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Behavioural effects of methanol stem bark extract of *Ficus sycomorus*

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Abstract

Background: Neuropsychiatric disorders are characterized by anxiety, altered locomotion and altered sleep pattern characterized by insomnia. *Ficus sycomorus* is a widely used medicinal plant used locally in Northern Nigeria for calming agitated patients.

Aim: The aim of the current study was to provide scientific evidence to support its continuous use in the management of neuropsychiatric conditions.

Methods: The effect of *Ficus sycomoros* stem bark extract (50, 100 and 200 mg/kg body weight orally), Normal saline (l0 ml/kg orally) and diazepam (1 mg/kg body weight orally) on anxiety in mice were evaluated on Elevated plus Maze (EPM), Elevated Zero Maze (EZM) and Open Field Apparatus (OFA).

Results: The extract at doses of 50,100 mg/kg body weight and diazepam (2.5 mg/kg orally) produced significant ($p<0.05$) increase in time spent in the opened arm of Elevated plus Maze and Elevated Zero Maze when compared to Normal saline treated control. At 200 mg/kg body weight orally the extract paradoxically produced significant ($p<0.05$) increase in time spent by treated mice in the closed arm of Elevated Plus Maze and Elevated Zero Maze when compared to Normal saline treated control. The extract at 50, 100 mg/kg body weight orally and diazepam (2.5 mg/kg body weight orally) produced anxiolytic-like effects in treated mice on the open field apparatus. At 200 mg/kg body weight orally the extract produced sedative-like effects on treated mice in the opened field apparatus.

Conclusion: *Ficus sycomoros* stem bark extract possesses anxiolytic activity in mice which may be explored further for leads in the development of potent safe and effective anxiolytic agent for human use.

Keywords: Ficus sycomoros, agitation, calming, extract, open field apparatus

Introduction

Anxiety is the main symptom of many psychiatric disorder with rapid increase in prevalence globally (Naina *et al.*, 2013; Tijani *et al.*, 2012; Cassano *et al*., 1999) [16]. Benzodiazepines have remained the first drug of choice in the management of acute anxiety. However, inspite efficacy diazepam use is associated with intolerable side effects including development of tolerance, insomnia, memory disturbance, psychological dependence, ataxia (Naina, *et al.*, 2013; Goddard *et al*., 2001; Igbokwe, *et al*., 2010) [16, 12]. Thus the urgent need for effective, safe and well tolerated alternative agent for the management of anxiety. The WHO has acknowledged the utilisation of herbal medicine by approximately 80% of the population in developing countries for their primary health care need (Farnsworth *et al*., 1985) [8] through provision of guidance documents for safety and efficacy evaluation. Moreover, a number of plants are used in the treatment of psychiatric diseases and other central nervous system disorder (Galdino *et al*., 2010) [9]. Examples of such plants are *Datura metel, Acorus calamus* (sweet flag), *Argyreiants. speciosa* (convolvulaceae) (Suba *et al*., 2002) [24]. *Ficus sycomorus* is one of such plant whose use in management of neuropsychiatric disorders has not been well investigated.

F. sycomorus is a tropical plant belonging to the family Moraceae. It is locally known as 'Baure' in Hausa (Nigeria). It is a tree attaining up to a height of 20 meters and sometimes in breadth widely spreading branches and a massive crown (Dalziel, 1953) [7]. It is widely used in folk medicinal management of ailments including cough, diarrhoea, skin infections, stomach disorders, liver disease, epilepsy, tuberculosis, lactation disorders, helminthiasis, infertility and sterility (Pakia & Cooke, 2003; Malgras, 2008; Sofowora, 1993; Arnold, *et al*., 1984) [1] in Nigeria and many other African countries (Igbokwe, *et al*., 2010) [12]. In Northern Nigeria city of Zaria the hot decoction from the plant stem bark is used clinically in humans for management of diarrhoea, as astringent and diuretics (Bello, *et al*., 2013) [2]. The earlier report on the activity of *F. sycomoros* by Sandabe *et al*, (2006) [22] showed that similar to other Moracea, the stem bark extract of the plant also possess anticonvulsant activity. Members of Moracea family like *Ficus platyphylla* have been reported to ameliorate insomnia, epilepsy, muscle spasm, and cognitive deficit (Bokko, 1998; Onyili *et al*., 1998) [3, 18]. *F. sycomorus* is widely used for management of Neuro-psychiatric conditions characterized by agitations and insomnia (Muazzam, oral communication). There has been no research work done on the calming effect *F. sycomoros* stem bark to validate its acclaimed effectiveness in the management of these ailments. The objective of this study therefore was to evaluate the behavioural effects of the methanol stem bark extract of *F. sycomorus* in mice using Elevated plus Maze, Elevated zero-maze, and Open field apparatus.

Methods

Behavioural testing

The experiments were conducted in a room outside the breeding room between 9.00 h and 15.00 h. Data were manually observed and recorded. An independent group of mice was used for each behavioural test. The tables give details of the number of animals in each. Mice were naive to the test apparatus.

Elevated plus – Maze test

Anxiety activities were measured using the elevated plus-maze test (Lister, 1987). The elevated plus-maze consists of two open arms, 25 × 10cm, crossed with two closed arms, 25 × 5 × 10 cm, with an open roof; all four arms radiated from a central platform (5×5 cm). The maze is elevated to a height of 60 cm in a dimly lit room. The mice were divided into 5 groups of 5 mice each. All groups were given respective treatment of 50, 100 and 200 mg *F. sycomorus* /kg body weight. Normal saline (10 ml/kg, orally) and diazepam (2.5 mg/kg, orally) were administered as negative and positive control respectively. One hour post administration, each mouse was placed in turn in the centre of the maze facing one of the open arms. The frequency of entries into the open and closed arms, and the time spent in the respective arms over a 5 minutes test period were recorded and expressed in percentage. An arm entry into an arm is noted when all four paws cross the partition of respective arms.

Open Field Test

The effect of the *F. sycomorus* stem bark extract on anxiety and its related activities in the open field was carried out according to the method described by Archer (1973). Mice were observed in a square open field arena (45×45×40cm), made with wooden floor and glass sides. The floor was divided into 9 equally sized squares (15×15cm). Five groups of 5 mice each were orally administered with the respective treatments (10 ml/kg normal saline, 2.5 mg/kg diazepam, and 50, 100 and 200 mg/kg *F. sycomorus*. One hour after individual treatment, each mouse was placed at the corner of the apparatus. Records of locomotion (number of line crossings), latency to center square, numbers of rearing, freezing and defecation were taken over the 5 minute window period. The apparatus was cleaned with 70% ethanol after each test and allowed to dry.

Elevated zero maze

This study was carried out according to the method described by Tijani *et al*., (2014). Mice were randomly divided into five groups of six rats each. These mice were treated with graded doses of *F. sycomorus* (50, 100, and 200 mg/kg, orally). The negative control group received 10 ml normal saline/kg while the positive control group received standard reference drug diazepam (2.5 mg/kg, orally). Elevated zero maze consists of an elevated (50 cm above the floor) circular platform (6 cm width and 40 cm inner diameter) that is equally divided into four quadrants. Two quadrants on opposite sides of the platform are enclosed by 12 cm high walls while the other two quadrants are opened and bordered by 0.6 cm high lip. One hour after drug administration, each mouse was placed at the center of the open arm (facing toward the closed chamber). The times spent in both open and closed arms of the maze were manually recorded. The maze was thoroughly cleaned between tests with a tissue paper moistened with 70% ethanol.

Results

Effect of *F. sycomorus* on Elevated Plus Maze

F. sycomorus significantly increased the percentage time spent in the open arm relative to the control at doses of 50 mg/kg and 100 mg/kg ($p< 0.0001$). The most pronounced effect was produced at the dose of 100 mg/kg (29.00±1.00 %) though lesser than that produced by diazepam (93.80.40±3.51 %). At 200 mg/kg, the effect of *F. sycomorus* in increasing the time spent in the open arm diminished to a value (7.00±0.65 %) lower than the control (Figure 1). The frequency of entries into the open arms were significantly higher at doses of 50 mg/kg ($p<0.0001$) and 100 mg/kg ($p<0.0001$) of *F. sycomorus.* Comparing with normal saline, the percentage frequency of entry into the closed arms were significantly decreased at doses of 50 mg/kg and 100 mg/kg. (Figure 2)

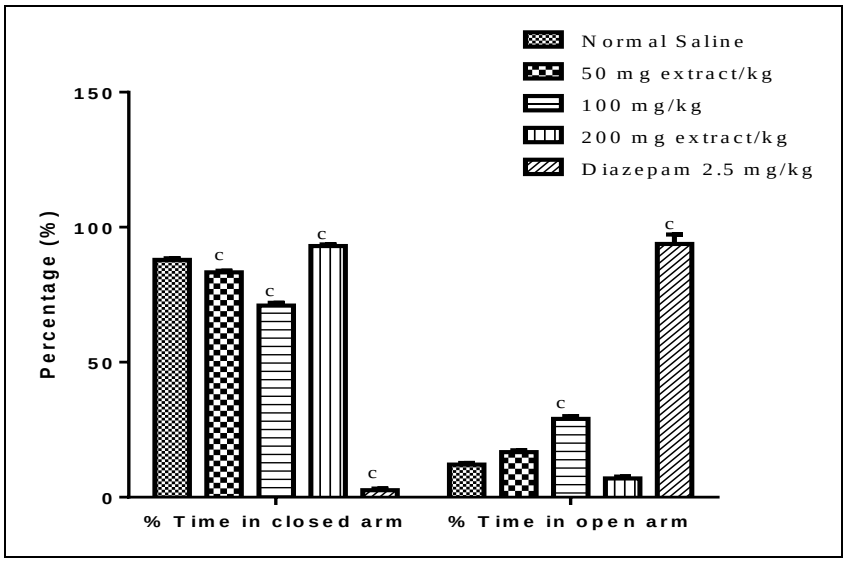

Fig 1: Effect of *F sycomorus* percentage time spent in open and closed arm by mice in the elevated plus maze test. Data are shown as means ± S.E.M., analysis of variance ANOVA followed by multiple comparison Dunnets test. $^{b}p < 0.01$, $^{c}p < 0.001$ compared to normal saline group.

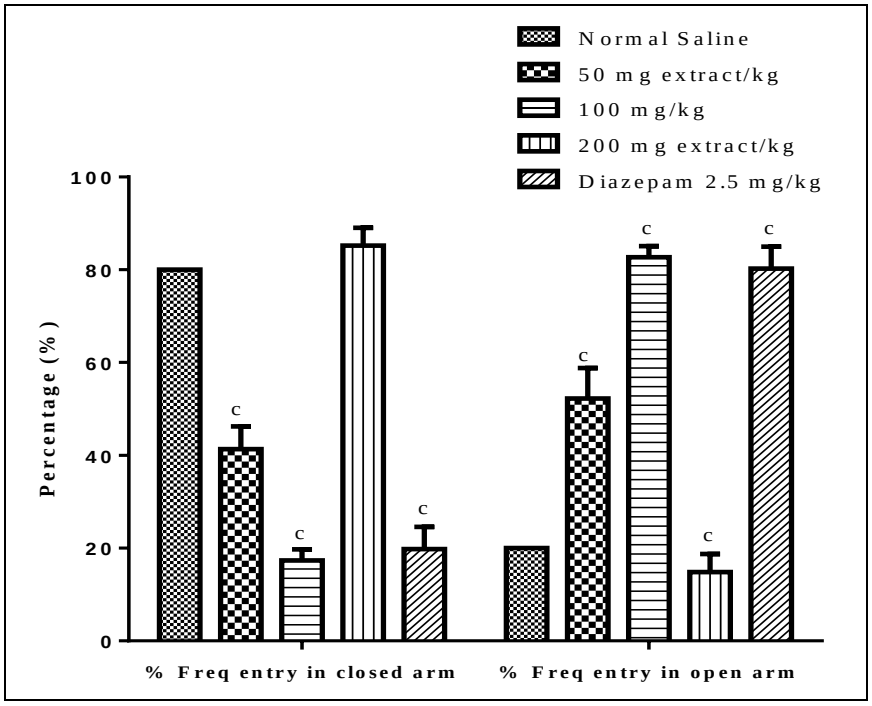

Fig 2: Effect of *F sycomorus* percentage frequency of entries into the closed and open arm by mice in the elevated plus maze test. Data are shown as means ± S.E.M., analysis of variance ANOVA followed by multiple comparison Dunnets test. $^{c}p < 0.001$ compared to normal saline group.

Effect of *F. sycomorus* in mice in the Open Field test

The extract at 50 mg/kg and100 mg/kg (respectively) significantly increased the locomotor activity in the open field as shown by the locomotion indexes total distance travelled ($p = 0.0016$; $p < 0.0001$), average speed of movement ($p = 0.0016$; $p < 0.0001$), decreased latency of first entry into the centre ($p < 0.0001$; $p < 0.0001$), number of rearing ($p < 0.0001$; $p < 0.0001$), and decreases in freezing ($p = 0.0059$; $p < 0.0001$) and frequency of defecation ($p = 0.0075$; $p < 0.0001$). However, in contrast, 200 mg/kg *Fs* produced significant sedative effects as evident in decreased total distance travelled ($p = 0.0003$), average speed of movement ($p = 0.0003$) and an increased latency to center time ($p = 0.0320$), while rearing, freezing and defecation were not significantly influenced. As expected, 2.5 mg/kg diazepam produced significant anxiolytic effects across board when compared to normal saline. (Fig. 3-6)

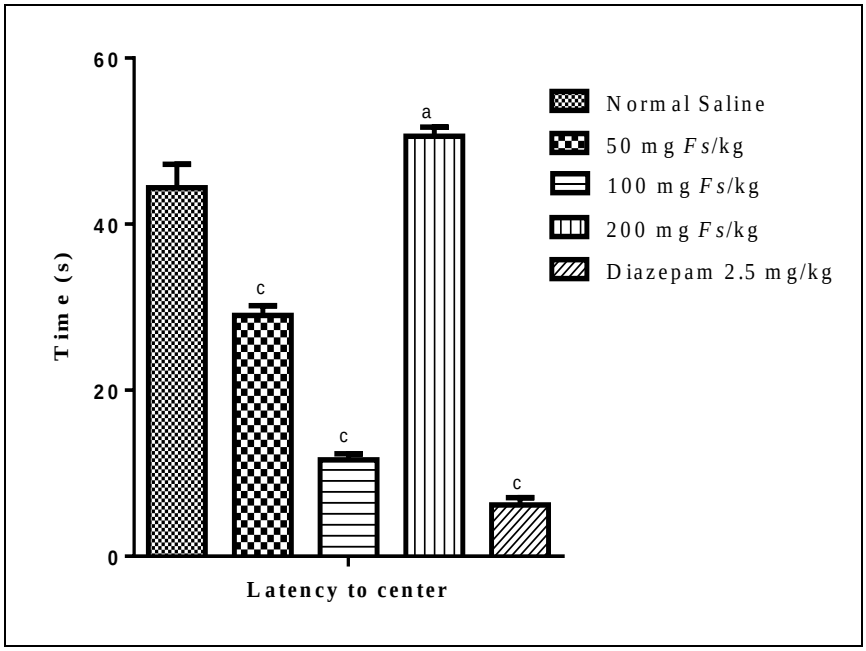

Fig 3: Effect of *F sycomorus* on the latency to center by mice in the open field test. Data are shown as means ± S.E.M., analysis of variance ANOVA followed by multiple comparison Dunnets test. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ compared to normal saline group

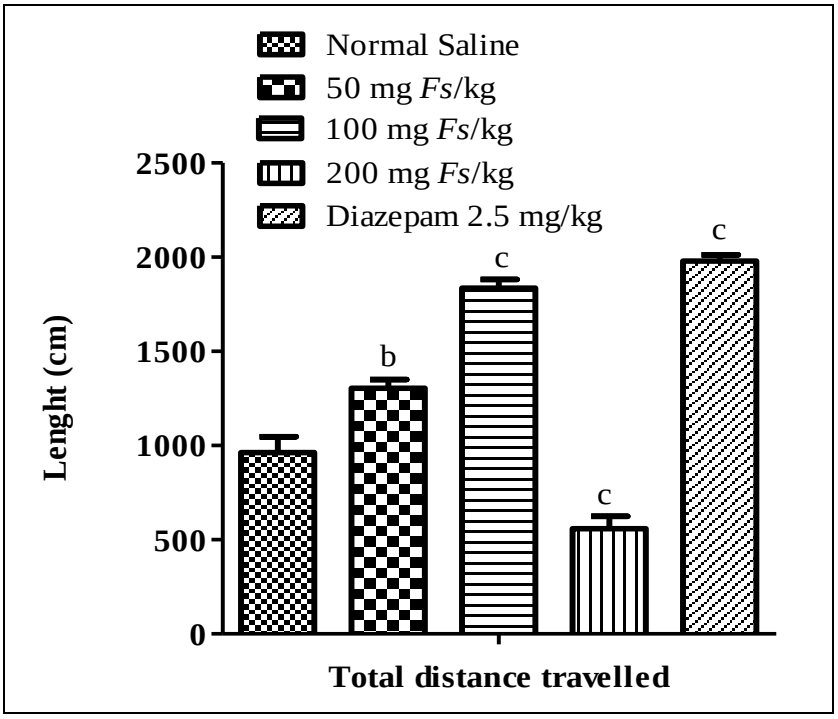

Fig 4: Effect of *F sycomorus* on total distance travelled by mice in the open field test. Data are shown as means ± S.E.M., analysis of variance ANOVA followed by multiple comparison Dunnets test. $^{b}p < 0.01$, $^{c}p < 0.001$ compared to normal saline group

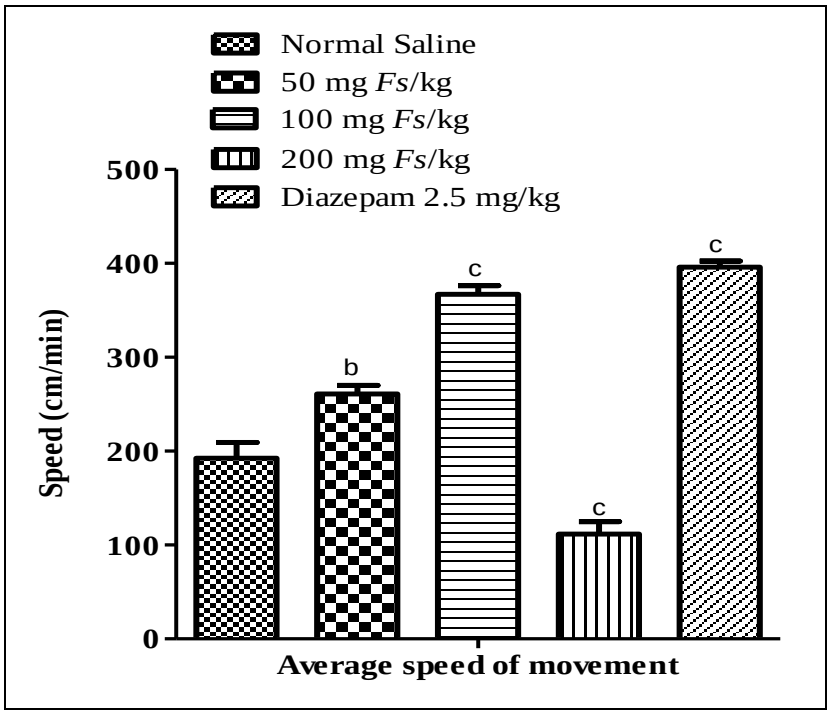

Fig 5: Effect of *F sycomorus* on the average speed of movement by mice in the open field test. Data are shown as means ± S.E.M., analysis of variance ANOVA followed by multiple comparison Dunnets test. $^{b}p < 0.01$, $^{c}p < 0.001$ compared to normal saline group

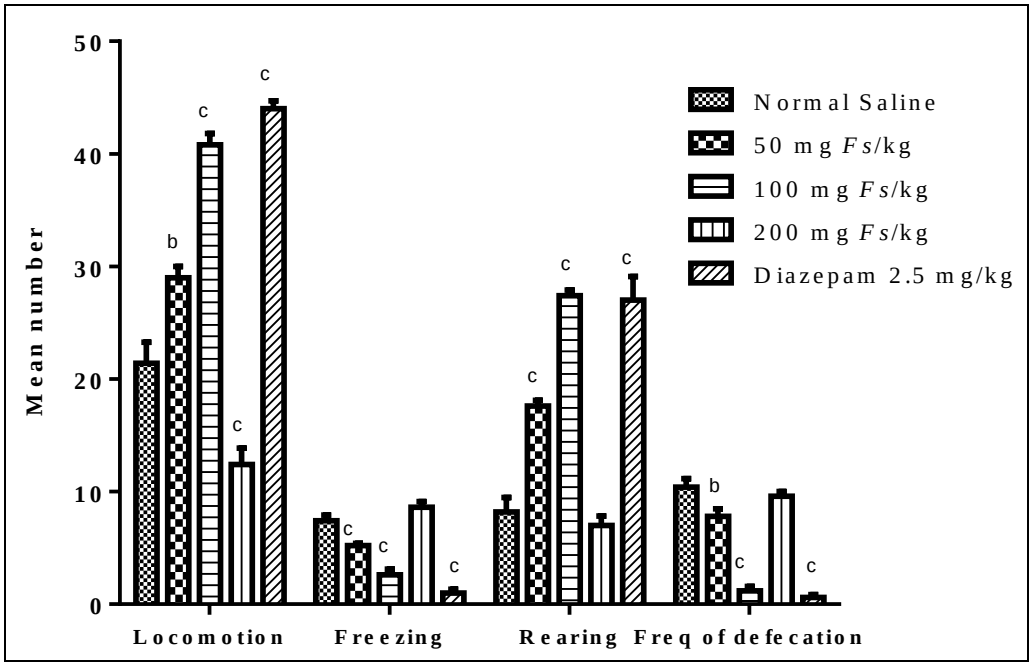

Fig 6: Effect of *F. sycomorus* on locomotion, freezing, rearing and frequency of defecation of mice in the open field test. Data are shown as means ± S.E.M., analysis of variance ANOVA followed by multiple comparison Dunnets test. $^{b}p < 0.01$, $^{c}p < 0.001$ compared to normal saline group.

Effect of *F sycomorus* on Elevated Zero Maze test

Administration of 50 mg/kg and 100 mg/kg extract of *F. sycomorus* produced significant ($p = 0.002$ and $p < 0.0001$ respectively) increase in percentage time spent in the open arm of elevated zero maze. The effect of 200 mg/kg *F. sycomorus* remain significantly unchanged both in the time spent and the frequency of entries into both arms. As expected, 2.5 mg/kg diazepam produced significant anxiolytic effects across board when compared to normal saline.

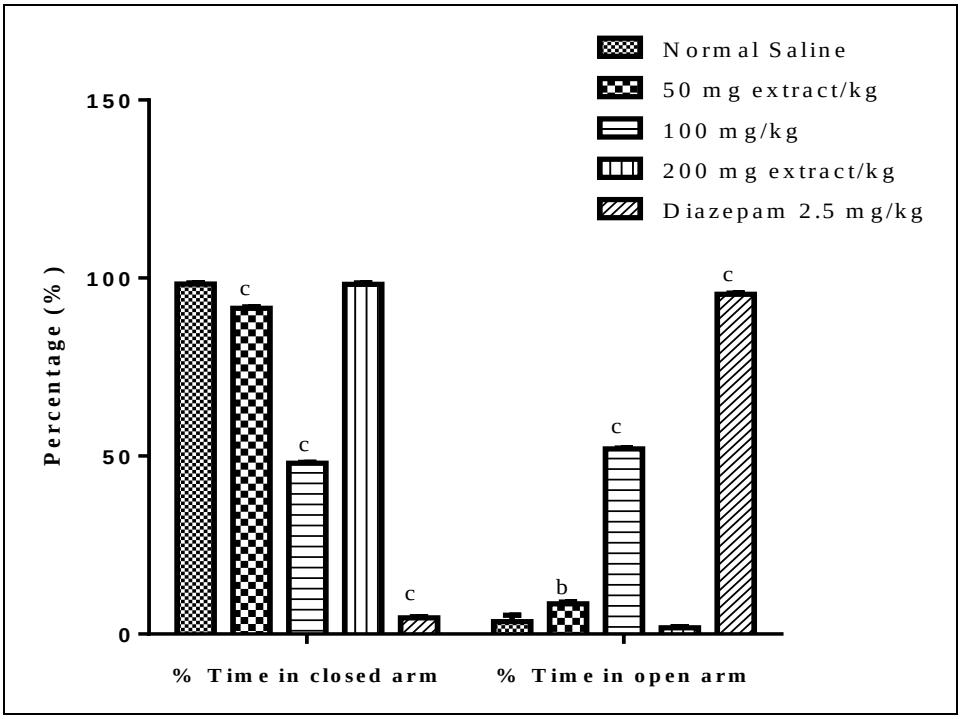

Fig 7: Effect of *F sycomorus* on locomotion, freezing, rearing and frequency of defecation of mice in the open field test. Data are shown as means ± S.E.M., analysis of variance ANOVA followed by multiple comparison Dunnets test. $^{c}p < 0.001$ compared to normal saline group.

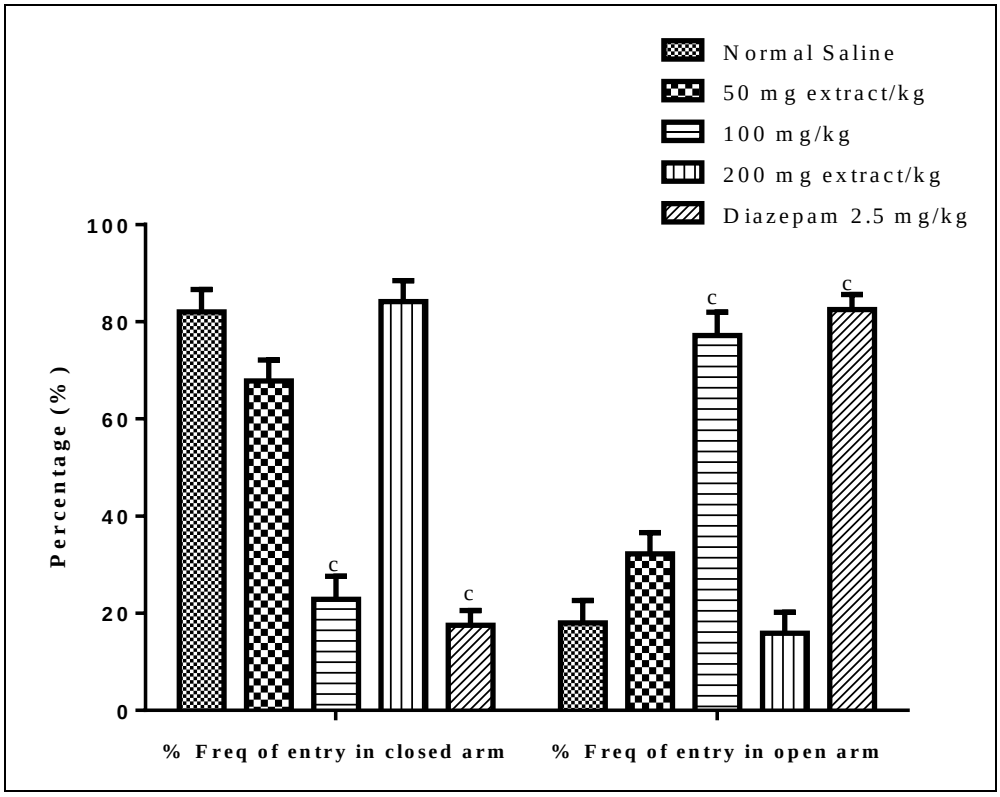

Fig 8: Effect of *F sycomorus* on locomotion, freezing, rearing and frequency of defecation of mice in the open field test. Data are shown as means ± S.E.M., analysis of variance ANOVA followed by multiple comparison Dunnets test $^{c}p < 0.001$ compared to normal saline group.

Discussion

The present study investigated the supposed anxiolytic effects of methanolic extract of the stem bark of *F. sycomorus*, a plant generally used in Nigerian folk medicine. This was carried out using elevated plus maze, open field and zero mazes. The results showed an alternation in mice behaviour after a single administration.

Mental disorders, like anxiety and depression, are major health challenges and can appear any time in life. Developing new compounds for anxiety and other mental disorders is desirable and requires good animal models. In studying anxiety in human, mice and mice are often used as translational models. This is based on the premise that basic physiological mechanisms underlying fear in rodent like the principal brain areas, brain circuits, and neurochemical substrates among others, can be equated to similar mechanisms operating in humans thus providing a degree of face validity for the many behavioural testing paradigms that have been developed (Mathew *et al*., 2008; Cryan and Holmes, 2005) [15].

Evaluation of rodent anxiety is from specific behavioural model tests, among which the elevated plus maze, the elevated zero maze and the open field test are mostly employed. Anxiolytic or anxiogenic causes a decrease or an increase in the anxiety-related behaviour of animals respectively, thus making these tests sensitive to pharmacological agents.

The EPM test as a rodent model for anxiety has been in use for over a decade, and is representative of tests that are based on the study of spontaneous behavioral patterns (Carobrez and Bertoglio, 2005; Skelly and Weiner, 2014) [23]. It has a high pharmacological, physiological and ethological validity. This assay essentially determines a preference between a comparatively safe and comfortable environment (the closed arms) and a risky environment (elevated open spaces). When placed on the EPM, the fear due to height induces anxiety in mice. The eventual manifestation of anxiety and fear is exhibited by decrease in motor activity, which is measured by the time spent and number of entries in the open arm (Pellow, *et al.,* 1985; Walf and Frye, 2009) [19]. Analyzed results of the EPM showed a significant increase in the percentage of time spent in the open arm and also the frequency of open arm entries at 50 and 100 mg/kg doses, thus revealing an anxiolytic effect.

The open field (OF) test is a measure of emotional behavior in rodent. It provides a unique opportunity to systematically assess novel environment exploration, general locomotor activity, and provides an initial screening for anxiety related behavior in rodents. The OF is influenced by social isolation resulting from physical separation of mouse from its cage mates and factor due to the stress created by the brightly lit, unprotected novel test environment (Landgraf and Wigger, 2002; Prut and Belzung, 2003) [20]. The number of line crossings, total distance travelled and rearing are measures of anxiety and overall exploratory/locomotor activity. A high frequency of these variables indicates a less emotional mice behavior. Analysis revealed that the stimulatory activities of *F. sycomorus* were higher at doses of 50 and 100 mg/kg owing to the significant increase in total distance travelled at a significant average speed of movement. Rearing was also significantly increased. On the other hand, 200 mg/kg produced significant decrease in total distance travelled, average speed, rearing, and total locomotor activities. Rodent will typically exhibits a significant thigmotaxic behavior, spending greater amount of time exploring the periphery of the arena than the unprotected central area. It is also thought

that the latency to the center, a measure of risk- taking behavior, is inversely correlated to the level of anxiety (Burn, 2008) [4]. Based on the premise that mice anxiety level changes with time, the latency to the center area was only recorded. Mice administered with 50 mg/kg and 100 mg/kg extract of *F. sycomorus* showed significantly shorter latency to enter the center arena. Defecation and freezing during behavioral testing has been used as a physiological measure of anxiety (Hall, 1934) [10]. Anxiolytic agents produces a calming effect leading to less freezing and defecation as observed in 50 and 100 mg/kg dose of *F. sycomorus.*

The EZM, like EPM is a widely validated tests which is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the gamma aminobutyric acid type A (GABA-A) benzodiazepine complex (Ohl, 2005) [17]. It offers a conceptually identical behavioral test that eliminates ambiguous behavioral variable in the EPM (Wall and Messier, 2000) [26]. Anxiety-related behavior is measured as preference for the closed arms in both models. The percentage of entries and time spent in the open arm indicates anxiety levels (Pellow, S., *et al*, 1985) [8]. *F. sycomorus* produced significant and dose dependent anxiolytic effect as evident in its increased percentage of time spent and number of entries into the open arm. This is replicated both in the EPM and EZM models in mice and mice respectively.

Diazepam acts as positive allosteric modulators on the gamma butyric acid (GABA)-A receptors. It is inhibitory in nature and thus reduces the excitability of neurones producing a calming effect on the brain. And as expected, diazepam produced significant anxiolytic effect across all front, with increases in exploratory and locomotor activities, and open arm times.

Taking together the effect of *Fs* in the three behavioural models of anxiety in mice, the present study clearly demonstrates a dose dependent and consistent effect. In a dose-related fashion, the actions of *Fs* varied from anxiolysis to sedative with increasing dose. Anxiolytic effect of 50 mg/kg and 100 mg/kg was reverted at a higher dose of 200 mg/kg. This could be due to the presence of sedative properties of the extract.

It is generally believed that locomotor activities results from brain activation, which manifest as an excitation of central neurones and as an increase in cerebral metabolism. Locomotor activity is considered as an index of alertness and a decrease leads to sedation due to reduced excitability of the central nervous system. With different neurochemical metabolisms involved in brain activation, there are at least five neurotransmitters that are believed to be involved in anxiety and anxiety-related behaviours, namely GABA, nor-adrenaline, serotonin, corticotropin-releasing hormone (CRH), and cholecystokinin. The interaction between these neurotransmitters is carefully orchestrated in the brain and changes in one neurotransmitter system elicit changes in another. All of these neurotransmitters are putative targets through which *F. sycomorus* could be eliciting its effects through.

These results suggest that the methanolic extract of *F. sycomorus* possess a dual pharmacological property which is capable of activating both excitatory and inhibitory neural mechanisms in the control of anxiety. Previous studies carried out on the stem bark of *F. sycomorus* revealed sedative properties (Sandabe *et al*., 2003) [21]. Whether this is a reason for the exact pattern of *Fs* remains to be further investigated.

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