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Effects of *Phyllantus amarus* administration on the open field locomotor activities in adult Wistar rats

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ABSTRACT: Effects of administration of *Phyllantus amarus* commonly used for the treatment of jaundice, diarrhea, dysentery, urogenital disease and wound on the locomotor activities of adult wistar rats was carefully studied. The rats of both sexes (n = 24), with average weight of 200g were randomly assigned into two treatments (A and B) and control (C) groups of 8 rats each. The rats in the treatment groups (A and B) received 400mg and 800mg of aqueous extract of phyllantus amarus per kg per body weight respectively through the orogastric tube administration daily for thirty days. The control group received equal volume of distilled water daily for thirty days through the same route. The rats were fed with growers marsh obtained from Edo Feeds and Flourmill Limited, Ewu, Edo State, Nigeria and given water liberally. Rats were taken from their home cages and placed randomly unto one of the four corners of the open field facing the center and allowed to explore the apparatus for five minutes as the various behavioural scored were measured.

The findings indicate that there was a steady significant difference ($P < 0.05$) in the frequencies of line crossing, walling and defeacation between the treatments and the control groups in this experiment.

Keywords: Phyllantus amarus, open field, locomotor activities, wistar rats

Introduction

Most of the population of the underdeveloped and developing countries depend on some form of traditional and herbal medicines since ancient times. One of the plants that is widely used traditionally for the treatment of many diseases in many countries is the Phyllantus amarus [1,2]. Phyllantus amarus has bitter, astringent, cooling, diuretic, stomachic, antiseptic, antiviral, antidiabetic, hypotensive, antinociceptive, febrfuge properties and is traditionally used in the treatment of jaundice, diarrhea, dysentery, diabetes, fevers, urogenital diseases, ulcers and wounds [3,1].

Herbal medicines are widely perceived by the public as being natural, healthful and free from side effects. Plants contain hundreds of constituents and some of them may elicit toxic side effects. Toxic effect of some herbal medicines has been reported [4, 5,6] .

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Open Field Test provides simultaneous measures of locomotion, exploration and anxiety [7]. The open field apparatus was constructed of plywood and measured 72x72cm with 36cm walls. The walls and floor were both white. Blue lines were drawn on the floor with a marker and were visible through the clear plexiglass floor. The lines divided the floor into sixteen 18x18cm square and a central square of equal size was drawn in the middle of the open field (18 x 18cm)[8]. The central square is often chosen because the mice have high locomotor activity and crosses the lines of the test chamber many times during a test session. Also the central square is required to have sufficient space surrounding it to give meaning to the central location as being very distinct from outer locations [9]. Stretch attend postures are "risk-assessment" behaviours which indicate that the animal is hesitant to move from its present location to a new position and thus a high frequency of these postures indicates a higher level of anxiety [10]. Grooming behavior is a displacement response and is expected to be displayed in a novel environment [11]. Grooming behaviours should, therefore, decrease with repeated exposure to the testing apparatus. Defeacation and urination are often used as measures of anxiety, but the validity of defeacation as a measure of anxiety has been questioned [12]. However, some other workers argued that there is no significant relation between fearfulness and urination and defeacation as measured in the open field test [13]. Nevertheless they agreed that defeacation and urination in a novel environment are signs of emotionality, which is not to be equated with fearfulness or timidity [13]. Repeated exposure to the open field apparatus result in time dependent changes in behaviours [14] . At first, when the apparatus is novel to the animals more fear-related behavior (such as stretch attends and activity in the corners and walls of the open field) are displayed. However, with repeated traits more exploration and locomotors activity (such as rearing and line crosses as well as more central square activity) is observed. There are, however, strain differences in behavior after repeated testing in the open field. With repeated exposure, some strains show such increased activity while others show habituation and decreased activity levels and others show no change [15].

The aim of this study was to elucidate the possible effects of the oral administration of phyllantus amarus on the open field locomotor activities in adult wistar rats.

Materials and Methods

ANIMALS: Twenty-four (24) adult wistar rats of both sexes with average weight 200g were randomly assigned into three groups: A, B and C of (n = 8) in each group. Group A and B of (n = 16) served as treatment group while group C (n = 8) was the control. The rats were obtained and maintained in the Animal Holdings of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city, Edo State, Nigeria. The animals were fed with grower's mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria and given water liberally. The phyllantus amarus leaves were obtained in Benin City, dried and processed into aqueous extract at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

PREPARATION AND ADMINISTRATION OF PHYLLANTUS AMARUS: The plant leaves were obtained in Benin City, cleaned and oven dried at 50^{o}c. this was macerated into dry powder. This phyllantus amarus powder was extracted with distilled water using Soxhlet apparatus and concentrated by rotary evaporator at 65^{O}c. this was transferred into a suitable container and freeze dried.

Animals in group A were given aqueous leaves extract of phyllantus amarus at a single dose of 400mg/kg body weight daily for thirty days through the orogastric tube, while animals in group B received 800mg/kg body weight daily via the same route and the same period. Animals in group C received equal ml of distilled water, for the same period and through the same route of administration

APPARATUS: The open field apparatus was constructed with plywood and measured 72 x 72cm with 36cm walls. The walls and floor were both white. Blue lines were drawn on the floor with a marker and were visible through the clear plexiglass floor. The lines divide the floor into sixteen 18 x18cm squares. A central square of equal size were drawn in the middle of the open field (18 x 18cm) [8].

PROCEDURE: The maze was located in a test room and lit by a fluorescent lamp for background lighting. The open field maze was cleaned between each rat using 70% ethyl alcohol to avoid odour cues. The rats

were carried to the test room in their home cages and tested one at a time for 5 minutes each. Rats were handled by the base of their tails at all times. Rats were taken from their home cages and placed randomly into one of the four corners of the open field facing the centre and allowed to explore the apparatus for 5 minutes. After the 5 minutes test, the rats were returned to their home cages and the open field was cleaned with 70% ethyl alcohol and permitted to dry between tests. To assess the process of habituation to the novelty of the arena, rats were exposed to the apparatus for 5 minutes on two consecutive days.

BEHAVIOURAL SCORE: The behavioural score measured in this experiment include:

1. Line crossing: Frequency with which the rats crossed one of the grid lines with all four paws
2. Rearing: Frequency with which the rats stood on their hind legs in the maze
3. Rearing against a wall: Frequency with which the rat stood on their hind legs against a wall of the open field.
4. Grooming: Frequency and duration of time the animal spent licking or scratching itself while stationary.
5. Defeacation: number of fecal boli produced

STATISTICAL ANALYSIS: The value obtained from the control and treatment groups were recorded and compared statistically using the paired sample T-Test and Symmetric Measured Test of the Statistical Package for Social Sciences (SPSS)

Results

There were significant ($P < 0.05$) differences in the behaviours of line crossing, walling and defeacation between the animals treated and the control groups during the period of the phyllantus amarus administration (as shown in Tables 1, 2, 3 & 5). There were no significant ($P < 0.05$) changes observed in the behaviours of hinding and grooming between the treatments and control groups (Tables 1 & 5).

Discussion

The result of the experiment revealed that oral administration of phyllantus amarus caused significant ($P<0.05$) changes in the behavioural scores of line crossing, walling and defeacation frequencies. It revealed no significant ($P<0.05$) change in the grooming and hinding frequencies. The result of the open field locomotor activities in this study is in consonance with the findings of some investigators that recorded suppression of exploration and locomotor activities following drug administration.

The number of line crossing and the frequency of rearing are usually used as a measure of locomotor activity. Ataxia and other gait disturbances have been implicated with such drug as the antibiotics, chloroquine and quinine [16]. A high frequency of these behaviours indicated increased locomotion and exploration activities. It has been reported that administration of central nervous stimulants, such as Strychnine, picrotoxin, theosemicarbazide, nikethamide, caffeine and amphetamine to rats resulted in suppression of exploration and locomotion [17], which is in consonance with this study.

Behavioural studies have been shown that intracerebroventricular injection of glucagons diminished spontaneous locomotor activity in rats and mice, impaired exploratory activity and reduced amphetamine – induced hyperactivity. In this study, the significant difference between the control and treatments animals in the behaviours of line crossing and walling may have been attributed to phyllantus amarus toxicity in the treated rats. Effects of some central nervous system stimulants such as amphetamine, leptazol, picrotoxin, strychnine and nikethamide have been reported to significantly suppress the open field exploration and locomotor activity in mice treated with these stimulant drugs.

Table 1: The Mean Behavioural Scores of the animals in the Open Field Test.

No of Days	Open Field Test	Treatment A (n=8)	Treatment B (n=8)	Group C (Control) (n=8)
-6	Line crossing Walling Hinding Grooming Defeacation	*39.8 ± 14.0 *9.3 ± 6.0 10.2 ± 7.0 10.0 ± 5.0 *3.8 ± 8.0	*58.3 ± 4.0 *15.5 ± 3.0 8.7 ± 5.0 9.5 ± 6.0 *1.0 ± 2.0	*45.0 ± 14.0 *8.3 ± 6.0 9.3 ± 6.0 1.7 ± 2.0 *0
-3	Line crossing Walling Hinding Grooming Defeacation	*38.8 ± 23.0 *10.0 ± 4.0 7.5 ± 4.0 11.2 ± 7.0 *6.8 ± 2.0	*43.7 ± 16.0 *8.5 ± 3.0 10.7 ± 8.0 9.3 ± 4.0 *3.2 ± 3.0	*29.5 ± 10.0 *3.17 ± 3.0 13.33 ± 22.0 4.33 ± 2.0 *1.0 ± 2.0
0	Line crossing Walling Hinding Grooming Defeacation	*24.7 ± 11.0 *5.5 ± 4.0 6.7 ± 5.0 14.3 ± 7.0 *3.8 ± 2.0	*33.3 ± 15.0 *6.0 ± 5.0 5.7 ± 4.0 6.5 ± 4.0 *2.8 ± 3.0	*52.0 ± 13.0 *9.6 ± 6.0 14.5 ± 4.0 8.3 ± 4.0 *1.7 ± 2.0
3	Line crossing Walling Hinding Grooming Defeacation	*18.8 ± 15.0 *2.8 ± 2.0 4.7 ± 3.0 14.5 ± 6.0 *4.3 ± 3.0	*34.8 ± 14.0 *4.8 ± 3.0 5.3 ± 3.0 7.2 ± 3.0 *4.4 ± 4.0	*35.5 ± 21.0 *7.8 ± 9.0 10.8 ± 8.0 6.0 ± 5.0 *2.5 ± 2.0
6	Line crossing Walling Hinding Grooming Defeacation	*27.7 ± 8.0 *2.8 ± 2.0 3.3 ± 3.0 3.7 ± 4.0 *14.0 ± 5.0	*35.5 ± 11.0 *8.7 ± 4.0 5.7 ± 7.0 4.2 ± 3.0 *1.8 ± 1.0	*32.7 ± 22.0 *1.8 ± 2.0 4.2 ± 3.0 2.2 ± 1.0 *2.0 ± 4.0
9	Line crossing Walling Hinding Grooming Defeacation	*25.8 ± 9.0 *5.2 ± 3.0 6.0 ± 4.0 11.7 ± 9.0 *3.3 ± 3.0	*31.2 ± 13.0 *5.0 ± 3.0 5.8 ± 3.0 7.8 ± 2.0 *2.5 ± 2.0	*32.2 ± 22.0 *1.8 ± 2.0 4.2 ± 3.0 2.2 ± 1.0 *2.0 ± 4.0
12	Line crossing Walling Hinding Grooming Defeacation	*23.7 ± 12.0 *3.7 ± 4.0 4.0 ± 6.0 10.2 ± 5.0 *4.0 ± 3.0	*3.7 ± 4.0 *1.3 ± 1.0 3.0 ± 3.0 5.8 ± 3.0 *3.2 ± 3.0	*20.7 ± 15.0 *4.5 ± 3.0 4.8 ± 3.0 2.5 ± 1.0 *1.7 ± 1.0
15	Line crossing Walling Hinding Grooming Defeacation	*23.4 ± 9.0 *4.0 ± 3.0 2.4 ± 2.0 7.8 ± 5.0 *6.6 ± 4.0	*22.8 ± *3.8 ± 2.0 6.2 ± 4.0 7.8 ± 3.0 *1.7 ± 1.0	*25.3 ± 22.0 *2.8 ± 5.0 1.7 ± 1.0 14.0 ± 8.0 *2.8 ± 4.0
18	Line crossing Walling Hinding Grooming Defeacation	*22.6 ± 16.0 *2.6 ± 2.0 9.6 ± 6.0 9.2 ± 4.0 *2.0 ±	*11.3 ± 5.0 *1.3 ± 3.0 3.0 ± 4.0 10.0 ± 5.0 *1.8 ± 1.0	*28.7 ± 17.0 *9.3 ± 5.0 3.0 ± 2.0 5.9 ± 4.0 *2.0 ± 3.0

No of Days	Open Field Test	Treatment A (n=8)	Treatment B (n=8)	Group C (Control) (n=8)
21	Line crossing Walling Hinding Grooming Defeacation	*18.2 ± 6.0 *2.6 ± 2.0 4.0 ± 3.0 13.8 ± 4.0 *3.4 ± 3.0	*15.8 ± 7.0 *2.2 ± 2.0 3.5 ± 2.0 10.7 ± 7.0 *4.0 ± 3.0	*20.2 ± 11.0 *3.5 ± 4.0 7.8 ± 6.0 10.0 ± 5.0 *3.0 ± 3.0
24	Line crossing Walling Hinding Grooming Defeacation	*20.6 ± 9.0 *2.2 ± 1.0 5.4 ± 3.0 12.2 ± 5.0 *4.6 ± 3.0	*16.0 ± 12.0 *2.0 ± 1.0 4.6 ± 5.0 10.4 ± 5.0 *4.0 ± 3.0	*22.7 ± 9.0 *6.0 ± 3.0 7.2 ± 6.0 10.8 ± 2.0 *3.3 ± 3.0
27	Line crossing Walling Hinding Grooming Defeacation	*15.8 ± 9.0 *2.4 ± 2.0 3.4 ± 3.0 12.0 ± 5.0 *2.6 ± 4.0	*15.0 ± 9.0 *2.2 ± 1.0 6.0 ± 3.0 7.6 ± 2.0 *5.0 ± 3.0	*30.8 ± 15.0 *4.0 ± 3.0 6.5 ± 4.0 5.2 ± 6.0 *2.5 ± 3.0
30	Line crossing Walling Hinding Grooming Defeacation	*20.6 ± 11.0 *2.0 ± 2.0 3.2 ± 1.0 9.6 ± 5.0 *3.6 ± 3.0	*16.2 ± 6.0 *4.8 ± 4.0 2.8 ± 2.0 6.2 ± 4.0 *5.2 ± 3.0	*27.33 ± 11.0 *3.8 ± 4.0 5.0 ± 4.0 7.3 ± 3.0 *3.7 ± 2.0

* Significant (P<0.05)

Table 2: The Symmetric Measure Test of the Line Crossing Behaviour in Open Field Test Between the Control and Treatments Groups of Animals

		Value	Asymp. Std. Error(a)	Approx. T(b)	Approx. Sig.
Interval by Interval	Pearson's R	.841	.098	5.166	.000(c)
Ordinal by Ordinal	Spearman Correlation	.768	.177	3.971	.002(c)
N of Valid Cases		13			

a Not assuming the null hypothesis.
b Using the asymptotic standard error assuming the null hypothesis.
c Based on normal approximation.

Table 3: The Symmetric Measure Test of the Walling Behaviour in Open Field Test Between the Control and Treatments Groups of Animals

		Value	Asymp. Std. Error(a)	Approx. T(b)	Approx. Sig.
Interval by Interval	Pearson's R	.793	.089	4.315	.001(c)
Ordinal by Ordinal	Spearman Correlation	.697	.143	3.220	.008(c)
N of Valid Cases		13			

a Not assuming the null hypothesis.
b Using the asymptotic standard error assuming the null hypothesis.
c Based on normal approximation.

Table 4: The Symmetric Measure Test of the Hinding Behaviour in Open Field Test Between the Control and Treatments Groups of Animals

		Value	Asymp. Std. Error(a)	Approx. T(b)	Approx. Sig.
Interval by Interval	Pearson's R	.507	.185	1.949	.077(c)
Ordinal by Ordinal	Spearman Correlation	.225	.292	.765	.460(c)
N of Valid Cases		13			

a Not assuming the null hypothesis.
b Using the asymptotic standard error assuming the null hypothesis.
c Based on normal approximation.

Table 5: The Symmetric Measure Test of the Defeacation Behaviour in Open Field Test Between the Control and Treatments Groups of Animals

		Value	Asymp. Std. Error(a)	Approx. T(b)	Approx. Sig.
Interval by Interval	Pearson's R	.576	.178	2.334	.040(c)
Ordinal by Ordinal	Spearman Correlation	.541	.241	2.134	.056(c)
N of Valid Cases		13			

a Not assuming the null hypothesis.
b Using the asymptotic standard error assuming the null hypothesis.
c Based on normal approximation.

The decreases locomotor activity of the treated animals compared with the control in this experiment might be partly due to the toxic effects of phyllantus amarus on the neuronal cells of the brain and the associated neurotransmitter substances. Fear behaviours which include closed arm activity, stretch attends grooming, freezing, defeacation and urination implies a greater level of emotionality or fear [12]. Bindra and Thompson (1953) agree that defeacation and urination in a novel environment is a sign of emotionality which is not to be equated with fearfulness or timidity. In this experiment, there was a significant change in the frequency of defeacation. Since defeacation is a sign of emotionality as suggested by Bindra and Thompson (1953), it beholds the fact that the emotional status of the experimental animals may have been implicated in this experiment.

It is probable that the significant ($P< 0.05$) value on line crossing, walling and defeacation in this experiment may have been due to the toxic effect of phyllantus amarus on the adult wistar rats

Conclusion and Recommendation

In conclusion, this study revealed that long term administration of phyllantus amarus affects the open field locomotor activities in the adult wistar rats. The line crossing, walling and defeacation behaviours of the locomotor activities of the treatment group in the open field test were significantly ($P<0.05$) affected. It is recommended that further studies aimed at corroborating these findings be carried out.

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