Asian Journal of Medicine and Health

7(1): 1-9, 2017; Article no.AJMAH.36294
ISSN: 2456-8414

Effect of Crude Ethanol Leaf-extract of *Murraya koenigii* on Anxiety in Mice

S. A. Bisong[1*], U. E. Okon[1], E. A. Egbung[2], F. E. Abuo[1] and O. A. Sanya[3]

[1]Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Nigeria.
[3]Department of Physiology, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author SAB designed the study, performed the statistical analysis, wrote the protocol. Author UEO wrote the first draft of the manuscript. Authors EAE and FEA managed the literature searches. Author OAS managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJMAH/2017/36294
Editor(s):
(1) Godswill Nduka Anyasor, Department of Biochemistry, Benjamin S. Carson (Snr.) School of Medicine, Babcock University, Nigeria.
Reviewers:
(1) Juliana Maria de Paula, Universidade de São Paulo, Brasil.
(2) Dinithi Peiris, University of Sri Jayewardenepura, Sri Lanka.
Complete Peer review History: http://www.sciencedomain.org/review-history/21094

Original Research Article

Received 22nd August 2017
Accepted 10th September 2017
Published 22nd September 2017

ABSTRACT

Herbs are used the world over for culinary purposes and treating a host of ailments including mental disorders. Hence, this study was aimed at assessing the effects of curry plant (*Murraya koenigii)*, commonly used as spice, on anxiety in mice. Twenty CD1 mice (body weight 21.5 g -30 g) were randomly assigned to two (2) groups of ten mice each. Mice received vehicle (distilled water- control) and ethanol leaf-extract of *M. koenigii* extract (80 mg/kg body weight) orally for 7 days before behavioural tests were done. Anxiety related behaviour were assesd using the elevated plus maze (EPM) and the light/dark (LD) box. *M. koenigii* extract increased the duration the mice spent in the open arms of EPM compared to the control ($p<0.01$) while also decreasing the time the mice spent in the close arms compared to control ($p<0.01$). The frequency of stretch attend postures of *M. koenigii*-treated mice were significantly ($p<0.05$) lower both in the EPM and LD box compared to control. These results indicate that *M. koenigii* extract decreased anxiety in the mice.

**Corresponding author: E-mail: bisongsa@yahoo.com, bisongsa@dal.ca;*

Keywords: Muraya koeniji; anxiety; mice.

1. INTRODUCTION

Medicinal plants (herbs) have been used for healing purposes from prehistoric times and form the origin of much modern medicine. According to reports by both World Health Organization [1] and Willcox and Bodeker [2], as many as eighty percent of African populations use traditional herbal medicine for a significant part of their primary health care. This is principally because they are far cheaper and locally available than contemporary medicine [3]. In 1991, the 44th World Health Assembly approved its 44.34 resolution that encourages all member countries to promote the use of traditional harmless remedies for diagnosis and treatment of diseases [4].

One of the plants commonly used not only as spice but also as herbal medicine is the curry plant. The Curry tree (scientific name: *Murraya koenigii*) is a tropical to sub-tropical culinary plant (spice) in the family Rutaceae and is native to India [5]. It is a small spreading shrub, about 2.5 metres high with dark green to brownish main stem which has numerous dots on it. The girth of the main stem is 16 cm [6]. The leaves of this plant are 30 cm long and bear up to 24 leaflets and each of the leaflets are 2 cm to 4 cm long. The flowers of the Curry tree are small and have a sweet fragrance [6]. The ethnomedicinal plant *Murraya koenigii* has been used as an important herb in the Ayurvedic traditional system of Medicine for centuries [7]. According to a study by Bhandari [7], Carbazole alkaloids which are abundantly present in the leaves, fruits, roots and bark of this plant have been reported for their antibacterial, anticancer, antidiabetic, antinociceptive, and antioxidant activities [7] as well as analgesic activity [8]. Three Carbazole alkaloids were isolated by Ramsewak et al. [9]. They included mahanimbine, munayanol and mahanine. As reported by Chevallier [10], the leaves contain medically active constituents including tannins, a glycoside (Koenigin) which is an essential oil. The leaves can be used in treating diarrhea, dysentery, colic and constipation [11-13]. From the bark, a paste is made and can be applied to the sites of insect bites and other venomous animals [7]. The root extracts of the plant *Murraya koenigii* have also been reported to alleviate pain associated with kidney disorders [7].

Seligman et al. [14] defined anxiety as an unpleasant state of inner turmoil, often accompanied by nervous behaviours such as pacing back and forth and can occur with any identifiable triggering stimulus. It is also the dreadful feeling of anticipated menacing occurrence [15]. It is often accompanied by muscular tension, restlessness and problem in concentration [14]. As reported by Baxter et al. [16], anxiety is the most common of all mental disorders and it currently affects about one in 13 people (7.3%). About 12% of people are affected by an anxiety disorder in a given year and between 5-30% are affected at some point in their life time [17]. Anxiety disorders are a group of mental disorders characterized by significant feelings of anxiety and fear [18]. There are various classes of anxiety disorders including generalized anxiety disorder (GAD), specific phobia, social anxiety disorder, separation anxiety disorder and panic disorder of which people frequently have more than one of them [18]. Females suffer more from anxiety disorder than males and this commonly begins before the age of 25 [17].

Generalized anxiety disorder (GAD), which is a common disorder is characterized by long-lasting anxiety, difficulty in concentration or having blank minds [19]. This disorder commonly affects older adults and sufferers experience non-specific persistent fear [20]. Another class of anxiety disorders is panic disorder. According to National Institute of Mental Health, [19], people with panic disorder have sudden and repeated attacks of fear that last for several minutes or longer and these attacks are characterized by a fear of disaster or of losing control even when there is no real danger. For people with social anxiety disorder (social phobia) there is a noticeable phobia about being with other people and having a hard time talking to them [19].

There are many herbs/spices that we consume in our everyday diets that could help to relieve minor to moderate mental health issues. Some teas for instance have a calming effect [21]. Spice used in cooking or as food condiment have also been said to provide some soothing effect on the nervous system. It is therefore, not out of place to investigate whether or not the leaf extract of *M. koenigii* could affect fear and anxiety in mice. The aim of the study was therefore to study the effect of crude ethanol extract of *M. koenigii* on anxiety in the mice.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Twenty male and female CD1 mice weighing between 21.5 – 30 g were used for this study. They were procured from the animal house of the Department of Physiology of the College of Medical Sciences, University of Calabar, Nigeria. The mice were exposed to a normal 12/12 hours light/dark cycles in well ventilated cages at room temperature of 25+2°C. The animals had access to normal ration of rodent chow from Vital Feed Nigeria Ltd and clean drinking water *ad libitum*. Approval for use of the animals was obtained from the College ethical Committee on the use of experimental animals and it was in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC). The animals were randomly assigned to two (2) groups of ten (10) animals each. While group one (1) served as control, group two (2) was for *Murraya koenigii*. The animals were acclimatized one week before the start of the research.

2.2 Preparation/Administration of Ethanol Leaf-extract of *Murraya koenigii*

The leaves of *Murraya koenigii* were harvested from the botanical garden of the University of Calabar, Nigeria. They were air-dried at room temperature and grinded with a blender. The powdered samples were extracted with 400 mls of ethanol by reflux for four hours at 78°C. The extract was then filtered using Whatmann paper 1. The filtrate was then evaporated in the oven at 40°C. The gel-like paste was collected and stored in a sterile sample bottle and kept in the refrigerator prior to its use. The extract was reconstituted using normal distilled water for oral administration. A dose of 80 mg/kg of the extract was administered orally for 7day before behavioural tests were conducted.

2.3 Behavioural Protocol

2.3.1 The elevated plus maze

The elevated plus maze apparatus designed according to the description of Lister [22], and the test protocol adapted by Bisong et al. [23] were used. The maze consists of two open arms (45 x 5 cm^2) having 0.25 cm high edges and two closed arms (40 x 5 cm^2) having 15 cm high walls projecting from a central square (5 x 5 cm). The open arms have a slight ledge (4 mm high) to prevent the mice from slipping and falling off the edge [24].

Prior to the test, the apparatus' surfaces and sides were cleaned with methylated spirit to remove olfactory clues, fecal bolls and urine. The test room was shaded with dark coloured thick curtains to provide dim lighting. The mice were placed in the central square of the equipment such that the mice faced an open arm away from the experimenter upon placement. Right after placement, a stopwatch was started and the mice freely explored the apparatus for 5 minutes. All test sessions were recorded using a video camera. Behaviour scored included open arm entry frequency and duration, close arm entry frequency and duration, stretch attend posture, grooming duration, head dips and rearing frequency [23].

2.3.2 The light-dark transition box

The light and dark transition box checks for unconditioned anxiety. It is based on the clash between exploring in a new environment and aversion to bright light. This box is divided into two compartments of unequal size as described by Costal et al. [25]. It is made up of plywood. The small compartment which is painted black, has a measurement of 18 x 27 cm and constitutes 2/5 of the box. The larger (27 x 27 cm) compartment is painted white and makes up 3/5 of the box. Both compartments are linked by a door (7.5 x 7.5 cm) that is located at floor level in the centre of the wall separating the two compartments. The floor which is covered with Plexiglas is divided into 9 x 9 cm squares. The tests in this apparatus were conducted in a 2 x 5 m neurobehaviour laboratory which was lit by a 60 watts red lamps for background lighting. The mice were placed into the apparatus and allowed to explore for 5 minutes. The test sessions were recorded using video camera. Behaviours scored included; transitions, light box duration, dark box duration, line crosses, rearing frequency, stretch attend posture and grooming duration. Just like the elevated plus maze test, the test room was dimly lithe while bright light chamber was particularly lithe by a small 2watt energy bulb [23].

3 RESULTS

3.1 Comparison of Behaviour Scored in the Elevated Plus Maze (EPM) Test between Mice Treated with Crude Ethanol Extract of *Muraya koenigii* and Control

The frequency of arms entries were lower in both open and closed arms for the group of mice administered 80mg/kg of ethanol leaf extract of *M. koenigii* compared to control ($p < 0.05$; Fig. 1). The amount of time spent in each arm of the EPM (arms duration) is shown in Fig. 2. The duration in the open arms was significantly higher in the group of mice administered 80mg/kg of ethanol leaf extract of *M. koenigii* compared to control ($p < 0.01$). Conversely, the *M. koenigii* treated group of mice spent less time in the closed arms compared to control ($p < 0.01$). The frequency of stretch attend postures (SAP) in the *M. koenigii-treated* group of mice was significantly lower compared to control ($P< 0.05$, Fig. 3). The frequency of rearing (Fig. 4) did not differ from control.

3.2 Comparison of Behaviour Scored in the Light/Dark Transition Box Test between Mice Treated with Crude Ethanol Extract of *Muraya koenigii* and Control

The comparison between the frequency of transition between the light and dark chambers of the light/dark transition box for the mice treated with crude ethanol leaf extract of *M. koenigii* and control (Fig. 5) did not show any significant difference. Similarly, the frequency of line crosses and frequency of rearing did not differ significantly between the test group of mice and control (Fig. 6 and 7 respectively). Although the time spent in the light chamber of the box did not differ, (Fig. 8), the frequency of stretch attend postures (SAP) for the mice administered crude ethanol extract of *Muraya koenigii* (80 mg/kg, i.p.) was significantly lower when compared to their control ($p< 0.01$; Fig. 9).

4. DISCUSSION

The elevated plus maze and light/dark box tests were used to assess the effect of ethanolic extract of *Murraya koenigii* on fear and anxiety. The test exploits the clash between exploring in novel areas and aversion of open spaces [24]. In the elevated plus maze, behaviours scored included: Open and Close arm duration, stretch attend posture (SAP) and frequency of rearing were assessed. From the results, it is shown that *Murraya koenigii*-treated mice exhibited fearlessness by spending more time in the open arms of the maze. The SAP of the *M. koenigii* group was also observed to be lower when compared to the control, signifying that *Murraya koenigii* was able to reduce anxiety.

According to Lister [26], fearful mice tend to spend more time in the dark chamber of the Light-dark box. Behaviours such as stretch

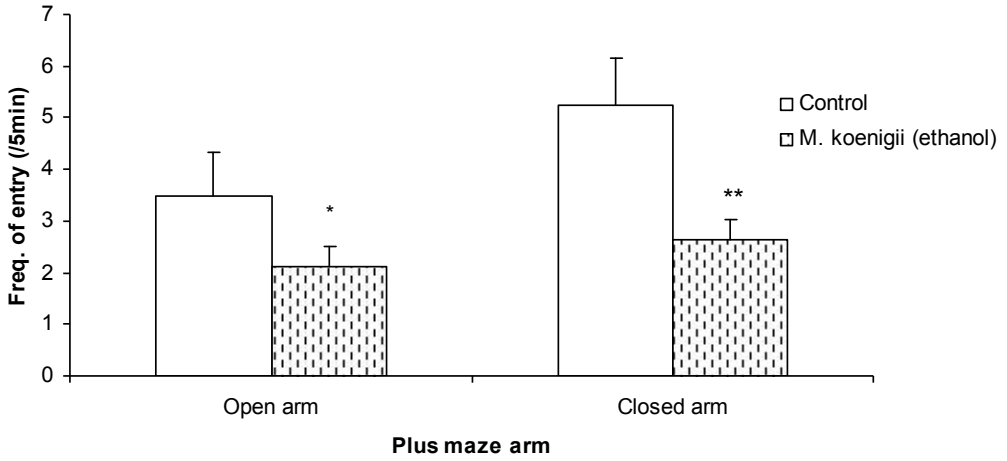

Fig. 1. Comparison between frequency of entry into the open and closed arms of the elevated plus maze for mice administered crude ethanol extract of *Muraya koenigii* (80mg/kg, i.p) and their control mice

** - Significant at p < 0.05 compared to control; ** - Significant at p < 0.01 compared to control*

attend posture (SAP), grooming, frequency of rearing, duration in the light and dark chambers were used as measures of anxiety. The higher the SAP, dark chamber duration and rearing frequency, the higher the level of fear and anxiety [8,26,27]. In this study, the *M. koenigii* treated group were intrepid and this was portrayed by decrease in frequency of stretch attend posture when compared to the control. These findings show that *M. koenigii* inhibited and/or reduced anxiety in the mice, thus confirming its anxiolytic effects.

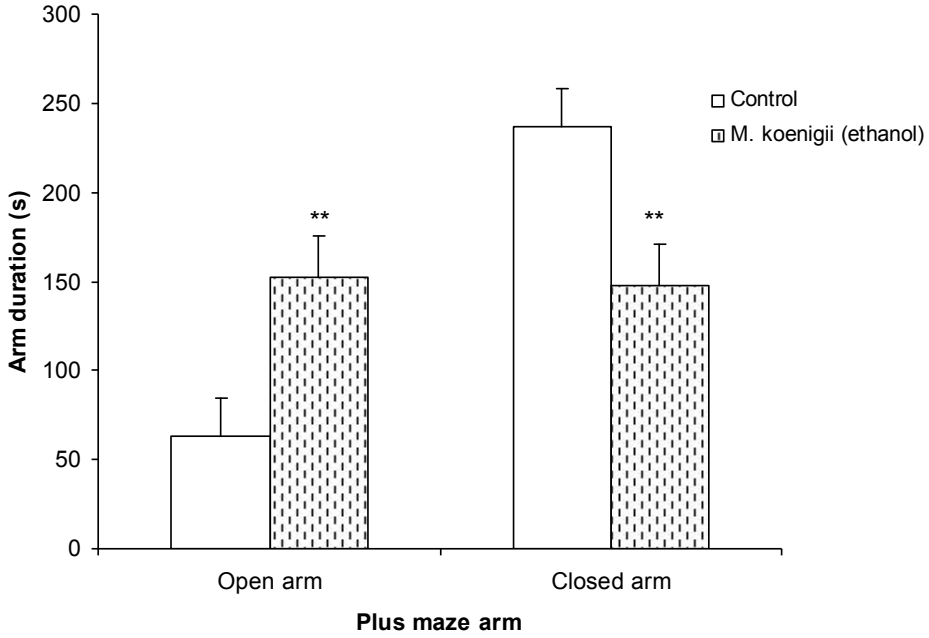

Fig. 2. Comparison between duration in the open and closed arms of the elevated plus maze for mice administered crude ethanol extract of *Muraya koenigii* (80 mg/kg, i.p) and their control mice

*** - Significant at p < 0.01 compared to control*

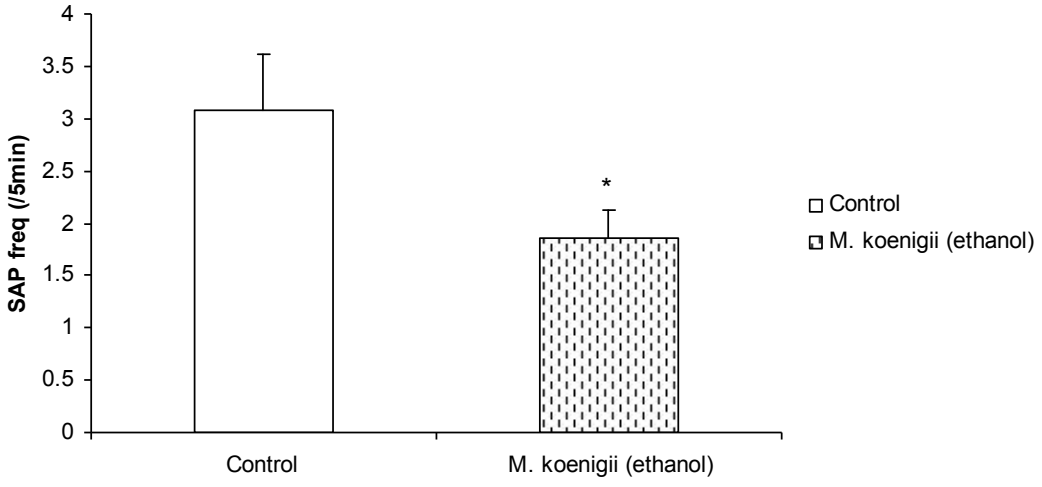

Fig. 3. Comparison between stretch attend postures (SAP) in the elevated plus maze for mice administered crude ethanol extract of *Muraya koenigii* (80 mg/kg, i.p) and their control mice

** - Significant at p < 0.05 compared to control*

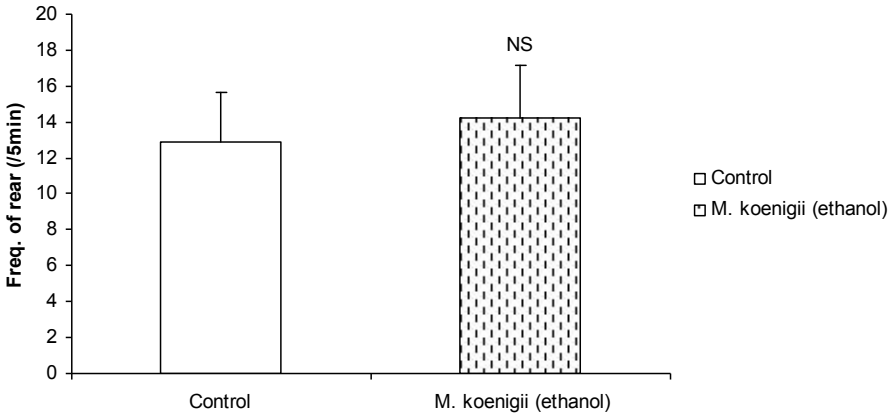

Fig. 4. Comparison between frequency of rearing in the elevated plus maze for mice administered crude ethanol extract of *Muraya koenigii* and their control mice

NS – Not significant compared to control

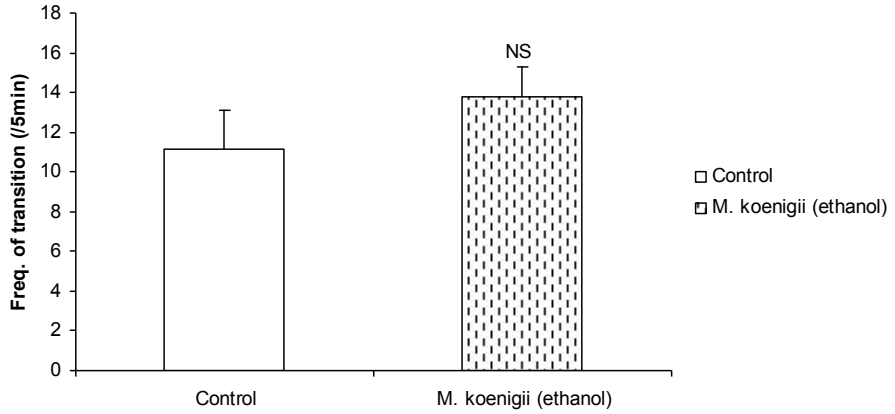

Fig. 5. Comparison between frequency of light/dark transitions in the light/dark transition box for mice administered crude ethanol extract of *Muraya koenigii* (80 mg/kg, i.p.) and their control

NS – Not significant compared to control

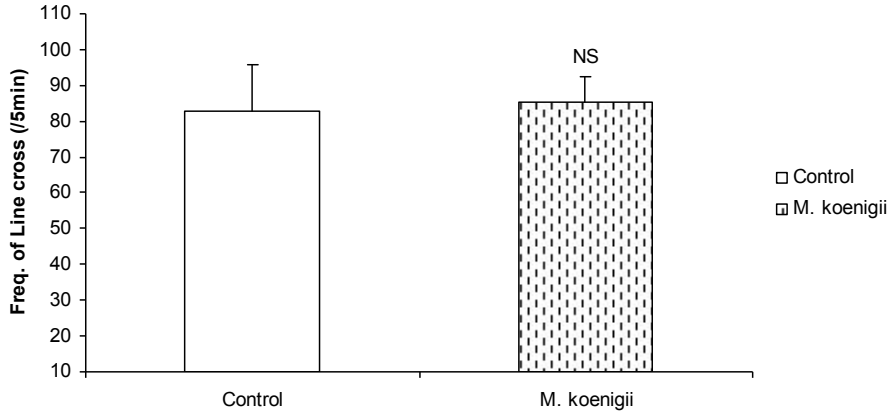

Fig. 6. Comparison between frequency of line cross in the light/dark transition box for mice administered crude ethanol extract of *Muraya koenigii* (80 mg/kg, i.p.) and their control

NS – Not significant compared to control

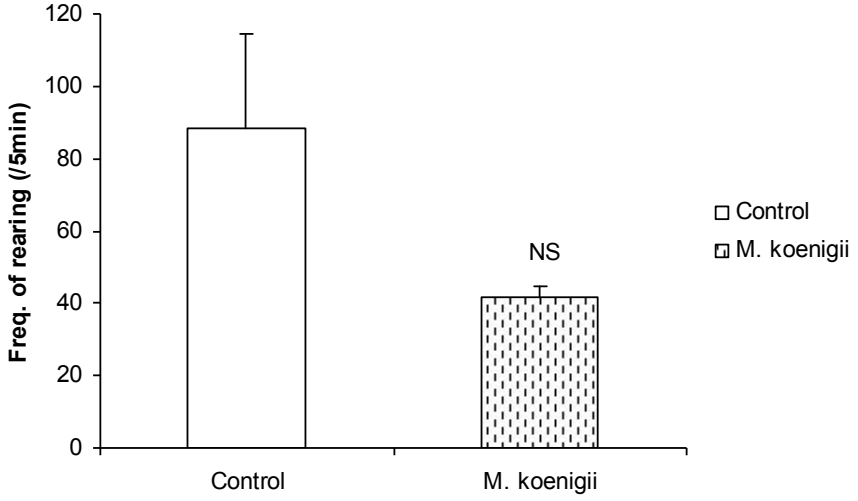

Fig. 7. Comparison between frequency of rearing in the light/dark transition box for mice administered crude ethanol extract of *Muraya koenigii* (80 mg/kg, i.p.) and their control

NS – Not significant compared to control

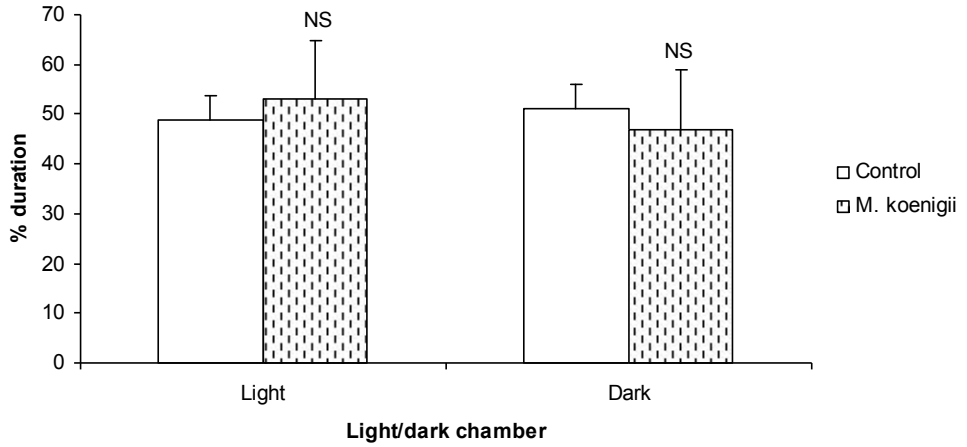

Fig. 8. Comparison between percentage duration in the light and dark chambers of the light/dark transition box for mice administered crude ethanol extract of *Muraya koenigii* (80 mg/kg, i.p.) and their control

NS – Not significant compared to control

Anxiety occurs as a result of excessive neurological activity and this is thought to stem from the fact that certain inhibitory neurons are not functioning properly by not releasing Gamma-aminobutyric acid (GABA) [28]. However, the ability to bind unto these receptors is influenced by the presence of benzodiazepines produced invivo. The binding of these substances to the sites on receptive neurons increases the ability of GABA binding to its own site [29]. Hence, the effectiveness of GABA is increased which invariably decreases neural activity and this subsequently reduces one's level of anxiety. The anxiolytic effects of *M. koenigii* (Curry tree) leaves may be due to the alkaloids (particularly mahanimbine), present in it. This is corroborated by the study authored by Dahiya et al. [30]. The alkaloid may have achieved this feat by mediating the GABA receptors to increase the efficacy of GABA, thus eventually leading to anxiety inhibition/ reduction.

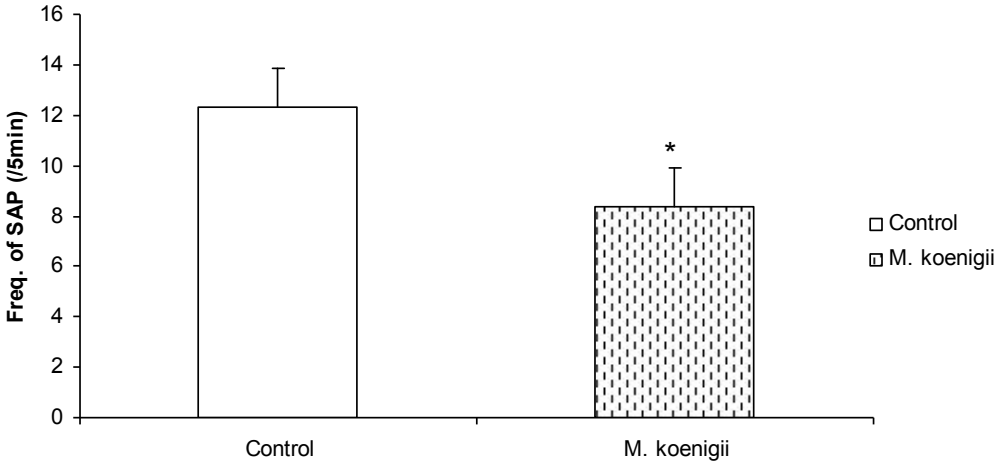

Fig. 9. Comparison between frequency of stretch attend postures (SAP) in the light/dark transition box for mice administered crude ethanol extract of *Muraya koenigii* (80mg/kg, i.p.) and their control

** - Significant at p < 0.05 compared to control*

5. CONCLUSION

This study reports that administration of ethanol extract of *Murraya koenigii* to decreased anxiety in mice. If these results apply to man, consumption of the curry leaf may help to produce a relaxing effect in the humans consuming it.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. WHO traditional medicine strategy 2002 –2005. Geneva, Switzerland; 2002.
2. Willcox ML, Bodeker G. Traditional herbal medicines for malaria. BMJ. 2004; 329(7475):1156-9.
DOI: 10.1136/bmj.329.7475.1156
3. Kamboj VP. Herbal medicine. Current Science. 2000;78(1):35–39.
4. World Health Organization. Guidelines for the assessment of herbal medicines. Geneva, Switzerland. 1991;1(2):7.
5. Noolu B, Rajanna A, Anitha C, Balakrishna N, Raghunath N, Ayesha I. *Murraya koenigii* leaf extract inhibits proteasome activity and induces cell death in breast cancer cells. BMC Complementary and Alternative Medicine. 2013;13:7.
DOI: 10.1186/1472-6882-13-7
6. Parmar C, Kaushal MK. *Murraya koenigii* – in wild fruits. Kalyani Publishers, New Delhi, India. 1982;45–48.
7. Bhandari PR. Curry leaf (*Murraya koenigii)* or cure leaf: Review of its curative properties. J Med Nutr Nutraceut. 2012;1: 92 – 7.
8. Ratnasooriya WD, Peiris LDC, Jayatunga YNA. Analgesic and sedative action of monocrotophos following oral administration in rats. Medical Science Res. 1996;23:401-403.
9. Ramsewak RS, Nair MG, Strasburg GM, Dewitt DL, Nitiss JL. Biologically active carbazole alkaloids from *Murraya koenigii.* J Agric Food Chem. 1999;47(2):444-447.
10. Chevalier A. The encyclopedia of medicinal plants. Dorling Kindersley, London; 1996.
ISBN: 0-7894-106-72
11. Barwick M, Van der Schans A. Tropical and sub-tropical trees – a worldwide

encyclopedic guide. Thames & Hudson. London; 2004.
ISBN: 0-500- 51181-0

12. Manandhar NP. Plants and people of Nepal. Timber Press. Oregon; 2002.
ISBN: 0-88192-527-6
13. Bown D. Encyclopedia of herbs and their uses. Dorling Kindersley, London; 1995.
ISBN: 0-7513-020-31
14. Seligman ME, Walker EF, Rosenhan DL. Abnormal psychology (4th Ed.). W.W. Norton & Company Inc; New York; 2000.
15. Davidson GC, Neale JM, Kring AM. Abnormal psychology (9th Ed.). Toronto: Veronica Visentin. 2008;154.
ISBN: 978-0-470-84072-6
16. Baxter AJ, Scott KM, Vos T, Whiteford HA. Global prevalence of anxiety disorders: A systematic review and meta-regression. Psychol Med. 2013;43(5):897-910.
DOI: 10.1017/S003329171200147X
17. Craske MG, Stein MB. Anxiety. Lancet. 2016;388(10063):3048-3059.
DOI: 10.1016/S0140-6736(16)30381-6
18. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. (5th Ed.) 2013;189-195.
ISBN: 978-0890425558
19. National Institute of Mental Health. Panic disorder: When fear overwhelms; 2016. Pub ID: 16-4679.
Available:https://www.nimh.nih.gov/health/topics/anxiety-disorders/index.shtml
20. Calleo J, Stanley M. Anxiety disorders in later life: Differentiated diagnosis and treatment strategies. Psychiatric Times. 2008;26(8).
21. Steptoe A, Gibson EL, Vuononvirta R, Williams ED, Hamer M, Rycroft JA, et al. The effects of tea on psychophysiological stress responsivity and post-stress recovery: A randomised double-blind trial. Psychopharmacology (Berl). 2007;190(1): 81-9.
22. Lister RG. The use of a Plus maze to measure anxiety in the mouse. Psychopharmacology (Berl). 1987;92(2): 180–185.
23. Bisong SA, Okon UA, Chukwu JAO, Sanya OA, Akinnuga MA, Unirere GN. Long term consumption of coconut oil diet increased anxiety related behaviour in CD1 mice. Journal of Complementary and Alternative Medical Research. 2017;2(1):1-13.
24. Trullas R, Skolnick P. Differences in fear motivated behaviours among in- bred mouse strains. Psychopharmacology. 1993;111(3):323–31.
25. Costal B, James BJ, Kelly ME, Naylor RJ, Tom Kins DM. Exploration of mice in a black and white test box: Klidation as a model of anxiety. Pharmacology, Biochemistry and Behavior. 1989;32:777-785.
26. Lister RG. Ethologically-based animal models of anxiety disorders. Pharmacol Ther. 1990;46(3):321–340.
27. Peiris LDC, Ratnasooriya WD, Jayatunga YNA. Analgesic and sedative effects of methamidophos. Medical Science Research. 1995;22:293-295.
28. Tallman JF, Paul SM, Skolnick P, Gallager DW. Receptors for the age of anxiety: Pharmacology of the benzodiazepines. 1980;207(4428):274-281.
29. Maren S. Neurobiology of Pavlovian fear conditioning. Annu Rev Neurosci. 2001;24: 897-931.
30. Dahiya J, Singh J, Kumar A, Sharma A. Isolation, characterization and quantification of an anxiolytic constituent – mahanimbine, from *Murraya koenigii* Linn. Spreng leaves. J Ethnopharmacol, Elvesier. 2016;193:706-11.
DOI: 10.1016/j.jep.2016.10.014

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21094