The Effect of *Mimosa Pudica* Root Extract on Cerebrum Histopathological of Rattus Norvegicus Induced with *Naja Sputatrix* Venom

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ABSTRACT

The aim of this study was to know the effect of *Mimosa pudica* root extract on histopathological appearance of *Rattus norvegicus* brain induced by *Naja sputatrix* venom. Thirty rats were divided into 5 groups. There were 2 control groups and 3 treatment groups, which was given 250, 500, and 1000 mg/kg BW of *Mimosa pudica root* extract orally. The first 7 days each group was adapted to the environment. On the 8th day, the treatment was started by injecting *Naja sputatrix* LD50 (0,13 □L/gram BW) IM in gluteus muscle, continued with giving *Mimosa pudica* root extract orally for the treatment groups 5 minutes after venom injection. 6 hours after the last treatment, rats were killed by cervical dislocation, injected with formalin 10% in the heart, then necropsied. Histopathological evaluation was done to score brain damage based on meningitis, perivascular cuffing, and necrotic cells using HE stain with 1000x magnification. The result showed 1000 mg/kg BW dosage of *Mimosa pudica* root extract can reduce brain damage based on meningitis, perivascular cuffing, and necroticcells in Rat (*Rattus norvegicus*) caused by *Naja sputatrix* venom and gave significant difference ($p < 0.05$) among the treatment groups.
Keywords: *Mimosa pudica*, *Naja sputatrix*, snake venom, brain damage

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INTRODUCTION

Snakebite envenoming is a global public health problem of such size and complexity that it deserves far more attention from national and regional healthauthorities than it has been given up until now. This environmental and occupational disease affects mainly agricultural workers and their children in some of the most impoverished rural communities of developing countries in Africa, Asia, Latin America and Oceania (World Health Organization, 2007). Asia is the continent where the majority of these bites take place, and also where most deaths occur (Chippaux, 1998; Kasturiratne *et al.,* 2008).

Venomous and poisonous snakes are a significant cause of global morbidityand mortality. They are found almost throughout the world, including many oceans and have evolved a variety of highly effective toxins and methods of delivery. Their impact on humans is considerable, most current data suggest that they cause in excess of 3 million bites per year with more than 150,000 deaths, particularly in rural tropical areas (White, 2000).

Snake bites and insect stings are most commonly encountered biotoxins (Mount, 1989). Snakes do not attack/prefer not to bite animals unless

they are disturbed / cornered. Among the domestic animals, dogs are most frequently attacked and killed by the snakes (Osweiler, 1996). Cattle and horses are also attacked while grazing. Sheep, goat, and pigs are occasionally struck, while cat is not often attacked because of its greater caution and superior agility when hunting (Shukla, 2009).

Currently there are 29 recognized extant species of terrestrial cobras assigned to the genus Naja (Uetz and Hosek, 2015). Of these, 11 species are found in Asia, and 19 inhabit Afrika (Uetz and Hosek, 2015; Wallach *et al.,* 2009). Cobra (*Naja sp.*) is characterized by local necrosis, neurological paralysis and cardiotoxicity (Yap *et al.,* 2011).

Snake venoms are complex mixtures containing predominantly proteins and polypeptides and small amount of organic compounds and minerals. Many of proteins exhibit enzymatic activities, whereas the polypeptides include neurotoxins, cardiotoxins, myotoxins, and cytotoxins (Yap *et al.,* 2011). Despite snake venoms being a depot of target-specific toxins, some of them may serve as drugs or prototypes for drug design, but the management and neutralization of fatal snakebites are of priority. Antivenom is the preferred and worldwide choice of treatment for snakebites. Therefore, polyvalent (prepared against the venoms of few snakes), bivalent (prepared against the venoms of two snakes) and monovalent (prepared against the venom of one snake) antivenoms are currently available for theurapeutic use (Chippaux *et al.,* 1991).

Tissue changes following snake envenomation depend on the species of snake responsible for the bite, the composition of its venom and also the susceptibility of the tissue for a particular component of the venom (Kamiguti *et al.,* 2000). The nervous system is a primary target for animal venoms as the impairment of its function results in the fast and efficient immobilization or death of a prey (Osipov and Yutkin, 2012). Histological changes in the brain tissues following snake bites are not widely documented compared to other organs. However, since snake venom is rich in neurotoxins significant microscopical changes are likely to be present in the brain tissue. Multiple small foci of haemorrhages and congestion of blood vessels have been reported following administration of collubrid snake venom to rats which was more marked with intravenous injection of venom (Peichoto *et al.,* 2006).

Antivenoms immunotherapy is the only specific treatment against snake venom envenomation. There are various side effects of antivenom such as anaphylactic shock, pyrogen reaction and serum sickns. Most of these symptoms may be due to the action of high concentrations of non-immunoglobulin proteins present in commercially available hyper immune antivenom (Maya Devi *et al.,* 2002). Over the years many attempts have been made for the development of snake venom antagonists from plant sources.

Several medicinal plants, which appear in old drug recipes or which have been passed on by oral tradition, are believed for the treatment of snakebite (Alam & Gomes, 2003). Mors et al. (1989) have found that β-sitosterol successfully antagonises the myotoxicity of South American Rattle Snake venom. Phytochemical analysis of *M. pudica* roots shows that the plant contains ascorbic acid, crocetin, D-glucoronic acid, linoleic acid, linolenic acid, palmitic and stearic acids, mimosine, D-xylose and β-sitosterols.

In preliminary screening of *Mimosa pudica* extract was found to exhibit anti-venom activity against common sea snake (*Enhydrina schistosa*) poisoning (Dnyaneshwar *et al.,* 2009).

Therefore, further work is necessary for better understanding of the mechanism of venom inhibition.

METHODS

This research conducted at the Laboratory Animals Model at Veterinary Medicine of Airlangga University for the treatment of experimental animals. Making of *Mimosa pudica* root extract was be done at the Laboratory of Pharmacology, Veterinary Medicine Airlangga University. *Rattus norvegicus* brain histopathological representation was be observed at the Laboratory of Pathology Veterinary, at Veterinary Medicine of Airlangga University. Implementation of this research was be carried out from May 2016 to June 2016.

The experimental unit used in this study are healthy rat (*Rattus norvegicus*) strain wistar with an average weight of 200 grams, maintained at the same place and were given the same feed.

Experimental design

In this research used *Rattus norvegicus* which randomly taken and then put into the cage maintenance that has been marked (C (-), C (+), T1 T2 and T3) by the same amount, each group contain 6 rats. Rat adapted and maintained for seven days in a cage, these rat was given food and drink ad libitum in the form of pellets and vegetables, replacing the litter when it's dirty.

Mimosa pudica extraction

Mimosa pudica root extraction using water extraction method (normal water extraction), as Mahanta and Mukherjee (2001) did. The fresh *Mimosa pudica* root left in the open air, dried under the sun, and pulverized to a powder. Four grams of the powder was added into beaker glass and was added 200 ml of distilled water, then stirred for about 3 hours at room temperature. The extract was filtered using muslin cloth then concentrated at 40° C. Then it was placed in freeze dry equipment.

Microscopic examination

Histopathological slide examination carried out using a light microscope 100 times magnification of the objective lens to 10 different field of view in a slide sample. The scoring was be observe in meningens dan cerebrum. The histopathologic scoring of vasculitis modified from (Kennedy *et al.*,1997) as follows:

	Score				
	0	1	2	3	4
Meningitis	None	Mild	Moderate	Severe	Severe
Perivascular Cuffing	None	None	Mild cuffing of some vessels	Prominent cuffing of some vessels	Prominent of most vessels
Necrosis Cell	None	0-25% cell necrosis	>25%-50%≤ cellnecrosis	>50%-75%≤ cellnecrosis	>75% cell necrosis

Data analysis

Data were analyzed with statistical test using Kruskal-Wallis and followed by Mann-Whitney test to compare the treatment effect of each treatment. (Attched in appendix).

RESULTS AND DISCUSSION

This experiment was conducted on 30 white rats (*Rattus norvegicus).* It divided into 5 groups of treatment with 6 replications. And then after passing through a period of adaptation for 7 days, the negative control (C-) was given aquadest 0,1ml then 10ml saline solution 5 minutes later per-orally, then

the positive control (C+) injected *Naja sputatrix* LD50 (0,13 L/gram BW) IM (*musculus gluteus*) and given saline solution 10ml per oral 5 minutes later, (T1 injected *Naja sputatrix* LD50 (0,13 L/gram BW) IM (*musculus gluteus*) and will be given 250 mg/kg BW *Mimosa pudica* root extract per-oral in 5 minutes later, (T2) injected *Naja sputatrix* LD50 (0,13 L/gram BW) IM (*musculus gluteus*) and will be given 500 mg/kg BW *Mimosa pudica* root extract per-oral in 5 minutes later (T3) were injected *Naja sputatrix* LD50 (0,13 L/gram BW) IM (*musculus gluteus*) and will be given 1000 mg/kg BW *Mimosa pudica* root extract per-oral in 5 minutes later. Then while doing the necroption, rat was injected with formalin in the heart to maintain the brain stay on the shape.

In this research, histological slide preparation were made from rat's brain on day after the last treatment, by fixating them in formalin 10%. The making of histopathology slide took approximately 2 weeks. Scoring of brain slides were done using Modified of Kennedy (Kennedy *et al.*, 1997).

Histopathology examination of white rat (*Rattus norvegicus)* brain given *Mimosa pudica* root extract post *Naja sputatrix* venom injection was done microscopically by *Hematoxylin Eosin* (HE) staining, using 1000 (100x) magnification. The variables observed in this observation were necrosis cells, meningitis and perivascular cuffing.

Meningitis is defined as inflammation of the membranes that surround the brain and spinal cord (Schuhat *et al.,*1997). In this experiment, meningitis can be found in piamater layer.

The result of meningitis scoring was analyzed using *Kruskal Wallis* and showing significant difference ($p<0.05$) between treatment groups, the analysis was continued afterwards using *Mann Whitney* test. Analytic result of total damage scoring is shown in the Table 1.

Table 1. Meningitis Damage Value

Treatment	Mean ± SD
C-	0.350^{a} ±0.104
C+	0.833^{c} ±0.816
T1	0.783^{c}±0.408
T2	0.750^{bc}±0.164
T3	0.550^{b}±0.187

The different superscript show there is significant difference between treatment groups ($p<0.05$).

Statistical analysis of total damage in Negative control (C-) shows significant difference to other treatment groups. Positive control (C+) shows no significant difference to *Mimosa pudica* root extract 250 mg/kg BW (T1) and *Mimosa pudica* root extract 500 mg/kg BW (T2),while *Mimosa pudica* root extract 500mg/kg BW(T2) shows no significant difference to *Mimosa pudica* root extract 1000 mg/kg BW (T3). Yet Positive control shows significant difference to *Mimosa pudica* rootextract 1000 mg/kg BW (T3).

The histopathological result was shown in Figure 1. It shows that there is not any inflammatory cell in negative control, C+ shows there is infiltration of PMN cell. Treatment with *Mimosa pudica* root extract 250mg/kg BW (T1) shows that there are some infiltration of PMN cell. While treatment *Mimosa pudica* root extract 500mg/kg BW (T2) shows that there are some PMN cell and haemorrhagic. Treatment with *Mimosa pudica* root extract 1000mg/kg BW shows that numbers of inflammatory cell is much reduced than prior treatments.

Perivascular cuffing scoring was analyzed using *Kruskal Wallis* and showing significant difference ($p<0.05$)

between treatment groups, the analysis was continued afterwards using *Mann Whitney* test. Analytic result of total damage scoring is shown in the Table 2.

Table 2. Perivascular Cuffing Damage Value

Treatment	Mean ± SD
C-	0.316^{a} ±0.117
C+	3.716^{d} ±0.147
T1	3.367^{c}±0.250
T2	1.583^{b}±0.386
T3	1.466^{b}±0.265

The different superscript show there is significant difference between treatment groups ($p<0.05$).

Statistical analysis of total damage in Negative control (C-) and Positive control(C+) shows significant difference to other treatment groups. *Mimosa pudica* 250 mg/kg BW (T1) shows significant different to both treatment groups (T2 & T3). While *Mimosa pudica* 500 mg/kg BW (T2) shows no significant difference to *Mimosa pudica* 1000 mg/kg BW.

From Figure 2 can be seen that there are necrotic cell and perivascular cuffing.

Necrosis has been defined as a type of cell death that lacks the features of apoptosis, and autophagy, and is usually considered to be uncontrolled. (Pierre and Guido, 2006).

Necrotic cell scoring was analyzed using *Kruskal Wallis* and showing significant difference ($p<0.05$) between treatment groups, the analysis was continued afterwards using *Mann Whitney* test. Analytic result of necrotic cell scoring is shown in the Table 1.

Table 3. Necrotic Cell Damage Value

Treatment	Mean ± SD
C-	0.400^{a}±0.632
C+	3.900^{d} ±0.167
T1	3.316^{c}±0.271
T2	2.216^{b}±0.444
T3	1.900^{b}±0.209

The different superscript show there is significant difference between treatment groups ($p<0.05$).

From Figure 3 can be seen that, histopathology in the negative control (C-) contained no inflammatory cell infiltration, and it can be seen that the size and shape of the cells is still relatively normal. In positive control (C+) shows that ganglion change to necrosis cell is more than 75%. And there is massive inflammatory cells in the blood vessel. Astrocyte, oligodendrocytes, pyramid cells does necrosis.

The histopathology of treatment 1 (*Mimosa pudica* root extract 250 mg/kg BW) shows that the amount of necrosis cell is about ≥ 75% and there is infiltration from inflammatory cell in the blood vessel.

Histopathology of treatment 2 (*Mimosa pudica* 500 mg/kg BW) shows necrosis cell is less than 75%. But, some massive inflammatory cells appears in the blood vessels.

Histopathology representation of treatment 3 (*Mimosa pudica* 1000 mg kg/BW) is less massive than any other treatment. It can be seen from appearance of total normal ganglion, pyramid cell, astrocytes. The inflammatory cells is rare to be found. The result of histopathological was shown in Figure 3.

Based on statistical analysis using *Kruskal-Wallis* test shows there are

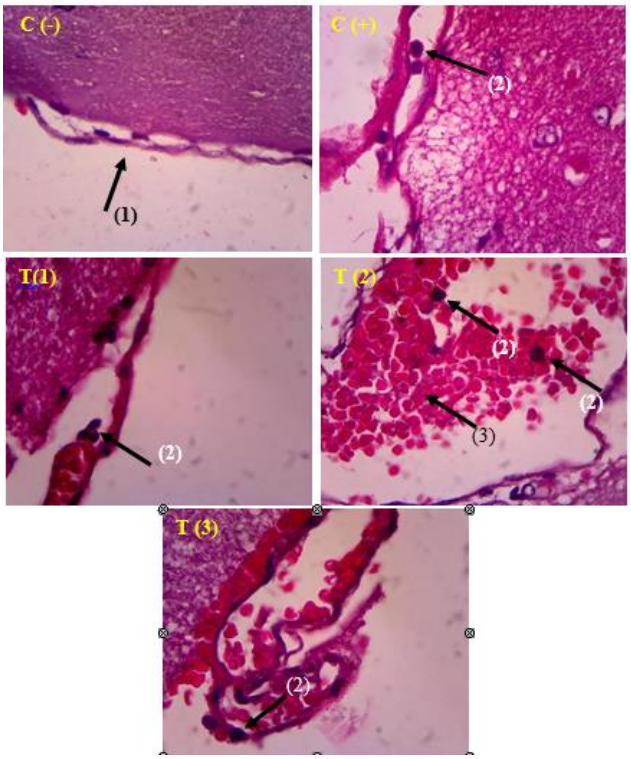

Figure 1. Picture of meningitis in the cortex cerebri in the brain. Showing: (1) Normal meningens. (2) PMN Cell. (3) Haemmorhagic. HE staining, 1000x magnification.

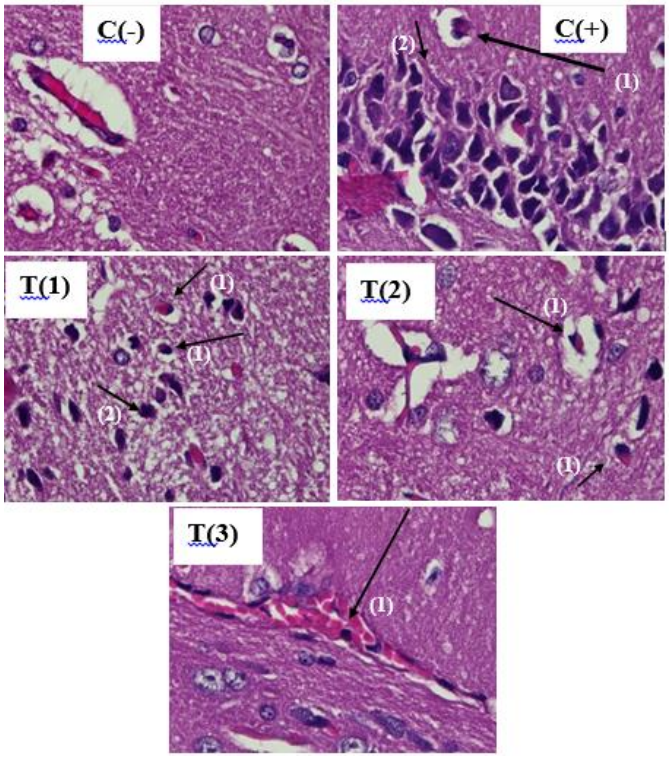

Figure 2. Picture of perivascular cuffing in the cortex cerebri in the brain. Showing: (1) Perivascular cuffing. (2) PMN Cell. HE staining, using 1000x magnification.

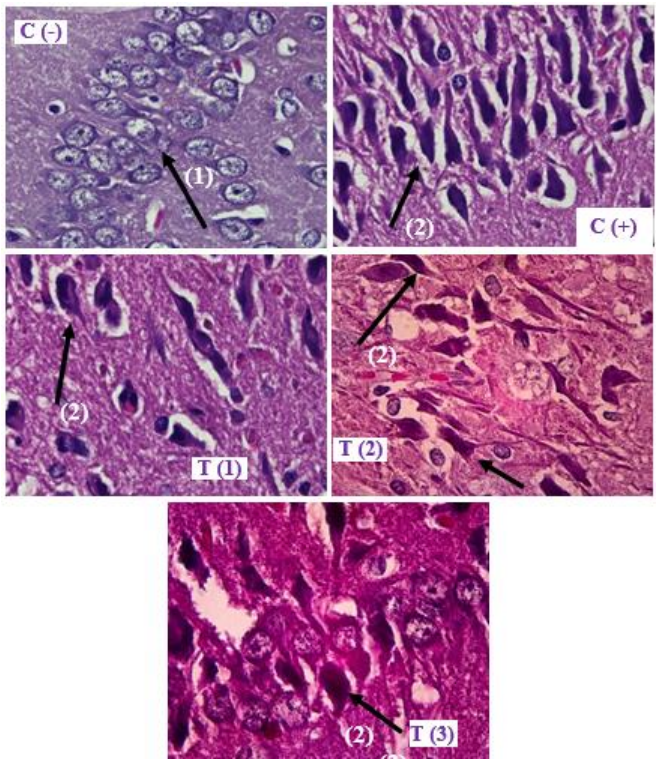

Figure 3. Picture of nectrotic cell in the cortex cerebri in the brain. Showing: (1) Normal cell. (2) Necrosis Cell. (3) PMN Cell. HE staining, 1000x magnification.

significant difference between treatment groups ($p<0.05$), then the analysis continued using *Mann Whitney* test. The result shown by observation and scoring of meningitis (inflammation of the membranes). The result is so moderate between positive control (C+) and *Mimosa pudica* root extract 1000mg/kg BW.

Cheng *et* al., (2008) said an α - neurotoxins, key components of neurotoxic venoms, recognize and bind nAChRs, which are widely expressed in CNS includingbrain capillary endothelial cells, which are the main constituent of the BBB. This property of α-neurotoxins might facilitate their penetration through the BBB. Zhang *et al.*, (2006) only 0.2% of the amount injected can reach the brain by subcutaneous. This means, snake venom succeed in damaging brain even for meningitis is not sosevere.

According to *Mann-Whitney* test, Negative control (C-) shows significant difference to all treatment groups. The result are negative control (C-) is 0.350, positive control (C+) is 0.833, *Mimosa pudica* root extract 250mg/kg BW (T1) is 0.783, *Mimosa pudica* root extract 500mg/kg BW is 0.750, and *Mimosa pudica* 1000mg/kg BW is 0.550.

Positive control (0.833) is significant difference with *Mimosa pudica* 1000mg/kg BW. It means *Mimosa pudica* root extract 1000mg/kg BW can reduce the damage caused by *Naja sputatrix* venom. According to Girish *et* al., (2004) Hyaluronidase and protease activities of the venom of some Indian snake (*Naja naja*, *Vipera russelii* and *Echis carinatus*) were found to be inhibited by the root extracted in water. In Joseph (2013), aqueous extract of dried roots of *M. pudica* was tested for inhibitory activity on lethality,

phospholipase activity, edema forming activity, fibrinolytic activity and hemorrhagic activity of *Naja naja* and *Bangarus caerulus* venoms. The aqueous extract displayed a significant inhibitory effect on the lethality, phospholipase activity, edema forming activity, fibrinolytic activity and hemorrhagic activity. About 0.14 mg and 0.16 mg of *M. pudica* extractswere able to completely neutralize the lethal activity of 2LD50 of *Naja naja* and *Bangarus caerulus* venoms respectively.

According to statistical analysis using *Kruskal-Wallis* test shows there are significant difference between treatment groups ($p<0.05$), then the analysis continued using *Mann Whitney* test. The result shown by observation and scoring of perivascular cuffing. The statistic result shows *Mimosa pudica* root extract 250 mg/kg BW (T1) 3.376 is significant to both *Mimosa pudica* root extract 500 mg/kgBW (T2) 1.583, and *Mimosa pudica* root extract 1000 mg/kg BW (T3) 1.466.

From the statistic data can be seen that the effective dose for *Mimosa pudica* is 500 mg/kg BW. Because it significant to T1 (3.376) and no significant to T3 (1.466). This could happen because in dose 500 mg/kg BW it can lessen the necrosishigher than T1 and T3.

Negative control (C-) 0.316 and positive control (C+) are significant to all treatment groups. It shows that *Naja sputatrix* venom succeed in damaging and causing haemmorrhage the brain. According to Peichoto *et* al., (2009) snake venomis rich in neurotoxins significant microscopical changes are likely to be present in the brain tissue. Multiple small foci of haemorrhages and congestion of blood vessels have been reported following administration of collubrid snake venom to rats which was more marked with intravenous injection of venom.

The presence of lymphocytes in vascular due to the success of the venom that can penetrate the BBB into the brain is considered a foreign substance, so thatthere lymphocytes to fagocyt this snake venom. This is what causes perivascular cuffing may occur. And also cobra venom factor (CVF) depleting complement system attenuates brain edema in intracerebral hemorrhage.

Nam *et al.* (2010) showed that crocin and crocetin contain in *Mimosa pudica* provide protection against neuroinflammation. This may explain the perivascular cuffing is lessen in treatment 2.

Kruskall-Wallis statistical analysis test shows there are significant difference between treatment groups ($p<0.05$), then the analysis continued using *Mann Whitney* test. The result shown by observation and scoring of necrosis cell.

The statistic result shows *Mimosa pudica* root extract 250 mg/kg BW (T1) 3.316 is significant to both *Mimosa pudica* root extract 500 mg/kg BW (T2) 2.216 and *Mimosa pudica* root extract 1000 mg/kg BW (T3) 1.900.

From the statistic data can be seen that the effective dose for *Mimosa pudica* is 500 mg/kg BW. Because it significant to T1 (3.316) and no significant to T3 (1.900). This could happen because in dose 500 mg/kg BW it can lessen the necrosishigher than T1 and T3.

In snake venom, there is a protein that can penetrate through the blood brain barrier (BBB) which is phospholipases A2.Phospholipase A2 and α-neurotoxins bind to postsynaptic α-neurotoxins to neuronal nicotinic acetylcholine receptors (nAChR), dendrotoxins - to voltage gated potassium channels, neurotoxic phospholipases A2 – to presynaptic membranes, and many other examples. It makes the neurotransmitter to other

organ is obstructed. It can cause respiratory failure andcardiac arrest.

α-neurotoxins, key components of neurotoxic venoms, recognize and bind nAChRs, which are widely expressed in CNS including brain capillary endothelial cells, which are the main constituent of the BBB. This property of α-neurotoxins might facilitate their penetration through the BBB. This may cause the necrosis cellin the brain such as ganglion spinal, astrocyte, oligodendrocyte etc.

Based on Nanayakkara *et al.*, (2009) study, necrosis was present, it was always associated with inflammatory infiltration and not vice versa. Periventricularfocal necrosis with associated inflammatory infiltration was the most prominent feature in the cerebrum which was seen only following administration of B. ceylonicus venom.

In Vejayan (2007) study the MP188ECT3 fraction was found to bind with certain protein components of the venom and precipitated them. This allowed the deactivation of potent venom components responsible for life-threateningconditions.

The aqueous extract displayed a significant inhibitory effect on the lethality, phospholipase activity, edema forming activity, fibrinolytic activity and hemorrhagic activity. About 0.14 mg and 0.16 mg of *M. pudica* extracts were able to completely neutralize the lethal activity of 2LD50 of *Naja naja* and *Bangarus caerulus* venoms respectively (Joseph *et al.*, 2013).

With these both theory may explain that why the presence of necrotic cell in the brain is lessen in the treatment 2.

CONCLUSION

The effect of *Mimosa pudica* root extract in histopathological representation of *Rattus norvegicus* cerebrum injected with *Naja sputatrix* venom shows that theextract can reduce necrotic cells and perivascular cuffing yet there isn't anysignificant difference in meningitis.

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