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Sedative and Anticonvulsant Effects of Ethyl Acetate Fraction of *Waltheria indica* in Mice

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Abstract: This study evaluated the central action of *Waltheria indica* extract. Aqueous ethanolic extract of the plant showed bioactivity in acetic-acid induced stretches in animal model. The central effects of the most biologically active fraction (ethyl acetate) of extract of *Waltheria indica* was evaluated in mice using the elevated plus maze paradigm and the strychnine and leptazol-induced convulsions. Sedative effect was studied using the amylobarbitone-induced sleeping time. The extract fraction significantly ($p < 0.05$) increased the amylobarbitone sleeping time and protected (100%) mice from death due to pentylentetrazole convulsion. The extract failed to protect mice against strychnine convulsion, even though it delayed the time of onset of death. The exploratory activity was also significantly ($p < 0.05$) decreased in the extract treated mice. The extract blocked leptazole-induced convulsion, potentiated amylobarbitone sleeping time and decreased exploratory activity, indicating anticonvulsant and sedative actions.

Key words: *Waltheria indica*, ethyl acetate, fraction, sedative, mice

INTRODUCTION

Waltheria indica (synonym *Waltheria americana*) is a plant widespread throughout the tropics (Trease and Evans, 1996; Burkill, 2000). It belongs to the family *Sterculiaceae* and is commonly called sleepy morning. In Nigeria, the plant is widely used to treat many diseases such as giddiness and diarrhoea (Heinrich *et al.*, 1992), cough, headache and fever (Gills, 1992). Aerial part of the plant is boiled and cooled and the resulting aqueous solution is used traditionally to treat convulsion in children (Mallam Bashir, personal communication). The present study was based on the hypothesis that *Waltheria indica* contains bioactive substances that have great potentials for treatment of various illnesses. Though, there are ample scientific and empirical evidences supporting the use of plant-derived formulations for treatment of diseases, medical professionals require scientific evidence of efficacy and safety of these herbal medicines (Gamaniel and Wambabe, 1996; Bizimenyera *et al.*, 2006). Hence there is the need for the scientific validation of *Waltheria indica* before it can gain wider acceptance and use in the orthodox medicine. The chemical characterization of the *Waltheria indica* leaf extract showed the presence of mucilages, tannins, reducing sugars, saponins, alkaloids, steroid derivatives and flavonoids (Harboune and Baxtler, 1993; Burkill, 2000). Manske (1975) has reported that the extract of *Waltheria indica* is physiologically active and scientific elucidation of its diverse bioactivities is required. As some of the diseases traditionally treated with *Waltheria indica* (headaches and convulsion) are of neural origin, this present study was carried out using standard methods to evaluate some effects of extract fraction of *Waltheria indica* on the Central Nervous System (CNS).

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MATERIALS AND METHODS

Plant Material

Fresh *Walteria indica* aerial parts (stem, leaves and flowers) were collected from fields around Samaru-Zaria, Kaduna State, Nigeria in August 2006 for this study. Taxonomic identification was confirmed by Dr. M.S. Abubakar of the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen (NPR/2006) has been deposited at the Departmental herbarium.

Preparation for Extraction

The plant material was washed, air-dried and oven-dried for 2 h in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. It was then pounded into powder and sieved.

Extraction

Five hundred grams of the powdered material was soxhlet extracted with aqueous ethanol (60% v/v). The aqueous ethanol extract, upon concentration, yielded a yellow greenish residue hitherto called the extract (15.3% w/w). The extract was suspended in water and defatted with petroleum ether. It was then successively partitioned with N-butanol, acetone and ethyl acetate and the respective fractions were concentrated over water-bath. The fractions were tested for bioactivity against acetic acid-induced writhes (Koster *et al.*, 1959) and tail immersion tests in mice (Turner, 1965).

Animals

Adult albino mice weighing 21.5-27 g were purchased from the animal house of the Department of Pharmacy, Ahmadu Bello University, Zaria-Nigeria. The animals were maintained under standard nutritional and environmental conditions, having access to water and food *ad libitum*. Feeding was withdrawn 12 h before experimentation.

Acute Toxicity Test

The acute toxicity (oral LD₅₀) of the extract of *Walteria indica* aerial portion was estimated in 25 albino mice using the standard method of Lorke (1983).

Exploratory Behavioural Studies

The elevated plus maze test was used to determine the effects of extracts on exploratory activities in mice (Lister, 1987; Espejo, 1997; Brown *et al.*, 1999). The elevated plus maze was built according to the description of Lister (1987) with two open arms (30×5×15 cm) and two closed arms (30×5×15 cm) extending from a central, open square (5×5 cm). The floor of the maze was painted black which was used to measure locomotion. A black lip (4 mm high) was attached to the floor of the open arms to prevent the mice from falling off (Trullas and Skolnick, 1993). The maze was elevated on a pedestal to a height of 45 cm above the floor. All the experiments were carried out under white light according to Lee and Rodgers (1990).

Twenty four mice were used for the study. They were divided into 3 groups, 1, 2 and 3 each comprising 8 mice. Mice in groups 1 and 2 were administered the extracts at a doses of 100 and 200 mg kg⁻¹, respectively. Each mice was placed in the central square of the maze facing an open arm and its behaviour (head dips and stretch attend postures) observed. The number of head dips and stretch attend postures were observed for 10 min each and recorded. The tests were carried out 30 min after pretreatment of the animals with doses of extract (100 and 200 mg kg⁻¹), groups 1 and 2, respectively. Standard drug, chlorpromazine (1 mg kg⁻¹) was given to the third group.

Effects of Extract on Amylobarbitone-Sodium Sleeping Time

Twenty mice of both sexes weighing 20.8-25.5 g were randomly divided into 4 groups of 5 mice each. They were treated with amylobarbitone sodium 30 min after varying doses of extract of *Waltheria indica* were administered. Mice in group 1 received only amylobarbitone sodium (20 mg kg^{-1}) and they served as control. Mice in group 2 were administered 50 mg kg^{-1} of extract 30 min before the administration of amylobarbitone sodium (20 mg kg^{-1}), while those in group 3 were given first 100 mg kg^{-1} of extract and 30 min later they were administered 20 mg kg^{-1} amylobarbitone sodium. Group 4 mice were administered first 200 mg kg^{-1} of extract and 20 mg kg^{-1} of amylobarbitone sodium 30 min later. All injections were given intraperitoneally and the duration of sleep was defined as the time between losing and regaining the righting reflex (Carlini and Burgos, 1979).

Effects of Extracts on Pentylentetrazole (Leptazol)-Induced Convulsion

Twenty adult albino mice of both sexes, weight 22.6-27.1 g were randomly divided into 4 groups of 5 mice each. They were housed in plastic cages and were given access to food and water *ad libitum*.

Group 1 which serve as control was given only the vehicle, 20 mL kg^{-1} normal saline. Group 2 received a convulsive dose of 100 mg kg^{-1} of leptazol only and served as reference. Group 3 received 100 mg kg^{-1} of leptazole 30 min after administration of 100 mg kg^{-1} extract, while group 4 was given 100 mg kg^{-1} of leptazole 30 min after treatment with 200 mg kg^{-1} extract. Leptazole was administered and all animals received 30 mg kg^{-1} pentazocine intraperitoneally before treatment with strychnine and pentylentetrazole to alleviate pains associated with convulsion.

Effects of Extracts on Strychnine-Induced Convulsion

Mice were randomly divided into 4 groups of 5 mice each. Group 1 received vehicle (20 mL kg^{-1} normal saline) to serve as control. Group 2 receive 2 mg kg^{-1} strychnine alone and served as reference. Group 3 was administered 2 mg kg^{-1} strychnine 30 min after extract treatment of 100 mg kg^{-1} , while animals in group 4 received 2 mg kg^{-1} strychnine 30 min after extract treatment of 200 mg kg^{-1} . Strychnine was administered intraperitoneally. The latency for convulsive episode in each mice was evaluated in minutes.

Statistical Analysis

Results were expressed as mean \pm SEM and analyzed using the Students' t-test and analysis of variance (ANOVA). Values of $p < 0.05$ were considered statistically significant.

RESULTS

The aqueous ethanolic extract of *Waltheria indica* was yellow greenish in colour. The acute toxicity study showed low toxicity of the extract (LD_{50} , 875 mg kg^{-1}) in mice when orally administered. The results of screening of the extract fraction for bioactivity showed that the ethyl acetate fraction conferred highest protection (69%) against the acetic acid induced-writhes compared to N-butanol (54%), acetone (50%) and aqueous residue (38%), respectively. The ethyl acetate extract fraction of *Waltheria indica* also significantly ($p < 0.05$) increased the sleeping time of amylobarbitone sodium dose-dependently in mice. The sleeping time in mice treated with 200 mg kg^{-1} of the extract was significantly ($p < 0.05$) higher than in those administered 100 mg kg^{-1} of the extract; 80.0 ± 1.02 and 69.6 ± 0.9 min, respectively. The sleeping time obtained in control mice was 55.6 ± 0.6 min and the value was significantly lower ($p < 0.05$) than those obtained in groups 3 and 4 mice. The result also indicated significant ($p < 0.05$) decrease in exploratory activities (head dip and stretch attend postures) in mice

Table 1: Effects of ethyl acetate aerial parts extract of *Waltheria indica* on exploratory behaviour in mice

Pre-treatment	Dose (mg kg ⁻¹)	Exploratory activity (mean±SEM, N = 10)	
		No. of head dip	No. of stretch attended posture
Control (vehicle normal saline)	0.2 mL	21.6±1.2	17.7±0.9
Extract	100	16.2±1.1	9.8±1.5
Extract	200	14.6±0.6*	8.1±0.8
Chlorpromazine	1.0	8.7±0.4*	5.0±0.8*

N = 10; Statistical analysis was evaluated using students t-test vs control; *: p<0.05

Table 2: Effects of ethyl acetate fraction of aerial parts extract of *Waltheria indica* on pentylenetetrazol-induced convulsion in mice

Pre-treatment	Group	Dose (mg kg ⁻¹)	Onset of convulsion in min (Mean±SEM)	Duration of convulsion in min (Mean±SEM)	Onset of death in min (Mean±SEM)	Mortality (%)	Protection (%)
Control (vehicle)	1	0.2 mL	0.0	0.0	0	0	0
Pentylenetetrazol (leptazol)	2	100	1.2±0.9	71.0±2.1	24.7±0.62	80	20
Extract	3	100	2.6±0.6	59.4±1.6*	0	0	100
Extract	4	200	3.5±1.0	47.0±1.1*	0	0	100

*: p<0.05 when compared with control group; Statistically analysis evaluation by students t-test vs control

Table 3: Effects of ethyl acetate fraction of extract aerial parts of *Waltheria indica* on pentylenetetrazol-induced convulsion in mice (N = 5)

Pre-treatment	Group	Dose (mg kg ⁻¹)	Onset of convulsion in min (Mean±SEM)	No. of convulsion per min (Mean±SEM)	Onset of death in min (Mean±SEM)	Mortality (%)	Protection (%)
Control (vehicle)	1	0.2 mL	0.0	0.0	0.0	0	0
Pentylenetetrazol (leptazol)	2	2	1.4±1.4	17.9±1.7	3.0±1.2	100	0
Extract	3	100	1.4±1.6	17.0±1.1	3.2±1.1	100	0
Extract	4	200	1.5±1.0	12.5±1.0*	3.5±1.1	100	0

*: p<0.05 when compared with control group; Statistically analysis evaluation by students t-test vs control

administered 200 mg kg⁻¹ of the extract fraction (Table 1). The effects of ethyl acetate fraction of *Waltheria indica* on leptazol-and strychnine-induced convulsion in mice respectively (Table 2, 3).

The extract at doses, 100 and 200 mg kg⁻¹ protected the mice from death due to pentylenetetrazole seizures. However, the extract at similar doses could not protect the mice from death due to strychnine seizures even though it significantly (p<0.05) reduced the number of convulsions in mice treated with 200 mg kg⁻¹.

DISCUSSION

The ethyl acetate fraction of the aqueous ethanolic extract of aerial portion of *Waltheria indica* showed central nervous system depressant activity. This action was demonstrated by its effects on amylobarbitone sodium sleeping time, pentylenetetrazole and strychnine-induced convulsions and exploratory activities. Marked decrease in exploratory activities in laboratory animals is a measure of depressant action (Lister, 1990; Harvey, 1991; Brown *et al.*, 1999). The extract treated mice in this study showed decreased gross behaviour, suggesting sedative effects of the extract. This result corroborates the findings of Ming-chin (1998) and that of Wakeel *et al.* (2004) in which decreased exploratory activity and potentiation of hexobarbitone-induced sleeping time was produced, following the administration of *Cistanche deserticola* and *Ficus platyhylla* stem bark respectively, in mice.

Decreased exploratory behaviour in rats treated with extracts of *Rauwolfia vomitoria* comparable to standard sedative drug, chlorpromazine 1 mg kg⁻¹ was similarly reported by Bisong and Osim (2006). The results obtained in this study also showed significantly (p<0.05) increased amylobarbitone sleeping time, corroborating the findings of Fujimori (1995) and Sandabe *et al.* (2003) that enhancement of amylobarbitone sodium sleeping time indicates central nervous system depression.

The extract was found to completely prevent death of animal resulting from pentylentetrazole convulsions, a finding which did not agree with the assertion that most drugs with anticonvulsant actions do not counteract pentylentetrazole convulsions, but retard them (Loscher *et al.*, 1991). However, the extract failed to protect mice from strychnine convulsions, suggesting differences in mechanisms of action of these convulsive drugs.

In conclusion, extract of *Waltheria indica* has low toxicity (LD_{50} 870 mg kg⁻¹). It also caused a reduction in locomotor activity and acted apparently synergistically with amylobarbitone to increase the duration of sleep. The findings of this study has established Sedative and anticonvulsant action of the plant. The data obtain will add to the large body of evidence collected scientifically to show the immense potentials of medicinal plants use in various traditional system. Further study is required in order to establish the mechanism of action of the extract on the central nervous system.

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