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STUDIES ON THE ANXIOLYTIC EFFECT OF *SPONDIAS MOMBIN L. (ANACARDIACEAE)* EXTRACTS

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Abstract

Spondias mombin L [Anacardiaceae] is a plant used by traditional medical practitioners in Nigeria in the treatment of various nervous disorders. In this study, the anxiolytic properties of the aqueous, methanol and ethanol extracts of the leaves were examined using aggressive-behaviour response and depression-related swimming behaviour activities. All the extracts administered orally were not toxic to mice up to a dose of 5 g/kg. On intraperitoneal injection, however, the LD_{50} values [mice/rats] were calculated to be 0.48 g/kg / 0.62 g/kg for ethanol extract, 1.10 g/kg / 1.08 g/kg for methanol extract and 1.36 g/kg / 1.42 g/kg for aqueous extract respectively. All residues from different extractions were dissolved in normal saline and administered intraperitoneally. It was found that the three extracts abolished the aggressive attacks by rats, and reduced swimming time in mice. These effects were found to be most potent with the administration of the ethanol extract. These effects of the extracts were blocked by flumazenil, an antagonist of $GABA_A$ receptor. The results suggest that the extracts of *Spondias mombin* possess anxiolytic effect mediated by GABAergic transmission.

Key words: *Spondias mombin*, neurological, muricidal, swimming despair, GABA receptor antagonist.

Introduction

A good proportion of the world population particularly those living in developing countries like Nigeria depend mostly on herbal medicines for their health needs. Medicinal herbs are indispensable part of the traditional medicine practised all over the world due to easy access, low cost and ancestral experience. *Spondias mombin L.* (Anacardiacaea) is a fructiferous tree growing in the rain forest and in the coastal area of Africa. All parts of the tree are medicinally useful (Irvine 1961, Daniel 1990). The fruits decoction is drunk as a diuretic and febrifuge, the

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decoction of the bark and the leaves is used as an emetic, anti-diarrhoea, dysentery recipe and for haemorrhoids as well as for gonorrhoea and leucorrhoea. A tea of the flowers and the leaves is taken to relieve stomachache. The gum is employed as an expectorant and to expel tapeworm (USDA, ARS 2002). Offiah and Anyanwu [1989] have reported the abortifacient activity of the aqueous extract. However, no activity of the plant parts has been examined for its CNS effects. The present study was undertaken to evaluate the anxiolytic activity of extracts of the plant leaves since it has been suggested to be useful in the treatment of psychiatric disorders (Adanlawo, personal oral communication).

Materials and methods

Plant material

Spondias mombin leaves were collected in June 2001 beside the Faculty of Pharmacy building, Obafemi Awolowo University Campus, Ile-Ife, Osun State Nigeria. Identification and authentication was done by Dr. H. C. Illoh of Department of Botany, OAU, Ile-Ife and Mr. A. Oladele - the Herbarium of the Faculty of Pharmacy, OAU, Ile-Ife.

The voucher specimen was deposited and compared with the already deposited specimen voucher number 4837 at the Department of Botany, Faculty of Science, OAU, Ile-Ife.

Extract preparation

The fresh leaves of *Spondias mombin* were collected and dried under shade and then reduced to coarse powder by grinding in a blender. A 110 g of dried powdered leaves was separately extracted with water, methanol and ethanol. Aqueous infusion of *Spondias mombin* leaves sample was prepared by pouring 50 ml of boiled distilled water upon 10 g of powdered sample. The mixture was allowed to stand for 30 minutes before filtration. The methanol and ethanol extracts were obtained with 1:1 ethanol/water ratio or methanol/water ratio by Sohxlet apparatus. The extracts were subsequently concentrated to dryness in vacuo at 40 ^{O}C using a rotary evaporator and stored in a dessicator. 0.5 g of the extract was dissolved in 5 ml of normal saline. Normal saline was used for the treatment of the control groups since it was the vehicle used in dissolving the extracts.

Drugs

Diazepam (Roche), Methanol and Ethanol (Sigma), Flumazenil (Sigma).

Animals

Albino Wistar rats (200 – 250 g) and Swiss mice (18 – 24 g) were used for all the investigations. All animals were maintained on suitable nutritional and environmental conditions throughout the experiment. All animals were fed with standard diet (Bendel feeds) and water was given *ad libitum* and were bred in the Post-graduate laboratory of the Department of Physiological Sciences, OAU, Ile-Ife. The “principle of laboratory animal

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care" (NIH publication No 85 – 23) guidelines and procedures were followed in this study (NIH publication revised 1985).

Observation cage

The observation cage used measured 30 cm by 30 cm by 25 cm with an open surface for maximal ventilation and illumination. The three sides were painted black to enhance easy observation of the animals, behaviour and ensure that the animals were not distracted in the course of the experiment. The base of the cage is not painted, but a known quantity of fine sawdust was placed there to absorb any assault done on the cage by the animals, thereby ensuring the cleanliness and dryness of the cage. A modified small cage (6 cm by 6 cm by 15 cm) that is transparent all over with an open top was introduced to study the muricidal effect of the extracts.

Phytochemical investigation

Basic phytochemical screening was performed to detect the presence of alkaloids, tannins, saponins, phenols, flavonoid, cardiac glycosides and phlobatannins in the extracts.

Toxicity test

Ten groups, each consisting of five albino mice of both sexes, were used for this test for each of the three extracts (i.e. aqueous, methanol and ethanol extracts). Groups 1-9 were injected intraperitoneally (i.p.) with varying doses (12.5, 25, 50, 100, 200, 400, 800, 1600 and 3200 mg/kg) of the extract dissolved in normal saline while group 10, which served as control, received 10 ml/kg of normal saline. After treatment with the extracts, the animals were allowed th have access to food and water *ad-libitum* in a clean cage and observed for clinical signs over a period of 48 hours (Hellion-Ibarrola et al; 1999). Death within this period was recorded and dose-response graph was drawn from which the LD_{50} was determined following the method of Bliss (1935). In rats, similar experimental protocol was followed to determine the toxicity of the intraperitoneal administration of the extracts. For oral toxicity test, the animals (mice/rat) were fed with the extracts (0.5, 1.0, 2.0, 3.0, 4.0 and 5 g/kg body weight) of the animals per group twice daily (9:00 am and 6:00pm) for six days and on the seventh day the animals were fed thrice (8:00 am, 12:00 noon and 4:00 pm) with the extracts with the aid of oral cannula. Alternatively, the extracts were given to the animals (mice and rats) as the only source of drink through the drinking bottle for three days and the effects were thereby observed for 24 hours after withdrawal.

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Effects of *Spondias mombin* extracts on the muricidal action of rats

Five groups, each consisting of 8 male rats (200-250 g) were used for this test for each of the three extracts (aqueous, methanol and ethanol extract of *Spondias mombin*). Male albino wistar rats were used since male rats are more aggressive than normal females (personal observation). Groups 1-4 were injected intraperitoneally with varying doses (12.5, 25, 50 and 100 mg/kg) of the extract dissolved in normal saline, while group 5, which served as control, received the same volume of normal saline by the same route. The rats were placed inside the observation cage (30cm by 30cm by 25cm) inside which a smaller cage (6cm by 6cm by 15cm) that contained a mouse was placed in a definite corner of the bigger cage. The number of aggressive attacks that the rats made on the mouse was recorded as a measure of muricidal effect of the *Spondias mombin* extracts. This is a modification of Harro et al 1993, and Griebel et al 1995 methods. In another set of experiments, rats were treated with flumazenil, a $GABA_A$ antagonist (2 mg/kg i.p.) 15 minutes before the administration of the extracts.

Effects of *Spondias mombin* extracts on the behaviour despair test in mice

The depression-related behaviour was tested with the Porsolt Swim test used to evaluate "behaviour despair", a measure of failure to seek escape from an aggressive stimulus (Porsolt et al 1977). The mouse was placed in a cylinder containing water at 32^OC and swimming behaviour was observed over ten minutes test session. The water was approximately 30 cm deep, such that the animal cannot balance on its feet or tail. The water surface is approximately 15 cm from the top of the cylinder, such that the animal cannot jump out. Rodents especially mice will generally swim, animals treated with anxiolytic drugs or depressive drugs or lesions will stop swimming and will float. Floating time was considered a measure of depression-like behaviour as the animal stopped swimming and "gave up" on finding no escape route. The floating time is measured by stopwatch. Any mouse that does not swim or float is immediately removed from the water. At the end of the 10 minutes swim test, the mouse is dried with a towel and returned to a home cage. In this test, five groups, each consisting of eight mice were used, following i.p. treatments of either the control solution (normal saline) group 1 or the extracts in groups 2-5 (12.5, 25, 50 and 100 mg/kg of ethanol, methanol or aqueous extracts). The mouse was introduced into the water-filled cylinder at room temperature as described by Crawley (1999) for its depression-related behaviour. Any mouse that did not swim or float is immediately removed from the water. At the end of the 10 minutes swim test the mouse is dried with towel and returned to the home cage. In another set of experiments, mice were treated with flumazenil, a $GABA_A$ antagonist (2 mg/kg i.p.) 15 minutes before the administration of the extracts.

Statistical analysis

Results are expressed at the mean $\pm$ S.E.M. The significance of differences between groups was analyzed using one-way analysis of variance (ANOVA) followed by

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Student – Newmann- Keul test Glantzl (1992). Probability level = or less than 0.05 was considered as statistically significant (Steel and Torrie, 1960).

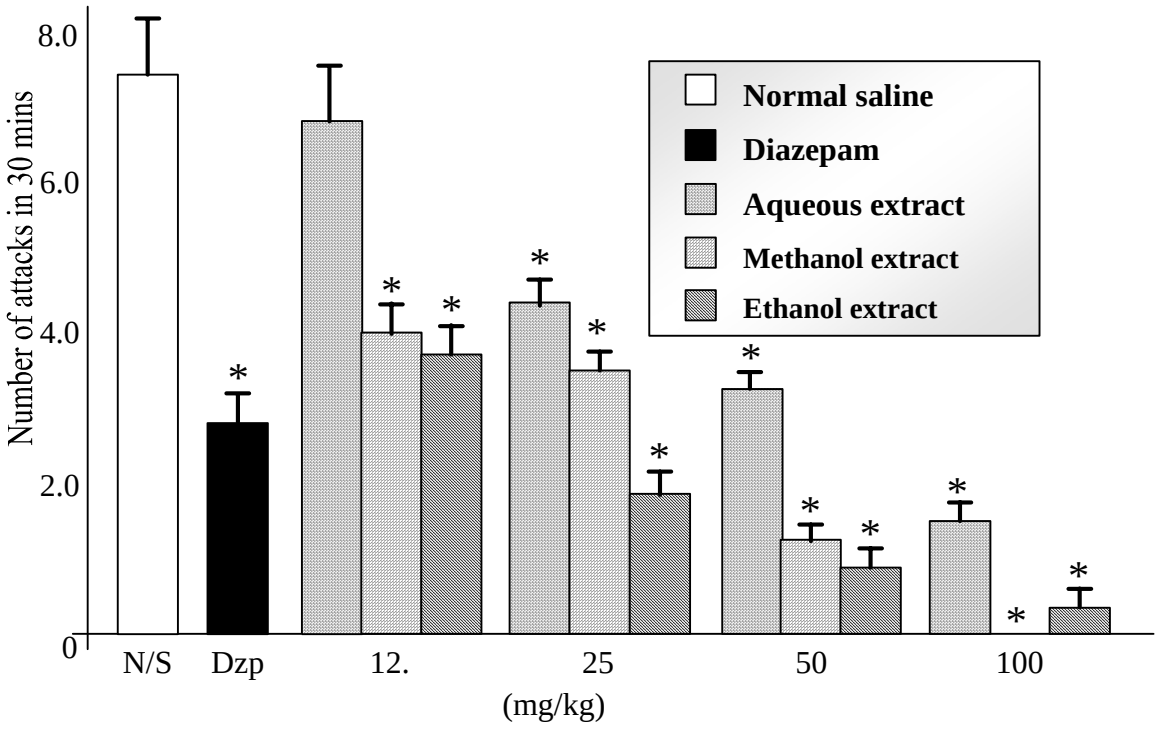

Figure 1: Effect of *Spondias mombin* extracts on the muricidal action of rats

* indicates significant difference from control.

Results

The weight of the extracts obtained were 1.65 g for hot water infusion, 7.85 g for methanol extraction and 9.60 g for ethanol extraction representing 16.5 %, 15.70 % and 19.2 % respectively. The preliminary phytochemical studies conducted revealed that the extracts contained tannins, anthraquinones, flavonoids, cardiac glycosides and saponnins. Phlobatannins and alkaloids were absent from the extracts. The aqueous extract did not contain phenol, while ethanol and methanol extracts contained phenol.

Toxicity

Oral administration of aqueous, methanol and ethanol extracts of *Spondias mombin* (≤5 g/kg) did not produce any toxic symptom in mice. Intraperitoneal (i. p.) administration

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of the aqueous extracts (≤200 mg/kg) also did not produce any toxic effects. However, the ethanol and methanol extracts (≥100 mg/kg) produced toxic symptoms. Lethal effects were observed in mice and rats with all the extracts (aqueous, methanol and ethanol) at the dose of 3.2 g/kg administered i.p. The LD_{50} in mice for the ethanol extracts was found to be 480 mg/kg body weight i.p., while it was 1100 mg/kg i.p. for the methanol extract and 1360 mg/kg i.p. for the

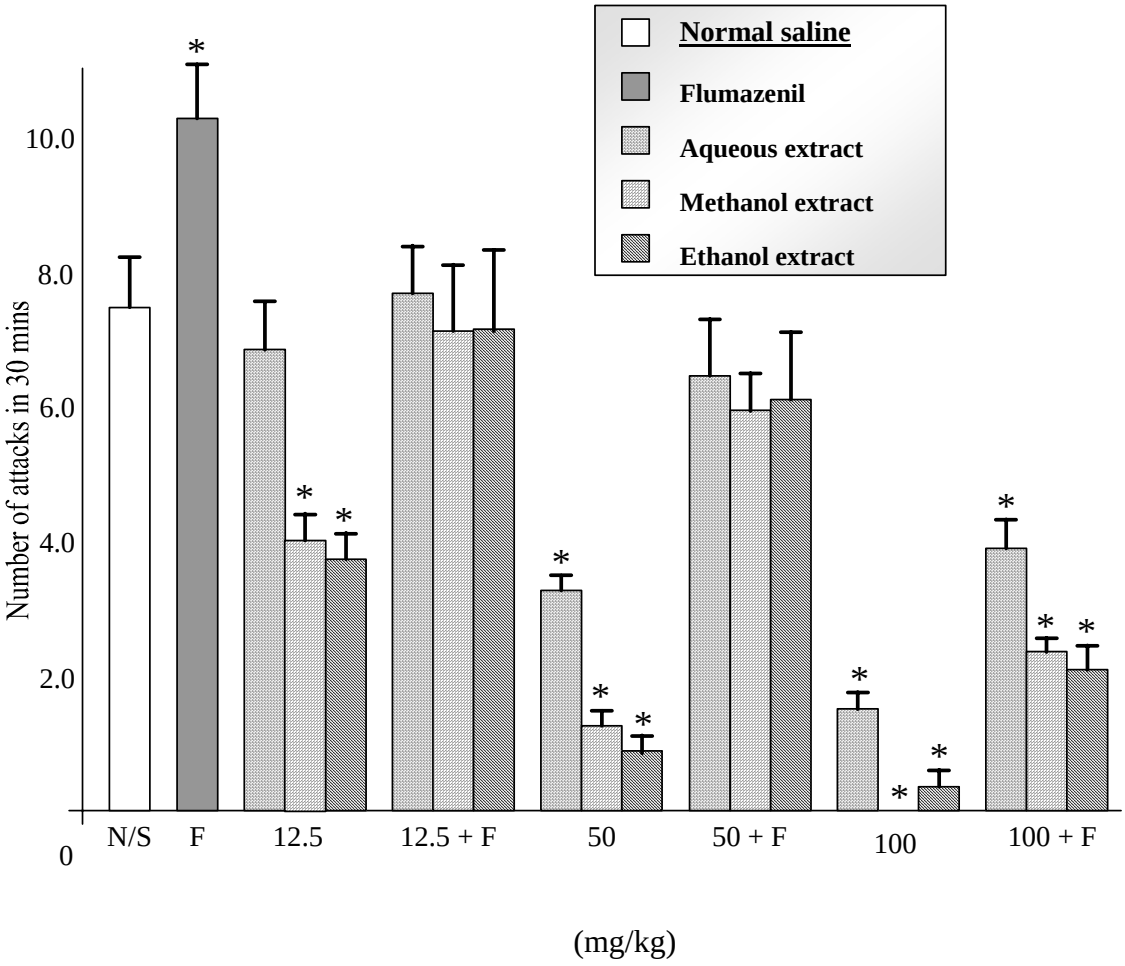

Figure 2: Effect of pre-treatment with flumazenil (2mg/kg i.p.) on the effect of *Spondias mombin* extracts on the muricidal action of rats

* indicates significant difference from control.

aqueous extract. Also the LD_{50} in rats for the ethanol, methanol and aqueous extracts were 620 mg/kg i.p., 1080mg/kg i.p. and 1420mg/kg i.p. respectively. The LD_{50} determinations of the extracts were carried out in a 48-hour continuous observation.**Toxic symptoms:** Animals locate a corner of the cage to stay, will not border to move, even when threatened and the two hind limbs become immovable for the animals**.**

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Effects of *Spondias mombin* extracts on the aggression behaviour of rats

Aqueous, methanol and ethanol extracts (12.5 –100 mg/kg.) injected i.p. into rats were observed to exhibits muricidal action. The muricidal effect was dose dependent. The ethanol extract is most potent at 12.5, 25 and 50 mg/kg i.p. However, at 100mg/kg i.p, the methanol abolished the number of aggressive attacks (Figure 1). This result shows that the extract was more potent as an anxiolytic agent than 0.5mg/kg diazepam used as standard drug.

Effects of *Spondias mombin* extracts on the aggression behaviour of rats pre-treated with Flumazenil (2mg/kg)

Intraperitoneal administration of flumazenil (2 mg/kg i.p.) alone increased the aggressive behaviour of rats compared with normal saline treatment (Figure 2). The extracts inhibited this aggressive behaviour induced by flumazenil (Figure 2).

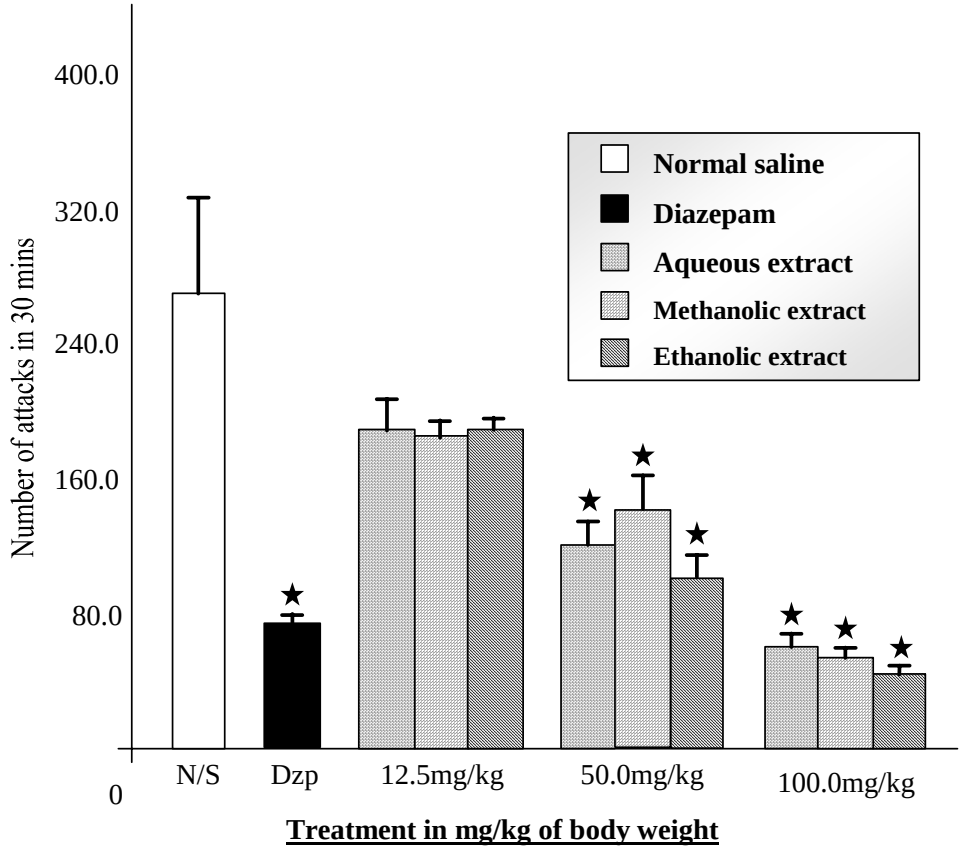

Figure 3: Effect of *Spondias mombin* extracts on swimming despair in mice

* indicates significant difference from control.

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Effect of *Spondias mombin* extracts on swimming despair

Figure 3 showed the depression-related behaviour result where the swimming period of the mice was observed to decrease dose-dependently. The result showed that the aqueous extract was more potent for this observed behaviour. This result also shows that the extract was more potent as an anxiolytic agent than 0.5mg/kg diazepam used as standard drug.

Effect of *Spondias mombin* extracts on swimming despair after pre-treated with Flumazenil

Intraperitoneal administration of flumazenil (2mg/kg i.p.) alone increased the swimming time in mice compared with normal saline treatment (Fig 4). The extracts decreased the swimming time induced by flumazenil (Figure 4).

Discussion

Anxiety may be regarded as a particular form of behavioural inhibition that occurs in response to environmental events that are novel. It has been established that there are lot of plant secondary metabolites being employed in the treatment of psychotic disorders especially for anxiety in traditional medicine practice, most of which directly or indirectly affect the central nervous system noradrenaline, serotonin, GABA and BDZ neurotransmitters activities (Wolfman et al, 1994; Viola et al, 1996; Salgueiro et al, 1997 Paladini et al, 1999; and Dhawan et al, 2003). This has made us to examine the possible anxiolytic effect of *Spondias mombin* leaves. Traditionally, *Spondias mombin* is commonly used among native doctors in Western Nigeria for the treatment of some forms of mental disorders. Palm wine (local alcohol in Yoruba land) is used for the extraction. (Adanlawo, personal oral communication)

The results of the present study show that extracts of *Spondias mombin* leaves exhibited very low toxicity, as shown in the high LD_{50} values for intraperitoneal administration (according to Rodricks 1992). There is an absence of toxic symptoms for oral administration of *Spondias mombin* leaves extracts either on single administration or on repeated doses. However, in i.p. administration it was also observed that the ethanol extract produced higher toxic value which is an indication that it extracted more of the constituents, while the aqueous extract produced low toxicity. This observation showed that the constituents in the leaves of the *Spondias mombin* are better extracted in alcohol than in both methanol and aqueous media. This finding therefore justifies why the plant is being extracted with local alcohol (Adanlawo, personal oral communication). Muricidal effect is a sign of aggression mediated by either norepinephrine and or serotonin in the brain (Kozak et a1, 1984; Miyamoto and Nagaoka 1987), which point to the fact that aggression is a form of anxiety (Kozak et a1 1984, Bhattacharya, 1994). The anxiolytic activity of extracts from natural products was suggested to be based on the fact that constituents from plants extracts could modify muricidal actions exhibited in rats (Bhattacharya, 1994). This study demonstrated

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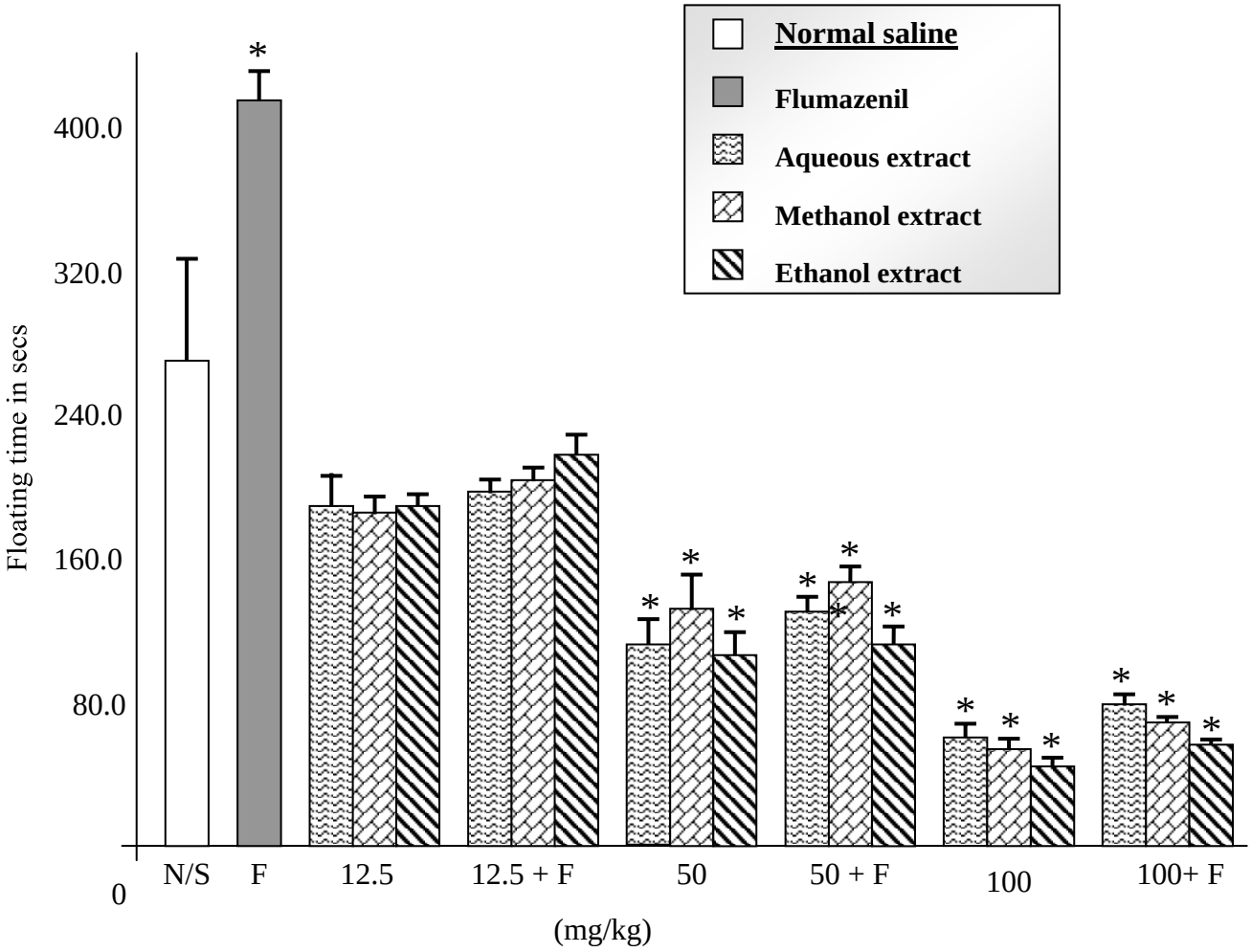

Figure 4: Effect of pre-treatment with flumazenil (2mg/kg i.p.) on the effect of *Spondias mombin* extracts on the swimming despair

* indicates significant difference from control.

that the extracts of *Spondias mombin* abolished aggressive behaviour in rats. This effect may be the interaction of the extracts with the neural substrates or chemical mediators like noradrenaline, serotonin, GABA, BZD, hormones (testosterones) and magnesium which are implicated to be responsible for aggressive and anxiety-like condition. The mechanism of its anxiolytic action may be by interacting with some of the natural endogenous mediators in the body (Siqueira et al 1998, Contarino et al 1999, Shih et al 1999). Contarino et al (1999) showed that mice lacking corticotropin-releasing factor receptor I, for example, exhibited reduction in anxiety-like behaviour. Also there could be a linkage in the interaction of the extracts with serotonergic pathway since serotonin had been widely implicated in aggressive behaviour (Blackburn 1992, Sanchez et al 1993, Unis et al 1997, Kadaba 1994). The effect of most of the anxiolytic agents is to enhance the response to GABA, by facilitating the opening of GABA-activated chloride channels.

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$GABA_A$ receptors were involved in anxiety and their direct activation would have an anxiolytic effect (Vogel, 2002). Flumazenil, a specific $GABA_A$ receptor antagonist increased anxiety-like behaviour as measured by increased swimming time in mice and increase in aggressive attacks recorded in rats. This was completely reversed by the extracts, demonstrating that the extracts may be facilitating GABA transmission (Walting, 1998).

There are various ways of explaining the mechanisms of action of anti-anxiety agents because of the involvement of many CNS chemical mediators. This is a reflection of the fact that drugs that relieve anxiety generally cause a degree of sedation and drowsiness, which is the main side effect of anxiolytic drugs. There is no doubt, therefore that the *Spondias mombin* leaves extract used in this study affected certain mediators to reduce anxiety as shown in the anti aggressor/muricidal property exhibited. However, in the context and the scope of this study, involvement of GABA was only explored. Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter known to counterbalance the action of the excitatory neurotransmitters: glutamate and noradrenaline (Lydiard, 2003). Several pharmacologic agents target the GABA system and modulate the overall effect of GABA. The activity of GABA was implicated in this study, bearing in mind the inhibitory nature it has on the excitations induced by aggressive behaviour of the rats. In the same vein, the effects of flumazenil which increase the state of anxiety behaviour of the animals was blocked by the extract. This therefore shows that GABAergic transmission could be involved. These results could therefore lend credence to or confirmed the basis for the use of *Spondias mombin* in traditional medicine for the management of psychiatric illnesses in Nigeria.

It is hereby concluded that the active principle(s) involved in the neuropharmacological effects of *Spondias mombin* is/are better extracted in ethanol alcohol) and that the extract of *Spondias mombin* contains constituents that ameliorate psychiatric disorders.

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