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ORIGINAL ARTICLE

Ameliorative Effects of Methanolic Leaf Extracts of *Azadirachta indica* and *Spondias mombin* : A Study on the Pons of Wistar Rats Induced with Zidovudine

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ABSTRACT
The effect of combined methanolic leaf extracts of Azadirachta indica and Spondias mombin on the histology of Zidovudine stress induced Wistar rats' pons was investigated. Thirty Wistar rats, weighing between 150 – 280g were randomly divided into five groups made up of six animals. The groups were designated A, B, C, D and E. Group A animals served as the control (negative) group and were fed with rat chow and water, ad libitum. Group B animals were treated with 400mg/kg body weight of Zidovudine for a period of 21 days and served as the positive control group. Group C animals received 400mg/kg body weight of Zidovudine for 7 days and were administered with 500mg/kg body weight of Azadirachta indica methanolic leaf extract for 14 days. Group D animals received 400mg/kg body weight of Zidovudine for 7 days and were administered with 500mg/kg body weight of Spondias mombin methanolic leaf extract for 14 days. Group E animals received 400mg/kg body weight of Zidovudine for 7 days and were subsequently treated with 500mg/kg body weight of combined methanolic extracts of Azadirachta indica and Spondias mombin for 14 days. Histological findings revealed normal cytoarchitechture of the pontine tissue in Group A animals, showing neurons made up of three types; large, medium and small neurons alongside blood vessels and numerous branching nerve fibers. Group B animals seemed to have undergone a surge of neuroglial cells when compared to that of Group A. Photomicrograph of the pontine tissue section of animals in Group B also showed the presence of large, medium and small neurons as well as hyper vascularity and the presence of numerous branching nerve fibers. Groups C and D both showed considerable differences when compared to Group B as there was hypoplasia of neuroglial cells and reduced hyper vascularity. Large, medium and small neurons as well as distinct branching nerve fibers were also present. In Group E animals, there were almost no neuroglial cells present, normal vascularity was shown, large, medium and small neurons remained intact and distinct numerous branching nerve fibers were seen. Therefore, combined methanolic extract of Azadirachta indica and Spondias mombin in short term administration has favourable effect in averting the damaging outcomes of Zidovudine on Wistar rats pons. Hence, this shows the anxiolytic effect of the combined methanolic leaf extracts of Azadirachta indica and Spondias mombin.
***Keywords**: Azadirachta indica, Neuroglial, Pons, Spondias mombin, , Stress, Zidovudine*

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INTRODUCTION

Plants have been found to play important roles in the treatment of diseases Worldwide [1, 2]. Medicinal alternatives from plants used by humans to improve their state of health have increased in recent years and are the number one go to remedy for a majority of people in most countries [3]. *Azadirachta indica* (neem) is one of the two species in the genus *Azadirachta*, native to India and the Indian subcontinent,

typically grown in tropical and semi-tropical regions[4]. Biswas *et al* [5] recently reviewed the biological activities of some of the neem compounds, pharmacological actions, clinical study and probable medical applications of neem along with their safety evaluation. Neem leaf extract has been shown to exhibit anti-inflammatory, anti-hyperglycemic, anti-ulcer, immune-modulatory, antiviral, anti-fungal, anti-bacterial, nematicidal, anti-malarial, insecticidal, anti-mutagenic and anti-oxidant properties [5-7]. Studies have shown that *Spondias mombin* bark and leaves are used in the treatment of athlete's foot in parts of Edo State, Nigeria [8]. Anti-inflammatory, expectorant and febrifuge actions of *Spondias mombin* fruit were also reported [9]. Anathematic, sedative, antiepileptic and antipsychiatric effects of *Spondias mombin* during various trial studies have also been reported [10-11]. It's antifertility, locomotor and haematinic effects have also been documented [12-16].

Stress according to Sutano and de Kloet [17] refers to the disturbance in homeostasis. Throughout life, all living organisms undergo this threatening factor [18]. There has been an increased incidence of a number of psychiatric illnesses, including Post Traumatic Stress Disorder (PTSD) and other anxiety disorders, mood disorders and substance related disorders shown to be closely related to stress [19]. Nociceptive thresholds have been shown to increase due to acute stress [20-23]; leading to the assumption that stress, in general, produces stress – induced analgesia (SIA) [24].). Stress had been established to contribute to the development of both neurological and psychiatric diseases [25]. While the role of the blood – brain barrier is increasingly recognized in the development of neurodegenerative disorders such as Alzheimer's disease, dysfunction of the blood – brain barrier has been linked to stress related psychiatric diseases only recently [26-28].

The pons is an important division of the brainstem; it contains nuclei that relay signals from the forebrain to the cerebellum, along with nuclei that deal primarily with sleep, respiration, swallowing, bladder control, hearing, equilibrium, taste, eye movement, facial expressions, facial sensations and posture. The Locus Coeruleus is involved with physiological response to stress and panic, this therefore calls for investigation of short term administration of combined methanolic extract of *Azadirachta indica* and *Spondias mombin* on the histology of Wistar rat pons after induction of oxidative stress using zidovudine.

MATERIAL AND METHODS

Extract Preparation

Fresh matured leaves of *Azadiractha indica* were harvested from the University of Calabar Botanical Garden and *Spondias mombin* from Ugep, Yakurr Local Government Area of Cross River State. Both leaves were authenticated by the Botanist in the botanical garden in the University of Calabar, Calabar. The leaves were rinsed with distilled water to make pure, removing dirt and other particles. The leaves were dried in a shade and then ground to powder and later used for extraction. The preparation of the methanolic extract of *Azadiractha indica* and *Spondias mombin* was carried out at the Department of Biochemistry Research Laboratory, University of Calabar. 400g each of the ground powder was dissolved in methanol (80% methanol) and homogenated for about 10 minutes with an electric blender. The homogenate was allowed to cool for 24 hours in a refrigerator and then filtered. First using a chess cloth and thereafter using a Whatmann no. 1 filter paper. The filtrate was then concentrated in vacuo using a rotary evaporator at 40°C to complete dryness and the residues obtained were stored in a freezer at -80°C until when needed for further test.

Ethical Consideration

Approval was given by the Faculty of Basic Medical Sciences Committee on animal use and care, University of Calabar to carry out this research work following laid down rules and guidelines of the institution in the use of medicinal plants and animal models.

Stress Induction

Oxidative stress was induced using Zidovudine obtained from the Plan President Emergency for Aids and liberation section, Teaching University of Calabar Hospital, Calabar town, Cross-River State, Nigeria. The animals in all the experimental groups received 450 mg/kg body weight of the Zidovudine. The drug was dissolved in 150 mls of distilled water and administered once daily to group C, D, and E for a period of seven days, while group B received the drug for a period of three weeks.

Procedures

A total of thirty rats were used in this experiment. The rats were divided into five groups A, B, C, D, E. Zidovudine was used to induce stress in the animals. Group A served as the control group. Animals in groups B, C, D and E were grouped based on their weight and consequent dose of the extract and Zidovudine administered. Administration of the plant extracts and stress inducing drug (Zidovudine) was done orally with the use of an orogastric tube. The tube was gradually lowered through the oesophagus and into the stomach of the animal to ensure that accurate concentration of the extract was administered.

Administration lasted for a total of 21 days. Group A was the control group (neither plant extract nor zidovudine was administered to this group). Group B received 400mg/kg body weight of Zidovudine for the total duration of administration (21 days). Group C received 400mg/kg body weight of Zidovudine for 7 days and subsequently treated with 500mg/kg body weight of *Azadiractha indica* methanolic leaf extract for 14 days. Group D received 400mg/kg body weight of zidovudine for 7 days and subsequently treated with 500mg/kg body weight of *Spondias mombin* methanolic leaf extract for 14 days. Group E received 400mg/kg body weight of Zidovudine for 7 days and subsequently treated with 500mg/kg body weight of combined methanolic extract of *Azadiractha indica* and *Spondias mombin* for 14 days.A day after the last administration (22nd day) the animals were sacrificed using the chloroform inhalation method. This method entailed placing the animals in an enclosed jar containing a tissue soaked in chloroform. The animals were thereafter removed.The skull was decapitated and the whole brain excised and fixed immediately in freshly prepared 10% buffered formalin.The staining technique employed for the purpose of this research work was the routine H/E staining method.

RESULTS

Group A animals served as the control (negative) group and were fed with rat chow and water, given *ad libitum*. They were not treated with Zidovudine nor plant extracts. Histological sections of pons of group A animals showed presence of neurons made up of three types; large, medium and small neurons as well as oligodendrocyte and blood vessels. Neurons were seen to be scattered around in no particular order within the tissue section. Distinct numerous branching fibers were also seen. (Plate 1)

Group B animals were treated with 400mg/kg body weight of Zidovudine for a period of 21 days and served as the positive control group. Sections from this group showed the presence of three types of neurons; large, medium and small neurons, unevenly distributed throughout the tissue section. The neural cytoplasm of the majority of the neurons were seen to be hyper chromic and neural hyperplasia of all three types of neurons was seen when compared to the tissue section of Group A animals. Neuroglial cells were present and hyper vascularity was seen, with cells suspected to be astrocytes (since astrocytes are known to form the blood – brain barrier) concentrated around blood vessels. Increased vacuolation was also evident throughout the tissue section and numerous branching nerve fibers were seen. (Plate 2)

Group C animals received 400mg/kg body weight of Zidovudine for 7 days and were subsequently treated with 500mg/kg body weight of *Azadirachta indica* methanolic leaf extract for 14 days. The large, medium and small neurons remained intact although they appeared to be hypoplasia of large neurons as compared to that in the tissue section of Group B animals. Neuroglial cells were also seen, although they also seemed to have undergone drastic hypoplasia. Some neurons were hyper chromic while others were hypochromic and hyper vascularity seemed to have been reduced when compared to the tissue section of Group B animals. There was also hypoplasia of cells around blood vessels and reduced vacuolation as compared to that observed in Group B. (Plate 3)

Group D animals received 400mg/kg body weight of Zidovudine for 7 days and were subsequently treated with 500mg/kg body weight of *Spondia smombin* methanolic leaf extract for 14 days. There was significant hypoplasia of large, medium and small neurons as well as hypotrophy of large neurons. Most neurons appeared to be hyperchromic, although some were hypochromic. Hypoplasia of neuroglial cells was also seen as well as significant decrease in vascularity and cells surrounding blood vessels when compared to that in Group B animals. Decreased vacuolation was also observed. Distinct branching nerve fibers were present. (Plate 4)

Group E animals received 400mg/kg body weight of Zidovudine for 7 days and were subsequently treated with 500mg/kg body weight of combined methanolic extract of *Azadirachta indica* and *Spondias mombin* for 14 days. Three types of neurons were seen; large, medium and small, although more large were neurons present. Neural cytoplasm became hypochromic with rounded centrally placed nucleolus, interspersed with normal cell bodies. Neuroglial cells seemed to have undergone drastic hypoplasia and were almost totally absent. Vacuolation was also seen and blood vessels appeared to be almost totally absent from the tissue section. Numerous branching nerve fibers were also seen. (Plate 5).

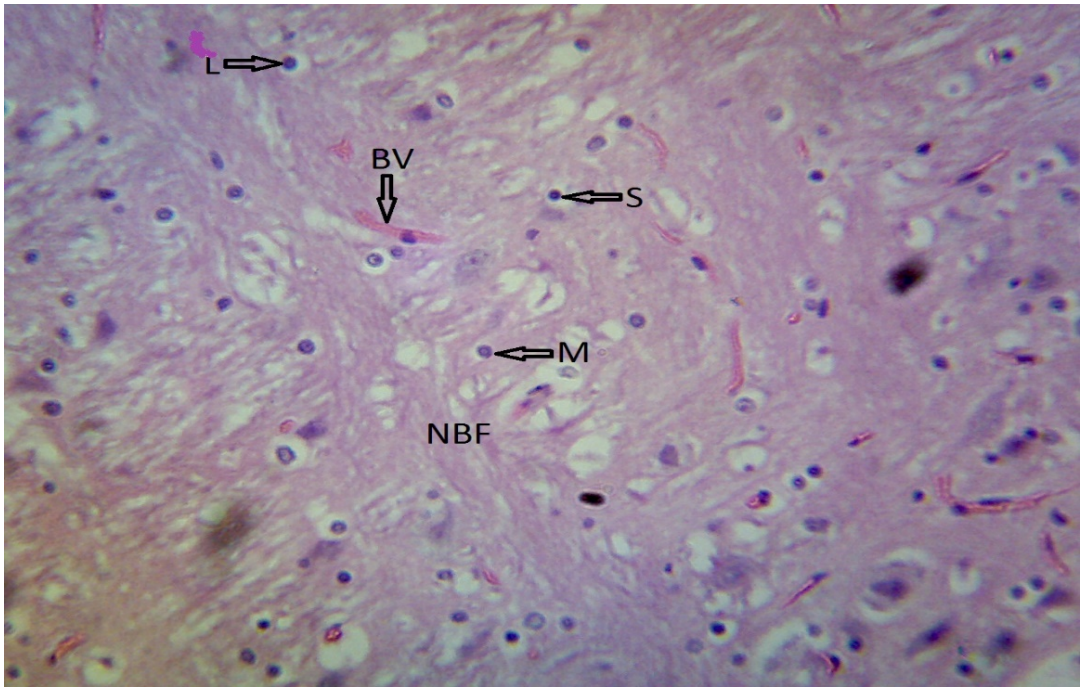

Plate 1: Photomicrograph of negative control pons (Group A) showing normal histological features of the pons which include Large neurons (L), Medium neurons (M) and Small Neurons (S) as well as blood vessels (BV) and numerous branching nerve fibers (NBF). (Haematoxylin& Eosin [x400])

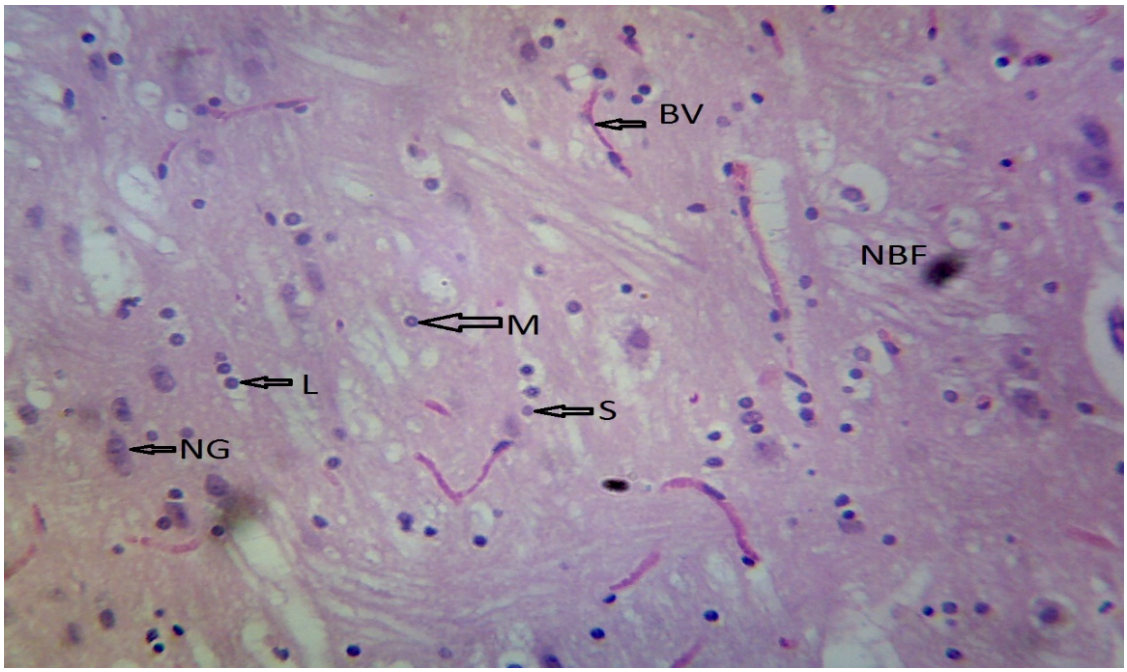

Plate 2: Photomicrograph of positive control pons (Group B) treated with 400mg/kg body weight of Zidovudine showing large neurons (L), medium neurons (M) and small neurons (S) alongside blood vessels (BV), Neuroglial cells (NG) and numerous branching nerve fibers (NBF). (Haematoxylin& Eosin [x400])

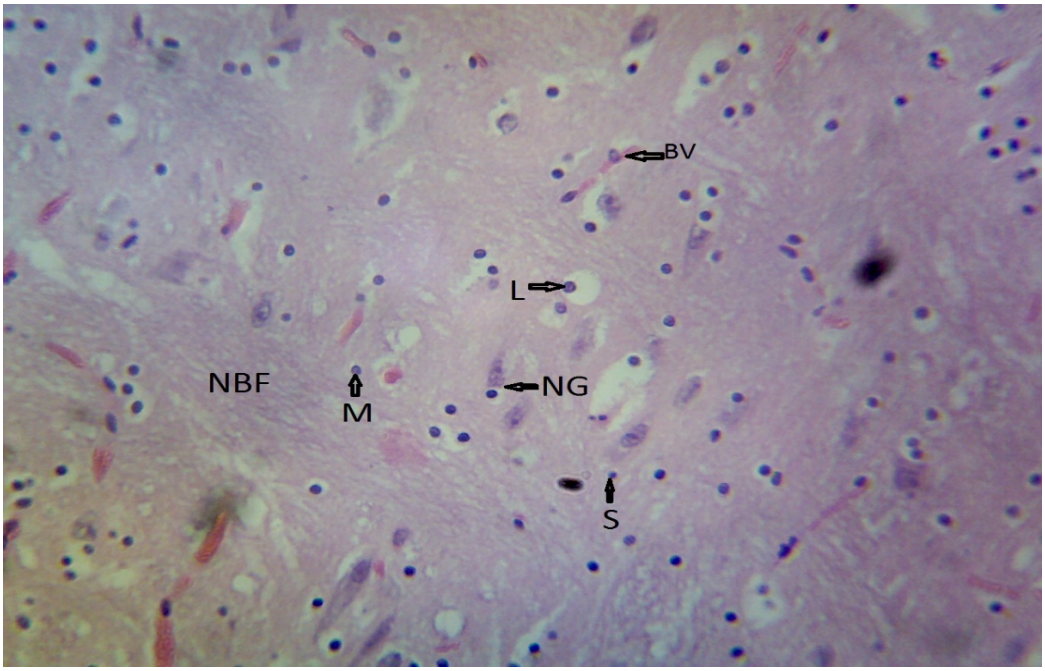

Plate 3: Photomicrograph of pons section treated with 400mg/kg body weight of Zidovudine and 500mg/kg body weight of *Azadirachtaindica* methanolic leaf extract showing large neurons (L), medium neurons (M) and small neurons (S) alongside blood vessels (BV), Neuroglial cells (NG) and numerous branching nerve fibers (NBF). (Haematoxylin& Eosin [x400])

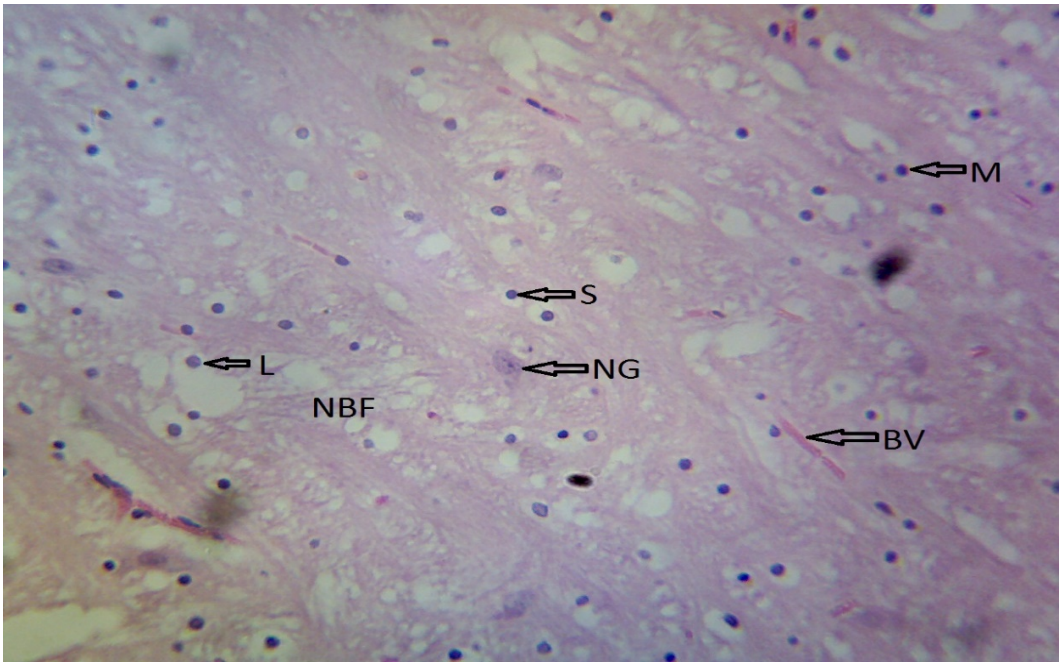

Plate 4: photomicrograph of pons section treated with 400mg/kg body weight of Zidovudine and 500mg/kg body weight of *Spondias mombin* methanolic leaf extract showing large neurons (L), medium neurons (M) and small neurons (S) alongside blood vessels (BV), Neuroglial cells (NG) and numerous branching nerve fibers (NBF). (Haematoxylin& Eosin [x400])

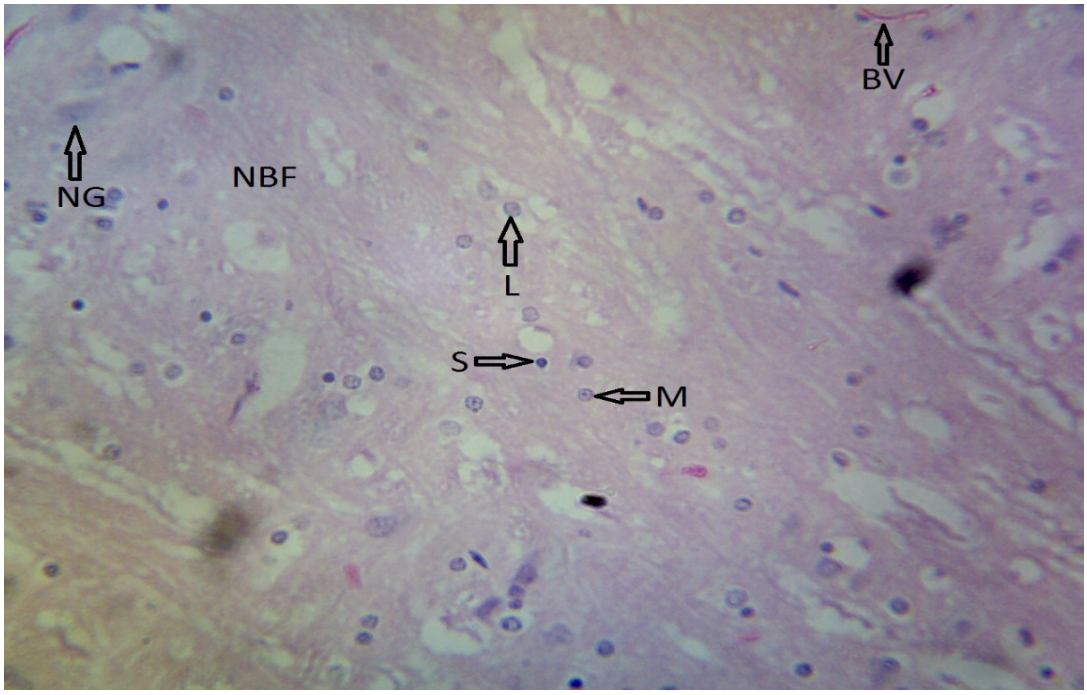

Plate 5: Photomicrograph of pons section treated with 400mg/kg body weight of Zidovudine and 500mg/kg body weight of combined methanolic extract of *Azadirachta indica* and *Spondias mombin* showing large neurons (L), medium neurons (M) and small neurons (S) alongside blood vessels (BV), Neuroglial cells (NG) and numerous branching nerve fibers (NBF). (Haematoxylin& Eosin [x400]).

DISCUSSION

The locus coeruleus is the largest nucleus of noradrenergic cell bodies in the brain [29] and is the major source of forebrain Norepinephrine [30]. The locus coeruleus is highly stress-reactive, as assessed by electro-physiological activity [31]. Antidepressants and some anticonvulsants block the effects of stress and / or promote neurogenesis in animal studies [32]. Corticotropin-Releasing Factor (CRF) acts centrally to mediate fear- related behaviours [33] and triggers other neurochemical responses to stress such as the noradrenergic system through the brainstem locus coeruleus [34]. The majority of noradrenergic cell bodies are located in the locus coeruleus, a nucleus in the dorsal pons region of the brainstem, with a dense network of axons that extends throughout the cerebral cortex and to multiple cortical and subcortical areas, including the hippocampus, amygdala, thalamus and hypothalamus, red nucleus of stria terminalis, nucleus accumbens as well as descending projections which synapse at the level of the thoracic spinal cord [35]. It has been shown that gastric ulcerogenesis, a manifestation of stressful condition, could be protected by *Azadirachta indica* [36]. *Azadirachta indica* offers promise as an oxidative agent due to its beneficial effects on health [37]. From this study, methanolic extract of *Azadirachta indica* showed significant anti-stress activity by causing hypoplasia of neuroglial cells which is an indication of reversal of trauma to the neural system, thus backing up the claims of previous studies which advocated the plant's anti-stress and anxiolytic activity, including that of Jaiswal et al [38] which stated that fresh leaf extracts of *Azadirachta indica* has been found to possess anxiolytic activity in rat models of anxiety.

Flavonoids and other derivatives have been identified in *Spondias mombin* plant with anti-herpes, antioxidant and anti-aging properties [39]; while flavonoids have been reported to be free radical scavengers, super antioxidants and with strong anti-cancer activity [40], they also provide anti-inflammatory activity as antioxidants [41]. The stem bark and leaf extracts of *Spondias mombin* also contain the antioxidant vitamins C and E [42]. Antioxidants repair free radical damages for the cells [43]. The presence of antioxidant molecules suggests that *Spondias mombin* can be used as vitamin supplements, probably during oxidative stressed conditions [42]. The results of a study by Ayoka *et al* [44] showed that *Spondias mombin* has high nutritive value which could attenuate physiological oxidative stress due to its high concentration of vitamin E and C as well as flavonoids content. This could be why the methanolic extract of *Spondias mombin* has shown to also ameliorate the expression of zidovudine induced stress in the pons as shown in this study

From this research, it has been seen that methanolic extracts of *Azadirachta indica* and *Spondias mombin* when used individually, avert the cellular effects of acute stress in the pons, thus creating a need for methanolic extracts of both plants to be investigated when used in combination. This is evident by their ability to reduce the number of neuroglial cells in the pons after they had been triggered with the administration of Zidovudine in order to induce stress. *Azadirachta indica* methanolic leaf extract not only caused hypoplasia of neuroglial cells but also led to reduced hyper vascularity of the pontine tissue, caused by the reaction to the acute stressor (Zidovudine) administered. This corroborates the claim by Jaiswal *et al* [38] that *Azadirachta indica* has proven to have significant anxiolytic activity. *Spondias mombin* methanolic leaf extract also elicited similar effects on the pontine tissue of the stressed animals when compared to the tissue section of animals in Group C, only that it seemed to have more effectively ameliorated the cellular reactions to Zidovudine induced stress as neuroglial cells were almost totally absent from the section, thus backing up the claim by Ayoka *et al* [45] which stated that leaves of *Spondias mombin* have been reported to be responsible for anxiolytic activity. These observations are similar to a study by Raghavendra *et al* [46] and Maduka *et al* [42] where they corroborated that medicinal plants have been recognized to be of great importance to the health of individuals and communities..

CONCLUSION

Histological findings in this research work showed that administration of combined methanolic extracts of *Azadirachta indica* and *Spondias mombin* is effective at ameliorating the effects of Zidovudine induced stress on the histology of wistarrats pons. Therefore, the short term administration of combined methanolic leaf extracts of *Azadirachta indica* and *Spondias mombin* may possess anti stress properties.

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