Effects of *Spondias Mombin* Leaf Extract on the Cytoarchitecture of the Cerebal Cortex and on Learning and Memory in Wistar Rats

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Abstract: ***This study was carried out to determine the effects of aqueous extract of Spondias mombin on the histology of the cerebral cortex and its possible learning and memory enhancing properties in adult male Wistar rats. 24 matured male Wistar rats (180g) were randomly divided into three groups; 1 control and 2 treatment groups. Group A served as control, group B and C were administered with 4oomg/kg and 800mg/kg body weight of Spondias mombin aqueous extract respectively. The administration lasted for 28days. Morris water maze was employed for the learning and memory protocol. Histopathology of the cerebral cortex was investigated. Section of the cerebral cortex in the 400mg/kg rats showed no degenerative changes whereas the 800mg/kg showed degenerative changes and intercellular degradation. Using the Morris water maze method, extract at 400mg/kg improved learning and retention with a significant improvement established in memory tests using the reversal training. At 800mg/kg, the extract had no significant effect on learning and memory, aqueous extract of Spondias mombin has a learning and memory enhancing potential at low dose. Caution must be taken in its usage at high dose to avoid possible cerebral injury***

Keywords: Cerebral cortex, Degenerative, Injury, Learning, Memory, Retention.

1. Introduction

Herbal medicinal preparations have become very popular in developing countries due to the unavailability and costs of orthodox medicines. A lot of the population in these Countries do not have adequate financial freedom to procure orthodox drugs and therefore rely mostly on herbal plants especially in the rural areas. Several herbal preparations have been employed in most developing Countries including Nigeria for treating and alleviating ailments. Preparations are made from herbs, fruits and spices; seeds of *Abrus precatorius* can be used to treat diabetes [1]. Fruits of *Ananas comosus* are employed for typhoid fever [2]. Ripe seeds of *Carica papaya* are an effective treatment for malaria [3]. *Aspilia affricana* leaves are used for wound healing [4]. *Sida acuta* is used for the management and treatment of ulcers[5].

Herbs have been known to have wide therapeutic usage, suitable for chronic treatments and also acceptable by the population [6]-[7]. *Spondias mombin* (SpM) is one of such medicinal plants that have been investigated to have a wide range of therapeutic use; it is antimicrobial [8], anti-inflammatory [9], antimalarial [10], antihelmintic [11], antispasmodic [12], has a lipid lowering effect [13and antigonadotrophic [14. Ayoka et al, [15] had demonstrated the hypnotic and sedative properties of the plant, also, Pauly and Fleury [16] reported that it acts as an anti-aging agent; it is possible therefore that it can improve learning. The ability to acquire knowledge and retain the acquired knowledge can be defined as learning and memory [17]. Several conditions such as age, stress and emotions may lead to impairment of learning [18]-[20]. However, ageing have been implicated to affect learning and memory more [21]. It has been shown to lead to memory loss, dementia and amnesia that may lead to life threatening diseases such as schizophrenia and Alzheimer's disease. There is no record in literature on the effect of *Spondias mombin* on the histology of the cerebral cortex as well as learning and memory, hence, this research was carried out.

2. Materials and Methods

Twenty-four adult male Wistar rats were bred in the animal house of the department of Human Anatomy, University of Calabar, Nigeria. The rats were kept under standard condition at a temperature of 25-27^{o}C. The animals were divided into three groups A, Band C with each consisting of eight rats. The group A animals served as control and groups B and C were the experimental animals. They were fed with rat chow and water given *ad libitum*.

2.1 Preparation of herb extract

The leaves of *Spondias mombin* were procured from a local community in Akpabuyo, Cross River State, Nigeria and authenticated by the Chief Botanist in the University of Calabar. The leaves were washed and air dried for 5 days, blended into fine powder using a Binatone kitchen blender and kept in a glass container with a cover lid. The extraction method involved aqueous extraction, where 100g of powdered sample was soaked in distilled water for 24 hours. The extract was then filtered and evaporated to dryness at room temperature to obtain a crude residue (yield of 40%).

2.2 Experimental protocol

400mg/kg and 800mg/kg per body weight of aqueous leaf extract of *Spondias mombin* (SpM) were orally administered to rats in groups B and C respectively for 28 days. Animals in group A received corresponding volumes of distilled water. Schedule of administration is represented in Table 1 below.

Table 1: Oral administration of the control and *Spondias mombin* treated groups.

Group (8)	*Treatments*	Duration (days)
Control	Distilled water	28
B	400mg/kg	28
C	800mg/kg	28

2.3 Learning and memory test

Morris water maze method of Brown [22] was used to test for learning and memory in the animals. Testing in the Morris water maze lasted eight days. The behaviors scored during the test were swim latency and duration in each quadrant

3. Results

3.1 Histological

The 6 cell layers of the cerebral cortex were well defined in the control animals (Fig 1). The group B animals treated with 400mg/kg SpM showed distinct cell layers similar to that of control (Fig 2). Distorted cell layers with all the cell types scattered within in the cytoplasm were seen in group C treated with 800mg/kg SpM.

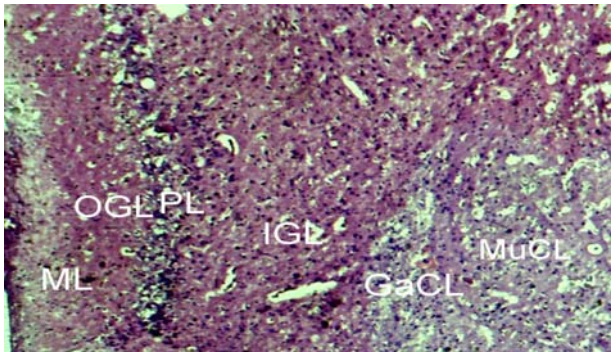

Figure 1: Cross section of cerebral cortex of control animals showing normal appearance. ML- Molecular layer; OGL- Outer granular layer; PL- Pyramidal layer; IGL- inner granular layer; GaCL- Ganglionic cell layer; MuCL- Multiform cell layer. (H & E X 100).

3.2 Learning and memory

Figure 4 shows the swimming latency of the control and experimental groups during the 3 days acquisition in the Morris water maze. Mean values for the control were 39.55±4.52; 25.05±6.11 and 20.47±3.19. Group B treated with 400mg/kg SpM that served as low dose recorded mean values of 54.26±3.72; 34.01±6.34 and 30.77±6.59, while group C treated with 800mg/kg (high dose) had values of 46.14±1.54; 38.33±2.76 and 38.38±2.76. The swimming latency of the low dose were significantly ($P<0.05$)

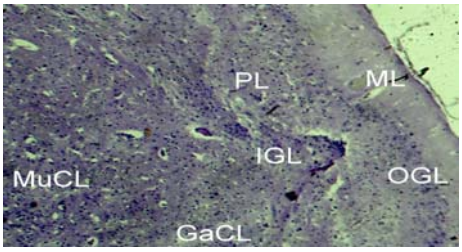

Figure 2: Cross section of cerebral cortex of animals treated with 400mg/kg SpM showing distinct features. ML- Molecular layer; OGL- Outer granular layer; PL- Pyramidal layer; IGL- inner granular layer; GaCL- Ganglionic cell layer; MuCL- Multiform cell layer. (H & E X 100).

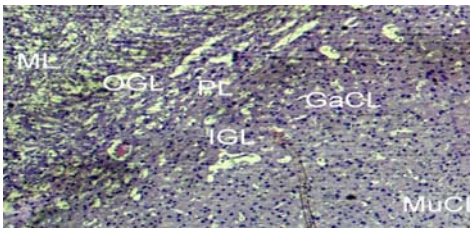

Figure 3: Cross section of cerebral cortex of animals treated with 800mg/kg SpM showing distortion of cell layers and scattered cell types. ML- Molecular layer; OGL- Outer granular layer; PL- Pyramidal layer; IGL- inner granular layer; GaCL- Ganglionic cell layer; MuCL- Multiform cell layer. (H & E X 100

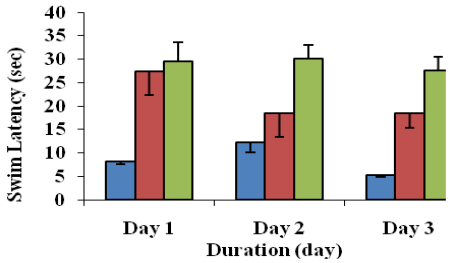

Figure 4: Comparison of the swim latencies during the acquisition training of the Morris water maze task in the different experimental groups of rats. Values are mean ± SEM, n = 8. – Control, - 400mg/kg, - 800mg/kg.

The swim latency of reversal training for 3 days of control and experimental groups is shown in Figure 5. The mean values of control animals were 8.26±0.69secs, 12.21±1.97secs and 9.31±0.41secs respectively. Group B recorded values of 12.41±8.01secs, 10.84±8.08secs and 8.72±2.58secs while group C had values of 29.53±5.59secs, 34.05±4.11secs and 37.50±4.36secs. Group C recorded a significantly ($P<0.05$) higher time.

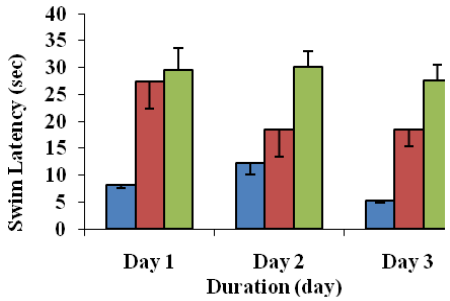

Figure 5: Comparison of the swim latencies during the reversal training of the Morris water maze task in the

different experimental groups of rats. Values are mean ± SEM, n = 8. ■ – Control, ■- 400mg/kg, ■- 800mg/kg.

Figure 6 shows the swim latency of animals in the quadrant during the probe trial. The groups from control to group C had values of 11.76±0-51secs, 15.43±0.40secs and 18.76±0.34secs respectively. These values were not significantly (P>0.05) different.

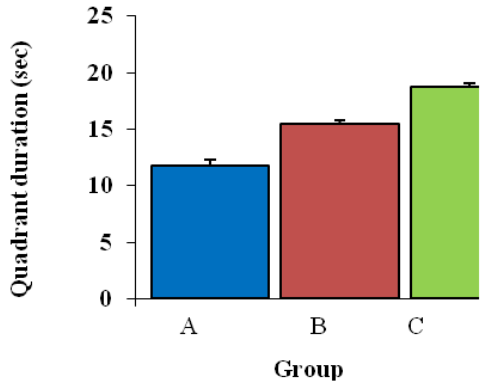

Figure 6: Comparison of the South East quadrant duration in the probe trial of the Morris water maze task in the different experimental groups of rats. Values are mean ± SEM, n = 8.

The swim latency during the visible platform task of the Morris water maze is shown in Figure 7. Mean values were 5.62±0.4secs for control, 6.27±0.79secs for group B and 14.02±2.51secs for group C. The swim latency of group C (800mg/kg) were significantly (P<0.05) higher compared to control.

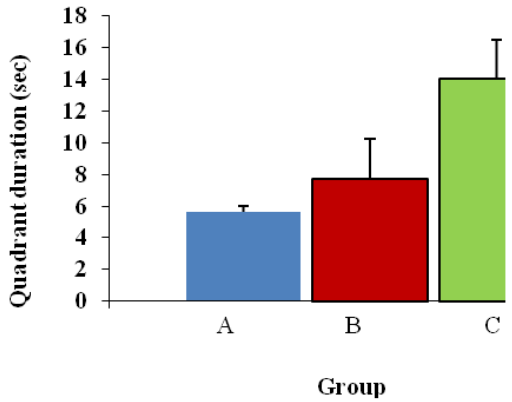

Figure 7: Comparison of swim latencies during the visible platform task of the Morris water maze task in the different experimental groups of rats. Values are mean ± SEM, n = 8.

4. Discussion

The cerebral cortex is involved and responsible for the coordination of learning and memory. Any distortion of the cytoarchitectural design of the cerebrum may manifest in a defect in memory and coordination of learning [23]. In this study, SpM aqueous leaf extracts was administered at varying doses (400mg and 800mg/kg) to adult Wistar rats with adverse effect found in the histological appearance and ability to learn and memorize at 800mg/kg with little or no effect at 400mg/kg. The mechanism through which this is affected is not clear, however, improved learning behavior and enhanced memory at 400mg/kg may be due to the structural changes observed in the cerebrum. Improved learning and enhanced memory have been linked to structural changes of the limbic system [24]-[26]. SpM may also have probably positively affected the biosynthesis of neurotransmitters such as acetylcholine, noradrenaline, dopamine and 5HT that have been reported to be involved in learning and memory mechanism [27]-[29]. Dashti and Morshedi [30] have also stated that ‘’neuronal activities are associated with learning and this leads to the expression of several genes whose protein products play vital roles in the process of memory formation’’. The use of traditional medicines has been confirmed to improve cognitive function and prevent the onset of age related deficits [31].

5. Conclusion

From this study aqueous extract of SpM has been found to improve the cytoarchitecture of the cerebrum and also enhances learning and memory capabilities. Therefore, caution must be taken in its use in the treatment of various ailments not to exceed a calculated safe dosage and duration so as not to lead to impairment in learning and memory.

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