

## Comparative Study of Aqueous and Ethanolic Leaf Extracts Of *Spondias Mombin* on Neurobehaviour in Male Rats

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**Abstract:** Remedies for anxiety and fear have been investigated through both orthodox and traditional medicine; this study compared the effects of chronic administration of graded doses of aqueous and ethanolic extracts of *Spondias mombin* on anxiety and fear in Albino Wistar rats. Fifty rats of Wistar strain (100-120g body weight) were divided into five major groups A-E with each group made up of ten rats (n=10). *Spondias mombin* (250, 400, 500 and 800mg/kg) were orally administered for 4 weeks before testing. The elevated plus maze was used to assess anxiety behaviour. Following administration of graded doses of aqueous and ethanolic extracts of *Spondias mombin* for a period of four weeks, decreased anxiety and fear in the albino rats was revealed ( $p < 0.05$ ). The order of the anxiolytic property following these graded dose of administration was; duration and frequency in open arm of the 500mg/kg group was significantly higher ( $90.2 \pm 37.2s$  &  $4.4 \pm 1.29/5min$  respectively) compared to control, and other treatment groups of *Spondias mombin* ( $P < 0.05$ ). Duration in closed arm ( $260.33 \pm 15.97s$ ) was significantly lower compared to control. Closed arms frequency with 250mg/kg and 500mg/kg ethanol extract were  $3.83 \pm 1.74/5min$  and  $3.16 \pm 1.40/5min$ . The extracts were significantly ( $P < 0.05$ ) lower compared with control ( $8.67 \pm 1.05/5min$ ). However, the 500mg/kg ethanol extract showed a lower frequency. The duration of grooming following four weeks of treatment with 250mg/kg and 500mg/kg ethanol extract of *Spondias mombin* were  $8.68 \pm 2.10s$  and  $12.1 \pm 0.001s$  respectively and these were significantly lower compared to control. *Spondias mombin* dose-dependently decreased anxiety and fear with significant decrease observed in rats administered with ethanolic extracts of *Spondias mombin*. Anxiolytic effect is shown by decrease in the frequency of grooming and duration of grooming, decrease in frequency of head dipping and stretch attempt posture (SAP), and a lower frequency of close arm entry by the experimental groups. Ethanolic extract from *Spondias mombin* produced better behavioral effects with decreased anxiety and fear compared to the aqueous extract and so has a potential as an alternative anxiolytic and sedative agent compared to its aqueous extract.

**Keywords:** *Spondias mombin*, Fear, Anxiety, Frequency, Duration, Ethanol, Elevate plus maze.

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### I. Introduction

Neurobehavioral test is a non-invasive method employed to evaluate the performance of the central nervous system in an individual or group with similar exposures. Herbs are an integral part of the development of modern civilization, much of the medicinal use of plants seems to have been observed through observation of wild animals, and by trial and error (1). Medicinal plants are distributed worldwide, but they are most abundant in tropical countries (2). People from developing Countries especially Africans still resort to the use of herbs despite advances and availability of Orthodox medicine. Herbal medicines are used for the treatment of mental disorders in Nigeria due to its affordability and the belief that the main causes of mental disorders are supernatural (3,4). Plants have been known for their medicinal properties in the central nervous system and used to influence brain function (5). Reports from other Continents have also shown the use of traditional medicine especially in China and the United States of America in the treatment of psychiatric disorders. (6,7).

*Spondias mombin* (*Sp*) is one of the herbs used for the treatment of psychiatric disorders in Nigeria; it belongs to the family *Anacardiaceae*. Herbal preparations of *Spondias mombin* are used by traditional medicine practitioners in the treatment of mental disorder in Western Nigeria (8). *Sp* is relied on for various herbal remedies for numerous conditions and virtually every part of the tree is used; from its thick corky bark, to its leaves, fruits, roots to even its flowers. The leaves are considered to be antiviral, antibacterial, anticandidal, and antiseptic. It is also used in numerous microbial problems; colds and flu, cystitis, urethritis, sore throats, herpes, yeast infections, gonorrhea, eye and ear infections, and used externally for infected wounds, cuts, burns and rashes. Researches have been carried out on *Spondias mombin*, and it was found that most of the results correlated with its traditional uses. It has been reported to be non-toxic (9). Its pharmacological activities have been reported (10-23). Recently we reported its antigonadotrophic and antifertility effects (24-25).

Therefore this research was aimed at comparing the effect of graded doses of ethanolic and aqueous extracts of *Spondias mombin* on behavioural parameters such as fear and anxiety in rats.

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## II. Materials and method

Fifty male rats of Wistar strain (100-120g body weight) purchased from the Department of Zoology, University of Calabar, Nigeria, were housed in a standard facility of the Department of Human Anatomy, University of Calabar, Nigeria. The animals had access to rat chow from Agrofeed Nigeria Limited and water *ad libitum*. The rats were divided into five main groups A-E made up of control and four experimental groups. Each group was made up of ten rats.

### 2.1 Preparation of ethanolic and aqueous leaf extracts of *Spondias mombin*

The plant *Spondias mombin* was identified in the botanical garden of the University of Calabar, Nigeria and a sample deposited in the University herbarium with the voucher number MIA 313. The leaves of the plant were harvested, washed and sun-dried before blending to fine powder which was stored in a cool dry place, until required for use. 110g of dried powdered leaves was separately extracted with water and ethanol. Aqueous infusion of *Spondias mombin* leaves was prepared by mixing 100mls of distilled water upon 20g of powdered sample. The mixture was allowed to stand for 30 minutes before filtration. Ethanol extract was obtained with 1:1 ethanol/ water ratio by Soxhlet apparatus. The mixture was then centrifuged at about 3000xg for 5 min and the supernatant collected. The supernatants collected were cleaned of particles by suction filtration using Whatmann no 1. Filter paper and cellulose filter paper. The extracts were subsequently concentrated to dryness in vacuo at 40°C using a rotary evaporator and stored in a dessicator. These extractions gave a percentage yield of 0.5% and 3% for aqueous and ethanol extracts respectively.

### 2.2 Herb preparation and treatment

The aqueous and ethanolic leaf extracts of *Spondias mombin* were reconstituted to a stock concentration of 1mg/ml. Graded doses according to body weights of rats were administered. Group A served as control (0.5% saline), while groups B and C animals received 400mg/kg body weight and 800mg/kg body weight of the aqueous extract of *Spondias mombin*. Groups D and E received 250mg/kg body weight and 500mg/kg body weight of ethanolic extract of *Spondias mombin*. The extracts were administered orally with the aid of an orogastric tube and lasted for 28 days. The last treatment was 24 hours before behavioural testing for *Spondias mombin*.

### 2.3 Behavioural assay

The elevated plus maze (EPM) test, which provides test of anxiety and exploration was used for this study. The elevated plus maze apparatus consists of two (2) open arms and two (2) closed arms in the sign of a plus the open arms are aversive to animals since they are open (26), the closed arms provide a sense of safety due to the enclosure. The arms measure (30x 5x 15 cm). The floor of the maze was constructed from a 5cm plank. The same plank was also attached to the floor of the open arms to prevent the rodent from falling off (27). Behaviour scored includes frequency of open arms entry, open arm duration, closed arm entries, close arm duration, grooming, and head dipping, frequency of stretch attend postures and rearing (28).

### 2.4 Statistical analysis

Data among the groups with different doses of extracts *Spondias mombin* was analysed by one- way analysis of variance (ANOVA) followed by post hoc Student's t-test. Data were presented as means  $\pm$  SEM, p-value less than 0.05 was considered statistically significant.

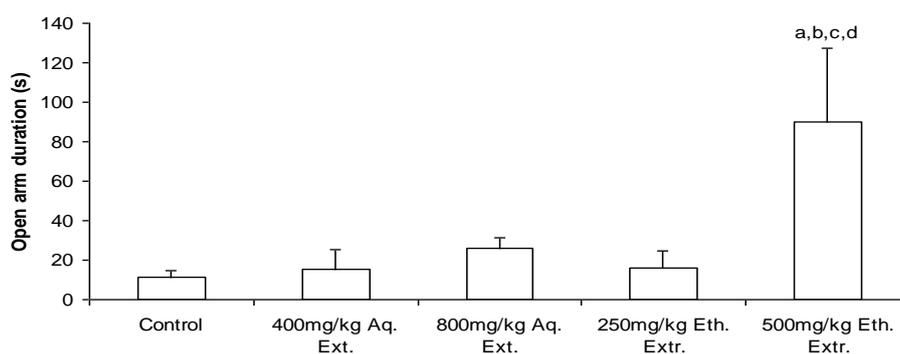
## III. Results

### 3.1 Comparison of duration and frequency of open arms entry in the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanolic extracts of *Spondias mombin*.

The duration in open arms following four weeks treatment with 400mg/kg and 800mg/kg aqueous extract of *Spondias mombin* were  $15.25 \pm 10.40s$  and  $26.2 \pm 5.29s$  respectively. These did not differ significantly when compared with control ( $11.5 \pm 3.50s$ ). The duration in the open arms following four weeks of treatment with 250mg/kg ethanol extract of *Spondias mombin* was  $15.8 \pm 9.01s$  and this was also not significantly different from control. However, following treatment with 500mg/kg ethanol extract of *Spondias mombin*, the duration in open arm ( $90.2 \pm 37.2s$ ) was significantly higher compared to control, 400mg/kg, 800mg/kg aqueous extract and 250mg/kg ethanol extract of *Spondias mombin* ( $p < 0.05$ ).

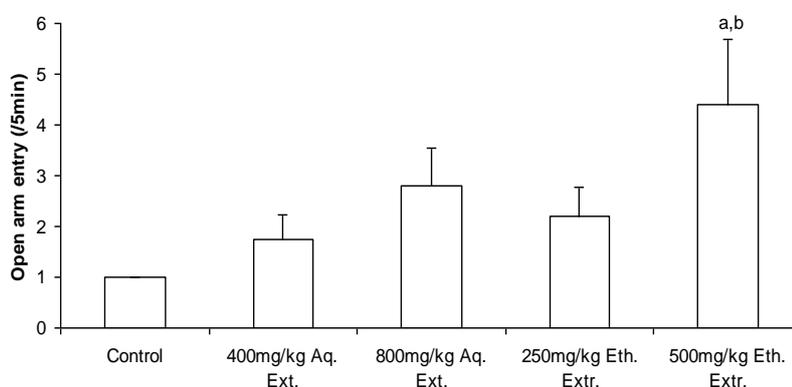
The frequency of open arms entry with 400mg/kg and 800mg/kg aqueous extract of *Spondias mombin* were  $1.75 \pm 0.48/5min$  and  $2.8 \pm 0.58/5min$  respectively and did not differ significantly from the control which was  $1.0 \pm 0.001/5min$ . The frequency of open arms entry following four weeks of treatment with 250mg/kg ethanol extract of *Spondias mombin* was  $2.2 \pm 0.58/5min$  and this was also not significantly different from control. Following treatment with 500mg/kg ethanol extract of *Spondias mombin* however, the frequency of

open arms entry ( $4.4 \pm 1.29/5\text{min}$ ) was significantly higher compared to control and 400mg/kg aqueous extract ( $p < 0.05$ ). This is shown in figs 1 & 2.



a =  $p < 0.05$  compared to control; b =  $p < 0.05$  compared to 400mg/kg Aqueous extract; c =  $p < 0.05$  compared to 800mg/kg Aqueous extract; d =  $p < 0.05$  compared to 250mg/kg Ethanol extract.

Fig. 1: Comparison of duration in open arms of the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*



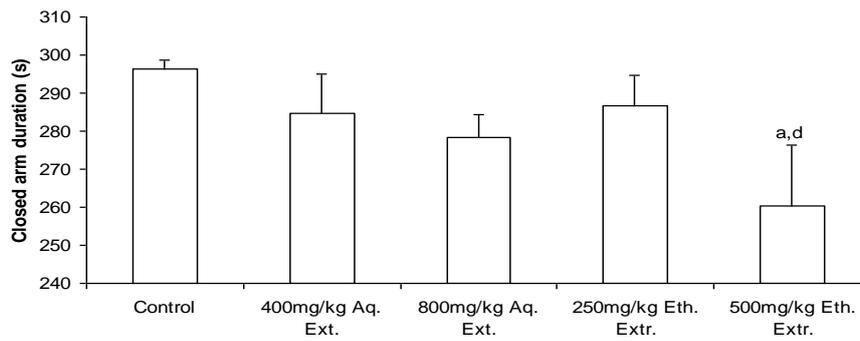
a =  $p < 0.05$  compared to control; b =  $p < 0.05$  compared to 400mg/kg Aqueous extract

Fig. 2: Comparison of frequency of open arms entry in the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*.

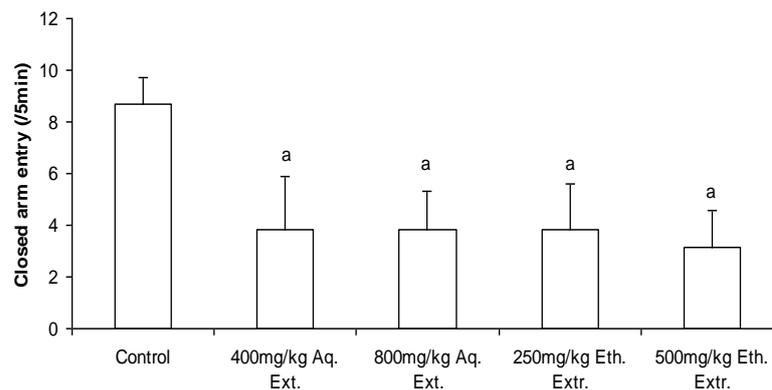
### 3.2 Comparison of duration and frequency in closed arms of elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*.

The duration in closed arms following four weeks treatment with 400mg/kg and 800mg/kg aqueous extract of *Spondias mombin* were  $284.75 \pm 10.40\text{s}$  and  $278.17 \pm 6.14\text{s}$  respectively and did not differ significantly when compared with control ( $296.17 \pm 2.58\text{s}$ ). The duration in the closed arms following four weeks of treatment with 250mg/kg ethanol extract of *Spondias mombin* was  $286.83 \pm 7.81\text{s}$  and this was also not significantly different from control. However, following treatment with 500mg/kg ethanol extract of *Spondias mombin*, the duration in closed arm ( $260.33 \pm 15.97\text{s}$ ) was significantly lower compared to control, and 250mg/kg ethanol extract of *Spondias mombin* ( $p < 0.05$ ). See fig 3

The frequency of closed arms entry with 400mg/kg and 800mg/kg aqueous extract of *Spondias mombin* were  $3.83 \pm 2.06/5\text{min}$  and  $3.83 \pm 1.50/5\text{min}$  respectively. Closed arms frequency with 250mg/kg and 500mg/kg ethanol extract were  $3.83 \pm 1.74/5\text{min}$  and  $3.16 \pm 1.40/5\text{min}$ . The extracts were significantly ( $p < 0.05$ ) lower compared with control ( $8.67 \pm 1.05/5\text{min}$ ). However, the 500mg/kg ethanol extract showed a lower frequency. Fig 4.



a =  $p < 0.05$  compared to control; d =  $p < 0.05$  compared to 250mg/kg Ethanol extract  
 Fig. 3: Comparison of duration in closed arms of the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*.

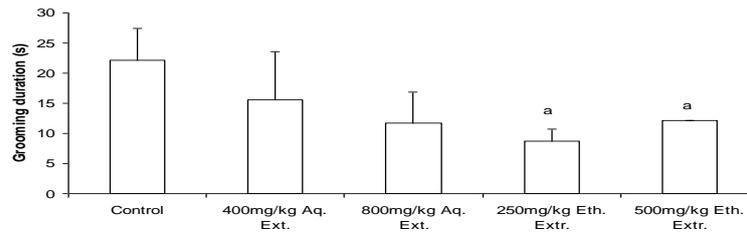


a =  $p < 0.05$  compared to control  
 Fig. 4: Comparison of frequency of closed arms entry in the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*.

### 3.3 Comparison of duration and frequency of grooming in the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*.

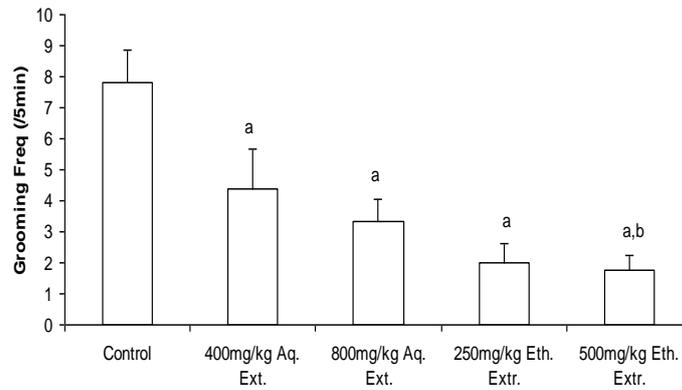
The duration of grooming following four weeks treatment with 400mg/kg and 800mg/kg aqueous extract of *Spondias mombin* ( $15.57 \pm 7.97s$  and  $11.73 \pm 5.07s$  respectively) were not significantly different compared to control ( $22.2 \pm 5.26s$ ). The duration of grooming following four weeks of treatment with 250mg/kg and 500mg/kg ethanol extract of *Spondias mombin* were  $8.68 \pm 2.10s$  and  $12.1 \pm 0.001s$  respectively and these were significantly lower compared to control. See fig 5.

The frequency of grooming for the groups of rats treated with 400mg/kg and 800mg/kg aqueous extract of *Spondias mombin* were  $4.41 \pm 1.28/5min$  and  $3.33 \pm 0.7/5min$  respectively. These values were both significantly lower when compared to control ( $7.83 \pm 1.01/5min$ ;  $p < 0.05$ ). the frequency of grooming for the groups treated with 250mg/kg and 500mg/kg ethanol extract of *Spondias mombin* were  $2.0 \pm 0.63/5min$  and  $1.75 \pm 0.48/5min$  respectively and these were also significantly lower than control ( $p < 0.05$ ). The frequency of grooming following treatment with 500mg/kg ethanol extract was also lower than the 400mg/kg aqueous extract ( $p < 0.05$ ). This decrease seemed dose dependent even though it did not significantly decrease at all doses of treatment. Fig 6



a = p< 0.05 compared to control

Fig. 5: Comparison of duration of grooming in the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*.

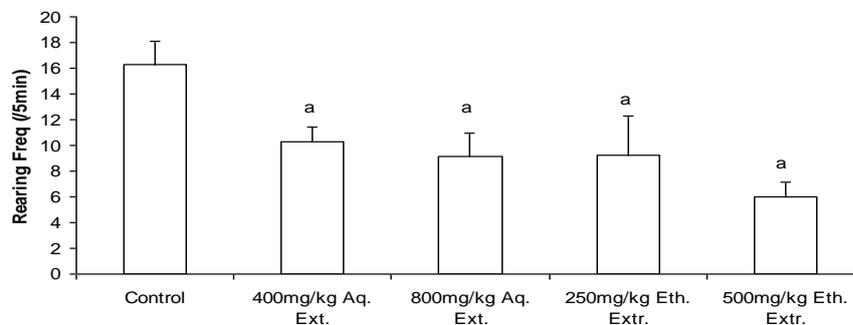


a = p< 0.05 compared to control; b = p< 0.05 compared to 400mg/kg Aqueous extract

Fig. 6: Comparison of frequency of grooming in the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*.

### 3.4 Comparison of frequency of rearing in the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*.

Figure 7 shows that the frequency of rearing following treatment with both aqueous and ethanol extracts of *Spondias mombin* were significantly lower when compared to control ( $16.33 \pm 1.75/5\text{min}$ ;  $p<0.05$ ). The frequency of rearing for the 400mg/kg and 800mg/kg aqueous extract of *Spondias mombin* were  $10.33 \pm 1.12$  and  $9.17 \pm 1.83/5\text{min}$  respectively while that for the 250mg/kg and 500mg/kg ethanol extract treated groups were  $9.2 \pm 3.09$  and  $6.0 \pm 1.15/5\text{min}$  respectively. that for control was  $4.5 \pm 1.11/5\text{min}$ .

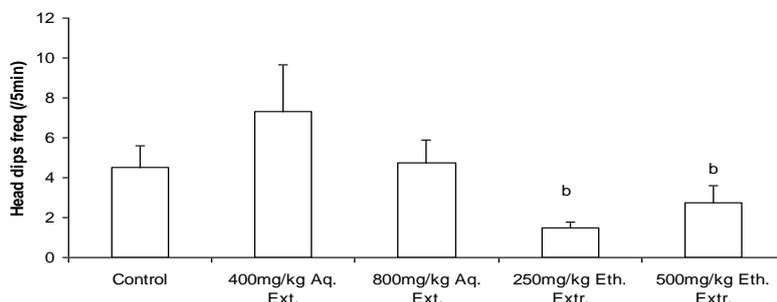


a = p< 0.05 compared to control

Fig. 7: Comparison of frequency of rearing in the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*.

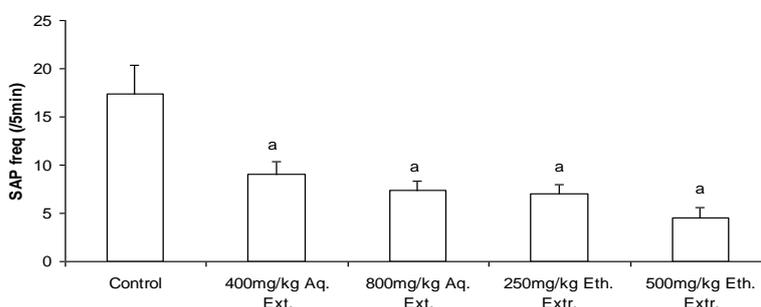
### 3.5 Comparison of frequency of head dips and stretch attend postures in the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*

The frequency of head dips for all the treated groups did not differ from control. However, this was lower in the ethanol extract treated groups when compared to the 400mg/kg aqueous extract group. Fig 8. Figure 9 shows that the frequency of stretch attends postures were significantly lower in all the treated groups of animals when compared to control.



b =  $p < 0.05$  compared to 400mg/kg Aqueous extract

Fig. 8: Comparison of frequency of head dips in the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*.



a =  $p < 0.05$  compared to control

Fig. 9: Comparison of frequency of stretch attend postures (SAP) in the elevated plus maze in rats following 4 weeks treatment with aqueous and ethanol extracts of *Spondias mombin*

## IV. Discussion

The elevated plus maze (EPM) is a typical test for anxiety / fear. At 4 weeks of treatment, the frequency of open arms entry and duration in open arms of the EPM were significantly higher for the 500mg/kg ethanol extract. The motivation for the EPM is the open space and height. Rodents have a natural aversion for open space and height (26). Thus, fearful/anxious rats would spend less time in the open. The group of rats treated with 500mg/kg ethanol extract was therefore less anxious compared to their control and lower doses of the extract. The closed arm entry and duration indicates the opposite effect. The closed arm duration is consistent with earlier results for the 500mg/kg extract. Anxious rats will spend more time in the closed arms which feel safer. Since the group of rats treated with 500mg/kg spent less time in the closed arm, they explored the open arms more.

Grooming is a displacement reaction (29). Rodents introduced into a novel environment would groom more as a sign of anxiety and apprehension. The frequency and duration of grooming was significantly lower for the groups of rats treated with 250mg/kg and 500mg/kg ethanol extract. The frequency of grooming in addition, showed a dose dependent decrease in the frequency of grooming. This decrease in frequency and duration of grooming for these doses indicate a decrease in anxiety.

The frequency of stretch attend postures is a risk assessment behaviour. An increase in this behaviour indicates increase hesitation to explore and therefore increased anxiety and vice versa. At all doses of treatment, the frequency of SAP was significantly lower than control. There was however no significant dose related effect even though it appeared dose related.

## V. Conclusion

The decreased anxiolytic effect of the leaf extracts of *Spondias mombin* may be due to its sedative and antipsychotic effect reported by Ayoka et al,(8) and Ayoka et al, (20). The anxiolytic activities observed with *Spondias mombin* leaf extracts may possibly be due to the presence of flavonoids, alkaloids and cardiac glycosides found in the plant extracts. These chemical constituents have been reported to be responsible for anxiolytic and sedative effects observed in different plant extracts (30-32).

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