ORIGINAL ARTICLE

Evaluation of central neuroprotective effects of anti-snake venom, methanolic extract of *Andrographis paniculata* and andrographolide in envenomation with *Naja naja*

Ashwini P. Aithal[1], Akshatha G Nayak[2], Maya Roche[3]*

[1]Division of Anatomy, [2]Division of Biochemistry, Department of Basic Medical Sciences, Manipal Academy of Higher Education, Manipal-576104 (Karnataka), India, [3]Department of Biochemistry, Faculty of Medicine, Manipal University College Malaysia, Melaka, Malaysia

Abstract

Background: Currently a partially purified polyvalent Anti-snake Venom (ASV) is the definitive treatment available against the venoms of snakes of India which is accompanied by allergic and anaphylactic reactions, especially at higher doses. No studies have reported the effectiveness of the Indian ASV on central nervous system dysfunction in envenomation with *Naja naja*. *Aim and Objectives:* This study investigated the effectiveness of the multipronged strategy of supplementation of ASV with Methanolic Extract of *Andrographis paniculata* (MAP), and Andrographolide (AP), to ameliorate deleterious changes in brain dopamine and histology in *Naja naja* envenomed rats. *Material and Methods:* Seventy female Wistar rats were divided into Group 1 (normal control), Group 2 (venom control), and Groups 3 to 7 were treated with ASV/MAP/AP/50% reduced ASV+MAP, or 50% reduced ASV+AP, respectively. For Groups 4 to 7, treatment with MAP/AP was continued for 14 days, after which animals were sacrificed. The brains were processed for biochemical and histopathological studies. *Results:* Of the envenomed rats, 60% survived. Their behaviour and physiological functions were drastically altered with blockade of sensory and motor pathways. Dopamine levels were significantly ($p = 0.001$) reduced with multifocal histopathological changes observed in all layers of the cerebral and cerebellar cortex. Rats treated with ASV showed persistent aggressive behaviour, decreased alertness, and slow reflexes. Dopamine levels were significantly ($p = 0.001$) improved. MAP/AP treatment reduced aggressiveness, fear response, and improved reflexes. Dopamine levels were 10-40% higher than the ASV-treated group ($p > 0.05$), along with the normal appearance of cells in the cerebrum and cerebellum. In animals treated with 50% reduced ASV+MAP/AP behavioural patterns, dopamine levels ($p = 0.001$) and brain cellular architecture were normalized. *Conclusion:* This study unequivocally demonstrated the central neuroprotective effect and the superiority of the multipronged strategy of addressing *Naja naja* envenomation and pave the way for more effective strategies to combat neurodegenerative diseases.

Keywords: *Naja naja*, *Andrographis paniculata*, andrographolide, dopamine, neuroprotection

Introduction

Envenomation by cobra (*Naja naja,* N.N, family: Elapidae) is responsible for a major proportion of mortality and morbidity associated with poisonous snakebites in India [1]. Due to its wide-ranging geographic distribution, the species exhibits significant intraspecies variation in venom composition [2]. All *in vitro* and *in vivo* studies with the venom document it to be primarily neurotoxic [3]. Peripheral neurotoxic effects of the venom are due to the presence of at least three types of toxins: Alpha-neurotoxins which bind to the post-synaptic acetylcholine receptors, acetylcholine esterases

which hydrolyze acetylcholine, and phospholipase A2s which digest the lecithin in neuronal membranes [3]. Many snake venoms also harm the Central Nervous System (CNS). Several reports of snake venom neurotoxins acting on the blood-brain barrier have been reported, which increase the likelihood of *in vivo* direct neurotoxic effects. As early as 1968, Krupnick *et al*. reported that N.N whole venom and its electrophoretically separated fractions affected physiological reactions in both cortical and subcortical areas [4]. Venom toxins can block central post-synaptic nicotinic acetylcholine receptors [5] by modifying the storage and release of neurotransmitters in central synaptosomes [6], interfering with GABAergic transmission [7], producing electrocortical convulsions, and neuronal damage [8].

Besides acetylcholine, snake venoms also interfere with neurotransmitters such as GABA, adrenaline, noradrenaline, dopamine, and γ-aminobutyrate [8]. Dopamine is a principal neurotransmitter in the CNS. Dopaminergic neurons are distributed in the brain through the mesolimbic, mesocortical, nigrostriatal, and tuberoinfundibular pathways. It is well established that dopamine plays a significant role in the CNS's hypothalamic-pituitary-adrenal axis functioning and integrating the information in sensory, limbic, and motor systems [9].

At present, the definitive treatment for envenomation with N.N is a partially purified polyvalent Anti-snake Venom (ASV), which is active against the venoms of 'big four' snakes of India (*Naja naja, Daboia russelli, Echis carinatus,* and *Bungarus caeruleus*) [10]. Treatment with ASV is accompanied by allergic and anaphylactic reactions, especially at higher doses [11]. There are no studies that have investigated the effectiveness of the Indian ASV on CNS dysfunction in envenomation with N.N, though studies on snakes such as kraits (Bungarus spp.) and taipans (Oxyuranus spp.) suggest that treatment with ASV may not be able to reverse CNS dysfunction [12].

Andrographis paniculata (AP) is an herbaceous plant, also known as the 'King of Bitters' which is used all over Southeast Asia to treat cobra bites. Earlier studies reported that the Methanolic Extract of AP (MAP) neutralized thromboelastographic changes induced by N.N venom in human blood, when measured in real-time [14]. The extract also corrected the abnormalities in secondary hemostasis measured as prothrombin time and activated partial thromboplastin time [14]. Toxic N.N venom enzymes such as acetylcholine esterase and hyaluronidase were effectively inhibited by the extract, and pan-proteinase inhibitor alpha 2 macroglobulin was rescued from inactivation by venom enzymes [15]. In all these studies, supplementation of reduced concentration of ASV with MAP conclusively proved this multipronged strategy's superiority in neutralizing the venom's toxic effects. Literature states that Andrographolide (AP) (diterpene lactone), a major constituent of MAP shows neuroprotective prophylactic and/or therapeutic effects in Alzheimer's disease, Parkinson's disease, multiple sclerosis, and cognitive impairment. The present study investigated the effectiveness of the multipronged strategy of supplementing ASV with MAP/AP, to ameliorate deleterious changes in brain dopamine and histology.

Materials

Animals

Four to five months old female Wistar albino rats (weighing 140–160 g) were used for this study.

Experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and after obtaining Institutional Animal Ethics Committee clearance (IAEC/KMC/80/2021). Animals were kept in cages in the central animal facility, which was maintained under standard conditions with a temperature of 26–30° C and humidity of 40–60%. Rats had continuous access to water and a regular pellet diet.

N.N venom

Lyophilized N.N venom was procured from KV Institute Ballia, Uttar Pradesh, India. A stock solution of N.N venom was prepared by dissolving 10 mg in 1 ml of saline (0.9%) and stored at 4° C.

ASV

Lyophilized polyvalent ASV was procured from Bharat Serums and Vaccines Pvt. Ltd, Maharashtra, India. The vial contents were dissolved in 10 ml of sterile water provided by the manufacturer and stored at 2-8° C. Each ml of the reconstituted ASV could neutralize 0.6 mg of N.N venom.

MAP and AP

MAP was obtained from Natural Remedies Pvt. Ltd. Bangalore, India, and AP from Essarkay Chemicals & Equipment Centre, Mangalore, India.

Methods

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

The LD_{50} of N.N venom was calculated using Organisation for Economic Co-operation and Development (OECD) guidelines and Acute Oral Toxicity (AOT) 425 statistical program, and was found to be 0.08 mg/kg.b.wt in experimental animals [15]. N.N venom at LD_{50} concentration was injected intramuscularly into the left thigh muscle of rats.

Estimation of the effective dose of MAP and AP

The dosage of MAP and AP used in this efficacy study was the same as the dose used in our previous study based on dose optimization [15] and was 280 mg/kg b.wt for MAP and 50 mg/kg b.wt for AP.

Experimental design

Seventy female Wistar rats were divided into seven groups, with ten animals in each group. Group 1 (normal control) received 0.25% w/v Carboxymethylcellulose (CMC) orally. Group 2 (venom control) was administered LD_{50} N.N venom intramuscularly. Groups 3-7 were the treatment groups. In group 3, animals were treated with a single dose of ASV 266.6 µl intraperitoneally after thirty minutes of administration of N.N venom. The dosage of ASV administered was calculated based on the concentration of N.N venom (LD_{50}) injected into the animals. Group 4 and Group 5 were treated orally with MAP (280 mg/kg b.wt) or AP (50 mg/kg b.wt), respectively after venom administration. Groups 6 and 7 were treated with a combination of reduced dosage of ASV (50% reduction) + MAP (280 mg/kg b.wt), or ASV (50% reduction) + AP (50 mg/kg b.wt), respectively. For Groups 4 to 7, treatment with MAP or AP was continued at the same dose mentioned above for 14 days. On day 15, animals were sacrificed by cervical dislocation. The head region was dissected, and the cerebrum and cerebellum from each rat were rapidly excised. Any presence/absence of blood clots/hemorrhage was noted. The specimens were washed thoroughly with isotonic saline and processed for biochemical and histopathological studies.

General behaviour test

The Irwin test is a systematic observational procedure for assessing and scoring the effects of drugs/chemicals on the behavioural and physiological state of rodents [16]. Irwin test with minor modifications was used to test the effect of N.N venom on rats. Experimental rats were observed at 30, 60, 120 minutes and 4 hours after administration of LD_{50} N.N venom. At each time point, the following parameters were assessed: Behavioural profile (awareness, mood, locomotor activity and tail elevation); neurological profile (body tone, limb position, grip strength, straub tail, startle response, righting reflex, gait, tremors, twitches, and convulsions); autonomic profile (writhing, exophthalmos, salivation, hypothermia, respiratory rate and heart rate) and death.

Estimation of dopamine (DA) concentration in the whole brain

The cerebral tissues from rats were minced into small pieces and rinsed in ice-cold phosphate buffered saline (PBS) (0.01M, pH = 7.4) to remove excess blood. Pieces of tissue were then weighed and homogenized on ice with a glass homogenizer using PBS. The homogenates were then centrifuged for 5 minutes at 5000×g, and the supernatant was collected. DA levels in the supernatant were estimated using Enzyme-Linked Immunosorbent Assay (ELISA) kit from Elabscience Biotechnology Inc. according to the protocol supplied by the manufacturer using the Competitive-ELISA principle. All the samples were assayed in duplicates.

Histopathology study

The cerebrum and cerebellum tissue samples from the rats were washed with saline and fixed in 10% formalin. The samples were then dehydrated in ascending grades of alcohol and embedded in paraffin. After the preparation of blocks, paraffin sections were obtained on clean glass slides. The slides were stained with hematoxylin and eosin and analyzed for histopathological changes under a light microscope. Relevant photomicrographs were captured.

Statistical analysis

The data were analyzed and tabulated using SPSS version 16.0. Quantitative data were expressed as mean ± SD. The mean values of experiment groups were compared using one-way ANOVA, followed by Tukey's post hoc test, and $p \leq 0.05$ was considered statistically significant.

Results

Gross examination of brain specimens

While the brain specimens were being dissected out of the cranium, it was observed that there were no blood clots around the brain. There was no evidence of increased hemorrhage. However, the brain specimens appeared shrunken in the venom control group, with no significant reduction in organ weight when compared to the normal control group.

Physiological and behavioural changes in rats

Group 1 (normal control) showed normal physiological functions and normal patterns in grooming, licking, gnawing, and burrowing with active locomotion. In group 2 (venom control), 60% of animals survived (after 6 hours of venom administration). Physiological changes observed were exophthalmos, increased salivation, increased respiratory rate and heart rate. A decline in reflexes was observed, indicating blockade of sensory pathways. This was exhibited as decreased alertness, touch, and pain response. Increased restlessness

and irritability/aggressiveness were also observed. Blockade of motor pathways was exhibited as the decrease in muscle tone and equilibrium. Animals also showed tremors, twitches, convulsions, and hypotonic gait responses. In Group 3 (ASV treated), 80% of animals survived (after 7-8 hrs of envenomation). The behaviour of rats treated with ASV alone did not normalize since aggressiveness and restlessness persisted. Decreased alertness and slow reflexes were also observed. In Group 4 (MAP treated), 80% of animals survived (after 7-8 hrs of envenomation). Oral administration of MAP reduced aggressiveness, fear response, and improved alertness. However, lethargy and decreased locomotion were observed. In Group 5 (AP treated), the survival of animals was 80% (after 7-8 hrs of envenomation). Compared to ASV treated group, MAP and AP-treated animals showed improved alertness and reflexes, and normal respiratory and heart rates. Animals administered with only MAP (group 4) showed normal behaviour (comparable to control) two days after administration of MAP, while treatment with AP (group 5) significantly normalized the altered behavioural changes just after one day, indicating significant protective therapeutic activity. In Group 6 (ASV+MAP treated) and Group 7 (ASV+AP treated), all animals survived the entire study duration. Respiratory rate and heart rate were normalized, and there were no convulsions or tremors observed. In addition, these animals were awake and aware, with typical combing, touching, and pain responses. The animals' vigilance, motor function, limb tone, grip strength, and locomotion were normal and comparable to normal control animals.

Brain DA levels

The DA level in group 1 (normal control) was 48.38 ± 3.15 pg/mL. DA levels in group 2 (venom control) significantly decreased (60%) compared to normal control ($p = 0.001$). Treatment with ASV increased the DA levels by 87% ($p = 0.04$) and MAP by 96% ($p = 0.03$) when compared to venom control group. The best results were seen when ASV+ MAP or AP alone or ASV+AP were used, with the DA levels significantly ($p = 0.001$) increasing by 144% when compared to venom control group (Table 1).

Table 1: Comparison of brain DA levels

Groups (n=10)	Dopamine concentration in whole brain homogenate (pg/mL) (Mean ± SD)	Percentage change (# decrease, $ increase)
Group 1 (Normal control)	48.38 ± 3.15	-
Group 2 (Venom control)	19.21 ± 1.76 *	60%#
Group 3 (V+ASV)	36.09 ± 1.07 **	87%$
Group 4 (V+MAP)	37.78 ± 3.24 **	96%$
Group 5 (V+AP)	43.65 ± 2.47 **	127%$
Group 6 (V+ASV+MAP)	43.58 ± 2.13 **	127%$
Group 7 (V+ASV+AP)	46.82 ± 2.02**	144%$

**p=0.001(normal control v/s venom control group); **p=0.001 (venom control group v/s treated groups).*
(V – Venom; ASV – Anti-snake Venom; MAP - Methanolic Extract of Andrographis paniculata, AP – Andrographolide)

Histopathology study

Histopathological examination of motor area of the cerebral cortex of Group 1 (normal control) showed normal histology. Individual cells were well stained with the molecular, granular, and pyramidal cell layers showing normal thickness. The pyramidal and granule cells expressed normal morphology with pale nuclei and clear cytoplasm. Neuroglial cells with dense nuclei were also seen (Figure1A, 2A). Examination of sections obtained from Group 2 (venom control) showed multifocal histological changes in all layers of the cerebral cortex. Many vacuoles of variable sizes appeared between and inside most of the cells in all layers. The pyramidal cell layer appeared less dense. The pyramidal cells had lost their processes, appeared shrunken and irregular in shape with deeply stained nuclei. Granule cells were lightly stained with ill-defined boundaries. Pyramidal cells and granule cells had pericellular halos and were surrounded by vacuoles (Figure1B, 2B). In Group 3 (ASV), pyramidal cells appeared irregular in shape with ill-defined margins (Figure 1C, 2C). Group 4 (MAP), Group 5 (AP), and Group 6 (ASV+MAP) showed fewer vacuoles between the cells and improvement in cellular architecture (Figure 1, 2). Group 7 (ASV+AP) showed significant improvement in the cortical architecture, which was comparable to that of the normal control group. No shrinkage was observed in the pyramidal and granule cells, with the absence of vacuoles (Figure 1G, 2G).

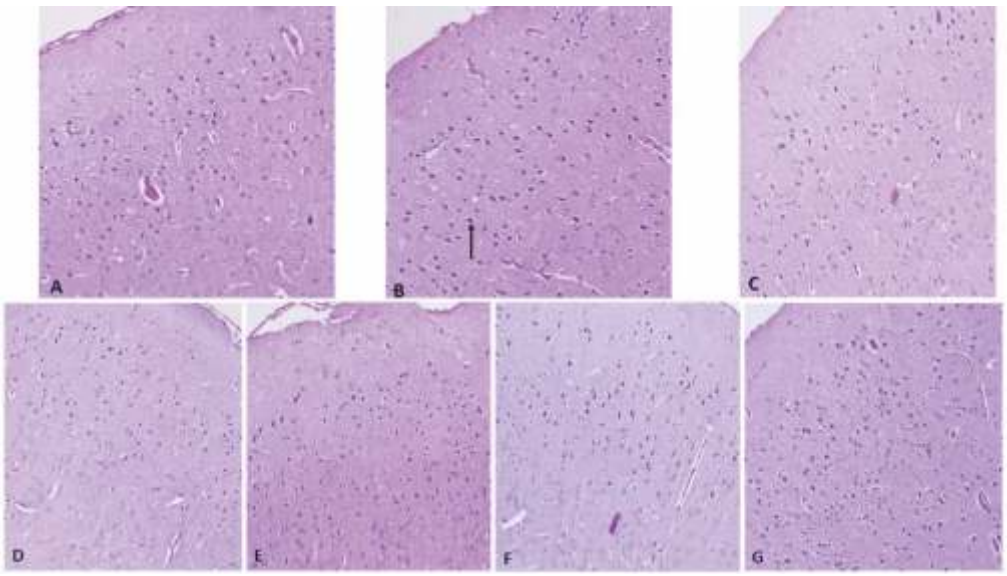

Figure 1: Photomicrograph of a section in the cerebral cortex of Group 1 (normal control) showed the general histological structure of the cerebral cortex with granular cell layer and prominent pyramidal cell layer (A). Section of the cortex of Group 2 (venom control) showed many vacuoles. Most of the pyramidal cells were irregular in shape and surrounded by pericellular halos (arrow) (B). In Group 3 (ASV), pyramidal cells had ill-defined margins (C). Treatment with MAP and AP showed nearly normal pyramidal cells (D, E, F). Treatment with reduced ASV+AP showed normal cerebral cortex comparable to normal control (G) (H&E 10×).

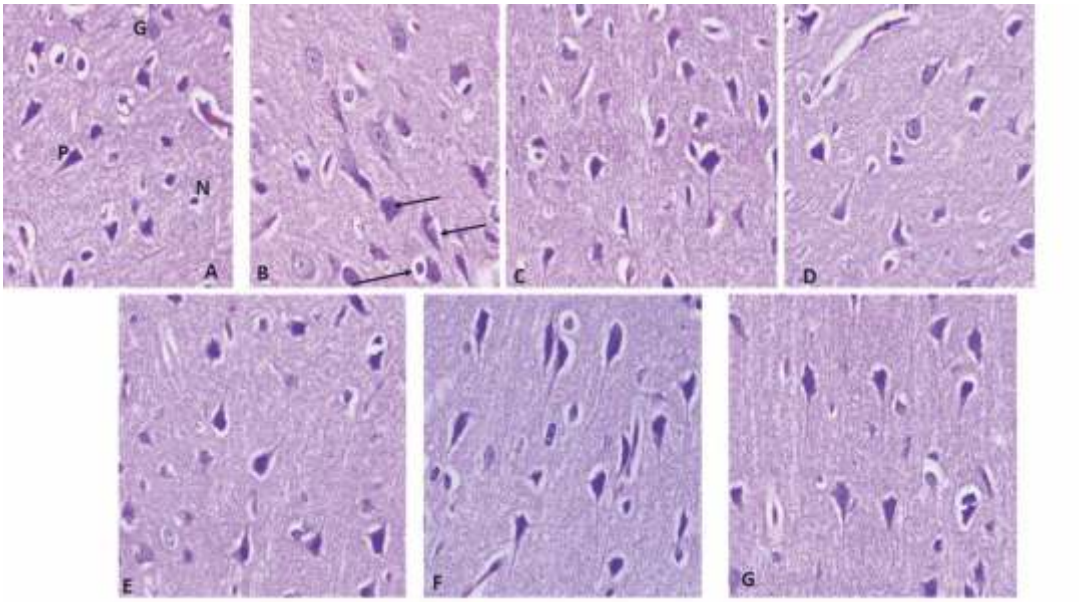

Figure 2: Photomicrograph of the section in the cerebral cortex of Group 1 (normal control) showing the showing pyramidal cell (P) and granule cell (G) with pale nucleus and neuroglia (N) with dense nuclei (A). Section of Group 2 (control venom) treated animals showed irregular pyramidal cells and intracellular vacuoles (arrows) (B). In Group 3 (ASV), pyramidal cells were affected with ill-defined margins (C). Treatments with MAP, AP, and reduced ASV+MAP/AP showed normal cortical architecture which was comparable to normal control (D, E, F, G). (H&E 40×).

Histopathological examination of the cerebellum of Group 1 (normal control) showed normal cellular architecture with distinct cortical layers with outer molecular and inner granular cell layers. Between these, a monolayer of large pear-shaped neurons identified as Purkinje cells with a uniform distribution of cytoplasm around the nucleus was also seen. The central medullary region of white matter was visualized (Figure 3A, 4A). The granular cell layer was dense with cells, while the molecular layer had a large number of unmyelinated fibers with few cells. In Group 2 (venom control), differences pertaining to the layering pattern of Purkinje cells could be discerned. Purkinje cells were seen as multiple pseudo-layers with unevenly distributed cytoplasm and decreased thickness. There was a reduction in the size of the cortical layers with a significant decrease in the number of cell bodies and white matter in the medulla (Figure 3B, 4B). In Group 3 (ASV), Purkinje cells appeared shrunken with few vacuoles (Figure 3C, 4C). Group 4 (MAP), Group 5 (AP), and Groups 6 and 7 (reduced ASV+ MAP/AP) showed signs of protection as the sections appeared to have normal cerebellar architecture with a well-defined, single layer of Purkinje cells. Uniform distribution of cells in the granule layer and molecular layer was observed (Figure 3, 4).

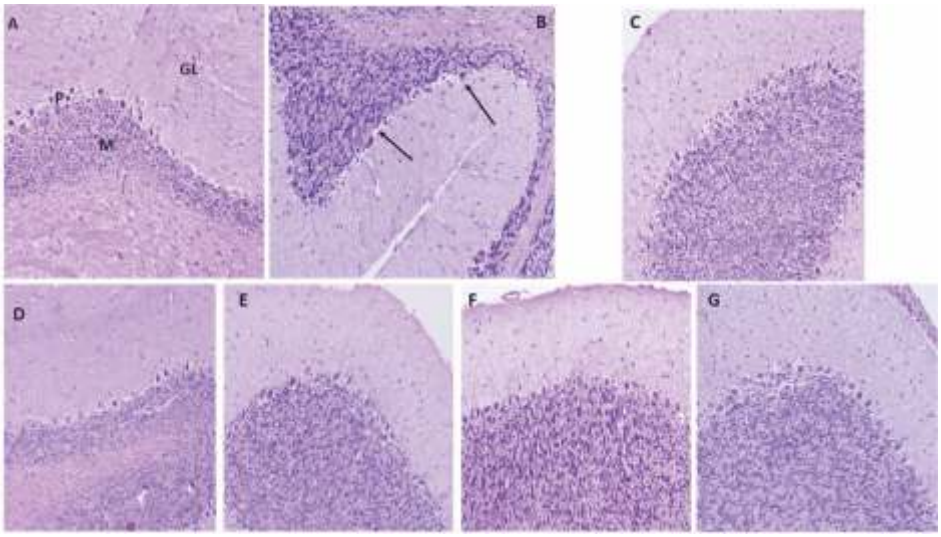

Figure 3: Photomicrograph of the cerebellum of control rat (A) showing highly cellular granular cell layer (GL), single Purkinje cell layer (P), molecular layer (M) with fewer cells, and the central medulla of white matter. Photomicrograph of the cerebellum of rats treated with venom showed decrease in thickness of the granular cell layer, decrease in the number of cell bodies in the molecular cell layer, Purkinje cells (arrows), and loss of white matter (B). In Group 3 (ASV), Purkinje cells appeared shrunken with few vacuoles (C). Treatments with MAP and AP and reduced ASV+MAP/AP showed normal appearance of the cerebellum (D, E, F, G) (H&E 10×).

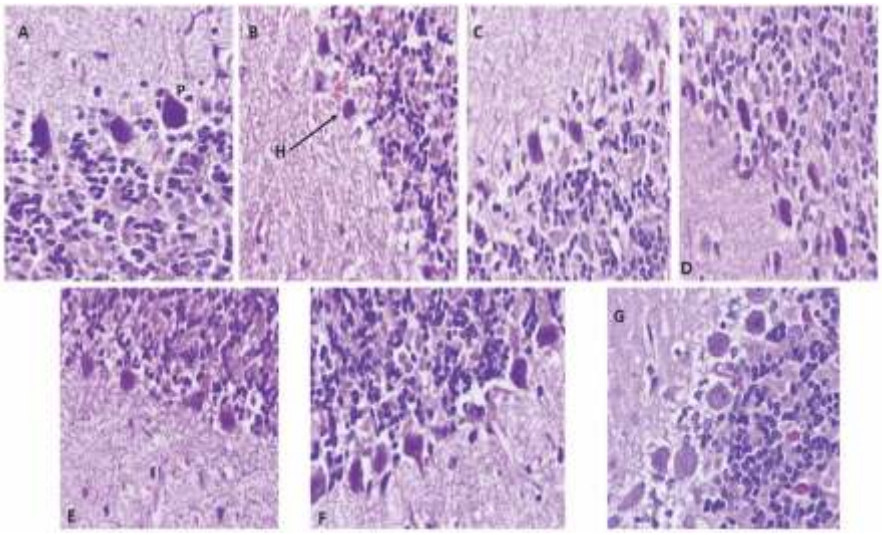

Figure 4: High magnification photomicrographs of cerebellar sections from normal control animals showed disc shaped/pear shaped Purkinje cells (P) with a thin layer of uniformly distributed cytoplasm around the nucleus (A). Representative sections from the venom treated group showed multi-layered pattern of Purkinje cells (H) between the outer molecular layer and inner granular layer. It also showed decreased number of Purkinje cells (B). In Group 3 (ASV), Purkinje cells appeared shrunken (C). Photomicrographs from MAP, AP and reduced ASV+MAP/AP treated groups showed monolayered pattern of prominent Purkinje cells (D, E, F, G) (H&E 40×).

Discussion

The study explored the effect of N.N envenomation on physiological and behavioural changes in rats, brain DA levels and cellular architecture. Since there was no evidence of extradural or subdural hemorrhage or thrombosis in the experimental rats, the changes observed in brain dopamine levels and histology can be attributed to direct central neurotoxicity. The Blood Brain Barrier (BBB) is a complex structure with multiple functions, and its disruption usually leads to structural and functional disorders within the CNS [17]. Several compounds such as bradykinin, 5-hydroxytryptamine, adenosine monophosphate, adenosine diphosphate, adenosine triphosphate, phospholipase A2, leukotriene C4, arachidonic acid, and oxygen-derived free radicals can increase the permeability of BBB. Some of these are found in snake venoms [18] while others can be produced by action of venoms *in vivo*. Snake venom components can produce large quantities of pro-inflammatory cytokines and nitric oxide and mediate tissue damage due to their synergetic actions in initiating and promoting inflammatory responses [19]. These include effects on neurotransmitter storage and release in central synaptosomes [6], inhibition of GABAergic transmission, and causing multi-focal brain damage leading to significant neuronal loss [20]. Earlier studies stated that rats with a marked decrease in brain DA levels exhibited decreased motor activity and aggressiveness [21], as observed in this study. Vast damage to the neo-striatal DA system in rats leads to bradykinesia, adipsia, aphagia, akathisia, short-step locomotion, excessive bracing and clinging reactions, and cognitive dysfunction [22]. DA regulates movement, emotional response, and sensations of pain and pleasure. Therefore, low DA levels in the brain could result in behavioural changes and motor in coordination, which was evident in this study. According to Miller *et al.*, the levels and function of neurotransmitters are broadly modulated by proinflammatory cytokines at multiple levels [23]. The neurotoxins in snake venoms produce large amounts of such proinflammatory cytokines affecting the glial cells. These, in turn, produce and release pro-inflammatory cytokines and mediators, exacerbating the inflammatory response and contributing to resultant brain injuries. Activation of microglia causes persistent neuroinflammation leading to alteration of dopamine synthesis, release, and reuptake [24]. Thus, toxins in the venom have a cascading effect in producing drastic changes in DA levels, causing the death of neurons and affecting the behaviour of rats.

Screening medicinal plants as antidotes to improve snake venom-induced toxicity plays a key role in snakebite management due to the limitations of anti-venom. MAP and its constituent, AP, also have immunomodulatory, anti-inflammatory, and antioxidant activities, neutralizing various toxicities induced by snake venoms, such as myotoxicity, neurotoxicity, and cardiotoxicity [25]. Studies have shown that AP can cross the BBB [26] and possibly enter the cells via passive diffusion. It improves neurological function and attenuates brain oedema and oxidative stress [27]. AP is known to decrease the production of cytokines and pro-inflammatory factors by inhibiting NF-kB activation to protect against cerebral ischemia [28]. Additionally, it has been reported that AP reduces inflammation-

mediated dopaminergic neurodegeneration by inhibiting the activation of microglia [29]. Therefore, inhibition of the inflammatory response by AP treatment can prevent the degeneration of nigrostriatal dopaminergic neurons, attenuate microglial activation, and improve neurological function. Our results demonstrated that along with the improvement in behavioural impairment, brain DA levels were elevated in MAP and AP-treated groups, and the levels were comparable to the normal control group conferring a neuroprotective effect in rats. The effect of AP in reducing neuronal apoptosis in the cerebrum and cerebellum was conspicuous. This finding corroborates with the previous study [30], which proved that AP protected dopaminergic neurons.

The Indian ASV is an IgG manufactured by hyperimmunizing horses. In envenomed rats, it appears that the ASV might have gained access to the brain due to increased permeability of the BBB, as mentioned earlier. This accounts for the recovery of brain DA levels and histology on treatment with ASV. However, the best results in reducing central neurotoxicity were obtained when the administration of reduced doses of ASV was followed by MAP or AP, which points to a synergistic action of these biomolecules. While the ASV can neutralize some of the protein/peptide toxins, MAP and AP have multiple mechanisms to reduce venom toxicity, as mentioned above. The effective supplementation of reduced ASV with MAP/AP once again proved its superiority over the traditional treatment, which uses only ASV. This multipronged strategy is a positive refinement because, it is more effective in reducing neuronal damage in the brain and boosting dopamine levels than just the use of ASV. In addition, it also reduces the dosage of ASV, which putatively would reduce the cost of treatment and minimizes the incidence of anaphylactic reactions in cases of N.N bite.

Conclusion

The present study demonstrated that the multipronged treatment strategy of N.N envenomation with ASV+MAP/AP has a central neuroprotective effect, as evidenced by marked improvement in animal behaviour and locomotor patterns. The strategy was also successful in minimizing deleterious histopathological changes in cerebral and cerebellar cellular architecture and boosting brain DA levels in envenomation. In a country like India, where the burden of consequences of snake bites is heavy, this approach could translate to better care in terms of cost and treatment outcome in cases of N.N bites. Similar multipronged strategies might also prove to be effective in neurodegenerative diseases associated with reduced DA levels.

References

1. Mukherjee AK, Maity CR. The composition of Najanaja venom samples from three districts of West Bengal, India. *Comp Biochem Physiol A Mol Integr Physiol* 1998;119(2):621–627.
2. Senji Laxme RR, Attarde S, Khochare S, Suranse V, Martin G, Casewell NR, *et al*. Biogeographical venom variation in the Indian spectacled cobra (Najanaja) underscores the pressing need for pan-India efficacious snakebite therapy. *PLoS Negl Trop Dis* 2021;15(2): e0009150.
3. Ferraz CR, Arrahman A, Xie C, Casewell NR, Lewis RJ, Kool J, *et al*. Multifunctional toxins in snake venoms and therapeutic implications: From pain to hemorrhage and necrosis. *Front Ecol Evol* 2019;7:218.
4. Krupnick J, Bicher HI, Gitter S. Central neurotoxic effects of the venoms of Najanaja and Vipera-palaestinae. *Toxicon* 1968;6(1):21-24.
5. Chiappinelli VA, Hue B, Mony L, Sattelle DB. Kappa-bungarotoxin blocks nicotinic transmission at an identified invertebrate central synapse. *J Exp Biol* 1989; 141:61–71.
6. Wernicke JF, Oberjat T, Howard BD. Beta-neurotoxin reduces neurotransmitter storage in brain synapses. *J Neurochem* 1974;22(5):781-788.
7. Ye JH, Ren J, McArdle JJ. Waglerin-1 inhibits GABA(A) current of neurons in the nucleus accumbens of neonatal rats. *Brain Res* 1999; 837(1-2): 29–37.
8. Kularatne SAM, Senanayake N. Venomous snake bites, scorpions, and spiders. *Handb Clin Neurol* 2014;120:987–1001.
9. Hoffman BJ, Hansson SR, Mezey É, Palkovits M. Localization and dynamic regulation of biogenic amine transporters in the mammalian central nervous system. *Front Neuroendocrinol* 1998; 19(3):187-231.
10. Chakma J, Menon J, Dhaliwal R. White paper on venomous snakebite in India. *Indian J Med Res* 2020; 152(6):568-574.
11. de Silva HA, Ryan NM, de Silva HJ. Adverse reactions to snake antivenom, and their prevention and treatment. *Br J Clin Pharmacol* 2016;81(3):446-452.
12. Silva A, Hodgson WC, Isbister GK. Antivenom for neuromuscular paralysis resulting from snake envenoming. *Toxins (Basel)* 2017;9(4):143.
13. Nayak AG, Ahammad J, Kumar N, Shenoy S, Roche M. Can the methanolic extract of Andrographis paniculata be used as a supplement to anti-snake venom to normalize hemostatic parameters: A thrombo-elastographic study. *J Ethnopharmacol* 2020;252: 112480.
14. Nayak AG, Kumar N, Shenoy S, Roche M. Evaluation of the merit of the methanolic extract of Andrographis paniculata to supplement anti-snake venom in reversing secondary hemostatic abnormalities induced by Najanaja venom. *3 Biotech* 2021;11(5):228.
15. Nayak AG, Aithal PA, Kumar N, Shenoy S, Roche M. Augmented rescue of macroglobulins by supplementation of anti-snake venom with methanolic extract of Andrographis paniculata in Najanaja envenomation. *3 Biotech* 2022;12(11):310.
16. Irwin S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia* 1968;13(3):222–257.
17. Kadry H, Noorani B, Cucullo L. A blood–brain barrier overview on structure, function, impairment, and biomarkers of integrity. *Fluids Barriers CNS* 2020; 17(1):69.
18. Villar-Briones A, Aird SD. Organic and peptidyl constituents of snake venoms: the picture is vastly more complex than we imagined. *Toxins (Basel)* 2018;10(10):392.
19. Frangieh J, Rima M, Fajloun Z, Henrion D, Sabatier JM, Legros C, *et al*. Snake venom components: tools and cures to target cardiovascular diseases. *Molecules* 2021;26(8):2223.
20. Bagetta G, Nisticó G, Dolly JO. Production of seizures and brain damage in rats by alpha-dendrotoxin, a selective K+ channel blocker. *Neurosci Lett* 1992; 139(1):34–40.
21. van Erp AMM, Miczek KA. Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. *J Neurosci* 2000;20(24):9320-9325.
22. Odewusi OO, Ogberetitinor OB, Ijeh WA, Obadire OS, Olaniyan OO. Evaluation of neuronal inflammation and oxidative DNA damage in different haptoglobin phenotypes in Nigerian type-2 diabetes mellitus population. *J Krishna Inst Med Sci Univ* 2023;12(3): 50-62.
23. Miller AH, Haroon E, Raison CL, Felger JC. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. *Depress Anxiety* 2013;30(4):297- 306.
24. Felger JC, Treadway MT. Inflammation effects on motivation and motor activity: Role of dopamine. *Neuropsychopharmacology* 2017;42(1):216-241.

25. Shivashankar S, Murali A, Sangeetha MK. Molecular interaction of phytochemicals with snake venom: Phytochemicals of Andrographis paniculata inhibits phospholipase A2 of Russell's viper (Daboiarusselli). *Biocatal Agric Biotechnol* 2019;18:101058.
26. Gong P, Zhang W, Zou C, Han S, Tian Q, Wang J, *et al*. Andrographolide attenuates blood-brain barrier disruption, neuronal apoptosis, and oxidative stress through activation of Nrf2/HO-1 signaling pathway in subarachnoid hemorrhage. *Neurotox Res* 2022;40(2): 508-519.
27. Mussard E, Cesaro A, Lespessailles E, Legrain B, Berteina-Raboin S, Toumi H. Andrographolide, a natural antioxidant: An update. *Antioxidants (Basel)* 2019;8(12):571.
28. Chan SJ, Wong WSF, Wong PTH, Bian JS. Neuroprotective effects of andrographolide in a rat model of permanent cerebral ischaemia. *Br J Pharmacol* 2010;161(3):668-679.
29. Wang T, Liu B, Zhang W, Wilson B, Hong JS. Andrographolide reduces inflammation-mediated dopaminergic neurodegeneration in mesencephalic neuron-glia cultures by inhibiting microglial activation. *J Pharmacol Exp Ther* 2004;308(3): 975–83.
30. Ahmed S, Kwatra M, Ranjan Panda S, Murty USN, Naidu VGM. Andrographolide suppresses NLRP3 inflammasome activation in microglia through induction of parkin-mediated mitophagy in in-vitro and in-vivo models of Parkinson disease. *Brain Behav Immun* 2021;91:142-158.

Author for Correspondence:
Dr. Maya Roche, Department of Biochemistry, Faculty of Medicine, Manipal University College Malaysia, Bukit Baru, 75150 Melaka, Malaysia
Email: maya.roche@manipal.edu.my

How to cite this article:
Aithal AP, Nayak AG, Roche M. Evaluation of central neuroprotective effects of anti-snake venom, methanolic extract of *Andrographis paniculata* and Andrographolide in envenomation with *Naja naja*. *J Krishna Inst Med Sci Univ* 2024; 13(4):31-42.

Submitted: *14-June-2024* ***Accepted:*** *26-Aug-2024* ***Published:*** *01-October-2024*