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Neurobehavioural activities of the ethanol leaf extract of *Calophyllum inophyllum* in rodents

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Abstract

Aim: The present study was designed to investigate the central nervous system activities of the ethanol leaf extract of *Calophyllum inophyllum* in rodents.

Methods: The neurobehavioural tests used include the hole board, open field, elevated plus maze and the light/ dark box tests. The animals were divided into the control (normal saline, 10ml/kg) experimental (50-150mg/kg) and the reference groups (indomethacin, 10mg/kg) after which they were subjected to the above tests.

Results: The results showed that in the hole board test, the 100 and 200mg/kg doses resulted in a significant ($p < 0.01$) reduction in the number of head dips compared with the control, while in the light and dark box test there was a significant ($p < 0.01$) increase in the time spent in the dark arena compared with the control. The open field test revealed a reduction in the total locomotion compared with the control although not statistically significant, while the time spent in the closed arm increased significantly ($p < 0.05$) at all doses, the rearing frequencies were significantly ($p < 0.05$) reduced at all doses, only the 200mg/kg dose reduced the grooming frequencies significantly ($p < 0.05$).

Conclusion: These results suggest that the ethanol leaf extract of *Calophyllum inophyllum* contains some active principles which may be sedative/ anxiogenic in nature.

Keywords: Sedation, anxiogenic, exploratory behaviour, locomotion.

Résumé

Objective : Cette présente étude était désignée pour investiguer les activités du système nerveux central de l'extrait d'éthanol des feuilles de *Calophyllum inophyllum* dans les rongeurs.

Méthodes : Les épreuves neurologique-comportemental comprenaient les épreuves de la planche à trou, le champ libre, élevé plus troublé et la boîte claire/noire. Les animaux étaient divisés dans les groupes de contrôle (saline normal, 10ml/kg)

expérimental (50-150mg/kg) et référence (indométacine, 10mg/kg) après lesquels ils étaient mis aux épreuves ci-dessus.

Résultats: Les résultats montraient que dans l'épreuve de la planche à trou, les doses de 100 et 200mg/kg résultaient à une réduction significative ($p < 0.01$) dans le nombre de plonges par la tête en comparaison avec le groupe de contrôle, tandis que dans l'épreuve de la boîte claire/noire il y avait un accroissement significatif ($p < 0.01$) dans le temps passé dans l'arène noire en comparaison avec le groupe de contrôle. L'épreuve du champ libre révélait une réduction dans la locomotion totale en comparaison avec le groupe de contrôle bien que pas statistiquement significatif, tandis que le temps passé dans le clos endroit augmentait de manière significative ($p < 0.05$) à toutes doses, les fréquences d'élevage réduisaient de manière significative ($p < 0.05$) à toutes doses, seulement la dose de 200mg/kg réduisait les fréquences de passage de manière significative ($p < 0.05$).

Conclusion : Ces résultats suggèrent que l'extrait d'éthanol des feuilles de *Calophyllum inophyllum* contient certains principes actifs qui peuvent être sédatif/anxiogène en nature.

Mots clé: Sédatif, anxiogène, comportement exploratoire, locomotion.

Introduction

Calophyllum inophyllum belongs to the family Clusiaceae. It is an evergreen tree found in the tropical areas of Africa, America and Asia [1]. The tree is medium sized and averages 8- 20cm in height with a broad spreading crown of irregular branches. It is used in carving, cabinet making and boat building. The leaves are dark green, shiny and hairless with bold elliptical blades. Phytochemical analysis of the plant revealed that it contains poly unsaturated fatty acids, lipoproteins, coumarins, flavonoids, tocopherols, tocotrienols and xanthenes [2].

Previous studies on the plant revealed that the fresh bark of the plant was used for the treatment of diabetes [3], its fresh fruits for rheumatism [4] and neuralgia [5]. Based on these ethnobotanical and pharmacological reports presented above, we postulate that the plant extracts could have potential activities on the central nervous system (CNS) and the administration of these extracts could result in behavioral modifications.

To test this hypothesis, we evaluated the CNS activities of the ethanol extract of *Callophyllum inophyllum* leaves in rats and mice using some behavioural models of anxiety.

Materials and methods

Plant material and preparation of extract

The leaves of the plant were collected from the botanical garden of the University of Ibadan, Nigeria in July, 2011 after identification and authentication in the herbarium of the department of Botany where a voucher specimen with reference no FHI 106877 was also deposited for future reference.

The leaves were air dried at room temperature for about 2 weeks and then grounded in an electric mill to obtain particles less than 4mm in diameter. This material (3kg) was extracted by maceration in 60% ethanol solution at 50°C for 2hr. The extraction procedure was repeated once again under the same conditions with a new solvent. Extracts were then filtered through a Whatman No. 1 filter paper and concentrated using a rotary evaporator under reduced pressure. The yield of the extract was quantified (14.45%) and the material obtained protected from light and stored under refrigeration at 4°C until ready for use.

Drugs

Diazepam (F. Hoffmann- La Roche, Basel, Switzerland) was used as reference drug (positive control) for anxiolytic activities.

Animals

Male Swiss mice (25-30g) and rats (220-250g) were used for the study. They were obtained from the pre-clinical animal house of the College of Medicine, University of Ibadan, Nigeria. They were housed six per cage and kept for a minimum of 7 days prior to the pharmacological experiments with free access to standard rodent pellet and water. They were kept in an environment with 12hr/12hr light- dark cycle at room temperature. Each experimental group consisted of at least six animals. Behavioural observations took place in a sound-proof room and at the same period of the day to reduce the influence of diurnal variations of spontaneous behaviour. Each animal was tested once. All experiments were conducted in accordance with international standards of animal welfare recommended by the society for neuroscience [6]. The regulation of the minimum number of animals and duration of observations required to obtain consistent data were strictly adhered to.

Treatment

The extract of *Callophyllum inophyllum* was freshly dissolved in distilled water before administration.

Doses of the extract and the time intervals were determined in preliminary tests. Diazepam (1mg/kg) was dissolved in 40% propylene glycol. All administrations were performed intraperitoneally (i.p) after which the animals were subjected to the behavioural tests after a latent period of thirty minutes.

Behavioural evaluation

The hole board test

Anxiety was evaluated in mice using the hole board apparatus (35× 35× 15) cm with 16 evenly spaced holes [7]. Mice were grouped (n= 6) and treated with normal saline (10ml/kg) or extract (50, 100 and 200mg/kg) or diazepam (1mg/kg). Thirty minutes later, the mice were placed singly on the board and the number of times the mice dipped their heads into the holes during the 5 mins interval was counted [8]. Results were expressed as means for the various treatment groups.

The Light/ dark box test

The box comprises a small dark safe compartment (one third) and a large illuminated aversive compartment (two thirds). The animals were grouped (n= 6) and treated with normal saline (10ml/kg), extract (50, 100, 200mg/kg) or diazepam (1mg/kg). Thirty minutes later, the mice were placed in the lit compartment and allowed to move freely through the opening between the two compartments and the time spent in each compartment was recorded for 5mins.

The Open field test

The open field box is a rectangular area composed of a hard floor measuring 36× 36 × 26cm made of a white painted wood. The floor is divided by permanent red markings into 16 equal squares at the bottom. Generally, spontaneous motor activity was monitored for 30 mins in the open field [9]. After the various treatments as explained earlier, each mouse was introduced into the field and the total locomotion (number of squares crossed with the four limbs), rearing frequency (number of times the animals stood on its hind limbs or with fore limbs against the wall of the observation chamber or free in air) and frequency of grooming (number of body cleaning with paws, picking of the body and pubis with mouth and face washing actions) were recorded for 10 mins. The arena was cleaned with 70% alcohol to eliminate olfactory bias and allowed to dry before introducing another animal.

Elevated plus maze test

This test is widely used to assess anxiety in rodents [10 ,11]. The apparatus constructed from Plexiglas consisted of two open arms (50× 10)cm each, two

enclosed arms (50× 10× 40)cm each and a central platform (10× 10)cm, arranged in such a way that the two arms of each type were opposite each other. The maze was elevated 70 cm above the floor. Thirty minutes after the animals were given intraperitoneal *Calophyllum inophyllum* (50, 100 and 200mg/kg) or diazepam (1mg/kg), each animal was placed at the centre of the maze facing one of the enclosed arms. During the 5 mins test period, the number of open and closed arm entries, plus the time spent in each of these arms were recorded. Entry into arm was defined as the point when the animal places all four paws into the arm. After the test, the maze was carefully cleaned with 70% alcohol solution to eliminate olfactory bias.

Statistical analysis

The data was analyzed and expressed as means ± SEM. Statistical comparison was done using Student's t-test. Values with $p < 0.05$ were considered significant.

Results

Hole board test

Fig. 1 showed that there was a significant ($p < 0.01$) reduction in the number of head dips at the 100 and 200mg/kg dose levels of the extract and 1mg/kg of the reference drug. However, reduction was observed at 50mg/kg dose of the extract compared with the control, although not statistically significant.

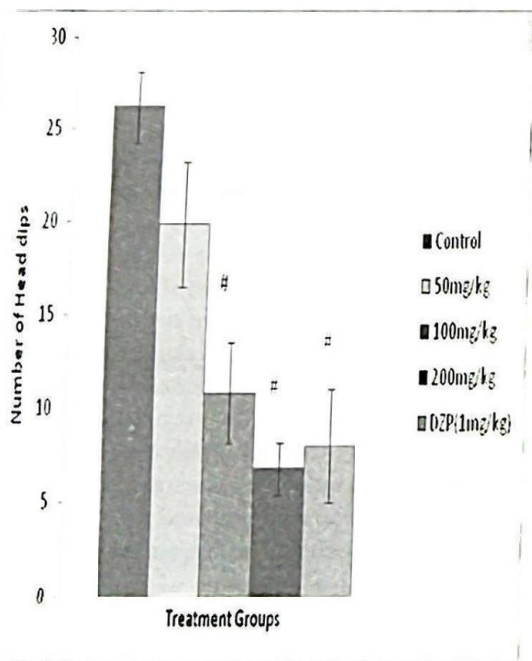


Fig 1: Effect of graded doses of ethanol leaf extract of *Calophyllum inophyllum* on the number of head dips in the hole board test. Values are expressed as means ± SEM. * $P < 0.01$ compared with control

Light/ dark box test

There was a significant ($p < 0.01$) increase in the time spent in the dark compartment and a corresponding fall in the time spent in the lit area. Fig 2

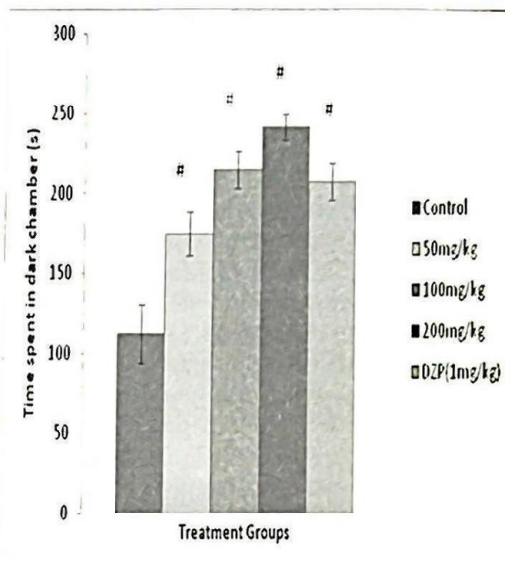


Fig 2: Effect of graded doses of ethanol leaf extract of *Calophyllum inophyllum* on the time spent in dark chamber in the light/ dark box test. Values are expressed as means ± SEM. * $P < 0.01$ compared with control.

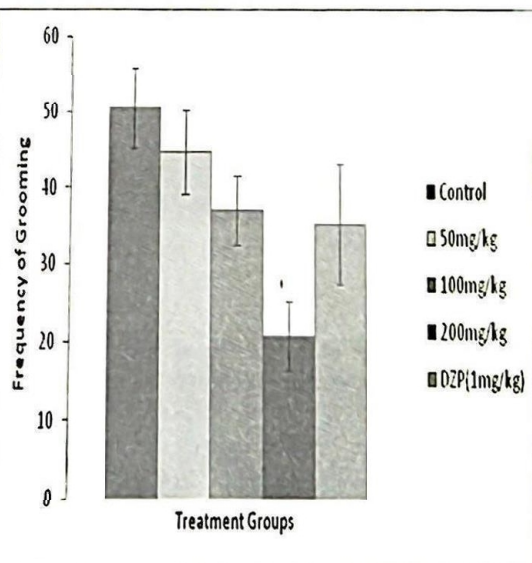


Fig 3a: Effect of graded doses of ethanol leaf extract of *Calophyllum inophyllum* on the frequency of grooming in the open field test. Values are expressed as means ± SEM. * $P < 0.01$, $n = 6$, compared with control.

Open field test

There was a significant ($p < 0.05$) reduction in the frequency of grooming only at the 200mg/kg dose

level, reduction in the frequency of grooming at the other dose levels of the extract and even the reference drug were not significant (fig. 3a). A significant reduction ($p < 0.05$) in rearing frequency was observed at 50 and 100 mg/kg of the extract. The 200mg/kg dose of the extract resulted in a greater inhibition ($p < 0.01$) of the rearing frequency than the reference drug ($p < 0.05$) compared with control (fig 3b). The extract at all doses (50- 200mg/kg) and the reference drug have no significant effect on locomotion activity of the animals (fig 3c).

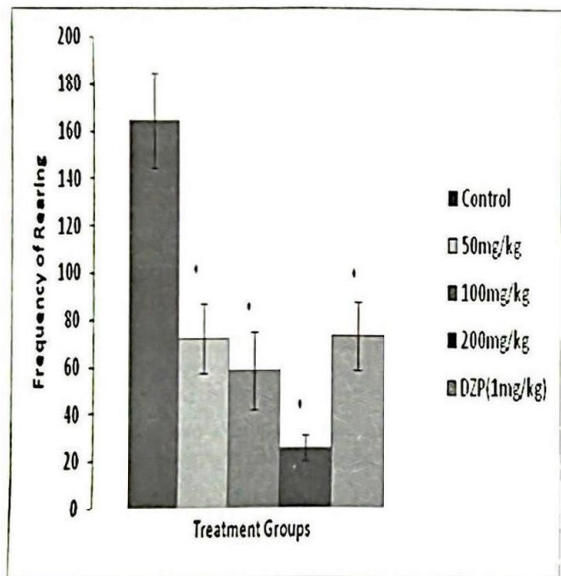


Fig 3b: Effect of graded doses of ethanol leaf extract of *Calophyllum inophyllum* on the frequency of rearing in the open field test. Values are expressed as means ± SEM. * $P < 0.05$ compared with control.

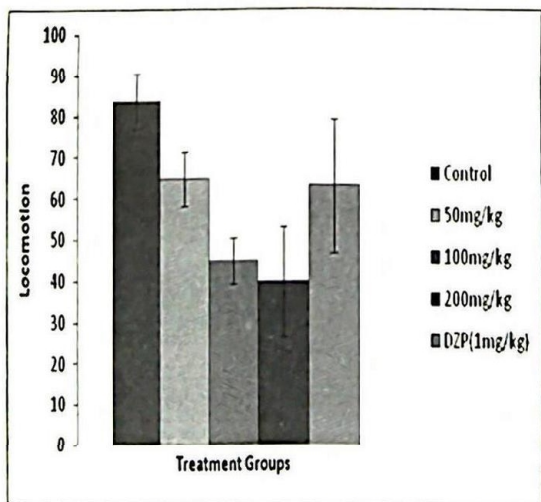


Fig 3c: Effect of graded doses of ethanol leaf extract of *Calophyllum inophyllum* on the locomotion activities in the open field test. Values are expressed as means ± SEM. NS not significant compared with control

Discussion

This study provided evidence that the ethanol extract of *Calophyllum inophyllum* leaves may contain psychoactive substances with sedative properties . Though, the extract insignificantly ($p > 0.05$) decreased locomotion activity, the decrease observed in the rearing frequency was significant at all doses ($p < 0.01$ at 50 and 100mg/kg) and ($p < 0.001$ at 200mg/kg). These observations taken together with the fact that there was a significant ($p < 0.05$) reduction in the grooming frequency at the 200mg/kg dose point towards a central depressant effect. According to Morais *et al* [12], mobile and rearing activities are functions of the CNS excitability and decrease in these parameters suggests sedative property.

The hole board test is a measure of exploratory behaviour. An agent that decreases this parameter reveals a sedative behaviour [10]. Anxiolytics have been shown to increase the number of head dips in the hole board test [13]. This is a further confirmation of the anxiogenic/ sedative nature of the extract under study since a decrease was observed in the number of head dips in this study. The extract remarkably diminished dose dependently the exploratory behaviour in mice, thereby suggesting that the extract possesses sedative rather than anxiolytic potentials.

The light/ dark box test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response. The test is useful to predict anxiolytic-like or anxiogenic- like activity in mice. We observed in this study that the extract has anxiogenic- like activity as revealed by the fact that the animals preferred to stay longer in the dark zone compared with the lit areas confirming their aversion for brightly illuminated areas.

The elevated plus maze represents one of the most widely used animal models for screening anxiogenics. It is a widely used test based on the natural aversion of rodents to height and open spaces and is sensitive to both anxiolytics and anxiogenics. The anxiogenic effect of the extract in this study was confirmed by the fact that a dose dependent decrease in time spent in the open arm of the elevated plus maze was observed compared with control. A probable explanation of this observation is based on the phytochemical studies of *Calophyllum inophyllum* showing the presence of xanthenes which has been shown to have a sedative effect [10]. In conclusion, the present study showed that the ethanol extract of *Calophyllum inophyllum* contains some psychoactive agents which may be sedative/ anxiogenic in nature.

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