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RESEARCH ARTICLE

INVESTIGATION OF THE AQUEOUS FRUIT EXTRACT OF *Lagenaria siceraria* for PHARMACOLOGICAL ACTIVITIES *IN VITRO* AND *IN VIVO*

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ABSTRACT

Some pharmacological activities of the aqueous fruit extract of Lagenaria siceraria (Mol) Standl (Cucurbitaceae) were investigated using *in vivo* and *in vitro* methods. The extract yielded 1.48 % w/w dry matter, which foams on shaken. The oral acute toxicity test gave LD_{50} of 181 mg/kg, while the brine shrimps lethality test gave LC_{50} of 66.79 ppm and EC_{50} of 6.68 at 95 % confidence interval. The extract significantly ($p<0.05$) increased pentobarbitone-induced sleeping time at 40 and 120 mg/kg. Also, the extract significantly ($p<0.05$) the number of acetic acid-induced writhing in a dose dependent manner. *L. siceraria* extract significantly ($p<0.05$) decreased amphetamine-induced stereotype behaviour in mice. These findings strongly suggest that the extract possessed central nervous system depressant effect. The extract neither evoked contraction nor inhibited spasmogen-induced contraction of isolated guinea pig ileum. Rather, it potentiated agonistic effect of histamine by 30 %. In conclusion, the aqueous extract of *L siceraria* has been shown to contain potent bioactive compounds with potent analgesic effect and non-specific CNS depressant activities among others and may be of value in psychotherapy as narcoleptic agents and also confirmed some of the folkloric uses.

INTRODUCTION

Medicinal plants have been used for the treatment of many diseases in developing countries where the cost of the conventional medicines presents a burden to the population (Ayensu, 1983; Zheng *et al.,* 2007). Natural products especially of medicinal plant origin have long been employed in the treatment of diseases and as a potential source for drug discovery (Rahman and Zaman, 1989; Anaga and Asuzu, 2010). Furthermore, ethnobotany and ethnomedical studies are today recognized as the most viable methods of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents (Adjanahoun *et al*, 1991). It is also believed that herbal remedies are safe and less damaging to the body than synthetic drugs (Williamson *et al* 1976). *Lagenaria siceraria* (Mol) Standl (Curcubitaceae) commonly known as "bottle gourd" has been an important economic medicinal plant in some parts of China, India and Africa. It is a climbing perennial vine widely cultivated as a vegetable crop in tropical countries such as India, Japan and Thailand with large leaves and lush appearance. It probably originated from Africa from where it was widely distributed to India, China, Indonesia, New Zealand, Egypt America etc (James, 2009). The Vine also called calabash is grown for its fruits which can either be harvested young

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and used as vegetable or harvested mature, dried and used as bottle, utensil or pipe. The fresh fruit has a light green smooth skin, white flesh and have a round or slim varieties (*http://en.wikipedia.org/wki/calabash*). The folkloric uses of the bottle gourd include: use of the young shoots and leaves as vegetables, fresh immature fruits are used in making cakes, or eaten in India (Jain, 2000). Economically, the Fulani tribe in Nigeria uses the gourd as a vase to sell fresh milk mixed with cereal gruel, a traditional meal known as "nono" (Kibel, 2000). It is also used as fishing floats, rafts, container for food, making musical instruments and popularly used for drinking palm wine in the Eastern part of Nigeria (Kibel, 2000). In traditional medical practices, the bottle gourd have been used as poultice, cardioprotective, diuretic, aphrodisiac, emetic, anti-asthmatic, cough suppressant and anti-tetanus agent (Duke and Ayensu, 1984; Moerman, 2004). It is also used for the treatment of head ache, sore throat, dropsy, and as an antidote to some poisons (Shah *et al*, 2010). It has also been used for the treatment of jaundice, surgical trephination in horses, enemas, colic (Heiser, 1979) and in the management of viral disease of poultry known as Newcastle disease (Burkill, 1985). The present study was designed to investigate some pharmacological activities of the aqueous fruit extract of *L. siceraria in vitro* and *in vivo* to confirm its folkloric uses.

MATERIALS AND METHODS

The experimental protocols used in this study were approved by the Ethics Committee of the University of Nigeria, Nsukka in accordance with the guide to the care and use of laboratory animals in research and teaching in the University. Also, Ethical conditions governing the use of laboratory animals for conduct of experiments as stipulated by ward and Elsea (1997) were strictly observed. Freshly prepared solutions of drugs and physiological solutions were used in all the experiments. All chemicals and reagents used in this study were of analytical grade.

Plant Collection and Extraction: Fresh mature fruits of *Lagenaria siceraria* were collected from Ubakala, Umuahia, Abia State, Nigeria and identified by Mr. A. Ozioko, a taxonomist in Pharmacognosy Unit of Bioresources Development and Conservation Programme (BDCP) Nsukka, Enugu State. The fresh fruits were cut into small bits and dried at room temperature (28°C) on top of the laboratory bench. The dried fruit material was pulverized into coarse powder (1.5 mm) using hammer mill. The plant material was extracted by cold maceration method. Briefly, 100 grams of the plant material was macerated in 120 ml of distilled water for 48 h with intermittent shaking at 2 h interval. The extract was filtered using Whatman number 1 filter paper. The extract was stored at 4°C throughout the duration of the study.

Determination of Concentration and Percentage Yield: A dry clean watch glass was weighed and 1ml of the extract was aspirated into the watch glass and evaporated to dryness on a hot plate at a constant weight of the extract and the concentration of the extract calculated as follows:

Concentration = (X – Y) mg/ml.

Where X = weight of watch glass + dry extract and

Y = weight of dry watch glass.

The percentage yield was further determined as follows:

$$\% \text{ yield} = \frac{(A \times B)}{C} \times \frac{100}{1}$$

Where A = concentration of extract

B = total volume of extract

C = original weight of plant material used for extraction

Experimental animals: White albino Wistar mice (20-30 g) of both sexes obtained from the Laboratory Animal Unit of Faculty of Veterinary Medicine, University of Nigeria Nsukka and adult female Guinea pig (400 g) obtained from Faculty of Pharmaceutical Science, University of Nigeria, Nsukka were used for the experiment. The animals were kept in stainless steal cages. The mice were fed on rodent pellets (Guinea feed® Nigeria Ltd, Nigeria), while the guinea pig were fed on fresh guinea grass (*Panicum maximum*). They were given access to clean drinking water, except in situations where fasting was required. The temperature varied between 25-30^{0}C and relative humidity of between 50-65%. They were allowed two weeks to acclimatize before the commencement of the experiment.

Brine shrimps lethality test (BSLT)

The method of McLaughlin and coworkers (1991) was used to study the toxicity *of L. siceraria* extract. Briefly, *Artemia salina* eggs obtained from a pet shop in Davis, California were incubated in natural sea water (from Bar Beach, Lagos, Nigeria) in a dam-well under room condition. About ten 48h - shrimp nauplii in 1ml of autoclaved sea water were put into each Bijou bottles using a Pasteur pipette under a stereo-microscope with a light source. They were separated into 4 groups in triplicate. Increasing concentrations (10, 100, 1000 ppm) of the aqueous fruit extract of L. siceraria were added into each of the triplicate and distilled water was added

into the control group. The nauplii were incubated at room temperature (28°C) for 24 h, after which the survivors in each well were counted. The results were analyzed using Finney Probit Analysis (MS-DOS-computer-program) to determine the LC_{50} at 95 % confidence interval. Weak nauplii were noted as an indication of central nervous system depression.

Acute Toxicity Test

Thirty albino Wistar mice of either sex were used for the study. They were randomly divided into five groups (A-E) of five mice each. They were kept in stainless steel cage and were provided feed and water *ad libitum*. The mice of the various groups were orally dosed with increasing doses (40, 80, 160, 400, and 800 mg/kg, b.w.) of the *L. siceraria* extract, while the control group received equal volume of distilled water. The mice were allowed free access to feed and water *ad libitum* and were observed for mortality and toxic signs for 48 h. The lethal dose (LD_{50}) was calculated (Anaga *et al.,* 2006).

Effect of *L. siceraria* on pentobarbitone induced sleeping time

This study was carried out following the methods of Shetty and Anika (1982). Mature albino mice were randomly grouped into five (A-E) with five mice per group and treated as follows: group A mice received distilled water (10 ml/kg), while groups B, C, D and E received 20, 40, 80 and 120 mg/kg of *L. siceraria* extract respectively. Thirty minutes post treatment, pentobarbitone sodium (35 mg/kg) was administered to each mouse intraperitoneally (i.p). The sleeping time was calculated as the interval between the time of commencement of sleep (loss of righting reflex) and the time of awakening (regain of righting reflex) of each mouse and was recorded.

Effect of *L. siceraria* extract on acetic acid-induced writhing reflex test

This study was carried out using the method of Koster *et al* (1959) as modified by Dambisya and Lee (1999). Twenty five albino mice of both sexes were randomly divided into five groups (A-E) of five mice per group. They were treated as follows: group A mice were given distilled water (10 ml/kg) which served as negative control. Group B mice were given morphine (2 mg/kg i.p) which served as positive control, while groups C-E mice received 20, 40 and 60 mg/kg of *L. siceraria* extract respectively by gastric lavage. One hour after administration of drug and extract, 0.7% glacial acetic acid (10ml/kg) was given intraperitoneally to all the mice to induce pain characterized by abdominal contortions or writhes. The number of writhes observed in each mouse was counted for 30 minutes and recorded. The degree of analgesia was calculated using the formula of Dambisya and Lee (1999) which represents the percentage of inhibition of abdominal constrictions:

$$\frac{Mean\ of\ the\ distilled\ water\ group - mean\ of\ the\ test\ group}{Mean\ of\ the\ distilled\ water\ group} x100$$

Effect of *L. siceraria* extract on amphetamine induced stereotype behaviours.

The method described by Gamaniel *et al* (2000) was adopted for this study. Twenty albino mice of both sexes were randomly divided into four groups (A-D) of five mice per group and treated as follows: group A mice were given distilled water (10 ml/kg) which served as control, while group B-D mice received 20, 40 and 60 mg/kg of *L. siceraria* extract respectively through gastric gavage. Thirty minutes later, amphetamine (2 mg/kg i.p) was given to each mouse. Stereotype behaviour which is exhibited by various reflexes such as sniffing, fore-paw licking (FPLK), jumping and cycling were observed for 2 hours and counted for each mouse and recorded.

Effect of *L. Sicerania* on Isolated Guinea Pig Ileum *in vitro*

The experiment was carried out using standard method (Perry, 1970), modified by Akah *et al* (2003). Briefly, a female Guinea pig (400 g) fasted overnight but was allowed free access to water was killed by a blow on the head and was exsanguinated and eviscerated. A segment of the ileum (2-3 cm) was cut and suspended in an organ bath containing tyrode solution maintained at 37 ± 1°C and aerated with air. Equilibration period of 60 minutes was allowed after which the contractile effects of acetylcholine (Ach, 1.0 µg/ml), 5-hydrotyptamine (5HT, 4.0 µg/ml) and histamine (1.0 µg/ml) on guinea pig ileum were tested. The contractile effect of increasing concentrations of the extract (1.8, 3.6 and 7.2 µg/ml) on guinea pig ileum was tested. Also, the effect of the extract (1.8µg/ml) on the contractile response induced by the agonists (Ach, 5HT, and Histamine) were also tested and recorded.

Statistical Analysis

The results obtained from the various experiments were presented as mean ± Standard Deviation (STD). These data were subjected to one-way analysis of variance (ANOVA) and the difference between the means were tested using Post hoc LSD to determine the level of

significance between the 'test' and 'control' group means. Values of $p < 0.05$ were considered statistically significant.

RESULTS

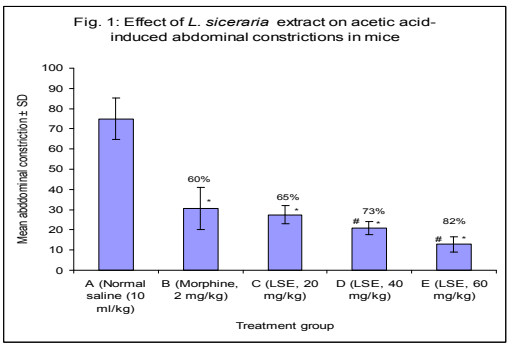
The aqueous extract of *L. siceraria* was amber in colour and the total solid recovered from the extract was 1.48 % (w/w) with a concentration of 18 mg/ml. In oral acute toxicity test, the mice showed the following toxicity sign: depression, clustering together and reduced activity for several hours and the extract produced LD_{50} of 181 mg/kg. The brine shrimp lethality test (BSLT) showed that *L. siceraria* contained bioactive compounds with LC_{50} of 66.79% at 95% confidence interval. Surviving nauplii were weak and dull in movement. The result of effect of *L siceraria* on pentobarbitone sleeping time is shown in Table 1. The result showed that the extract (40 and 120 mg/kg) showed a significant ($p<0.05$) increase in pentobarbitone sleeping time in mice when compared with the control group. The extract (40 mg/kg) gave the highest activity of 70 % increase in sleeping time compared with the control group. The result of the effect of *L siceraria* extract on acetic acid-induced abdominal constrictions is presented in Fig. 1. Morphine (2 mg/kg) and *L sicerania* extract in all the doses significantly ($p<0.004$) reduced the acetic-acid-induced abdominal constrictions in a dose dependent manner when compared to the negative control. All the doses of the extract had a better analgesic effect than the reference drug (morphine). Also the percentage protection increased with increase in the dose of extract from 0% in the negative control group to 82% in the 60mg/kg treated group with the reference drug morphine (2 mg/kg) producing 60% inhibition of writhing.

Table 1. Effect of *L Sicerania* on Pentobarbitone Sleeping Time

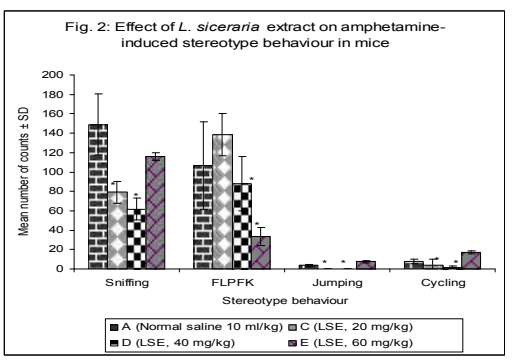
Group	Treatment	Sleeping Time ± SD (min)
A	Normal saline (10 ml/kg)	30 ±1.66
B	LSE 20 mg/kg	26 ± 5.51
C	LSE 40 mg/kg	51 ± 13.46*
D	LSE 60 mg/kg	27 ± 4.30
E	LSE 120 mg/kg	44 ± 8.41*

* P < 0.05 when compared to the negative control
LSE = *Legnaeria sicerania* extract,
SD = Standard deviation

The effect of *L sicerania* extract on amphetamine induced stereotype behaviour is present in Fig. 2. The result showed that the extract (20 and 40 mg/kg) significantly ($p<0.05$) decreased amphetamine induced sniffing, while at the doses of 40 and 60 mg/kg, the extract significantly ($p<0.05$) reduced the number of fore paw licking in mice. Also, the extract (20 and 40 mg/kg) significantly ($p<0.05$) decreased the number of amphetamine-induced jumping and cycling. However, at the dose of 60 mg/kg extract, the number of jumping and cycling was increased when compared to the control group. The result of the effect of *L sicerania* on isolated guinea pig ileum is presented in Table 2. The result showed that the extract in all the concentrations used had no effect on the smooth muscle of the ileum. The extract also had no effect on the contractions elicited by agonists like acetylcholine and 5HT but potentiated the contractions evoked by histamine by abort 30%**.**



* $p<0.004$ when compared to the negative control (group A), # $p<0.05$ when compared to the positive control (group B).



*$p<0.05$ when compared to group A (Normal saline, 10 ml/kg), LSE = *L. siceraria* extract, FPLK = Fore paw licking, SD = Standard deviation

DISCUSSION

The aqueous extract of *L sicerania* was amber colored and was foaming profusely when shaken which suggested the presence of saponins as was reported by Schultes (1990). The yield was 1.48% w/w dry matter which was quite low. The acute toxicity test of the plant

extract in mice gave an LD_{50} of 181mg/kg. This informed the choice of the test doses.

Table 2. Effect of *L Sicerania* on Isolated Guinea Pig Ileum

Drug/Extract	Concentration µg/ml	Increase in Contraction (mm)	% Stimulation
Acetylcholine	1.0	63	
5HT	4.0	15	
Histamine	1.0	46	
Extract	1.8	0	-
Extract	3.6	0	-
Extract	7.2	0	-
Acetylcholine + Extract	1.0 ± 1.8	62	1.6
5HT + Extract	4.0 ± 1.8	15	0
Histamine + Extract	1.0 ± 1.8	60	30.4

The animals showed signs of depression and were clustering together which suggests the involvement of the central nervous system. The brine shrimps lethality is a rapid, inexpensive and a single bioassay for testing bioactivity of plant extracts which in most cases correlates reasonably with the cytotoxicity and anti-tumour properties of natural products (Alluri *et al*, 2005). The LC_{50} was 66.79 ppm which was quite low, an indication of the presence of biological activity compounds in the extract since according to Lewis (1995), a wide variety of biologically active compounds are lethal to brine shrimps. This cytotoxic effect agrees with ribonucleolytic activity of the bottle gourd reported by Wang (2000). The ED_{50} value for general bioactivity is approximately one tenth of the value of the LC_{50} in BSLT (McLaughlin et al., 1991). Therefore, the ED_{50} of *L. siceraria* extract was approximated to 6.68 ppm (668 µg/ml). The surviving nauplii were dull and inactive, which is a sign of CNS depression (McLaughlin et al., 1991).

The aqueous extract of *L sicerania* caused CNS depression in mice because it significantly increased pentobarbitone sleeping time at the doses of 40 and 120 mg/kg by 70 and 46.7 % respectively. The CNS depressant effect of the extract corroborates the clinical observations in the acute toxicity study and BSLT. The increase in pentobarbitone sleeping time may be due to inhibition of the enzymes responsible for the biotransformation of pentobarbitone thereby prolonging the action of the barbiturate (Hardman *et al*, 1996) or could be through synergistic effect with pentobarbitone in C.N.S depression (Shetty and Anika 1982). Though the depression of CNS was not specific, it may justify the use of the gourd in surgical trephination in horses as reported by Heiser (1979).

The analgesic or antinociceptive effect of *L sicerania* was investigated using the acetic acid-induced writhing reflex model in mice. Acetic acid induced abdominal constriction is a sensitive method for screening analgesic effect of compounds (Bentley *et al* 1983). The extract showed a promising anti-nociceptive effect because it significantly decreased the number of acetic acid-induced abdominal constrictions in all the doses (20, 40 and 60 mg/kg) of the extract. The percent pain inhibition showed that the extract (40 and 60 mg/kg) was more effective than morphine (2 mg/kg, i.p) (Fig. 1). Since, the extract caused depression and clustering of the mice in acute toxicity test; increased pentobarbitone-induced sleeping time and weakened the movement of surviving nauplii in BSLT, it lends more credence to fact that the extract has central nervous system depressant effect, as central depressants and antihistamines are known to reduce the number of abdominal writhings (Onasanwo and Elegbe, 2006). Amphetamine induces stereotype behaviours such as sniffing, fore-paw licking, jumping, and cycling etc (Roffman and Raskin 1979). Stereotype behaviour consists of motor responses that are repetitive, invariant and seemingly without purpose or goal. The most classic behavioural pattern that is characteristic of stereotypy is that elicited by high doses of stimulants such as cocaine and amphetamine in rodents and the frequency of different behaviour is measured by scoring the presence or absence of a given behaviour during predetermined time bins (Ann, 2001). The extract of *L sicerania* at the doses of 20 and 40 mg/kg significantly ($p < 0.05$) reduced the amphetamine induced stereotype behaviours. This further confirms the CNS depressant activity of the extract. Though the exact mechanism through which the extract achieved this was not established, it may be through the inhibition of the action of amphetamine on synaptic junctions where it liberates catecholamine (Duke, 1992).

L sicerania extract had no effect on the guinea pig ileal smooth muscle. It also had no effect on the contractions evoked by known spasmogens such as acetylcholine and 5HT; however, it potentiated the contractions evoked by histamine. This suggests that the plant extract will be of little or no value in diseases involving gastro intestinal motility, however the potentiation of the contraction evoked by histamine may substantiate the use of the plant in folk medicine as a purgative (Shah, 2010). In conclusion, the aqueous extract of *L sicerania* have been shown to contain bioactive compounds with potent analgesic effects and

non-specific CNS depressant activities among others and may be of value in psychotherapy as narcoleptic agents, however, more work is required to test for other pharmacological activities of the extract and to determine the specific mechanism(s) of action.

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