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Full Length Research Paper

Anxiolytic and anti-nociceptive activity of hydroalcholic leaf extract of *Cnidoscolous acontifolius*

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The C*nidoscolous acontifolius* hydroalcholic leaf extract (CAHLE) has been described as exhibiting biological activity in the central nervous system assays. The objectives of this study were to investigate the anxiolytic and antinociceptive effects of CAHLE in mice. Swiss albino mice (18 to 22 g) were randomly allotted to thirteen groups (n = 5 each). Groups 1 to 5 and 9 to 13 were treated with graded doses of CAHLE (100, 200, 400, 800 and 1600 mg/kg), Groups 6 and 7 received normal saline (10 ml/kg) and diazepam (1 mg/kg), respectively, while group 8 received 5 mg/kg of pentazocine hydrochloride (HCl). All drugs and test compounds were administered via intraperitoneal route except for pentazocine which was given subcutaneously. Data analysis was by one factor analysis of variance (ANOVA) followed by post hoc analysis using Student Newman Kuels multiple comparison tests. CAHLE treated mice showed increase open arm entries and open arm exploration time compared to control group in the elevated plus maze test. CAHLE treated mice also exhibited a time-dependent and a dose-dependent increase in tail flick latency. CAHLE treated mice also exhibited longer lasting protection from pain compared to standard. The study concluded that CAHLE possesses mild anxiolytic and analgesic properties to warrant its use in treatment of anxiety and relief of pain.

Key words: Anxiolytic, anti-nociceptive, central nervous system.

INTRODUCTION

Anxiety in recent years, has been increasingly acknowledged not only because it is highly prevalent, but also that the burden of illness associated with these disorders is often considerable (Sommers et al., 2006). Anxiety affects millions of individuals all over the world with a prevalence of 7.2% and an incidence of 9.7 per 1000 person-years (Martin-Merino et al., 2009). Incidence and prevalence were highest in women and young adults (20 to 29 years) (Martin-Merino et al., 2009). It is a cause of significant morbidity and loss of work hours. Pain is also an important symptom associated with almost all disease conditions. Pain has high clinical incidence (Maier et al., 2002). It is associated with tissue damage and is often a protective mechanism to prevent further damage (Kandel et al., 2002). Therapeutic agents available for the treatment of anxiety and pain include benzodiazepines, opioids and non-steroidal anti-inflammatory drugs. These agents however are not without significant side effects that limit their use. Tolerance and dependence to some of these agents particularly opioids and benzodiazepines, make the search for potent alternatives very important. Therefore there is a strong need to identify newer agents that have fewer side effects.

Medicinal plants represent a growing and important source of new drugs for the treatment of different conditions. The use of these plants in natural medicine is widespread and many of these plants have been validated through scientific experiments (Olorunisola et

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Figure 1. *Cnidoscolous acontifolius*.

al., 2012). The chemical diversity of plants has made them one of the main sources for the isolation of bioactive organic compounds (Basso et al., 2005). *Cnidoscolous acontifolius* (CA), a large, fast growing and leafy perennial shrub native to the Yucatán Peninsula of Mexico, has been claimed by local healers to be useful in treatment of insomnia and pain (Molina-Cruz, 2000). Traditional healers have utilised this plant in treating pain of scorpion stings and gout for centuries (Molina-Cruz, 2000). They have also employed it in calming down patients and improving sleep in individuals with poor sleep. These uses suggest that CA may have the potentials of a central nervous depressant.

However, there has been no controlled experiment to test the usefulness and effectiveness of this plant in treating pain and to test the hypotheses that CA has anxiolytic and anti-nociceptive actions. The scientific evaluation of these claims is needed to verify CA's usefulness and efficacy in treating pain and anxiety or otherwise. This study sought to evaluate the anxiolytic and the anti-nociceptive effects of hydroalcholic leaf extract of *C. acontifolius (CAHLE)* in mice.

MATERIALS AND METHODS

The leaves of *C. acontifolius* (Figure 1) were collected in November 2008, during the dry season in the backyard garden of a family at Owode Estate, Apata, Ibadan, Southwest Nigeria. The leaves were identified and authenticated by Mr. T. K. Odewo, a taxonomist in the Forestry Research Institute of Nigeria (FRIN), Ibadan, where a voucher specimen (FHI; 107727) of the plant already exist. Diazepam (Roche, Switzerland) and pentazocine (Sigma Chemicals, USA) were used in this study.

Extract preparation

The leaves of *C. acontifolius* were air-dried at room temperature (28°C) powdered and weighed (200 g). The powdered leaves were cold-extracted with methanol and water (80:20) over a 72 h period. The marc leaves were sieved using the Whatman No. 1 filter paper and the residue collected and then concentrated in vacuo at 60°C and finally air-dried at room temperature until all the methanol/water had been removed. The residue was weighed to be 10.2 g; yield was 5.1% w/v. The dried extract was dissolved in normal saline (1: 50 w/v) to obtain a stock solution of 1600 mg/kg, which was further diluted to 100, 200, 400 800 and 1600 mg/kg. These doses were employed in our study to determine the anxiolytic and antinociceptive activities of *C. acontifolius* hydroalcholic leaf extract (CAHLE).

Animal and experiment groups

Male and female Swiss albino mice weighing 18 to 22 g obtained from the animal house of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria, were selected for this study. All animals were kept at room temperature (28 ±1°C, 70 to 80% humidity, 12 h light/dark cycle) in the animal holding unit of the laboratory of the Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, for one week. Thereafter, the mice were randomly allotted to thirteen groups of five mice each (n = 5). Groups 1 to 5 and 9 to 13 received graded doses of CAHLE, while groups 6 and 7 received intraperitoneally 10 ml/kg of normal saline and 1 ml/kg of diazepam, respectively. Meanwhile, group 8 received 5 mg/kg of pentazocine hydrochloride (HCl) via subcutaneous route. All other drugs and CAHLE were administered intraperitoneally.

Elevated plus-maze test

Experiment was carried out in a quiet laboratory to ensure that no external stimuli other than the height of the plus maze could invoke maze anxiety. Before the introduction of each animal into the elevated plus maze it was cleaned with 70% alcohol to eliminate possible bias due to odour that might have been left behind by the previous subject. Groups 1 to 5 received graded doses of CAHLE via intraperitoneal route, while Groups 6 and 7 served as control and standard. Each mouse after 30 min of administration of CAHLE, the vehicle or the standard was placed at the centre of the

Table 1. The effect of CAHLE on open arm entries and time spent in the open arms of elevated plus maze.

Treated groups	Open arm entries	Time spent in open arms
CAHLE 100 mg/kg	6.60 ± 0.51*#	138.23 ± 13.86*#
CAHLE 200 mg/kg	8.20 ± 0.80**#	144.20 ± 10.87*
CAHLE 400 mg/kg	6.20 ± 0.20	99.19 ± 2.40#
CAHLE 800 mg/kg	5.80 ± 0.58	86.46 ± 11.44
CAHLE 1600 mg/kg	5.20 ± 1.20	85.56 ± 9.58
Control (N/S)	5.00 ± 0.84	83.67 ± 3.22
Standard (1 mg/kg)	6.20 ± 0.80	130.4 ± 8.86

Values represent the mean+ S.E.M. (n = 5). **P<0.01; *P<0.05 (test dose compared with vehicle); #P<0.05 (test dose compared to diazepam).

elevated plus maze with its head facing the open arm for a period of 5 min. The behaviour of the mouse was recorded as the number of entries into the open or closed arms and time spent by the mouse in each of the arms. The experiment was carried out in a quiet laboratory to ensure that no external stimuli other than the height of the plus maze could invoke maze anxiety.

Tail flick latency

Six groups of Mice (n = 5) each were selected for this study. Group 8 received 5 mg/kg of pentazocine HCl via the subcutaneous route and Groups 9 to 13 received graded doses of CAHLE (100, 200, 400, 800 and 1600 mg/kg). Tail flick latency was measured by the new Ugo Basile tail flick unit 37360. The instrument measures the latency of the avoidance response when pain is induced by radiant heat applied to the mouse's tail. It basically consists of an I.R. source (50 W bulb), whose radiant energy of adjustable intensity is focused by an embodied parabolic mirror on the rodent's tail. An intensity of 5 (out of a possible 100) was selected for this study. The mice were held by the inclined mouse restrainer on the instrument unobstructed upper panel in such a way that its tail, placed over a flush mounted window, received the I.R. energy. The stimulus and the related reaction-time counter were started by a function key located on the front panel. When the mouse felt the pain and flicked its tail, a sensor detected it and stopped the reaction-time counter and switched off the bulb. The reaction time of the animal was automatically determined to the nearest 0.1 second. A cut off of 50 s was chosen automatically by the apparatus to prevent thermal injury to the tail of the experimental mouse.

Ethical considerations

This study was conducted in line with standard practices, procedures, international protocols and accepted principles for laboratory animal use and care as found in the guiding principles for the care and use of animals in research and teaching (Helsinki declaration, 2010 update).

Statistical analysis

CAHLE treated groups were compared with control by one factor analysis of variance (ANOVA) followed by post hoc analysis using Studentized Newman Kuels multiple comparison tests. CAHLE treated groups were also compared with the standard drug (diazepam) group. The results were analyzed using the Graph Pad Prism 4.0 statistical software. All results were expressed as mean ± standard error of mean (S.E.M.). Values of P less than 0.05 were taken as significant (that is, P<0.05).

RESULTS

Elevated plus maze test revealed that CAHLE treated mice had more open arm entries and spent more time in the open arm of the elevated plus maze compared to mice treated with normal saline (Table 1). However, Only 200 mg/kg treated group of mice showed significantly greater open arm entries and time spent in the open arm of the elevated plus maze compared with diazepam. There was a gradual decline in the in the number of open arm entries and time spent in the open arm of the elevated plus as the dose of CAHLE was increased from 200 to 1600 mg/kg.

In addition, CAHLE significantly increased tail flick latency in mice groups in a time-dependent fashion. At time 30, 60 and 120 min after administration of CAHLE, there was a significant increase in time taken to respond to the pain stimulus (Table 2). Treatment with 1600 mg/kg showed the greatest increase in tail flick latency after 120 min. On the other hand, 200 mg/kg of CAHLE showed the least increase in tail flick latency across the time frame employed in the experiments. All doses of CAHLE showed a progressive increase in tail flick latency as period increases. Overall, 1600 mg/kg showed comparable and even greater efficacy compared with pentazocine at all dose levels. All doses of CAHLE showed significantly lower elongation of tail flick latency compared to pentazocine after 30 min of administration of CAHLE and pentazocine, respectively.

Moreover, 400 and 1600 mg/kg of CAHLE showed higher percentage elongation of tail flick latency compared to pentazocine after 60 min post administration. Furthermore, 400 and 1600 mg/kg showed greater than 100% elongation of tail flick latency compared to pentazocine after 120 min of administration of test compound and pentazocine (Table 3).

Table 2. Time dependent tail flick latency of mice treated with graded doses of CAHLE.

Treatment groups	Dose (mg/kg)	Time (min)			
		0	30	60	120
Control	N/S	15.9 ± 3.48	15.3 ± 3.93	16.9 ± 2.98	15.6 ± 3.67
CAHLE	100	8.2 ± 1.39	19.1 ± 3.86	20.3 ± 3.06	21.7 ± 2.81
CAHLE	200	10.2 ± 3.45	19.5 ± 3.36	23.2 ± 3.11	29.5 ± 2.65*
CAHLE	400	14.8 ± 3.98	21.7 ± 3.09	27.6 ± 2.43	31.7 ± 3.23*
CAHLE	800	7.7 ± 1.32	22.3 ± 3.83	27.9 ± 2.75	31.9 ± 3.71*
CAHLE	1600	13.5 ± 2.90	25.2 ± 3.97	30.0 ± 4.14	32.6 ± 3.71*
Standard	5.0	13.7 ± 4.74	27.8 ± 4.13	22.1 ± 4.15	23.0 ± 4.59

Values represent the mean+ S.E.M (n = 5). *P<0.05 (CAHLE treated groups compared to control).

Table 3. Percentage elongation of tail flick latency in mice treated with graded doses of CAHLE.

Treatment groups	Dose (mg/kg)	Percentage elongation (min)		
		30	60	120
Control	N/S	0	0	0
CAHLE	100	24.84	20.11	31.10
CAHLE	200	25.84	7.69	25.00
CAHLE	400	41.83	63.31	103.21
CAHLE	800	42.68	23.67	34.64
CAHLE	1600	32.03	77.51	108.97
Standard	5.0	81.69	30.77	47.44

Values represent percentage change in tail flick latency over time (n = 5).

DISCUSSION

Anxiolytic agents are known to act by potentiating inhibitory influences in the central nervous system or by directly depressing central nervous activity. The evidence that CAHLE has some anxiety relieving action as presented is shown by the results of the elevated plus maze test where an increased open arm exploration time and increased open arm entry is seen in CAHLE treated mice. Drugs that increase open arm exploration time are considered as anxiolytics and the reverse holds true for anxiogenics (Hellion-Ibarrola et al., 2006). Since CAHLE increased the open arm exploration time and entry into the open arm in the elevated plus maze, one can then say that it may possess anxiolytic activity. These results suggest therefore that CAHLE exhibits anxiolytic action. Though CAHLE at 200 mg/kg dose showed the greatest anxiolytic effect, this effect is not statistically different from that produced by diazepam. It therefore means CAHLE may not produce more potent anxiety alleviating action than that already produced by diazepam though equally potent. The time dependent increase in tail flick latency by all doses of CAHLE also suggests the antinociceptive action in CAHLE treated groups. The longer reaction time to noxious stimulus shown by CAHLE treated mice demonstrated their increased capacity to withstand pain. CAHLE's longer lasting protection also suggests a longer duration of action compared to pentazocine.

Meanwhile, this study has not determined whether this pain relieving action is mediated centrally or locally. Further study along this line may help determine whether CAHLE exerts local pain block or centrally. It is also possible for CAHLE to exert it action via both mechanisms. This study has not also explained what role the different constituents of CAHLE play in the observed effects of CAHLE in mice. CAHLE is a complex heterogeneous mixture of secondary metabolites (Yakubu and Ogunowo, 2008). CAHLE contains flavonoids, alkaloids and terpenoids (Awoyinka and Balogun, 2007). These secondary metabolites, individually or in combination, would account for the observed pharmacological effects of this plant extract. Secondary metabolites present in plant extracts, through additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with physiological processes may be responsible for the observed effects (Briskin, 2000). The questions that arise out of this study then include: which of the variety of constituents in CAHLE produces these effects, or do they act singly or synergistically to produce the observed effects? These chemical constituents have been reported to be responsible for a variety

of observed effect among them are anxiolytic and sedative effects observed in different plant extracts (Houghton, 1999; Carlini, 2003).

Conclusion

From this study, it can be concluded that CAHLE has anxiolytic and antinociceptive actions comparable to known standards at some of the tested doses.

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