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Neuropharmacological evaluation of ethanol leaf extract of *Zea mays*

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Abstract

The ethanol leaf extract of *Zea mays* (170 - 510 mg/kg) was investigated for effect on the central nervous system (CNS) using open field, forced swimming, and tail suspension tests using mice. The extract was found to significantly ($p < 0.05$-0.01) increase the frequency of line crossing, rearing and walling activities of mice in open field test. The extract also decreased significantly ($p < 0.05$-0.001) the duration of immobility time of mice in forced swimming and tail suspension tests. The results of this study show that the leaf extract of *Z. mays* has CNS stimulatory activity and this justify its use in ethnomedicine for the treatment of central nervous system disorders.

Keywords: *Zea mays*, CNS stimulant, Open field test, Forced swimming test, tail suspension test

1. Introduction

Zea mays L. (Family: Poaceae) known as maize or corn, is an annual grass plant cultivated for its seeds which are edible and also used in animal feed. It originated from South America and was introduced to Nigeria in the 16th century [1]. It is tall with strong erect stalks and a fibrous root system. The long narrow leaves are spaced alternately on opposite side of the stem. It bears ears that are enclosed in modified leaves known as husks [2]. In addition to its nutritive values, maize grains, leaves, corn silks, stalk, and inflorescence are employed in traditional medicine for the treatment of several ailments. The corn silk is used as an antidiabetic or diuretic, and decoction of the silk is consumed for the treatment of urinary troubles and gallstones [3, 4, 5]. The ash of the cob is used for the treatment of cough [3] as well as inflammatory diseases. The husks are used in the treatment of pains and arthritis [6]. Warm tea made from the husk and leaf is taken for the treatment of malaria and other diseases in Ibibio traditional medicine. Biological activities reported on the leaf extract include; anticancer [7], antioxidant [8] and antioxidative stress [9, 10, 11], antiinflammatory and analgesic [12], antimalarial and antiplasmodial [13] activities. The LD_{50} value for the leaf extract was reported to be 1732.05 mg/kg [13]. Information on the biological activities of the leaf extract is scarce. We report in this study the CNS stimulatory activity of the leaf extract to confirm its use to treat central nervous system disorders in Ibibio ethnomedicine.

2. Materials and methods

2.1. Collection of plant materials

The fresh leaves of *Zea mays* were collected in August, 2015 at farmland in Uyo village in Uyo LGA, Akwa Ibom State, Nigeria. The leaves were identified and authenticated as *Zea mays* by Dr. Margaret Bassey, a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria. Herbarium specimen (FPH 614) was deposited at the Faculty of Pharmacy Herbarium, University of Uyo, Uyo, Nigeria.

2.2. Extraction

The plant parts (leaves) were washed and air- dried on laboratory table for 2 weeks. The dried leaves were pulverized using a pestle and mortar. The powdered leaf was macerated in 95% v/v ethanol for 72 hr. The liquid ethanol extract obtained by filtration was evaporated to dryness in a rotary evaporator 40 ˚C. The extract was stored in a refrigerator at 4 ˚C until used for experiments reported in this study.

2.3. Animals

The animals (Swiss albino mice) of either sex were used for these experiments. The animals were housed in standard cages and were maintained on a standard pellet feed (Guinea feed) and water *ad libitum*. Permission and approval for

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animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

2.4. Open field test

The animals were randomly divided into groups of 5 mice each and treated as follows for 5 days before open field test; control (normal saline, 10 mL/kg *p.o.*), imipramine (5.0 mg/kg, *p.o.*) and ethanol leaf extract of *Zea mays* (170, 340, and 510 mg/kg, *p.o.*). The open-field arena was made of acrylic (transparent walls and black floor, 30 cm (L) × 30 cm (W) × 15 cm (H)), divided into nine squares of equal areas. The open field was used to evaluate the exploratory activity of the animal [14]. The observed parameters were the number of squares crossed (with the four paws) and number of walling and rearing activities, recorded for 5 min testing period.

2.5. Forced swimming test

Mice were randomly divided into groups of 5 mice each and treated as follows for 5 days before the behavioural test; control (normal saline, 10 mL/kg *p.o.*), imipramine (5.0 mg/kg, *p.o.*) and ethanol leaf extract of *Zea mays* (170, 340, and 510 mg/kg, *p.o.*). For assessing antidepressant activities, we employed the method described by Porsolt et al. [15, 16] . The development of immobility when mice were placed inside an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. Briefly, mice were individually placed in a circular tank (46 cm tall × 20 cm in diameter) filled with tap water (25 ± 1 °C) to a depth of 20 cm and left there for 5 min. During this period, the behavior of the animals was recorded by an observer. Mice were considered immobile when remained floating without struggling and making only slight movements necessary to maintain the head above the water.

2.6. Tail suspension test (TST)

Mice of either sex were randomly divided into groups of 5 mice each and treated as follows for 5 days before tail suspension test; control (normal saline, 10 mL/kg *p.o.*), imipramine (5.0 mg/kg, *p.o.*) and ethanol leaf extract of *Zea mays* (170, 340, and 510 mg/kg, *p.o.*). The total duration of immobility induced by tail suspension was measured according to the methods described by Steru *et al.,* [17]. Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Mice were considered immobile only when they hung passively and were motionless.

2.7. Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using ANOVA (One-way) followed by a post test (Tukey-kramer multiple comparison test). Differences between means were considered significant at 5% level of significance ($p \leq 0.05$).

3. Results

3.1. Open field test

Administration of leaf extract of *Zea mays* (170 – 510 mg/kg) for 5 days caused significant ($p < 0.05 – 0.01$) dose-dependent increase in the frequency of line crossing when compared to control. The standard drugs, imipramine (5 mg/kg), caused a significant ($p < 0.001$) higher increase in the locomotor activity of the mice as evident in the frequency of the line crossing (Table 1).

Zea mays leaf extract (170 – 510 mg/kg) caused prominent increase in walling frequency of the mice which was significant ($p < 0.05$) when compared to control. These effects were dose-dependent. The standard drug, imipramine (5 mg/kg), produced a significant ($p < 0.001$) increase in the walling frequency of the animals.(Table 1).

The leaf extract of the *Zea mays* (170 – 510 mg/kg) caused significant ($p < 0.001$) dose-dependent increase of the rearing frequency of mice administered with the extract for five days. Similarly, the standard drug, imipramine (5 mg/kg), exerted a significant ($p < 0.001$) increase in the rearing frequency when compared to control.(Table 1).

Table 1. Effect of ethanol leaf extract of *Zea mays* on locomotive behavior of mice during open field test.

Treatment	Dose mg/kg	Line crossing	Walling	Rearing
Control normal saline	-	29.23 ± 2.78	6.28 ± 1.30	2.21± 0.14
Immipramine	5	84.24 ± 3.18[c]	24.30 ± 2.17[a]	7.20 ± 0.15[c]
Crude extract	170	68.95 ± 2.76[c]	14.12 ± 1.34	6.25 ± 0.25[c]
	340	76.34 ± 2.75[c]	22.45 ± 2.54[a]	7.24 ± 1.45[c]
	510	81.38 ± 4.39[C]	30.14 ± 5.12	10.62 ± 0.16

Data are expressed as mean ± SEM, significant at [a]$p < 0.05$, [c]$p < 0.001$, when compared to control. (n=6).

3.2. Effect on forced swimming test

Administration of the ethanol leaf extract of *Zea mays* (170 – 510 mg/kg) to mice for five days significantly ($p <$ 0.05-0.01) reduced immobility duration dose-dependently in mice during forced swimming test when it was compared

Table 2. Effect of ethanol leaf extract of *Zea mays* on behavior of mice during forced swimming test.

Treatment	Dose mg/kg	Duration of immobility (sec)
Control normal saline	-	120.24 ± 6.92
Imipramine	5	88.56 ± 5.60[a]
Extract	170	101.22 ± 6.55[b]
	340	95.14 ± 4.28[b]
	510	90.27 ± 6.29[b]

Data are expressed as mean ± SEM, significant at [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, when compared to control. (n=6).

to control. The standard drug, imipramine (5 mg/kg) similarly produced a significant ($p < 0.001$) reduction in the immobility time of the mice when compared to control.(Table 2).

3.3. Effect on tail suspension test

Administration of the ethanol leaf extract of *Zea mays* (170 – 510 mg/kg) to mice for five days significantly ($p < 0.001$) reduced immobility duration dose-dependently during tail suspension test when it was compared to control. The standard drug, imipramine (5 mg/kg), exerted a significant ($p < 0.001$) reduction of the immobility time of the mice when compared to control (Table 3).

Table 3. Effect of ethanol leaf extract of *Zea mays* on behavior of mice during tail suspension test.

Treatment	Dose mg/kg	Duration of immobility(sec)
Control normal saline	-	120.10 ± 6.39
Imipramine	5	76.37 ± 5.38[c]
Crude extract	170	92.36 ± 5.84 [a]
	340	87.15 ± 4.38[c]
	510	71.19 ± 6.45 [c]

Data are expressed as mean ± SEM, significant at [b]$p < 0.01$; [c]$p < 0.001$, when compared to control. (n=6).

4. Discussion

In this study, evaluation of the effect of ethanol leaf extract of *Zea mays* on central nervous system was carried out in mice using different models; Open field test, tail suspension test and forced swimming test. The leaf extract (170 – 510 mg/kg) was found to cause significant non dose-dependent increases in the frequency of line crossing, walling and rearing activities of the pretreated mice. It also reduced significantly the immobility time of the mice in forced swimming and tail suspension tests.

Monitoring of locomotor activity of animals has been used in assessing effect of drug on the CNS. An increased movement is a measure of the level of excitability of the CNS [18] and its decrease may be intimately related to sedation resulting from depression of the CNS [19]. Central nervous system stimulants are known to increase locomotor activity, while agents with depressant activity cause reduction in movements [20]. The leaf extract was found to increase significantly line crossing, walling and rearing activities during open field test demonstrating increased psychomotor activity and stimulatory effect on the CNS.

The CNS stimulatory effect of the leaf extract was further supported by its potential to reduce immobility time of mice during forced swimming and tail suspension tests. Forced swimming and tail suspension tests are two of the most commonly used animal models for evaluation of effect on the CNS especially depression. In the forced swimming test, the development of immobility when mice are placed into an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior [21]. The tail suspension test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. Some drugs are capable of reversing the immobility and promote the occurrence of escape related behavior.

Forced swimming and tail suspension tests which represent the behavioural despair model, claimed to reproduce a condition similar to human depression [15, 17, 22]. The tests are based on the observation that animals, following initial escape oriented movements, develop an immobile posture when placed in an inescapable chamber. The immobility is thought to reflect either a failure of persistence in escape-directed behaviour (i.e. behavioural despair) or the development of passive behaviour that disengages the animal from active forms of coping with stressful stimuli [21]. Clinically effective antidepressants (such as imipramine) typically increase the swimming efforts of the animal seeking a solution to the problem and, therefore, they decrease the duration of immobility in the forced swimming test [15]. This extract was observed in this study to also decrease the immobility time.

The results of this study suggest that the leaf extract exhibited significant CNS stimulatory activity with a strong psychomotor activity. Phytochemical constituents such as flavonoids have been implicated in stimulatory action on the CNS [23]. The leaf extract of *Z. mays* have been reported to contain some phenolics such as *p*-hydroxycinnamic acid and polyunsaturated fatty acids such as 9-Octadecenoic acid (Z)-, 2- hydroxyethyl ester, ethyl 9,12,15-octadecatrienoate,9,12,15-Octadecatrien-1-ol, ethyl (9Z,12 Z)-9,12-octadecadienoate and hexadecanoic acid, ethyl [13]. Omega-3 polyunsaturated fatty acids (PUFAs) have been suggested to provide a range of neurobiological activities through modulation of neurotransmitters, anti-inflammation, anti-oxidation and neuroplasticity [24, 25, 26, 27]. These phytochemical constituents may be responsible for the observed activity of the leaf extract in this study.

The results of this study suggest that the leaf extract of *Zea mays* possess a strong CNS stimulatory activity which maybe due to the phytochemical compounds present in the leaf.

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