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Pharmacological evaluation of the central nervous system activity of *Aframomum melegueta* seed extract in mice

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Abstract

Objective: To study the effects of intraperitoneal injection of aqueous seed extract of *Aframomum melegueta* (AM) on the central nervous system (CNS) in mice. Materials and methods: The study sought to evaluate the effects of the extract on the general behaviour of the animals (Irwin test), on pentobarbitone-induced sleeping time, on methamphetamine-induced stereotyped behaviour, on motor coordination, and on convulsive seizures induced by isoniazid and picrotoxin. AM was tested at a dose range of 5-400 mg/kg. Results: AM (50–200 mg/kg) produced a significant decrease in spontaneous motor activity and also caused a dose-related prolongation of pentobarbital-induced sleeping time. At a dose range of 100-400 mg/kg, a significant inhibition of methamphetamine (35 mg/kg, i. p) induced stereotyped behaviour was observed. Furthermore, it offered a significant protection against convulsions induced by isoniazid (200 mg/kg, i.p). However, it failed to modify the convulsive action of picrotoxin (10 mg/kg, i.p) and did not cause any significant effect in the motor coordination of animals on the rota-rod machine. Conclusion: The results of this study suggest that *A. melegueta* seed extract possesses central nervous system depressant activity.

Keywords: *Aframomum melegueta,* sedative, anti-stereotypic, anticonvulsant, pentobarbital, methamphetamine, isoniazid

1. Introduction

The seed of *Aframomum melegueta* K. Schum (Zingiberaceae), popularly known as the 'grain of paradise', is commonly used as a condiment in African dishes. It is a herb with narrow leaves and ovate fruits, found in the coastal regions of Nigeria. The fruit contains reddish-brown seeds, which are strongly aromatic and pungent [1]. The pungency of the seed is due to the presence of alkaloid- and flavoniod-like chemical substances such as 6-paradol and related compounds, which have been shown to be responsible for its pharmacological actions [2]. The seed extract of this plant is used by natives of various regions of the world for diarrhoea, stomachache, inflammatory painful conditions, as carminative and it is believed to

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possess mild CNS stimulant activity [3]. The seeds of this plant is reported to be widely, chewed as a CNS stimulant by the natives of Northern Nigeria [4].

Although, the anti-ulcer, cytoprotective, anti-microbial and analgesic properties of the seed extract of this plant have been described previously [2, 3, 5], no detailed experimental studies have shown the effects of the seed extract of this plant on the central nervous system. This study therefore presents the results of detailed investigations of the effects of the seed extract of *A. melegueta* on the central nervous system in mice.

2. Materials and methods

2.1 Drugs

Pentobarbital and Chlorpromazine (May and Baker Ltd., England), Diazepam and Methamphetamine (Roche Ltd., England), Picrotoxin (Sigma Chemical Co., USA) and isoniazid (Hamex Medica Ltd., United Kingdom) were used as experimental drugs. Experiments were conducted between 10.00 AM and 4.00 PM.

2.2 Plant material

The dried fruits of *A. melegueta* were obtained from Mushin Market, Lagos, Nigeria and identified by Prof. J.D Olowokudejo, of the Department of Botany and Microbiology, University of Lagos, Nigeria. Voucher specimen of the fruits, were deposited in the herbarium of the Department of Pharmacognosy, College of Medicine, University of Lagos, Nigeria.

2.3 Extraction procedure

The seeds were ground into fine powder and 150 g of the powdered seeds was soaked in 500 ml of distilled water for 48 h. The solution was filtered and the filtrate was evaporated to dryness at less than 40°C. The yield of the extract was 10.6%, with reference to the powdered seeds. On any experimental day, 500 mg of the residue was dissolved in 10 ml of distilled water and made ready for use. It is referred to as AM.

2.4 Animals

Albino mice (18-30 g) used in the study, were obtained from Laboratory Animal Center, College of Medicine, University of Lagos. They were allowed free access to food and water. The extract, normal saline (control group) and standard drugs were administered to the animals intraperitoneally.

The following experimental procedures were used:

2.5 General behavioural assessment

The modified procedure described by Irwin was used for the assessment of the general behavioural effects produced by the extract in mice [6]. Adult albino mice were divided into five groups each group containing 6 animals. The first 4 groups were injected with aqueous extract of AM at different doses (10, 50, 100 and 200 mg/kg) and the fifth group was treated with saline (10 ml/kg). The effects were observed at 30 min intervals in the first hr and at hourly intervals for the next 4 h for the following parameters:

2.5.1 Spontaneous motor activity

This was tested by placing each mouse in an observation chamber (21 x 18 x 15 cm). Mice usually show a moderate degree of inquisitive behaviour.

2.5.2 Awareness

This was tested by assessing, the ability of the animal to right itself, when placed on the back. Loss of righting reflex indicates loss of consciousness.

2.5.3 Touch response

This was carried out with a touch at the side of the neck, on the abdomen and on the groin.

2.6 Rota-rod test

The effect of AM seed extract on motor coordination was assessed as described by Dunham and Miya [7]. The animals were trained by placing them on the rotating rod (18 rev./min), twice daily for three consecutive days, before the experiment. Those animals that performed on the rotating rod for 180 s were selected for further testing. Selected animals (10 mice/group) were tested 30 min after administration of normal saline or AM (10-200 mg/kg).

The results were expressed, as the duration of time the animals were able to maintain themselves on the rotating rod. The cut-off period of 180 s was used in the study. Statistical analysis was performed using ANOVA followed by post hoc test.

2.7 Sodium pentobarbital induced sleeping time

The animals (10 mice/group) were pretreated with either normal saline or AM (5-200 mg/kg), 30 min before i.p injection of pentobarbital (45 mg/kg). Sleeping time was considered to be the interval between loss and gain of righting reflex. Statistical analysis was carried out using ANOVA followed by post hoc test.

2.8 Amphetamine-induced stereotypy

The ability of AM to interfere with the stereotyped behaviour induced by methamphetamine (35 mg/kg) was carried out according to the method of Bourin *et al.* [8]. The animals (10 mice/group) were pretreated either with AM (100-400 mg/kg), diazepam (2 mg/kg) or chlorpromazine (2.0 mg/kg) or normal saline prior to administration of methamphetamine.

Each mouse was observed in a cage (21 x 16 x 14 cm) for 2 min at 10, 30, 90, and 120 min after methamphetamine injection. Stereotypy was scored as: (0) absence of stereotyped behaviour, (1) presence of stereotyped movements of the head and intermittent sniffing, (2) sniffing and chewing and (3) chewing and intense licking. The results were expressed as the mean of these values in each group. Statistical analysis was performed, by using Mann-Whitney U test.

Table 1.
Effect of *A. meleguata* seed extract on the motor performance in mice.

Treatment	Dose (mg/kg)	Performance on the rota-rod (s)
Saline-control	-	176 ± 1.02
A. melegueta	10	177.10 ± 0.32
A. melegueta	50	176.5 ± 0.40
A. melegueta	100	175.2 ± 1.0
A. melegueta	200	174.3 ± 2.32

Each value represents the mean ± SEM of 10 mice/group. The differences with respect to saline-control group was not significant at $p < 0.05$ (ANOVA followed by post hoc test).

Table 2.
Effect of *A. melegueta* on pentobarbital (45mg/kg, i.p) induced sleeping time in mice.

Drug	Dose mg/kg	Sleeping time (min)
Pentobarbital-control	-	58.4± 8.4
A. melegueta	5	65.0±6.7
A. melegueta	10	67.0±2.4
A. melegueta	25	89.6±3.8*
A. melegueta	50	90.2±4.0*
A. melegueta	100	97.2±9.4*
A. melegueta	200	106.7±7.6*

*$p < 0.05$ compared to control group (ANOVA followed by post hoc test). Values are expressed as mean ± SEM of 10 animals/group

2.9 Anticonvulsant assay

The animals (10/group) were pretreated with either AM (50, 100 and 200 mg/kg), normal saline or diazepam (2 mg/kg) 30 min before administration of picrotoxin (10 mg/kg) or isoniazid (200 mg/kg). The animals were observed for the onset of convulsions for 30 min and 2 h after injection of picrotoxin and isoniazid respectively. Data obtained were analysed using ANOVA followed by post hoc test.

3. Results

3.1 Behavioural effects

The animals pretreated with AM (50-400 mg/kg) showed decrease in spontaneous motor activity and touch response. There was no loss of righting reflex, at the tested doses of the extract.

3.2 Motor coordination

AM did not cause any significant effect in the motor coordination of animals tested on the rota-rod machine (Table 1).

3.3 Pentobarbital sleeping time

At a dose range of 5-10 mg/kg, AM did not significantly prolong sleeping time induced by pentobarbital (45 mg/kg, i.p) in mice. However, at higher doses (25-200 mg/kg), it significantly ($p < 0.05$) prolonged pentobarbital-induced sleeping time in a dose-related manner (Table 2).

3.4 Anticonvulsant properties

Isoniazid (200 mg/kg, i. p) induced tonic–clonic convulsions and death within 60 min of administration. However, oral administration of AM (100-200 mg/kg) delayed significantly the onset of convulsions or death and also reduced the mortality rate induced by the agent. However, it did not modify the convulsive effects of i.p injection of 10 mg/kg of picrotoxin. On the other hand, diazepam (2.0 mg/kg) offered significant protection against convulsions induced by isoniazid and picrotoxin (Table 3).

3.5 Methamphetamine-induced stereotypy

Stereotypy induced by methamphetamine (35 mg/kg, i.p) was significantly inhibited by AM

Table 3
Anticonvulsant properties of an aqueous seed extract of *A. melegueta* (AM) in mice.

Drug	Dose mg/kg i.p.	Picrotoxin mg/kg i.p.	Isoniazid mg/kg	Onset of convulsions (min)	No. of mice that convulsed /No. Used	Onset of death (min)	% Mortality	% Protection
Saline	-	-	200	45.0 ± 2.0	10/10	59.8 ± 1.8	100	-
AM	50	-	200	52.1 ± 6.2	10/10	69.0 ± 9.1*	90	10
AM	100	-	200	69.5 ± 8.7*	10/10	82.6 ±11.4*	50	50
AM	200	-	200	58.5 ± 7.8*	10/10	75.8 ± 4.1*	60	40
Diazepam	2.0	-	200	73.0 ± 5.2*	6/10	-	-	100
Saline	10	-	-	7.2 ± 0.4	10/10	12.6 ± 1.0	100	-
AM	50	10	-	7.8 ± 0.4	10/8	13.0 ± 0.4	100	-
AM	100	10	-	8.5 ± 0.4	10/10	13.5 ± 0.3	100	-
AM	200	10	-	9.0 ± 0.7	10/10	14.8 ± 1.2	100	-
Diazepam	2.0	10	-	13.4 ± 0.7*	10/10	19.0 ± 0.9*	60	40

*$p < 0.05$ with respect to saline-control group (ANOVA followed by post hoc test).

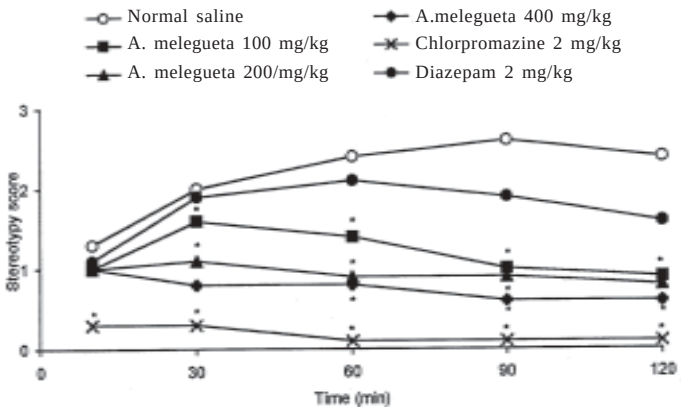

Fig. 1.
Effect of Aframomum melegueta on steraotype induced by methamphetamine (35 mg/kg, i.p.) in mice. Each point represents the mean ± SEM of 10 animals/ group *p<0.05 compared to saline-treated group (Mann-Whitney U test).

(100-400 mg/kg) dose-dependently and by i.p injection of 2 mg/kg of chlorpromazine (Fig. 1).

4. Discussion

The results of the present study demonstrated that the seed extract of *A. melegueta* produced a significant decrease in spontaneous motor activity and prolonged sodium pentobarbital induced sleeping time and inhibited the stereotypy produced by methamphetamine in mice. Furthermore, it reduced the number of animals that died and also delayed the onset of convulsions and death elicited by isoniazid in mice. However, it did not cause significant motor non-coordination in mice.

The inhibitory effect of *A. melegueta* on spontaneous motor activity as well as prolongation of pentobarbital-induced sleeping time, suggest that it has central nervous system depressant action. This observation is further supported by its ability to inhibit stereotyped behaviour and hyper-locomotion induced by methamphetamine. It is well known that the stereotypic effect of methamphetamine is due to the release of dopamine [8,10] and the ability of *A.melegueta* to inhibit this behavior in the same manner as chlorpromazine, therefore suggests that this plant has antidopaminergic activity.

According to Kendal *et al.* [11] the anticonvulsant potential of an agent, is not only measured by it ability to prevent convulsions but also to delay the onset of seizures. In this study, *A. melegueta* showed significant anticonvulsant activity, as it reduced the number of animals that died and also delayed the onset of convulsions as well as death elicited by isoniazid.

However, it did not modify the convulsive actions of picrotoxin. Dissimilarity in their mode of action may be responsible for this observation. It has been shown that isoniazid induces tonic-clonic seizures, typically related to status epilepticus by inhibiting glutamic acid decarboxylase, the enzyme responsible for the synthesis of gamma-aminobutyric acid [12-14].

In fact, isoniazid- treated animals, has been described as a suitable model for investigating the potential efficacy of agents that directly or indirectly enhances GABAergic transmission [13]. On the other hand, picrotoxin acts by blocking the chloride ion gate, which has been reported to be resistant to most anticonvulsant drugs [15-16].

Therefore, it may be speculated that *A. melegueta* did not influence the chloride ion channel whereby picrotoxin exerts its action, but rather enhances the function of GABAergic transmission in isoniazid-treated animals.

The seed of *A. melegueta* has been reported to contain active chemical substances such

6-paradol and related compounds [2], which have been shown to be responsible for its pharmacological activities [2,3]. Previous studies have also shown that flavonoids and alkaloids possess CNS depressant activity [1,9]. It is our belief that 6-paradol and related compounds which are either alkaloids or flavonoids in nature may be responsible for the CNS depressant activity, produced by the seed extract of *A. melegueta* in this study.

In conclusion, the results of this study, suggest that the seed extract of *A. melegueta* possess central nervous system depressant effect.

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