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EFFECTS OF NUTMEG CONSUMPTION ON THE OPEN FIELD LOCOMOTOR ACTIVITIES IN ADULT WIST AR RATS.

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ABSTRACT

Effects of Nutmeg consumption commonly used as a spice m various dishes, as components of teas and soft drinks or mixed in milk and alcohol on the locomotor activities of adult will star rats was carefully studied. The rats of both sexes (n = 24), with average weight of 200g were randomly assigned into two groups - Treatment (A & B) (n=16) and Control (c) (n=8) groups. The rats in the treatment groups (A & B) received 1g and 2g of nutmeg thoroughly mixed with the feeds respectively on a daily basis. The control group (c) received equal amount of feeds daily without nutmeg added for thirty two days. The growers mash feeds was obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo state, Nigeria and the rats given water liberally. Rats were taken from their home cages and placed randomly into one of the four comers of the open field apparatus facing the center and allowed to explore the apparatus for five minutes as the various behavioral scored was measured and subject statistically using the paired sample T-Test and Symmetric Measured Test of the Statistical Package for Social Sciences (SPSS) The findings indicate that there was a steady significant difference ($p < 0.05$) in the behaviours of line crossing and walling. There was no much significant changes ($P<0.05$) in the behaviours of hinding, grooming and defeacation between the Treatments and Control groups of animals.

Keywords: Nutmeg, Wistar rats, Open field, Locomotor activities

The Nutmeg plant, Myristica fragrans Houtt, is a member of the small primitive family called Myristicaceae, taxonomically placed between the Annonaceae and Lauraceae (Joseph 1980). At Present, Myristicaceae is considered as a member of Magnotiales or its taxonomical equivalents (Cronquist 1981, Dahlgren 1983), Nutmeg has long been known for its psychoactive properties (producing anxiety/fear, Hallucination), from as early as 16th century writings to current internet based site (Brenner 1993, Kelly 2003 and Forrester 2005).

Nutmeg is widely accepted as flavoring agents, are used in higher doses for their aphrodisiac and psychoactive properties in male rat (Tajuddin 2003 & 2005). Nutmeg and its Oleoresin are used in the preparation of meat products, soaps, sauces, baked foods, confectioneries, puddings, seasoning of meat and vegetables, to flavour milk dishes and punches. Powdered nutmeg is rarely administered alone, but enters into the composition of numerous medicines such as aromatic adjuncts. Medicinally, nutmeg is known for its stimulative and carminative properties (Madsen and Bertelsen 1996, Lagouri and Boskou 1995). In pregnancy and lactation, traditionally Nutmeg has been used as an abortifacient. Although this use has been largely discounted but it remains a persistent cause of nutmeg intoxication in women (De Malto 2005). The active ingredient in nutmeg is called Myristicine and is a naturally occurring insecticide and acaricide With possible neurotoxic effects on dopaminergic neurons and a monoamine oxide (Truitt, 1963; Lee, 2005). Cytotoxic and apotoxic effects of Myristicine have been reported such that cell viability was reduced by exposure to Myristicine in a dose and dependent manner (Lee 2005).

The Open Field Test provides simultaneous measures of locomotion, exploration and anxiety (Walsh and Cummins 1976). The open field apparatus was constructed of plywood and measured 72x72cm with 36cm walls. The walls and floor were both white and 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) (Brown et al 1999). The central square is often chosen because the rats 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 (Carrey et al 2000). 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 (Blanchard et al 2001). Grooming behavior is a displacement response and is expected to be displayed in a novel environment (Espej 1997). 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 (Lister 1990). However, some other workers argued that there is no significant relation between fearfulness, urination and defeacation as measured in the open field test (Bindra, and Thompson 1953). 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 (Bindra and Thompson 1953). Repeated exposure to the open field apparatus result in time dependent changes in behaviours (Choleris et al 2001). At first, when the apparatus is novel to the animals more fear-related behaviour (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 behaviour 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 (Bolivar et al 2000). The aim of this study was to determine the possible effect of Nutmeg on the Open field locomotor activities in adult wistar rats.

MATERIALS AND METHODS

ANIMALS: Twenty-four adult wistar rats of both sexes with average weight of 200g were randomly assigned into three groups: A, Band C of (n=8) in each group. Group A and B served as treatment groups (n=16) while group C (n=8) served as the control. The rats were obtained and maintained in the Animal Holding 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 Nutmeg seeds were obtained from Oba Market, Benin City, Edo State, Nigeria. They were dried and graded into powder at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City.

NUTMEG ADMINISTRATION: The rats in the treatment groups (A and B) were given

1g and 2g of Nutmeg thoroughly mixed with the growers mash respectively on a daily basis for thirty-two days. The control group (c) received equal amount of feeds without Nutmeg added for the same period of thirty-two days.

APPARATUS: The open field apparatus was constructed with plywood and measured 72 x 72cm with 36cm walls. The walls and floor were both white and 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) (Brown et al 1999).

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.

BEHA VIOURAL 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 against a wall: Frequency with which the rat stood on their hind legs against a wall of the open field.
3. Rearing against a wall: Frequency with which the rat stood on their hind legs against a wall if 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 version 15)

RESULTS

There were significant differences ($p<0.05$) in the frequencies of line crossing and walling between the animal treated and control groups during the period of the nutmeg consumption (as shown in table 1 and 2 below). There was no significant change ($p<0.05$) observed in the frequencies of hinding, grooming and defeacation between the treatment and control groups (table 1 and 2)

Table 1: The Mean Behaviour 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)
-4	Line crossing	44.7±210	44.7 ± 210	45 ± 14.0
	Walling	9.3 ± 7.0	10.7 ± 4.0	8.3 ± 6.0
	Hinding	3.6 ± 7.0	3.2 ± 1.0	9.3 ± 6.0
	Grooming	3.5 ± 3.0	3.2 ± 1.0	1.7 ± 2.0
	Defeacation	6.7 ± 1.0	1.2 ± 2.0	0
0	Line crossing	34.7 ± 10.0	29.8 ± 10.0	29.5 ± 10.0
	Walling	9.8 ± 5.0	9.3 ± 6.0	3.2 ± 3.0
	Hinding	4.5 ± 6.0	5.8 ± 4.0	13.3 ± 22.0
	Grooming	6.5 ± 2.0	6.2 ± 2.0	4.3 ± 2.0
	Defeacation	6.7 ± 1.0	0	1.0 ± 2.0
4	Line crossing	53.0 ± 12.0	47.3 ± 6.0	52.0 ± 12.0
	Walling	11.8 ± 4.0	13.0 ± 4.0	9.6 ± 6.0
	Hinding	7.8 ± 4.0	5.0 ± 2.0	14.5 ± 4.0
	Grooming	3.2 ± 2.0	5.0 ± 2.0	8.3 ± 4.0
	Defeacation	2.7 ± 2.0	1.7 ± 2.0	1.7 ± 1.0
8	Line crossing	44.3 ± 17.0	49.0 ± 9.0	39.7 ± 22.0
	Walling	5.8 ± 3.0	10.8 ± 2.0	8.0 ± 5.0
	Hinding	5.2 = 1.0	6.2 ± 3.0	6.0 ± 3.0
	Grooming	4.2 ± 1.0	4.7 ± 2.0	6.5 ± 2.0
	Defeacation	2.1 ± 2.0	1.7 ± 2.0	1.2 ± 1.4
12	Line crossing	25.5 ± 13.0	38.2 ± 12.0	35.5 ± 20.0
	Walling	5.5 ± 4.0	8.5 ± 4.0	7.8 ± 8.0
	Hinding	6.2 ± 4.0	1.7 ± 7.0	10.8 ± 8.0
	Grooming	4.5 ± 4.0	4.0 ± 2.0	7.0 ± 5.0
	Defeacation	2.0 ± 2.0	1.2 ± 2.0	2.5 ± 3.0
16	Line crossing	25.5 ± 10.0	33.5 ± 8.0	31.0 ± 23.0
	Walling	2.7 ± 2.0	5.8 ± 2.0	1.2 ± 2.0
	Hinding	2.8 ± 2.0	7.7 ± 7.0	4.2 ± 3.0
	Grooming	6.2 ± 3.0	4.2 ± 4.0	2.2 ± 1.0
	Defeacation	1.2 ± 2.0	3.7 ± 4.0	2.0 ± 4.0
20	Line crossing	29.5 ± 12.0	52.7 ± 19.2	40.0 ± 20.0
	Walling	6.8 ± 4.0	9.8 ± 6.0	8.0 ± 6.0
	Hinding	5.0 ± 2.3	13.2 ± 7.0	6.2 ± 4.0
	Grooming	5.6 ± 3.0	4.5 + 3.0	6.5 ± 2.0
	Defeacation	8.3 ± 2.0	2.8 ± 3.0	0
24	Line crossing	21.0 ± 4.0	24.8 ± 9.0	24.8 ± 13.0
	Walling	3.3 ± 2.0	4.5 ± 2.0	4.5 ± 3.0
	Hinding	2.9 ± 3.0	5.7 ± 2.0	4.8 ± 3.0
	Grooming	6.0 ± 3.0	5.2 ± 2.0	2.8 ± 1.0
	Defeacation	1.0 ± 2.0	0.5 ± 1.0	1.3 ± 2.0

28	Line crossing	19.7 ± 10.0	37.3 ± 12.0	25.3 ± 22.0
	Walling	2.8 ± 2.0	7.5 ± 4.0	1.7 ± 1.0
	Hinding	4.3 ± 4.0	5.5 ± 4.0	14.0 ± 8.0
	Grooming	5.0 ± 3.0	8.2 ± 3.0	2.8 ± 4.0
	Defeacation	1.7 ± 4.0	1.5 ± 2.0	0
32	Line crossing	10.5 ± 6.0	24.8 ± 16.0	37.7 ± 22.0
	Walling	1.3 ± 9.0	3.7 ± 2.0	4.3 ± 5.0
	Hinding	2.0 ± 2.0	3.4 ± 5.0	10.2 ± 9.0
	Grooming	8.3 ± 4.0	4.2 ± 4.0	4.2 ± 4.0
	Defeacation	0	3.3 ± 3.0	2.0 ± 2.0

* Significant ($P<0.05$)

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

		Value	Asymp. Std. Error (a)	Approx. T (b)	Approx. Sig
Nominal by Nominal	Contingency Coefficient	.943			.242
Interval by Interval	Pearson's R	.701	.177	2.783	.024©
Ordinal by Ordinal	Spearman correlation	.693	.219	2.719	.026©
N of Valid Cases		10			

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

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

		Value	Asymp. Std. Error (a)	Approx. T (b)	Approx. Sig
Nominal by Nominal	Contingency Coefficient	.943			.242
Interval by Interval	Pearson's R	700	.111	2.771	.024©
Ordinal by Ordinal	Spearman correlation	.748	.176	3.185	.013©
N of Valid Cases		10			

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

Table IV: The Symmetric Measure Test of the Grooming Behaviour in Open Field Test Between the Control and Treatments Groups of Animal

		Value	Asymp. Std. Error (a)	Approx. T (b)	Approx. Sig
Nominal by Nominal	Contingency Coefficient	.935			.099
Interval by Interval	Pearson's R	-.272	.279	-.799	.447©
Ordinal by Ordinal	Spearman correlation	.257	.377	-.752	.474©
N of Valid Cases		10			

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

Table V: The Symmetric Measure Test of the Grooming Behaviour in Open Field Test Between the Control and Treatments Groups of Animal

		Value	Asymp. Std. Error (a)	Approx. T (b)	Approx. Sig
Nominal by Nominal	Contingency Coefficient	..884			.476
Interval by Interval	Pearson's R	428	.216	1.338	.218©
Ordinal by Ordinal	Spearman correlation	.366	.283	1.111	.299©
N of Valid Cases		10			

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

DISCUSSION

The result of this research revealed that nutmeg consumption showed a significant ($P<0.05$) changes in the behavioural score of line crossing and walling 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 (hinding and walling) 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 (Eisenhaver et al 1998). A high frequency of these behaviours indicates increased locomotion and exploration activities. In line with this study, it has been reported that administration of central nervous stimulant such as strychnine, picotoxin, caffeine, the osemicarbazide, nikethamide and amphetamine to rats resulted in suppression of exploration and locomotion (Agarwal 1995).

Microanalysis of locomotor patterns of group-reared mice in the staircase and methamphetamine increased the number of climbing from the first to third step. This result suggested that isolation rearing causes an anxiety-like state with increased exploratory behaviour in mice (Yukio et al 2007). Open field activity monitoring provides a comprehensive assessment of the motor as well as behavioural activities of mice and rat. It is an ideal method for assessing the degree of locomotor impairment in myopathic muscle function and locomotor (Nagaraju et al 2007 and Raben et al 1998). Open field exposure is commonly used as a measurement of anxiety- related behaviour (Belzung et al 2001 and Prut et al 2003). Behaviour in the open field test is influenced by genetic variation (Sakai et ill 2001 and Van der Staay et at 1990), sex (Ramos et ill 2002) age (Colorado et at 2006) and exposure to illumination (Bowman et al 2006) in the chamber. Bright light can be used as an aversive stimulus in the open- field paradigm leading to an increase in anxietyrelated behaviours (Cunha et at 1978 and Valle 1970).

A study carried out to investigate the relationship between noveltyseeking behaviour and operant oral ethanol self- administration in wistar rats suggested that there was no relationship between novelty seeking and operant ethanol self administration in wistar rats (Przemyslaw et ill 2001). Goeders and Guerin, (1996) has shown that there is no association between the locomotor responses to a novel environment and cocaine self administration. There is a rather moderate relationship between novelty.seeking and alcohol consumption observed only under specific experimental conditions (Nowak et at 2000). Matemal separation of neonatal rats increased locomotion and exploration behaviour of male adult rats and enhances the anxiolytic effects of diazepam (Noppamars et al 2008). There is evidence that maternal separation of neonates' rats may influence the adult rat behaviours and the responsibility to psychotropic drugs (Lovic et at 2004; Wongwitdecha et ill 2005, Wongwitdecha, 2007 and Zimmerberg et at 2003).

Behavioural studies have shown that intra-cerebro-ventricular injection of glucagon diminished spontaneous locomotor actiVity in rats and mice, impaired exploratory activity and reduced amphetamineinduced hyperactivity. In this study, the significant changes between the control and treated animals in line crossing and walling frequencies may have been attributed to the effect of nutmeg toxicity in the treated rats. Several studies have shown that administration of nutmeg can produce psychoactive properties (Brenner 1993, Kelly 2003 and Forrester 2005). Effects of some central nervous system stimulants such as amphetamines, 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. The significant difference in the locomotor activity of the treated animal compared with the control in this experiment might be partly due to the neurotoxic effects of nutmeg on the neuronal cells of the brain and the associated neurotransmitter substances.

In this study, the significant difference between the control and treatment animals in the behaviour of line crossing and walling may have been attributed to nutmeg 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.

The decreased locomotor activity of the treatments animals compared with the control in this experiment might be partly due to the neurotoxic effects of nutmeg on the neuronal cells of the brain and the associated neurotransmitter substances. Fear behaviors which include closed arm activity, stretch attends grooming, freezing, defeacation and urination which implies a greater level of emotionality or fear (Lister, 1990) was not observed. In this experiment, there was no 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 not been implicated.

Since the neurons of the central nervous system is affected by nutmeg, it is probable that the significant ($P< 0.05$) value on line crossing and walling together with the slight change value on defeacation in this experiment may have been due to the neurotoxic effect of nutmeg on the neuronal cells of the brain of adult wistar rats. It is probable that the significant value obtained in this experiment in the line crossing and walling frequencies may have been due to the neurotoxic effects of nutmeg on the brain cells of adult wistar rats.

CONCLUSION AND RECOMMENDATION

In conclusion, this study revealed that chronic administration of nutmeg has a significant ($P<0.05$) effect on the locomotor activities of the adult wistar rats. The line crossing and walling behaviours of the locomotor activities of the treated group in the open field test were significantly ($P<0.05$) affected as compared to the control group.

It is recommended that further studies aimed at corroborating these findings should be carried out most especially on the histological, histochemical and biochemical studies.

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