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Excitatory Effect of *Urtica dioica* on Locomotor Behaviour of Mice Using the Open Field Maze Task

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: It is generally believed that the presence of some neuroactive phytochemicals such as acetylcholine, histamine, serotonin, tannins etc. in *Urtica dioica* may yet possess the key to the management of certain neurological and behavioral conditions at an easily accessible and affordable rates. The growing acceptance of alternative medicine in Nigeria and the world at large has necessitated this research work so as to ensure that the general/consuming public is properly guided.

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Objective: This study was carried out to investigate the effect of administration of *Urtica dioica* on locomotor behavior. The amount of *Urtica dioica* reaching the Central Nervous System (CNS) is affected by the extent to which it is converted/stimulates any of the excitatory agents like acetylcholine in the periphery.
Methods: A total of 30 apparently healthy Swiss male albino mice weighing 20-25g were used in the study. After the 14 days of acclimatization, the mice were randomly separated into three (3) groups of ten (10) animals each and were housed in separate cages. The amice in group one (1) serves as the control for the research work and were given water and normal animal feed. Group two (2) of the mice were given feed mixed 400mg/kg of the extract while group three (3) mice were given feed mixed with 800 mg/kg of the extract. The open field maze was employed for the evaluation of locomotor behavior while the extracts were mixed with the animals' feed. So, the extracts were administered to the mice orally. The extracts were administered for a duration of 28days (4 weeks).
Results: The frequency of rearing in the open field was significantly increased in the *Urtica dioica* fed group compared to control ($p<0.05$). There is also a significant increase in the frequency of Line Crosses, Centre Square Entry and Rearing in the test groups compared to control ($p<0.01$). At a higher dosage, the Frequency and Duration of Grooming significantly increased ($p<0.05$). The Frequency of Defecation also significantly increased ($p<0.05$).
Conclusion: These results indicate that repeated administration of *Urtica dioica* enhances locomotory behavior in mice.

Keywords: Open field maze; Urtica dioica; locomotor; mice.

1. INTRODUCTION

"*Urtica dioica* (stinging nettle), is a perennial plant belonging to the family of Urticaceae, genus Urtica. Despite the use of nettle in folk veterinary medicine is well documented, *Urtica dioica* is today an underestimated and frequently neglected plant, considered by the contemporary agriculture as a weed to be eliminated. Therefore, this research focuses on the effect of administration of Urtica dioica as a single herb to determine its effect on neurobehavior following the protective effect it had exhibited in an induced neurobehavioral change reported by Dhouibi" [1]. The laudable strides attained in the diagnosis and management of neuromuscular and mood disorders, remain an enigma as most of the drugs used in this context are either carce or very exorbitant for most individuals especially in third world countries like Nigeria hence the tendency to seek alternate medicine therapy.

The abundant presence of neuroactive phytochemicals such as acetylcholine, histamine, serotonin and tannins in *Urtica dioica* as asserted by Fu [2], may yet possess the key to the management of certain neurological and behavioral conditions at an easily accessible and affordable rates with the growing acceptance of alternative medicine in Nigeria and the world at large.

Scientific evidence from clinical trials to authenticate the neurophysiological importance of this herb is scanty. Hence, the need for further research to ascertain its efficacy and safety since Unregulated or inappropriate use of traditional medicines and practices can have negative or dangerous effects [3].

2. MATERIALS AND METHODS

Standard procedures in handling of experimental animals were observed in the course of this study. These include obtaining ethical approval from the department, allowing the animals to acclimatize for two weeks before commencement of experiments and careful handling of animals without being cruel.

2.1 Animals

A total of 30 healthy Swiss male albino mice weighing 20-25g were used in the study and they were acquired from Laboratory Animal House of Michael Okpara University of Agriculture Umudike, Nigeria and transported to the animal house in Abia State University Uturu, where they were used for the study. The animals were housed in hygienic and well-ventilated cages under standard environmental conditions of temperature (23±2°C), humidity (55±15%) and light (natural light). They were fed with normal commercial rodent chew and allowed access to drinking water for 14 days to allow them acclimatize.

2.2 Experimental Design

The Open Field Maze apparatus (OFM) was used to access locomotor behavior. The open field test was used to provide measures of

locomotion, exploration and anxiety [4]. "The experiment was performed in an enclosed laboratory to screen the animals from noise and provide dim light to avoid distraction of the animals. The animals were placed in the center of the maze and allowed to explore the open field for 5 minutes. Before introducing each animal; the floor of the maze was cleaned using 70% ethyl alcohol in order to eliminate olfactory influences" [4]. The following behaviors were scored during the 5 minutes to assess locomotor behaviors: line crossing, rearing, grooming, center square entry and frequency of defecation. Randomly, the mice were arranged into three groups of 10 mice each as follows;

GROUP I – Control received normal food and water,

GROUP II – Received 400 $mgkg^{-1}$ ethanolic extract of *U. dioica,* food and water*,*

GROUP III – Received 800 $mgkg^{-1}$ ethanolic extract of *U. dioica*, food and water*.*

2.3 Preparation of Extract

Fresh leaves (200g) of *U dioica* were obtained from a farmer in Okigwe LGA, Imo State and identified by botanists at Michael Okpara University of Agriculture, Umudike. The leaves were dried at room temperature over 7days and powdered using clean pestle and mortar after which it was sieved. The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature.

The fine particles were soaked in ethanol (2 L) for 72 hours so that alkaloids and flavonoids if present will be dissolved. The solution was filtered and the filtrate concentrated using the hot air oven to give a semisolid residue. This product was kept in the refrigerator (1.6°C or 35 F) for further use. The semi-solid extract was subsequently reconstituted in distilled water at appropriate concentrations for the various experiments.

2.4 Acute Toxicity Test

The mice were divided into five groups consisting of 5 mice per group. Each group of mice for *U. dioica* were given a different dosage of the extract (5000mg/kg, 4000mg/kg, 3000mg/kg, 2000mg/kg, 1600mg/kg, 800mg/kg, 400mg/kg, 200mg/kg, and 100mg/kg) orally and deaths in each group within 24-72hours was recorded. The LD_{50} was calculated using probit kill of the dose, a method patented by Lorke [5].

2.5 Administration of Extract

After the 14 days of acclimatization, the mice were randomly separated into three (3) groups of ten (10) animals each and were housed in separate cages.

Group 1 serves as control. Received only normal food and water. Group 11 receives low dose 400 $mgkg^{-1}$ of ethanol extract of *u. dioica,* food and water. Group 111 receives high dose 800 $mgkg^{-1}$ of ethanol extract of *u. dioica*, food and water*.*

The extract was mixed with 6 grams of normal rodent chew (6g=average mice food intake) for each mouse in the test group while the control received 6 grams of normal rodent chew per day. The two groups also received 15 milliliters of water per mouse each day. Their daily food and water intake were measured using electronic weighing balance and syringe respectively every 24 hours and their body weight measured every three days for each mouse. The extract was administered for 28 days (4 weeks).

Dose calculation; the calculation of the dosage was done using the standard formula as follows;
Dose = (dose to be administered x weight of the mouse) / (1000 x concentration of the stock solution). The stock solution was prepared by dissolving 0ne gram (1 g) of the extract in twenty milliliters (20 ml) of distilled water.

2.6 Experimental Protocol

2.6.1 Locomotion and exploratory behavior using open field maze (OFM) test

The open field (OF) test, which provides simultaneous measures of locomotion, exploration and anxiety (4) was used for this study. "The open field apparatus is constructed from white plywood with a 72 x 72 cm floor and 36 cm walls. One of the walls is made of clear Plexiglas, so that mice could be visible in the apparatus. Blue lines are drawn on the floor with a marker and are visible through the clear Plexiglas floor. The lines divide the floor into sixteen 18 x 18 cm squares. A central square (18 cm x 18 cm) is drawn in the middle of the open field [6]. The central square has sufficient space surrounding it to give meaning to thecentral location as being distinct from the outer locations" [7].

Picture 1. The open field apparatus

2.6.2 Procedure

Mice were carried to the test room in their home cages and tested one at a time for 5-minutes each. The video cassette recorder (VCR) was turned on to record and the mouse's number indicated before testing.

Mice were scooped up in a small plastic container from their home cage and placed at the centre square of the open field, then allowed to explore the apparatus for 5-minutes while the experimenter scored the behaviours live using a video camera- and manually also. After the 5-minute test, the mice were scooped up from the open field with the plastic container and returned to their home cages. The number of urinations and defecations for each mouse were counted and recorded before the open field is cleaned with 70 % ethyl alcohol and permitted to dry between trials. Mice were placed in the anteroom or back into the colony room while cleaning the apparatus in bright light conditions.

The behaviors scored [6] included:

1. Line Crossing: Frequency with which the mice crossed one of the grid lines with all four paws.
2. Centre Square Entries: Frequency with which the mice crossed one of the red lines with all four paws into the central square.
3. Center Square Duration: Amount of time the mice spent in the central square.
4. Rearing: (aka rearing center) Frequency with which the mice stood on their hind legs in the maze without aid of a wall.
5. Rearing Against a Wall: Frequency with which the mice stood on their hind legs against a wall of the open field.
6. Stretch Attend Postures: Frequency with which the animal demonstrated forward elongation of the head and shoulders followed by retraction to the original position.
7. Grooming: Frequency and duration of time the animal spent licking or scratching itself while stationary.
8. Freezing: Duration and frequency with which the mouse was completely stationary.
9. Urination: Number of puddles or streaks of urine.
10. Defecation: Number of fecal boli produced [8,9].

2.7 Statistical Analysis

The values were expressed as mean ± SEM. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc performed with Least Significant Difference (LSD) dunnett. P value of less than 0.05 was considered to indicate statistical significance and 0.001 as highly significant respectively.

3. RESULTS AND DISCUSSION

The study observes the effect of Urtica dioica on some neuro behavioral parameters which are indicators of excitation in Swiss mice. The neuro behavioral parameters considered in this include locomotor activities like Line Crossing, Centre Square Entry, Frequency of Grooming and Rearing etc. “The open field test (OFT) which was developed by Calvin S. Hall, is an experiment used to ascertain general locomotor activity levels and anxiety in rodents in scientific research and willingness to explore in rodents” [10]. “However, the extent to which behavior in the open field measures anxiety is controversial. Rodents display a natural aversion to bright areas” [11]. “They also have a drive to explore a perceived threatening stimulus. Decreased anxiety leads to increased exploratory behavior. Increased anxiety will result in less locomotor motion and preference for the edges of the field” [12].

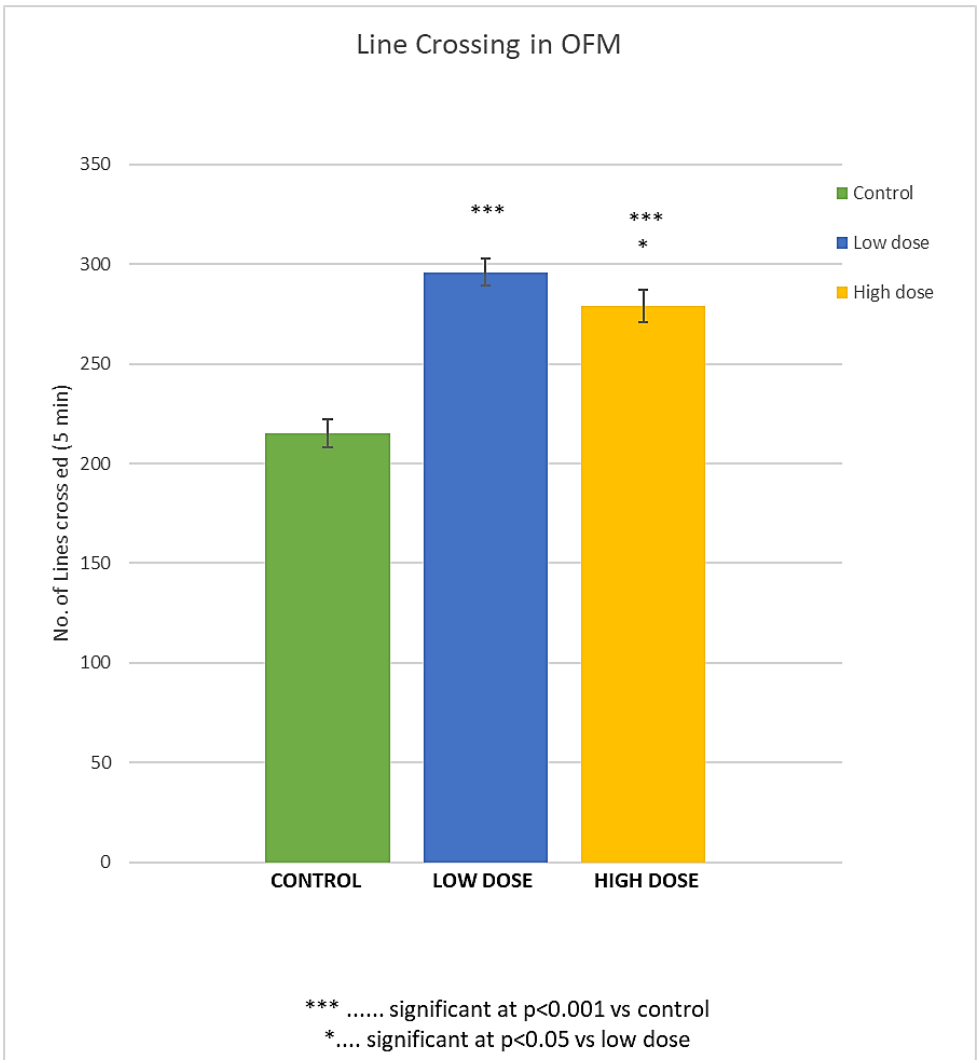

Fig. 1. Frequency of line crossing in the different experimental group in the open field test. Values are expressed as mean ± SEM, n = 10

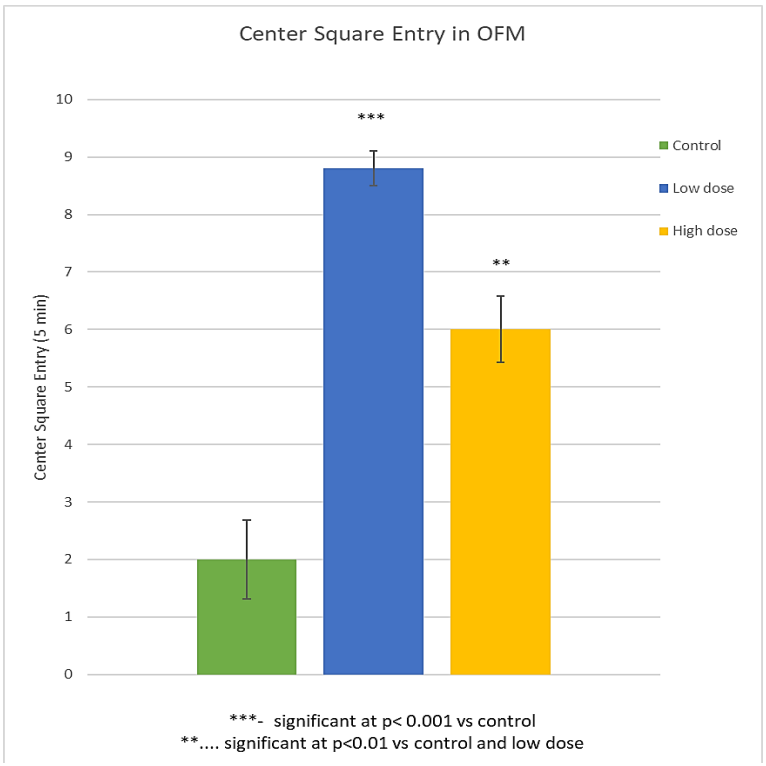

Fig. 2. frequency of center square entry in the different experimental group in the open field test. Values are expressed as mean ± SEM, n = 10

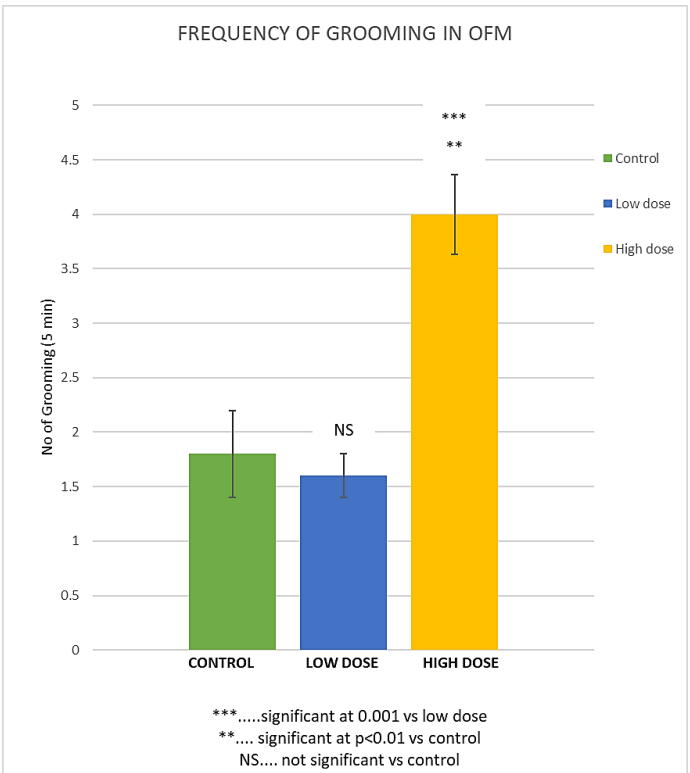

Fig. 3. frequency of grooming in the different experimental groups in the open field test. Values are expressed as mean ± SEM, n = 10

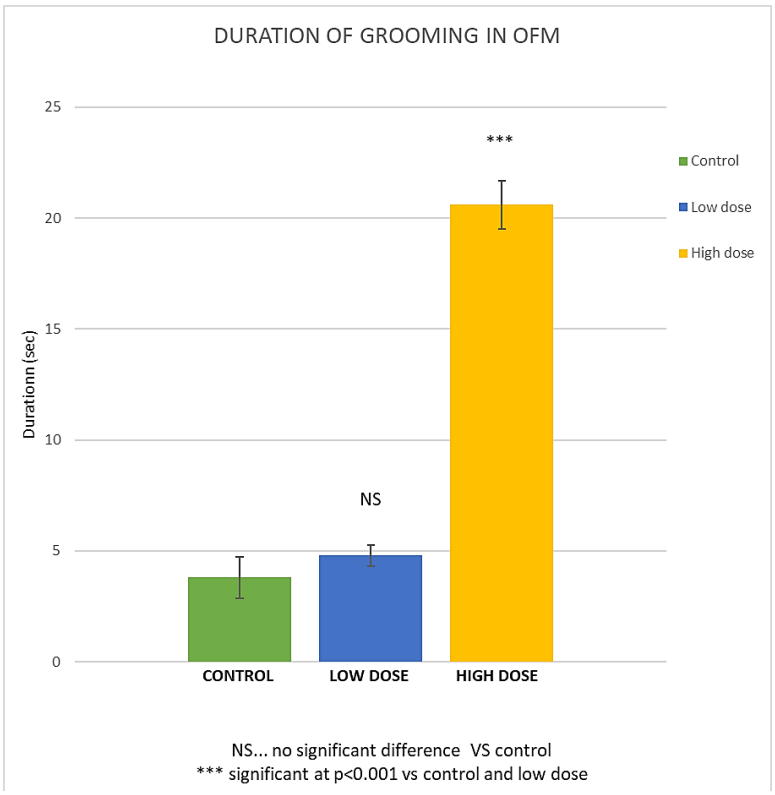

Fig. 4. Duration of grooming in the different experimental groups in the open field test. Values are expressed as mean ± SEM, n = 10

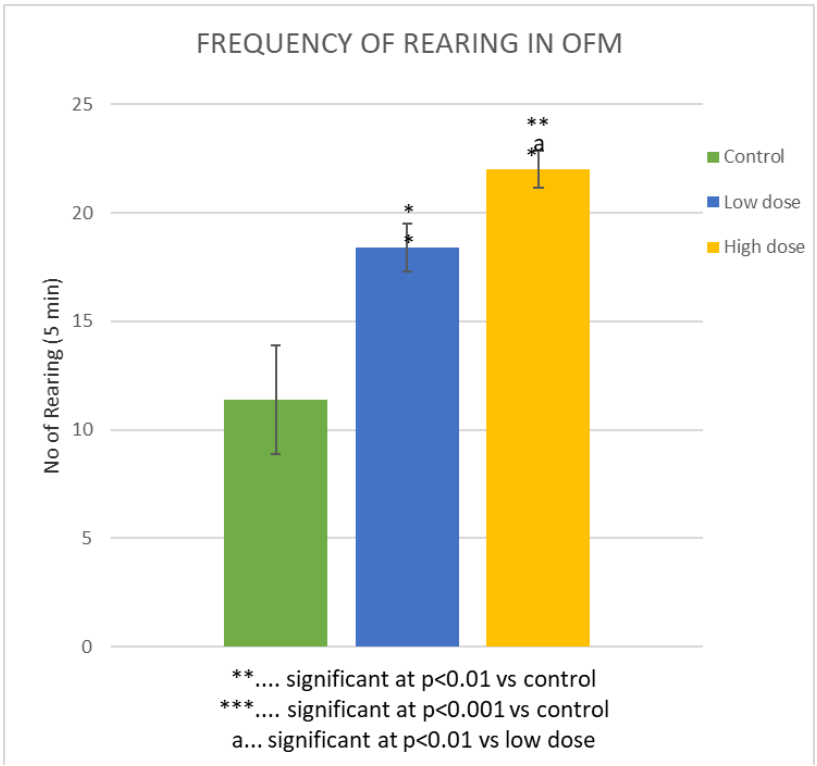

Fig. 5. frequency of rearing in the different experimental groups in the open field test. Values are expressed as mean ± SEM, n = 10

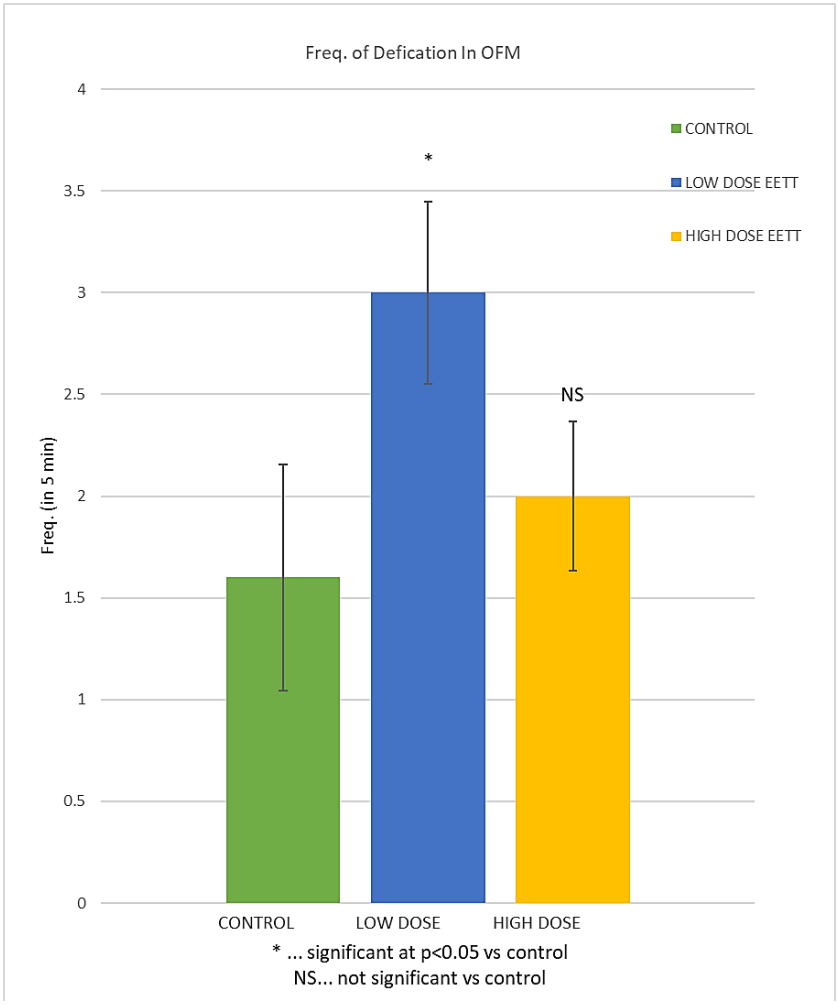

Fig. 6. frequency of defecation in the different experimental groups in the open field test. Values are expressed as mean ± SEM, n = 10

"The open field test has been used as a test that assesses locomotion behaviors. It provides simultaneous measures of locomotion, exploration as well as anxiety" [13]. "Behaviors such as the number of line crosses and stretch attend postures are used as measures of exploration and anxiety. A high frequency of these behaviors indicates increased locomotion and exploration. The frequency of line crosses (number of lines crossed per unit time) measures the horizontal locomotor behavior. The number of lines crossed following administration of low doses of *Urtica dioica* showed a significant increase ($P<0.05$) from the control group. The high dose group also showed a significant increase from the control group and recorded an increase in the number of line crossings compared to the control group. An increase in exploratory behavior in the open field of the apparatus indicates decreased fear. This decreased fear (anxiolytic effect) could be caused by anxiolytic drugs/agents" [14]. This trend was also seen in the high dose group. The result of this experiment has shown that *Urtica dioica* has impressive or excitatory effect as the extract increased locomotion activity in the open field box.

Furthermore, from the results shown above, it is observed that extract of *U. dioica* significantly enhanced Line Crossing, Centre Square Entry, Frequency and Duration of Grooming when compared to the test control. Same pattern is also seen in Rearing and Frequency of Defecation. However, for grooming, *U. dioica* exerted more effect when administered at a higher dose of 800mg/kg while in the case of defecation, *U. dioica* exerted more effect at a lower dose of 400 mg/kg than at high dose of 800 mg/kg.

The frequency of defecation of the mouse during experimentation could also be indicative of fear and anxiety. However, more studies need to be done to ascertain this effect of *U. dioica* on fear and anxiety in mouse.

4. CONCLUSION

The significant increase in line crossing, Centre Square Entry, Frequency/Duration of Grooming and Rearing indicates that ethanolic extract of *U. dioica* enhances locomotor activities in mouse.

Furthermore, from the results obtained from this study, it can be concluded that consumption of *Urtica dioica* increases locomotion and exploratory activity in swiss mice. It also enhances motivation. More so, *Urtica dioica* also decreases fear and anxiety. If these results are to be applicable to humans, then this result is a good one and people should be encouraged to consistently consume it moderately.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dhouibi R, Affes H, Ben Salem M, Charfi S, Marekchi R, Hammami S, Zeghal K, Ksouda K. Protective effect of *Urtica dioica* in induced neurobehavioral changes, nephrotoxicity and hepatotoxicity after chronic exposure to potassium bromate in rats. Environ Pollut. 2021;15(287):117657.
2. Fu Stuart I. Human Physiology. 9th edition. Pierce college, Los Angeles McGraw-Hill, ISBN 0 – 07 – PMID 6207592; 2006.
3. Wells CE. (Rev.) The central nervous system and behavior. American Medical Association Archives of Neurology. 2003; 1(4):464–466.
4. Walsh V RN, Cummins RA. The open field test: A critical review. Psychological Bulletin. 1976;83:482-504.
5. Lorke DA. New approach to practical acute toxicity testing. Archives of Toxicology. 1983;54:275-287.
6. Brown RE, Corey SC, Moore AK. Differences in measures of exploration and fear in MHC-congenic C57BL/6J and B6-H-2K mice. Behavior; 1999.
7. Carrey T, Taylor K. Biological flora of the british isles: *Urtica dioica* L. Journal of Ecology. 2000;97(6):1436–1458.
8. Burger A, Stadler K, Sasvari M, Jakus J, Jung KJ. The effect of exercise and nettle supplementation on oxidative stress markers in the rat brain. Brain Res Bull. 2004;65:487–493.
9. Naidu R, Skolnick P. Differences in fear motivated behaviors among inbred mouse strains. Psychopharmacology. 2006;111: 323-331.
10. Stanford SC. "The open field test: Reinventing the wheel". Journal of Psychopharmacology. 2007;21(2):134–4.
11. Lalonde R, Strazielle C. "Relations between open-field, elevated plus-maze, and emergence tests as displayed by C57/BL6J and BALB/c mice.". Journal of Neuroscience Methods. 2008;171(1):48–52.
12. Ennaceur A. Tests of unconditioned anxiety — Pitfalls and disappointments. Physiology and Behavior. 2013;135:55–71.
13. Chi KJ, Schmitt D. Mechanical energy and effective foot mass during impact loading of walking and running. Journal of biomechanics. 2005;38(7):1387-1395.
14. Sandi CE. Healing anxiety disorders with glucocorticoids. Proceedings of the National Academy of Sciences. 2001; 108(16):6343-6344.

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