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Study of the Effect of Hydro-Ethanolic Extract of *Commiphora africana* (Stem-bark) on Sleeping Time and Convulsion in Mice

[1]I. Ezekiel, [2]M.A. Mabrouk, [3]J.O. Ayo, [1]A.D.T. Goji, [1]A.O. Okpanachi,
[1]A. Mohammed and [1]Y. Tanko
[1]Department of Human physiology, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Medical physiology, Faculty of Medicine AL-Azhar University Cairo, Egypt
[3]Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria

Abstract: The hydro-ethanolic extract of *Commiphora africana* (Engl Rich A.) (stem-bark) was investigated for sleeping time and anti-convulsive activity. The anti-onvulsant activity was studied using the penylenetetrazole-induced (PTZ) seizures in ice.The sleeping time was studied using the diazepam-induced sleep in mice. The results showed that the hydro-ethanolic (stem-bark) extract of *Commiphora africana* possess significant ($p<0.05$) sleep activity. The total sleep time rose ($p<0.01$) from 38±0.71min in the control group to 61.8±1.07, 72±1.05 and 94.2±1.11min in the groups treated with the extract at the doses of 50, 100 and 200 mg/kg, respectively. The result showed that hydro-ethanolic (stem-bark) extract of *Commiphora africana* possess significant ($p<0.05$) anti-convulsant activity.The extract significantly ($p<0.05$) prolonged the onset of convulsion in the non-protected mice that were administered 100 and 200 mg/kg of the extract, which showed about 60% protection in the mice. The results of the preliminary phytochemical screening of *Commiphora africana* stem bark extract revealed the presence of flavonoids, tannin, anthraquinone, cardiac glycosides, triterpenoids, saponins, alkaloids and reducing sugars. The LD_{50} was calculated as 471.2 mg/kg in mice. It was concluded that the plant may be good in treatment of sleep disorders and may be beneficial in the management of absence and tonic-clonic seizures.

Key words: *Commiphora africana*, convulsion, diazepam, phytochemical screening, sleeping time

INTRODUCTION

Sleep is a transient reversible lose of consciousness. Epilepsy is one of the most common serious neurological diseases. In contemporary society, the frequency and importance of epilepsy can hardly be overstated from the epidermiologic studies. However, in most studies, the overall incidence of epilepsy in developed societies has been found to be around 50 cases per 100,000 persons per year, and rises steeply in older age (Poole *et al*., 2000; Ropper and Brown, 2005). The current therapeutic treatment of epilepsy with modern antiepileptic drugs are associated with side effects, dose-related and chronic toxicity, teratogenic effects, and approximately 30% of the patients continue to have seizures with current Antiepileptic drugs therapy (Poole *et al*., 2000; Samren *et al*., 1997).

Commiphora africana belongs to the family of Burseraceae and a group of plant called Myrrh (Hanus *et al*., 2005). The specific name simply means African. (Martinetz, 1993). *Commiphora africana* is a small tree, not more than 5 m high. It can be recognized unmistakably from a distance by its outline- a spherical top and a short trunk with low branches. Crown is rounded, with the branches ascending and then curving downwards. Many of the branchlets end in spines. The bark is grey-green, sometimes shiny, peeling in membranous scales; slash red, pleasantly scented, exuding a clear gum. It has a creeping root system that spreads several meters around the tree. Aliyu *et al.* (2002) reported the anti-microbial activity of *Commiphora africana* ethanolic leaf extract. Fruits are used for the treatment of typhoid fever and as a remedy for stomach problems. The powdered bark is mixed with porridge to cure malaria. The resin also has medicinal uses, including sealing and disinfecting wounds. It is applied as a plaster and used for spasms. There is a dearth of information on sedative, hypnotic and convulsive effects of *Commiphora africana* in the available literature hence the need to investigate the above-mentioned parameters.

MATERIALS AND METHODS

Animals: 45 Swiss albino mice (weighing between 19 to 30 g) of both sexes were used. They were obtained from the Animal House of the Department of Pharmacology

Corresponding Author: I. Ezekiel, Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria

and Clinical Pharmacy, ABU, Zaria. The animals were maintained on Excel feeds, Ilorin and water *ad libidun,* were kept in plastic cages at room temperature throughout the study. The experiments were conducted in a quit laboratory between the hours of 900 and 1600 h.

Plant material: Samples of the stem bark of *Commiphora africana* was collected in the mouth of February 2009 within main campus of the Ahmadu Bello University (ABU) Zaria. The plant was identified and authenticated by M. Musa of the herbarium section of the Department of Biological Science, ABU Zaria, where a herbarium specimen (N_O. 900300) was deposited for future reference.

Preparation of the plant extract: The stem bark of *Commiphora africana* was collected and dried under shade and ground into powder. The powder (500 g) was macerated in 30% of distilled water and 70% ethanol at room temperature for 24 h. It was then filtered using a filtered paper (Whatmann size no.1), and the filtrate evaporated to dryness in water bath at 60ºC. A brownish residue weighing 30.5 g was obtained. This was kept in airtight bottle in a refrigerator until used.

Acute toxicity test: The lethal dose (LD_{50}) of the plant extract was calculated by the method of Lorke (1983) using 12 mice. In the initial phase, male and female mice were divided into three groups of three mice each. They were treated with the *Commiphora africana* stem bark extract at doses of 1, 10 and 100 mg/kg per intraperitoneally. Animals were observed for 24 h for signs of toxicity. No mortality was recorded. In the second phase of the toxicity study, the animals were divided into three groups of one mouse each. They were treated with the *Commiphora africana* stem bark extract at doses of 160, 290 and 500 mg/kg i.p., The median lethal dose (LD_{50}) was calculated using the second phase.

Phytochemical screening: The preliminary Phytochemical screening of Commiphora africana extract stem bark was carried out in order to ascertain the presence or absence of various constituents utilizing standard conventional protocol (Trease and Evans, 1983; Harbone and Baxter, 1993).

Drugs used: All chemicals and drugs were obtained commercially and were of analytical grade.

(a) Diazepam-induced sleep in mice: The method described by Beretz *at el.* (1978) and modified by Rakotonirina *et al.* (2001) was adopted in this study. 20 Adult mice of either sex were divided in to 4 groups of five mice in each group. The first group was administered normal saline (1 ml/kg), intraperitoneally (i.p.,) Second, third and fourth groups were administered the extract of *Commiphora africana* at the doses of 50, 100 and 200 mg/kg (i.p.,). Thirty minutes later, diazepam at a dose of (3 mg/kg) was administered to all the mice intraperitoneally. Each mouse was then observed for the onset and duration of sleep. The criterion for sleep is the loss of rightening, in which the mice cannot roll back when turned over (Miya *et al.*, 1973). The interval between loss and recorvery of rightening reflex was used as the index of hypnotic effect (Fujimori, 1965).

(b) Pentylenetetrazole (PTZ) test: The method of Swinyard *et al.* (1989) was employed. Clonic seizures were induced in male mice by the intrapreitoneal injection of 8.5 mg/kg Pentylenetetrazole (PTZ). 25 Swiss albino mice were divided into 5 groups of 5 mice each. Group 1 received normal saline 1 ml/kg intraperitonealy. Groups 2, 3 and 4 received the extract at the doses of 50, 100 and 200 mg/kg intraperitonealy respectively. Group 5 received valproic acid 20 mg/kg i.p., the protective effect of the plant was recorded in mice treated 1 h before with the extract. The time of onset of seizures in non-protected mice was also recorded. The general clonus was characterized by forelimb clonus followed by full clonus of the body. The time taken before the onset of clonic convulsions, the duration of clonic convulsions, and the percentage of seizure and mortality protection were recorded (Vogel and Vogel, 1997).

Statistical analysis: All data were expressed as mean ± SEM. The data were analyzed statistically using one-way analysis of variance (ANOVA) with multiple comparisons versus control group. Values of $p<0.05$ were taken as significant (Duncan *et al.*, 1977).

RESULTS

Phytochemical analysis: Freshly prepared extract was subjected to preliminary Phytochemical screening test for various constituents. This revealed the presence of flavonoids, tannin, anthraquinone, cardiac glycosides, triterpenoids, saponins, alkaloids and reducing sugars.

Acute toxicity studies (LD_{50}): The sign of toxicity were first noticed after 8-10 h of extract administration. There was decrease locomotor activity, decrease feed intake, and prostration after 8 h of extract administration. The median lethal dose (LD_{50}) in mice was calculated to be 471.2 mg/kg body weight intraperitoneally.

Table 1 The effect of hydroethanolic extract of *Commiphora africana* on sleep-induced by diazepam. The onset of sleep was decreased significantly in the groups treated with the extract when compared with the control group. The duration of sleep-induced by diazepam increased significantly from 38±0.71 min in the control

Table 1: Effect of hydro-ethanolic stem bark extract of *Commphora africana on diazepam*-induced sleep in mice.

Dose (mg/kg)	Onset of sleep (min)	Duration of sleep (min)
Extract 50	21.20±0.80*	61.80±1.07*
Extract 100	15.00±0.32*	72.00±1.05*
Extract 200	11.80±0.37*	94.20±1.11**
Diazepam 3.0	24.40±0.31	38.00±0.71

Values are given as mean ± SEM; experimental groups were compared with control. Values are statistically significant at
*: $p<0.05$, **: $p<0.01$

Table 2: Effect of hydroethanolic extract of *Commiphora africana* on PTZ-induced convulsion in mice

Treatment	Onset of convulsion	Protection (%)	Mortality (%)
Distilled water 1 ml/kg	3.00±1.79	0.0	100
Extract 50 mg/kg	$4.00\pm1.14_{ns}$	20	80
Extract 100 mg/kg	6.00±1.22*	60	40
Extract 200 mg/kg	6.00±1.55*	60	40
Valproic acid 20 mg/kg	0.0	100	0.0

Values are given as mean±SEM; experimental groups were compared with control. Values are statistically significant at *: $p<0.05$, ns = not significant

group to 61.80±1.07, 72.00±1.05 and 94.20±1.11 in the groups treated with the extract at the doses 50, 100 and 200 mg/kg, respectively. The 200 mg/kg of the extract showed greater decreased onset of sleep and longer duration of sleep when compared with the control group that received diazepam only.

Table 2 The effect of hydroethanolic extract of *Commiphora africana* on pentylenetetrazole-induced seizures in mice. The extract did not show significant ($p>0.05$) in onset of convulsion and protection in the non-protected mice that were administered 50 mg/kg of the extract which showed only 20% protection in the mice. However, the extract significanty prolonged the onset of convulsion in the non-protected mice that were administered 100 and 200 mg/kg of the extract, which show about 60% protection in the mice. The valproic acid showed 100% protection of the mice.

DISCUSSION

The extract of *Commiphora africana* significantly and dose-dependently reduced the onset and prolonged the duration of sleep-induced by diazepam. By potentiating diazepam-induced sleep, the extract of *Commiphora africana* seems to possess sleep-inducing properties (Guillemain and Tetau, 1980; Rakotonirina *et al.,* 2001). Sedative hypnotic agents act to increase Gamma Amino Butyric Acid (GABA) mediated synaptic inhibition either by directly activating GABA receptors or, more usually, by enhancing the action of GABA on $GABA_A$ receptors. Benzodiazepines and barbiturates are examples of widely used therapeutic agents that act as positive allosteric modulators at $GABA_A$ receptors (Johnston, 2005). The ability of the extract to potentiate the sedative property of diazepam suggests that it may possibly act by interacting with GABA-mediated synaptic transmission. It has been reported that saponins show a potent sedative activity when tested in similar models (D'ubios *et al*., 1986). The flavonoid, has been reported to act as a positive allosteric modulator across a range of $GABA_A$ receptor subtypes (Kavvadias *et al*., 2004). Alkaloids are the most important secondary metabolites in many plants that are held responsible for their sedative action (Elizabetsky and Costa-Campos, 2006). It is therefore plausible to suggest that the sedative activity of the extract may be due to the presence of alkaloids, saponins and flavonoids among other phytochemical constituents, which have been shown to be present in the extract. Standard drugs, diazepam acts selectively on $GABA_A$ receptor, which mediates fast inhibitory synaptic transmission throughout the central nervous system. Benzodiazepines bind to the gamma-sub-unit of the $GABA_A$ receptor that causes an allosteric (structural) modulation of the receptor results with an increase in $GABA_A$ receptor activity.

The hydroethanolic extract of *Commiphora africana* increased the threshold of PTZ-induced convulsion in mice and offered some level of protection (60%) against PTZ-induced convulsion in mice. This was significant compared to the normal saline group. Clonic seizures induced by PTZ are blocked by drugs that reduce T-type calcium currents (ethosuximide) and drugs that enhance inhibitory neurotransmission by GABA receptors (benzodiazepine, Phenobarbital and valproate) (White *et al*., 1997). Convulsants whose actions previously were unexplained (including penicillin and PTZ) may act as relatively selective antagonist of the action of GABA (Macdonald *et al*., 1992; Macdonald and Oslen, 1994). The fact that the extract of *Commiphora africana* protected the animals against PTZ-induced seizures, one may say that the plant extract contains compound(s) that facilitate GABAergic transmission. The result of this study showed that the hydroethanolic extract of *Commiphora africana* possess anticonvulsant properties which are possibly mediated partly via facilitation of GABA transmission. This result suggests that the stem-bark of Commiphora africana may be beneficial in the management of absence and tonic-clonic seizures.

CONCLUTION

From the study conducted Commiphora africana has hypnotic effect and it also potentiated sedation and sleeping induced by diazepam. It is postulated that *Commiphora africana has* anti-convulsive effects which could be mediated through GABA (gamma amino butyric acid) which is involved in neural impulse transmission, because substances which stimulate GABA are known to possess anticonvulsant, pain relieving and sedative activity.

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