MCV. The only

significant change observed was the significant increase in Red cell distribution width – CV. This is an index of variation in the cell size due to haemolysis. The test drug significantly reversed the changes observed in MCV, MCHC and RDW-CV. Further venom injection lead to 70% decrease in total WBC count. This was moderately reversed by antivenom and significantly reversed by the test drug. Similarly the test drug significantly elevated the platelet count. How these changes are brought about is not known- except that it is caused by haemolytic factors present in the venom. The test drug may be interfering with their effect. This may be one of the contributing factors for the protection observed at sub-lethal effect. Among the significant changes observed in the biochemical parameters are significant decrease in ALP activity, near significant increase in serum creatinine level, significant decrease in serum total cholesterol level, remarkable decrease in Creatinine-Kinase (CK-MB), significant increase in serum total bilirubin, Moderate non-significant increase was observed in SGOT activity, acetyl choline esterase activity and serum urea. The other parameters studied were not affected to significant extent. Alkaline phosphatase enzyme has an important diagnostic value in liver and bone diseases. Some authors have reported decreased ALP activity in haemolysed blood while others could not find such a decrease [16,17]. Haemolysis is one of the prominent features of envenomation hence the observed marked decrease may be due to this factor. Another possibility is decreased formation in the liver. It is curious to note that even the anti venom treatment failed to reverse this venom induced lowering of the ALP activity. Similar trend was observed with test drug also. Thus the changes in ALP activity did not corroborate with anti venom effect observed with both antivenom and test drug.

Serum creatinine is an important bio-marker for assessing the kidney function. Elevation indicates renal injury; this is further corroborated by the histopathological examination of the kidney in which kidney sections from some of the rats exhibited glomerular dilatation and fatty changes. This elevation was considerably decreased by both antivenom and test drug treatment. However, concurrent elevation in serum urea was not reversed in a statistically significant manner indicating probably the functional impairment in the kidney. The changes observed in this parameter along with histopathological examination can be considered to indicate nephro protection against the venom induced renal injury. Significant serum total cholesterol level lowering was observed in venom control group. There are not many reports related to the effect of snake venom on lipid metabolism. The observed decrease may be indicative of lipolytic activity of the venom components. Another contributing factor could be venom induced functional impairment of the liver. Both the antivenom and test drug produced only a weak reversal of this lipolytic effect. This indicates that lipolytic inhibition is not a major contributor to the observed antivenom effect. Phospholipase A2 id one of the major components of snake venoms belonging to Elapid (includes cobra, krait, sea snakes). It can produce several serious biological effects such as cardiotoxicity, myotoxicity, pre or postsynaptic neurotoxicity, edema, haemolysis, hypotension, convulsion, platelet aggregation inhibition and anticoagulation. Some of the toxic effects mentioned above can be attributed to this component. The effect of the treatments used in this study is required to be elucidated. Indirect data do not indicate major modulatory effect.

Another remarkable effect observed in the venom control group is the marked decrease in the activity of Creatinine-Kinase (CK-MB). This is used as the marker to distinguish the variation in necrosis that may occur in different muscles. CK is a dimeric globular protein consisting of two subunits with a molecular mass of 43 kDa. It buffers cellular ATP and ADP concentrations by catalysing the reversible exchange of high-energy phosphate bonds between phosphocreatine and ADP produced during contraction. At least five isoforms of CK exist: three isoenzymes in cytoplasm (CK-MM, CK-MB and CK-BB) and two isoenzymes (non-sarcomeric and sarcomeric) in mitochondria. CK-MM is found in several sites of the myofibre where ATP consumption is high and is used as a marker of muscle disease. CK-MB increases in acute myocardial infarction, and CK-BB increases in brain damage. Mitochondrial CK is elevated in mitochondrial myopathies. As mentioned above CK-MB is one of the most important myocardial markers, with well-established role in confirming acute myocardial infarction. Serum level of CKMB and SGOT enzymes increases when the tissue destruction is associated with the cardiac muscle, liver tissue, skeletal muscle and kidneys. In the present study marked decrease in the activity of these enzymes was observed this indicates interference with the ATP.ADP buffering role. This decrease was reversed to significant extent in the groups treated with antivenom and test drug. This may be considered as an index of reversal of the toxic effect of venom on muscular tissue. Moderate increase in SGOT activity was observed without SGPT elevation. This may indicate mild to moderate cardiac tissue injury. This was reversed to moderate extent in the antivenom group where as moderate nonsignificant further elevation was observed in test drug administered group. This did not correlate with the histological examination of the heart section in which good protection was observed in both the treated groups. The increase in total bilirubin in the venom control as compared to normal control may be due to the lesions caused by the venom from different tissues and haemolysis. This was not affected to significant extent indicating both antivenom and test drug are not effective against the haemolytic factor of the venom. AchE activity was found to be moderately elevated in venom control group in comparison to the normal control. This may be indicative of labialization from the affected muscle fibres. Another reason may be the venom induced blockade of neuromuscular receptors causing muscle paralysis. This elevation was found to be moderately reversed in antivenom treated group and comparatively less reversal was observed in test drug treated group. Analysis of the result show good protection in antivenom treated group and weak to moderate protection in test drug administered

groups. The exact mechanism of protection is not clear it may be due to general cytoprotective effect or interference with the activity of the above mentioned toxic factors. The most important finding of this study is the observation of complete protection against sub lethal dose of cobra venom by the test drug and moderate protection and survival period prolongation against lethal dose of venom by the test drug administered group.

The increase in total bilirubin in the venom control as compared to normal control may be due to the lesions caused by the venom from different tissues and haemolysis. This was not affected to significant extent indicating both antivenom and test drug are not effective against the haemolytic factor of the venom. AchE activity was found to be moderately elevated in venom control group in comparison to the normal control. This may be indicative of labialization from the affected muscle fibres. Another reason may be the venom induced blockade of neuromuscular receptors causing muscle paralysis. This elevation was found to be moderately reversed in antivenom treated group and comparatively less reversal was observed in test drug treated group.

CONCLUSION

Analysis of the result show good protection in antivenom treated group and weak to moderate protection in test drug administered groups. The exact mechanism of protection is not clear it may be due to general cytoprotective effect. Interference with the activity of the above mentioned toxic factors. The most important finding of this study is the observation of complete protection against sub lethal dose of cobra venom by the test drug and moderate protection and survival period prolongation against lethal dose of venom by the test drug.

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