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Mitigating cardiotoxicity of *Naja naja* venom: Evaluation of a multipronged strategy using anti-snake venom and methanolic extract of *Andrographis paniculata*

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ABSTRACT

This study assesses the effect of anti-snake venom (ASV), methanolic extract of *Andrographis paniculata* (MAP), and ASV+MAP (50% reduced dose of ASV+MAP) in mitigating cardiotoxicity in rats. Thirty female Wistar rats were divided into five groups (n = 6): normal control, venom control (VC), and three test groups treated with ASV or MAP or ASV+MAP. Electrocardiogram was recorded at baseline, 30, 90 minutes, 24 hours, and days 7 and 14. Serum creatine kinase (CKMB) levels were measured at baseline, 24 hours, and days 7 and 14. On day 15, animals were sacrificed for histopathological analysis. Bradycardia occurred at 90 minutes in all groups except the ASV+MAP group. The amplitude of the P wave improved maximally with ASV+MAP. CKMB, which was high in the VC group, was reduced by ASV to the extent of 20% and ASV+MAP by 30%. On day 14, a decrease in CKMB was observed in all groups, lowest being the ASV+MAP group. Histopathological changes observed in the VC group were attenuated by ASV+MAP. The multipronged approach of administering MAP along with a 50% reduced dose of ASV was shown to be the best strategy for mitigating cardiotoxicity of *Naja naja* venom.

INTRODUCTION

Snake bite has been designated as a neglected tropical disease by the World Health Organization. India is at the epicenter of the problem, with over 50,000 fatalities reported annually [1–3]. Most of the fatalities occur in rural areas [4] among agricultural workers. Snake bites also lead to limb deformities, sometimes requiring amputation. The treatment of snakebite is a huge economic burden on the country, especially when coupled with loss of livelihood [5]. Fifty-three species of poisonous snakes inhabit India. However, the majority of bites and consequent mortality are attributable to the "Big Four" snake species, *viz*., Indian spectacled cobra or *Naja naja* (N.N), *Daboia russelii* (Russell's viper), *Bungarus caeruleus* (krait), and *Echis carinatus* (saw-scaled viper) [6]. The present study focuses on the venom of N.N, which is known to be primarily neurotoxic, due to the presence of acetylcholine esterase, phospholipase A2, and a post-synaptic alpha-neurotoxin with curare-like activity. However, it has widespread toxic effects on vital organs such as the heart, kidney, liver, lung, and skeletal muscle [7,8].

Cobra venom cardiotoxins are well known for their direct cardiovascular effects [7,8]. Various changes in electrocardiogram (ECG), such as sinus tachycardia, sinus

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arrhythmia, sinus bradycardia, non-specific ST-T changes, atrioventricular block, hypertension, or hypotension, have been reported in envenomed patients [9,10], indicating the involvement of the autonomic nervous system. This implies that the disruption of cardiac muscle electrical activity could be a contributing factor to mortality. Envenomation causes an increased demand for myocardial oxygen, potentially resulting in cardiac ischemia characterized by coronary vasospasm, depolarized block, loss of cardiovascular reflexes, inhibition of cardiac conduction, and ventricular ectopics [11]. Considering the widespread toxic effects of N.N. venom on the heart, it is a crucial parameter to be considered in a case of snake bite.

At present, the only treatment options available in India for envenomation with *Naja naja* is a polyvalent anti-snake venom (ASV), which contains antibodies against the venoms of the "big four" snakes of India [12]. However, treatment with ASV results in hypersensitivity reactions such as itching, urticaria, dry cough, nausea, vomiting, diarrhea, tachycardia, fever, and hypotension in 20%–50% of cases [13–15]. Despite being a life-saving medication, ASV can lead to serious adverse effects (10%–15%), causing anaphylaxis and death [14].

Numerous medicinal plants have been explored for their pharmacological potential as antivenom agents [16–20]. One such herb widely used across Southeast Asia to treat cobra bites is *Andrographis paniculata* (A.P) [21,22]. Among the different extracts of this plant, the methanolic extract of A.P (MAP) was the best in prolonging the survival time in envenomed albino mice [23]. *In-vivo* experiments with MAP have demonstrated mitigation of edema and hemorrhage [18,24]. Compounds in plants with antivenom properties reported are the diterpene lactone andrographolide, flavonoids, steroids, tannins, and polyphenols [25]. An *in-vitro* study with the MAP showed neutralization of thromboelastographic changes induced by N.N. venom in human blood when assessed in real-time [26]. The extract effectively blocked the toxic N.N venom enzymes acetylcholine esterase, hyaluronidase, and rescued human pan-proteinase inhibitor alpha 2-macroglobulin from inactivation [27]. Secondary hemostatic abnormalities related to prothrombin time and activated partial thromboplastin time, induced by the venom, were corrected by MAP [28]. In each of these studies [26–28], supplementation of a 50% reduced concentration of polyvalent ASV with MAP demonstrated the superiority of this multipronged strategy in reducing the venom-induced deleterious effects. Hitherto, other workers have reported the neutralization of the toxic effects of various venoms by incubating them with herbal constituents *in vitro* [21]. The present study mimics the sequence of events in the natural setting, with the snake bite (venom injection) occurring first, followed by treatment using ASV or MAP or ASV+MAP, and studying their effectiveness in mitigating cardiotoxicity.

MATERIALS AND METHODS

Materials

Animals

Female Wistar rats weighing between 180 and 200 g were used for the study. Female animals were used because of their greater sensitivity to toxins compared to males. Animal Ethical Clearance was obtained from the Institutional Animal Ethics Committee (IAEC/KMC/80/2021). Guidelines from the Committee for the Purpose of Control and Supervision of Experiments on Animals and the Animal Welfare Division, Government of India, formed the basis of all experiments. The central animal facility of the Manipal Academy of Higher Education housed the animals in polypropylene cages, which were maintained under standard conditions (26°C–30°C, 40%–60% relative humidity, 12 hours of light and 12 hours of darkness). Over the duration of the trial, the animals had continuous access to water and received a regular pellet meal obtained from VRK Laboratory Animal Feed, Maharashtra, India.

N. N. venom stock solution

Dry, lyophilized powder of N.N venom was procured from K.V. Institute, Ballia, Uttar Pradesh, India. The stock solution of the venom was prepared by dissolving 10 mg of the venom in 1 ml of 0.9 % saline and stored at 2°C–8°C.

MAP

Natural Remedies Pvt. Ltd, Bangalore, India, provided the MAP. The sample was analyzed for the presence of heavy metals such as mercury, cadmium, arsenic, and lead by inductively coupled plasma mass spectrometry. The percentage of Andrographolide in the sample was estimated by high-performance liquid chromatography, and a certificate of analysis was obtained [26].

ASV

Bharat Serums and Vaccines Pvt. Ltd, Maharashtra, India, was the source of lyophilized polyvalent ASV. As indicated on the ASV vial by the manufacturer, 1 ml of reconstituted ASV could neutralize 0.6 mg of N.N venom. As indicated by the manufacturer, contents of the vial were dissolved in 10 ml sterile water and stored at 2°C–8°C.

Anesthetic agent: Ketamine and Xylazine

An anesthetic regimen containing ketamine hydrochloride (injection I.P, 250 mg/5 ml) and xylazine (23 mg/ml) was employed, serving as an anesthetic and muscle relaxant, respectively.

Methodology

Determination of median lethal dose (LD_{50}) of N.N venom

LD_{50} of N.N venom was calculated using OECD guidelines 425 and software AOT 425 StatPgm. Initially, 2,000 mg/Kg was administered, following which the dose for each successive animal was adjusted down by a factor of 3.2. Different concentrations (1, 0.9, 0.8, and 0.7 mg/kg.b.wt) of N.N. venom from venom stock (10 mg/ml), dissolved in 0.9% saline, were injected into the left thigh muscle of rats (n = 6 in each group). ECG was recorded under anesthesia. Animals were observed for different time periods ranging from 30 minutes, 6 hours, 24 hours, and after that, daily for 14 days. The number of deaths in 24 hours was noted.

Effective dose of anesthetic and muscle relaxant

Based on dose-dependent calculation, to a rat weighing 200 g, 0.2 ml of ketamine (10 mg) and 0.08 ml of xylazine (1.86

mg) were administered intraperitoneally after half an hour of venom injection. Though it is known that ketamine–xylazine anesthesia can influence heart rate (HR) and ECG parameters, since the same anesthetic protocol was applied uniformly across all groups, including the control group, it minimizes bias and allows valid comparisons between groups.

Effective dose of MAP and ASV

The dosage of MAP used in this study was 280 mg/kg body weight [27]. MAP was dissolved in 0.25% (w/v) carboxymethylcellulose (CMC) and given orally. ASV was administered at a concentration of 266.6 µl/rat, based on a dose optimization study by Nayak et al. [27]. The estimated LD_{50} was 0.8 mg/kg body weight, which amounts to 0.16 mg for a rat weighing 200 g. (0.8 mg × 200/1,000). As per the manufacturer's guidelines, 0.6 mg of venom can be neutralized by 1 ml ASV. Therefore, 0.16 mg of venom can be neutralized by 266.6 µl of ASV (1,000 × 0.16/0.6). In the present study, a dose of ASV equal to 133.3 µl has been denoted as '50% reduced dose of ASV' and was employed when ASV was used in combination with MAP. ASV was administered to the animals intraperitoneally (i.p), instead of intravenously (as in clinical use), because i.p. delivery is an established, reliable method for administering antivenom in rodents and ensures fast systemic absorption. As all groups received ASV by the same route, internal validity of the rescue comparisons is preserved.

Electrocardiographic assessment

The PowerLab® 26T data acquisition system with the LabChart® 7 software (AD Instruments, Australia) and a bio-amplifier with a 3-electrode ECG attachment was used for recording the ECG in anesthetized rats. The needle electrodes were used to record lead II ECG. All ECG parameters and HR were monitored.

Morphometric analysis of the heart

Morphometric analysis included the measurement of longitudinal diameter (distance between apex and the base of the heart) and transverse diameter (distance between left side and right side of the heart at the point of atrioventricular sulcus) [29].

The heart was fixed for 24 hours in a 10% solution of formaldehyde. After fixation, a transverse section in the atrioventricular sulcus was made, followed by measurements of the thickness of the interventricular septum (IS), free walls of the left ventricle (LV), and the right ventricle (RV). These values were used for the calculation of longitudinal cardiac diameter/transverse cardiac diameter ratio, heart weight/body weight ratio, and ventricular ratio [(LV + IS)/RV]. Measurements were taken using ImageJ software.

Histopathological analysis of the heart

Heart tissue was removed, fixed in 10% formalin, rehydrated with alcohol, and then embedded in paraffin. Paraffin slices were obtained on glass slides and stained with hematoxylin and eosin. The slides were analyzed for histopathological changes under a microscope. Relevant photomicrographs were recorded.

Experimental design

Thirty female Wistar rats were divided into five groups with six animals in each group. In all the groups, collection of blood samples for creatine kinase (CKMB) estimation and ECG recording was done under anesthesia. Blood samples (1 ml/bleed) were obtained from all the animals from the retro-orbital plexus. CKMB, which is the earliest and specific biochemical marker of myocardial injury, was estimated using SD BIOSENSOR F 200 and AGAPPE kit [30–32]. Group 1 served as a normal control (NC or sham-anesthesia control) and was injected with 0.1 ml of CMC intramuscularly into the left thigh muscle. Group

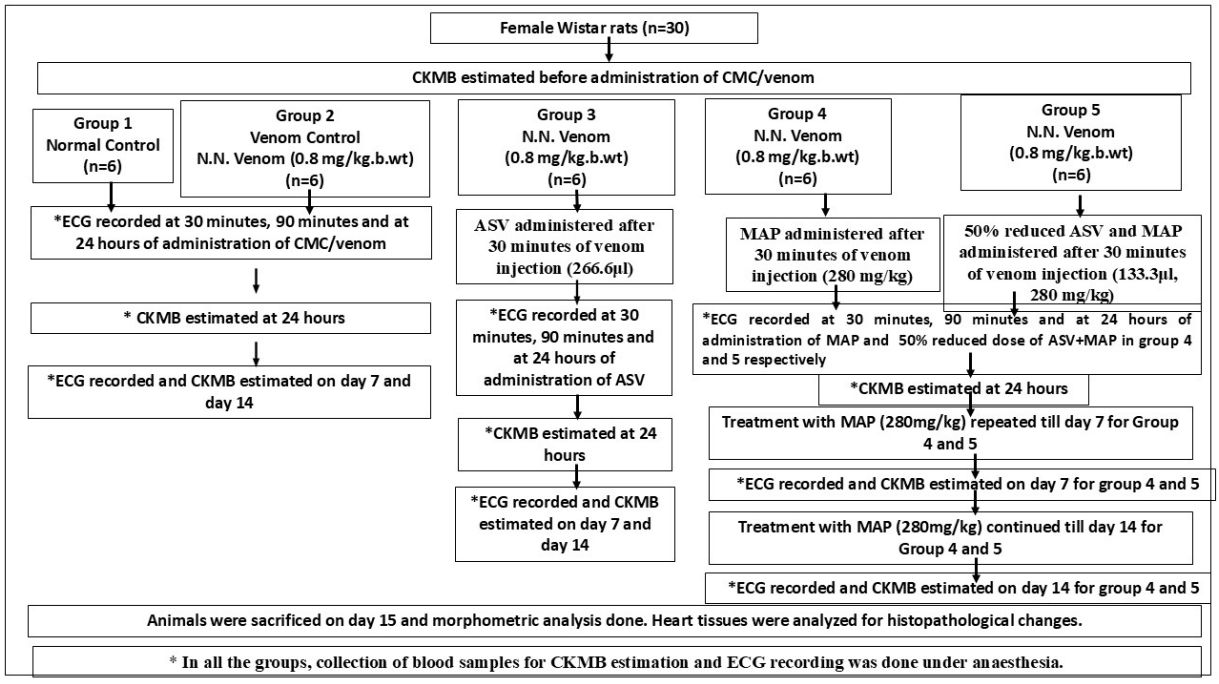

Figure 1. Detailed flow chart representing the experimental design.

Table 1. Effect of ASV, MAP, and 50% reduced ASV+MAP on HR in envenomed female Wistar rats.

	HR at 30 minutes (bpm) (Mean ± SD)	HR at 90 minutes (bpm) (Mean ± SD)	HR at 24 hours (bpm) (Mean ± SD)	HR on day 7 (bpm) (Mean ± SD)	HR on day 14 (bpm) (Mean ± SD)
Group 1	226 ± 26	229 ± 21	228 ± 29	209 ± 39	218 ± 21
Group 2	203 ± 31	146 ± 37 *(p = 0.027)	182 ± 21 *(p = 0.020)	197 ± 9	224 ± 34
Group 3	197 ± 16	132 ± 33 *(p = 0.009)	203 ± 5	201 ± 26	173 ± 46
Group 4	198 ± 15	158 ± 39 *(p = 0.028)	234 ± 26	213 ± 16	205 ± 12
Group 5	232 ± 18	226 ± 17 @(p = 0.034) ** (p = 0.011) *** (p = 0.027)	215 ± 13	266 ± 67	228 ± 20

Legends: Group 1: NC (Normal control); Group 2: LD50 N.N venom (Venom control); Group 3 (Test): LD50 N.N venom + ASV; Group 4 (Test): LD50 N.N venom + MAP; Group 5 (Test): LD50 N.N venom + 50% reduced ASV+MAP. *p < 0.05 compared to Group 1at 90 minutes and 24 hours, @p < 0.05 compared to Group 2 at 90 minutes; **p < 0.05 compared to Group 3 at 90 minutes; ***p < 0.05 compared to Group 4 at 90 minutes.

Abbreviations: ASV = polyvalent anti-snake venom; Bpm = beats per minute; HR = heart rate; LD50 = median lethal dose; MAP = methanolic extract of *Andrographis paniculata*; N.N = *Naja naja*; NC = normal control.

2 was injected intramuscularly with 0.1 ml of N.N. venom and designated as venom control (VC). Anesthesia was administered, following which an ECG was recorded at 30 minutes, 90 minutes, and 24 hours. Next, CKMB was estimated at 24 hours after administration of venom for both NC and VC groups. Groups 3–5 were designated as "treatment groups." As shown in Figure 1, for Group 3, a single intraperitoneal dose of ASV (266.6 µl) and for Group 4, MAP (280 mg/kg b.wt) was administered orally. For Group 5, a reduced dose of ASV (50% reduced dose compared to Group 3) was administered intraperitonially (i.p.), which was supplemented with MAP (280 mg/kg b.wt) orally, 30 minutes after administration of venom, respectively. ECG was recorded at 30 minutes, 90 minutes, and 24 hours in Groups 3–5. CKMB was estimated at 24 hours for all the groups. Treatment with MAP was continued at the same dose for 7 days for Groups 4 and 5. On day 7, an ECG was recorded, and CKMB estimation was done for all the groups (Groups 1–5). Treatment with MAP was continued for Groups 4 and 5 at the same dose until day 14. On day 14, an ECG was recorded, and CKMB estimation was done for all the groups (Groups 1–5). On day 15, animals were sacrificed with an overdose of pentobarbital. The tissues were washed with isotonic saline and processed for morphometric analysis and histopathological studies (Fig. 1).

Statistical analysis

SPSS 16.0 was used to analyze data. Quantitative data were represented as Mean ± SD. Significance between the groups at different time points for ECG and CKMB was measured using repeated measures of ANOVA followed by Tukey's post hoc test. Morphometric parameters were measured using One-way ANOVA followed by Tukey's post hoc test. Statistical significance was indicated at $p \leq 0.05$.

RESULTS

Determination of median lethal dose (LD_{50}) for N.N. venom

The LD_{50} of N.N. venom was determined to be 0.8 mg/kg body weight in female Wistar rats. This served as the basis for efficacy studies involving ASV, MAP, and their combination. All rats succumbed to venom exposure at a concentration of 0.9 to 1 mg/kg body weight. The survival rate was 50% at 0.8 mg/kg body weight and 80% at 0.7 mg/kg body weight.

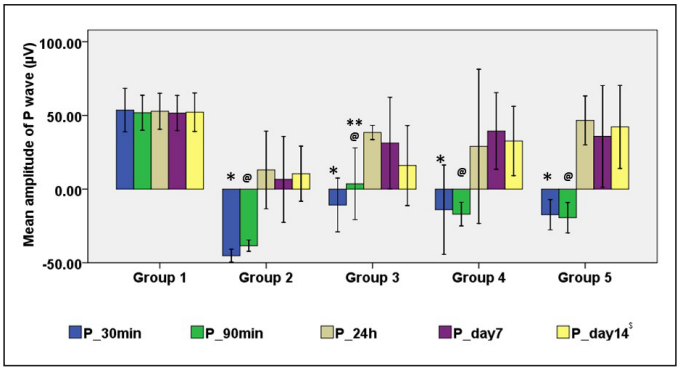

Figure 2. Effect of ASV, MAP, and 50% reduced ASV+MAP on the amplitude of the P wave in envenomed female Wistar rats. Legends: Group 1: NC (Normal control); Group 2: LD_{50} N.N venom (VC); Group 3: LD_{50} N.N venom + ASV; Group 4: LD_{50} N.N venom + MAP; Group 5: LD_{50} N.N venom + 50% reduced ASV+MAP, *p < 0.05 compared to Group 1 at 30 minutes, @p < 0.05 compared to Group 1 at 90 minutes; **p < 0.05 compared to Group 2 at 90 minutes. ASV = polyvalent anti-snake venom; LD_{50} = median lethal dose; MAP = methanolic extract of *Andrographis paniculata*; N.N = *Naja naja*; NC = normal control.

Electrocardiographic analysis

Table 1 illustrates the impact of envenomation with N.N. on HR. There was no statistically significant change in HR at 30 minutes post-envenomation among the control and test groups. However, at 90 minutes post-envenomation, HR significantly decreased in Group 2 (p = 0.027), Group 3 (p = 0.009), and Group 4 (p = 0.028) compared to Group 1. Notably, no significant change in HR was observed in Group 5 compared to Group 1. At 24 hours, a decrease in HR was observed only in Group 2 (p = 0.020). From day 7 onward, the HR was within normal range in all the groups.

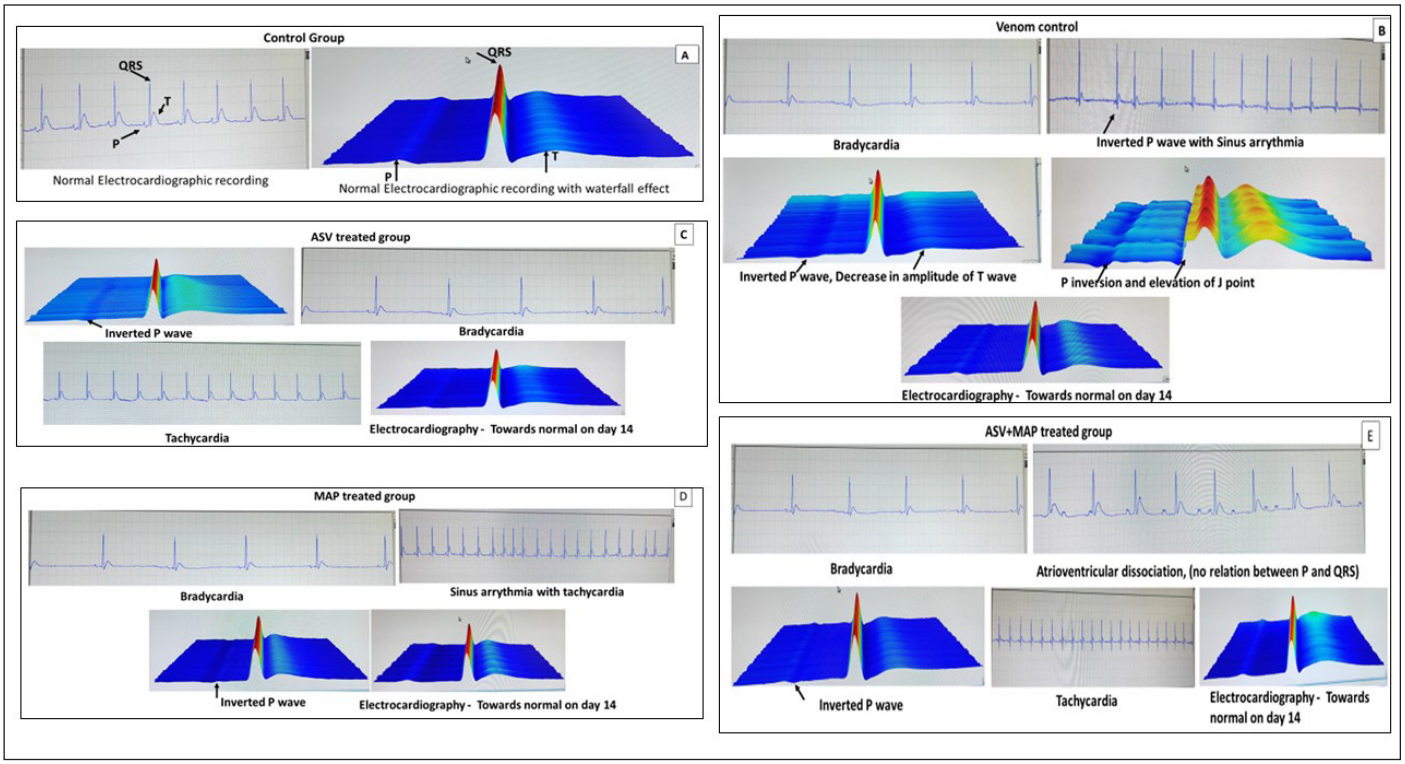

Figure 3. ECG recording in normal control, VC, ASV, MAP and 50% reduced ASV+MAP treated groups. A: Group 1-NC (Normal control); B: Group 2 - LD_{50} N.N venom (VC); C: Group 3 - LD_{50} N.N venom + ASV; D: Group 4 - LD_{50} N.N venom + MAP; E: Group 5 – LD_{50} N.N venom + 50% reduced ASV+MAP. ASV = polyvalent anti-snake venom; LD_{50} = median lethal dose; MAP = methanolic extract of *Andrographis paniculata*; N.N = *Naja naja*; NC = normal control.

Figure 2 shows the effect of the N.N. venom and treatment with ASV, MAP, and 50% reduced ASV+MAP on the amplitude of the P wave. At 30 and 90 minutes, the amplitude of the P wave decreased significantly in Groups 2–5 compared to Group 1 ($p < 0.005$). Though the amplitude of the P wave did not normalize completely, the best results were obtained with Group 5, which was treated with 50% reduced ASV+MAP.

No statistically significant differences were noted in the amplitude of the R wave, QRS duration, PR intervals, and ST segments up to 14 days in all the groups. Though the duration of the P wave was slightly prolonged at 90 minutes of envenomation in the VC and ASV groups, there were no statistically significant differences observed. A decrease in amplitude of the T wave was observed at 30 and 90 minutes in the VC group, though it was statistically not significant. Representative images of the ECG recordings in different groups were shown in Figure 3.

Estimation of CKMB

Figure 4 shows the effect of the N.N. venom and treatment of envenomation on CKMB. Administration of venom caused a 58% increase in CKMB at 24 hours in Group 2. ASV brought down the CKMB by 20% and the best results were obtained with the ASV+MAP-treated group, with a reduction of 30%. However, a further increase in CKMB was noted on day 7 (Group 2% – 123%, Group 3% – 75%, Group 4% – 105% and Group 5% – 85%). On day 14, a decrease in CKMB was observed in all the groups (Group 2% – 78%, Group 3% – 70%,

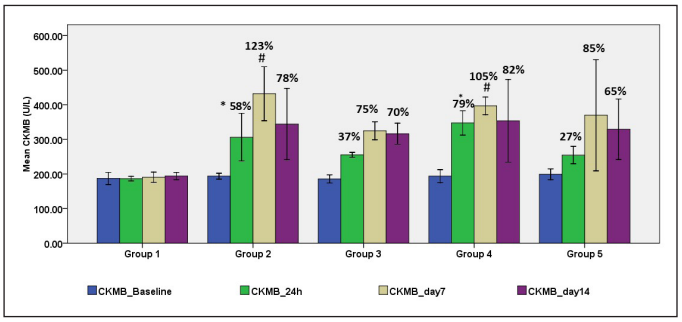

Figure 4. Effect of ASV, MAP, and 50% reduced ASV+MAP on Creatine Kinase (CKMB) in envenomed female Wistar rats. Group 1: NC (Normal control); Group 2: LD_{50} N.N venom (VC); Group 3: LD_{50} N.N venom + ASV; Group 4: LD_{50} N.N venom + MAP; Group 5: LD_{50} N.N venom + 50% reduced ASV+MAP; $^{*}p < 0.05$ compared to Group 1 at 24 hours; $^{\#}p < 0.05$ compared to Group 1 on day 7. ASV = polyvalent anti-snake venom; LD_{50} = median lethal dose; MAP = methanolic extract of *Andrographis paniculata*; N.N = *Naja naja*; NC = normal control.

Group 4% – 82% and Group 5% – 65%), with the lowest being recorded in the ASV+MAP group.

Morphometric analysis

Morphometric analysis of the animals regarding heart weight and heart weight/body weight ratio revealed a decrease ($p < 0.05$) after envenomation in all the groups. The consistent reduction in heart weight across all groups, along with the observed histopathological changes (mentioned

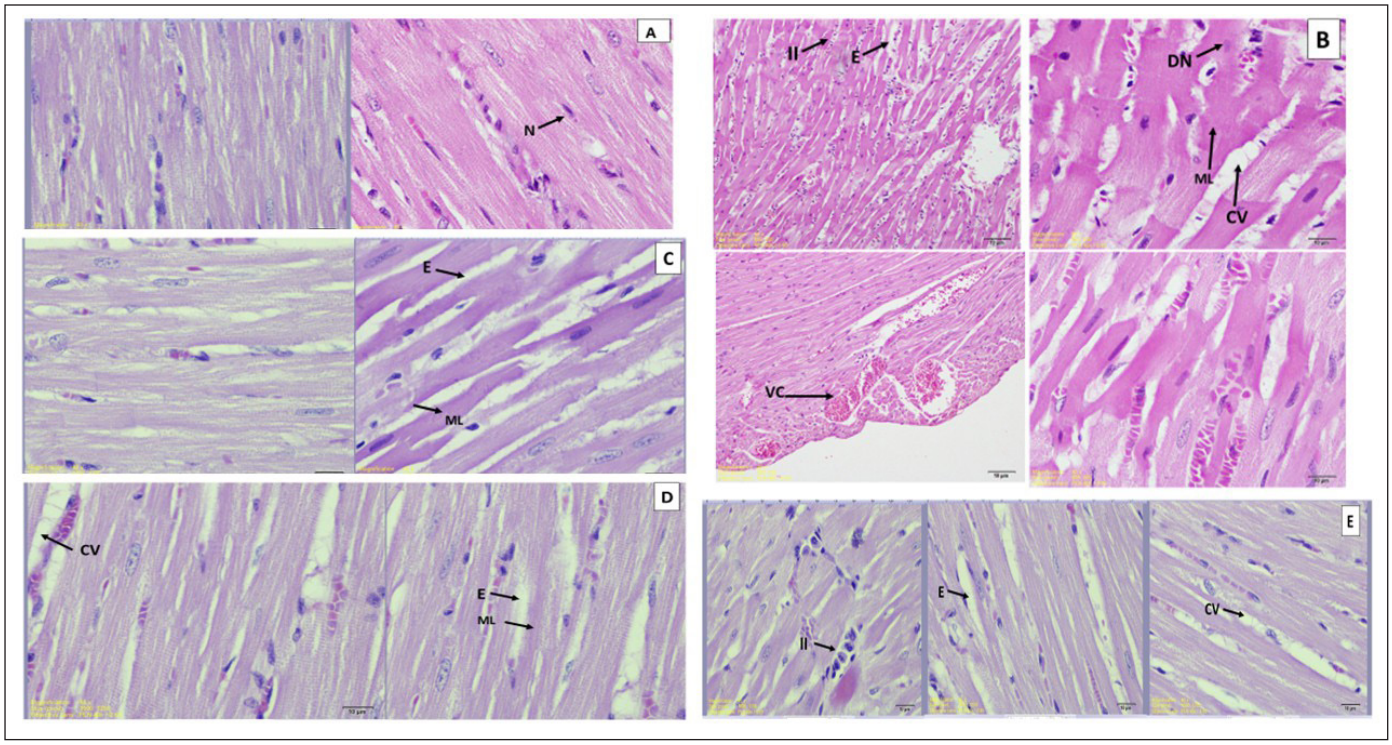

Figure 5. Effect of ASV, MAP, and 50% reduced ASV+MAP on cardiac muscle histology in envenomed female Wister rats. A: Group 1-NC (Normal control); B: Group 2 - LD_{50} N.N venom (VC); C: Group 3 - LD_{50} N.N venom + ASV; D: Group 4 - LD_{50} N.N venom + MAP; E: Group 5 - LD_{50} N.N venom + 50% reduced ASV+MAP. ASV = polyvalent anti-snake venom; CV = cytoplasmic vacuolation; DN = Degenerated nucleus; E = Edema; II = inflammatory infiltrate; LD_{50} = median lethal dose; MAP = methanolic extract of *Andrographis paniculata*; ML = Myofibrillar loss; N = Centrally placed nucleus; N.N = *Naja naja*; NC = Normal control; VC = Vascular congestion.

under histopathological analysis), suggests a correlation between heart weight and morphological alterations. Other morphometric parameters such as the thickness of the IS, LV, and RV. Longitudinal cardiac diameter/ transverse cardiac diameter ratio and ventricular ratio [(LV + IS)/RV] did not show any significant difference between control and treated groups.

Histopathological analysis

The effect of ASV, MAP, and 50% reduced ASV+MAP on cardiac muscle histology in envenomed female Wister rats is shown in Figure 5. A blinded qualitative histopathological evaluation of all the groups of animals was carried out by a pathologist. Histology of the heart from the control group (A) showed normal cardiomyocytes with centrally placed nuclei. VC (B) showed myofibrillar loss, disruption of myofibrils, intermuscular edema, cytoplasmic vacuolation, degeneration of the nucleus, inflammatory infiltrate, and vascular congestion. Treatment with ASV (C) largely preserved myocardial morphology compared to VCs, with residual myofibrillar loss, interstitial edema, and cytoplasmic vacuolation. Treatment with MAP and 50% reduced ASV+MAP (D and E) also showed attenuation of venom-induced changes, with residual interstitial edema, cytoplasmic vacuolation, inflammatory infiltrate, and myofibrillar loss.

DISCUSSION

The present study assesses the effectiveness of a multipronged approach in mitigating the cardiotoxic effects of *Naja naja* venom using ASV, MAP, and supplementation of 50% reduced dose of ASV with MAP. The bradycardia observed in envenomed rats aligns with previous animal studies [33,34]. Similar observations have been recorded in a study involving 96 snake bite patients [35]. The mechanism of bradycardia is attributable to interference with ion channels in cardiac myocytes, thus altering action potentials. Depolarized membranes or loss of the fast phase of action potentials can also cause low conduction [36]. In addition, myocardial inflammation, disruption of energy metabolism, is also known to cause bradycardia [37].

The P wave signifies atrial depolarization. An extended P wave duration is indicative of left atrial abnormality, which is linked to myocardial fibrosis and atrial fibrillation. Since the venom did not cause any significant changes in the duration of the P wave, it can be assumed that it lacks components that bring about such changes. The PR interval, serving as a measure of impulse conduction between the sinus node and AV node, is easily quantifiable in both rodents and humans. Prolonged PR intervals are indicative of conduction delays. However, in rodents, the normal range for PR intervals is broader, posing a challenge in distinguishing between normal and abnormal values. In the present study, no significant difference in the PR interval was observed. Similar results have been reported by others [33].

The QRS complex illustrates the propagation of depolarization through the ventricles. It is characterized by a high amplitude and short duration, enabling the monitoring

of conduction blocks and arrhythmias. In rats, Q waves are typically not discernible in normal ECGs, and measurements are predominantly based on RS complexes [38]. In the present study, the venom had no effect on QRS duration in the VC group, as reported by others [33].

The P wave is represented by a positive deflection in the ECG, reflecting atrial depolarization [39]. The venom caused a decrease in amplitude or inverted P wave, which typically characterizes an ectopic atrial rhythm not originating from the sinus node and often indicates a junctional rhythm [33]. The P wave amplitude in rats is inherently small, making it highly sensitive to anesthesia and venom toxins. High standard deviation reflects the inherent variability of rodent ECG measurements, and the consistent group-wise trends and significant *p* values confirm the reliability of our findings. In the treatment groups (ASV, MAP, and 50% reduced ASV+MAP), the amplitude of the P wave showed a tendency to return towards normal, with the best effect observed with the 50% reduced ASV+MAP-treated group.

The T wave represents ventricular repolarization. Administration of ASV, MAP, and 50% reduced ASV+MAP caused improvement in T wave values. In rats, the onset of the T wave occurs as a continuation of the QRS complex, with no clear ST segment, as highlighted in previous studies [40,41]. Consequently, studying ST-segment depression or elevation is challenging in this context, as these changes typically result from myocardial ischemia or infarction. This phenomenon could be attributed to alterations in myocardial cellular electrophysiology, bundle branch block, or ventricular hypertrophy. The inversion of the T wave is a commonly reported occurrence in snake bite victims [14,39]. However, in the current study, there was no evidence of T-wave inversion. An elevation at the J point, signifying the junction between the end of the QRS complex and the beginning of the ST segment, was observed in only one animal in the VC group. This elevation is indicative of early repolarization, an indication of myocardial ischemia. Although ECG recordings exhibited a return to a normal pattern on day 14 in all groups, complete reversal was not seen. The best results were observed in 50% reduced ASV+MAP-treated group. The results indicate a need for vigorous monitoring of cardiac health in envenomation.

In Group 2 (VC), CKMB levels rose early after venom exposure and remained elevated until day 7, with a slight reduction by day 14. There was an apparent discrepancy between CKMB reduction at day 14 and persistent histological damage in Group 2 (VC). CKMB is released early following sarcolemmal injury caused by venom phospholipases, 3-finger toxins, and oxidative stress by L-amino acid oxidases, which produce H_2O_2 [15–18,42–45]. The persistent elevation of CKMB up to day 7 post-envenomation, suggests a toxic effect of a longer time frame. Reports in the literature have mentioned CKMB elevation up to 48 hours. In Group 2 (VC), a slight decline in CKMB by day 14 was observed, while histopathologic analysis revealed persistent myofibrillar disruption, cytoplasmic vacuolation, interstitial edema, inflammatory infiltration, and vascular congestion. This highlights that CKMB reflects dynamic, ongoing membrane injury, whereas histologic analysis captures elements of slower structural damage.

In treated groups, administration of ASV or MAP or ASV+MAP stops further toxin action, limiting additional injury and resulting in improved myocardial morphology. While the ASV acts by blocking venom antigens, which are proteins in nature, the MAP has multiple mechanisms of action, as listed earlier, which complement the action of ASV. Future studies, with serial histopathologic evaluation and biomarkers, such as cardiac troponin, would allow more precise temporal correlation between biochemical and structural outcomes.

A reduction in both heart weight and heart weight/body weight ratio was observed 14 days after administration of venom, in VC as well as treatment groups, suggesting long-term toxic effects on the heart. The consistent reduction in heart weight across all groups, along with the observed histopathological changes, suggests a correlation between morphological alterations and loss of heart weight. The histopathological changes following envenomation may be due to the action of phospholipase A2, three-finger toxins, and L-amino acid oxidases, which cause damage to the myocardium [15–18]. Alterations in the coagulation cascade [26], vasospasms, altered vascular permeability, or combinations of these may all be contributing factors [35]. It has been reported that AP has cardioprotective and antioxidant properties by virtue of the presence of andrographolide, diterpenoids, flavonoids, quinic acid, xanthones, noriridoids, and andrographidoids [46–51]. These are involved in inhibiting toxic venom enzymes and reducing oxidative stress by neutralizing H_2O_2 [46,51]. The ASV-treated groups showed better results compared to the MAP. However, a combination of 50% reduced ASV along with the MAP was able to reduce cardiotoxicity to almost the same extent as that of ASV alone, giving credence to the multipronged strategy.

CONCLUSION

Naja naja venom-induced cardiotoxicity was associated with alterations in HR, ECG, and histology. These toxic effects were attenuated by ASV and a combination of 50% reduced ASV with MAP, indicating the potential of MAP as an adjunct therapy. While these findings are promising, the study was limited by a small sample size, the use of only female animals, and limited specificity of the CKMB assay. Further studies, eliminating the above-mentioned limitations, would add additional proof to the effectiveness of the multipronged strategy in attenuating cardiotoxicity of N.N. venom.

LIMITATIONS

The study used only female animals, measured CKMB as the sole biochemical marker, which is less specific than cardiac troponins, and employed a human CKMB assay kit without rat-specific validation. The histopathological evaluation was qualitative, without standardized morphometric scoring.

ABBREVIATIONS

A.P, *Andrographis paniculata*; AF, Atrial fibrillation; ASV, Anti-snake venom; CKMB, Creatinine kinase MB; CMC, Carboxymethylcellulose; ECG, Electrocardiogram; HR, Heart rate; IS, Interventricular septum; LD50, Lethal dose; LV, Left ventricle; MAP, Methanolic extract of *Andrographis paniculata*;

N.N, *Naja naja*; NC, Normal control; PLA2, Phospholipase A2; RV, Right ventricle; VC, Venom control; WHO, World Health Organization.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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Ethical approval details are given in the ‘Material and Method’ section.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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