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Behavioural effect of standardized aqueous whole plant extract of *Acanthospermum hispidum*: Ethnopharmacological Justification for its use in folkloric management of malaria

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Abstract

Acanthospermum hispidum DC is a medicinal plant commonly used locally for the treatment of malaria, cough, diarrhoea, typhoid, inability to sleep well and vomiting. The aim of the study was to evaluate the behavioural effects of the aqueous whole extract of *Acanthospermum hispidum* in young chicks and mice. The oral acute toxicity of *Acanthospermum hispidum* was carried out in young chicks and mice using the acute toxic class method. The antiemetic effect of the extract (50, 100 and 200 mg/kg body weight orally) and metoclopramide (5 mg/kg intramuscularly) was assessed using copper sulphate-induced retches in young chicks. The effect of the extract (50, 100 and 200 mg/kg body weight orally) on apomorphine-induced pecking in chicks, pentobarbitone-induced sleep, exploratory behaviour on hole board apparatus and motor coordination on rota rod were evaluated. The oral median lethal (LD_{50}) dose of the extract was greater than 2000 mg/kg in chicks and mice. *Acanthospermum hispidum* extract significantly ($F_{4, 49}$=222, $p<0.0001$) inhibited copper sulphate-induced retching when compared to negative control while metoclopramide significantly inhibited copper –induced retches. The extract (at 50, 100 and 200) and chlorpromazine at 2 mg/kg body weight significantly reduced ($F_{4, 49}$=331, $p<0.0001$) apomorphine-induced pecking in young chicks. The extract (50, 100 and 200 mg/kg) and diazepam (30 mg/kg) significantly ($F_{4, 29}$=18, $p<0.0001$) shortened onset but prolonged ($F_{4, 29}$=637, $p<0.0001$) duration of sleep in pentobarbitone –induced sleep. The extract significantly ($F_{4, 29}$=98, $p<0.0001$) reduced frequency of head dip in hole board apparatus but had no effect on motor coordination. The results suggested that *Acanthospermum hispidum* possesses potent anti-emetic and sedative effects but had no effect on motor function which explains its continued use for management of malaria symptoms in folk medicine. These effects may have been mediated by flavonoid and tannins present in the extract.

KEY WORDS: MALARIA, ACANTHOSPERMUM HISPIDUM, RETCHES, DOPAMINE, STEREOTYPY

Introduction

Malaria remains a devastating global health problem. Worldwide, an estimated 300–500 million people contract malaria each year, resulting in 1.5–2.7 million deaths annually (1, 2, 3). Its symptoms include fever, weakness, dry cough, diarrhea and vomiting. The use of plant remedies has steadily increased worldwide in recent years, as well as the search for new phytochemicals that potentially could be developed as useful drugs for the treatment of human diseases including malaria (4, 5, 6). One of such plant that is widely used for malaria management in traditional medicine is *Acanthospermum hispidum.*

Acanthospermum hispidum belongs to the *Asteraceae.* It is a branched herb up to 60 cm tall. The stem is covered with bushy hairs and smaller glandular hairs while the leaves are elliptic, obovate and 1.5 cm to 7 cm long. The plant bears yellow flowers which are typical of the Aster or Daisy Family. The petals (corollas) of the ray flowers are pale yellow and are about 1.5 mm long. The fruits are flattened and triangular in shape spiny and 5 cm to 10 cm in length. These fruits are covered with stiff, hooked hairs and have either a straight or curved pair of spines at the top. The bristly appearance and grouping of several fruits in each head provides the most frequently used common name, Bristly Starbur. These terminal spines supply yet an additional common name, Goat head. The aerial parts of *Acanthospermum hispidum* are often used in folk medicine for managing various ailments which include hepato-biliary disorders, cephalgias, headaches, abdominal pains, convulsions, cough, eruptive fever, snake bites, jaundice, epilepsy, constipation, blennorrhoea, diarrhoeas, malaria and vomiting (7, 8). Traditional healers often use a water decoction prepared with trona for management of vomiting associated with malaria (Muazzam, oral communication).

The phytochemical studies of this plant have yielded compounds identified as Terpenoids (9) saponins (10) and lipids (11). The authors however, did not come across any record of systematic scientific evaluation of its anti-emetic and central nervous system effects. Interest in its behavioural and antiemetic effects is apt to explain its continued use in symptomatic management of malaria in folk medicine and provide additional safety pharmacology data.

Materials and methods

2.1 Chemicals

Metoclopramide (Pfizer pharmaceuticals), Apomorphine HCl (Sigma Co., USA), chlorpromazine (Sigma Co., USA), normal saline (0.9% sodium chloride), Anhydrous copper sulphate (Sigma Co., USA).

2.2 Plant material

The whole plant part of *Acanthospermum hispidum* was collected by Mallam Shuaib Wanzam from Mubi in Mubi local government (Adamawa State, Nigeria) and identified by Mallam Ibrahim Muazzam an ethnobotanist and authenticated by Mrs. Grace Ugbabe a taxonomist of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Idu Industrial Area, Abuja, Nigeria. The whole plant was cleaned, air-dried at room temperature (60-80°F) away from sunlight and pounded into fine powder using mortar and pestle. The powder was stored in an air-tight container and kept at 39.2°F for subsequent use.

Animal

Male young chickens (35-40 g; 4 days old) obtained from a poultry local store in Kubwa, Abuja were used in the study. The chicks were fed with Pfizer growers' marsh and given water ad libitum. Adult Swiss albino mice (18–20 g) of either sex, obtained from Animal Facility Centre (AFC) of National Institute for Pharmaceutical Research and Development (NIPRD) were used for this study. The mice were housed in transparent plastic cages padded with wood shavings, under standard

conditions of temperature, relative humidity and light/dark cycles (12/12 h). They were fed with pelletized feeds obtained from Feeds cap limited Ibadan and water ad libitum. Mice were approved for use by the AFC committee after reviewing the protocol. We certify that all experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

2.3 Extraction method

The air-dried whole plant of *A. hispidum* was crushed into a fine powder. The powdered whole plant (200 g) was cold macerated with 1.0 L of water for 24 h with constant shaking using a GFL shaker. The resultant mixture was filtered using Whatman filter paper No. 1 (Cat. No. 100125) and the filtrate was freeze-dried.. The dried sample was stored in specimen bottle and kept in refrigerator until required for use.

Phytochemical analysis

Screening for phytochemical constituents of the aqueous extract of *A. hispidum* was done using standard methods (12, 13). The constituents and tests used are presented (Table 1)

High Performance Liquid Chromatography Analysis

High performance liquid chromatography analysis was performed on the aqueous extract of *A. hispidum* using method described by (14) with some modifications. The chromatographic system includes Shimadzu HPLC system consisting of Ultra-Fast LC-20AB prominence equipped with SIL-20AC autosampler; DGU-20A3 degasser; SPD-M20A UV-diode array detector; column oven CTO-20AC, system controller CBM-20Alite and Windows LC solution software (Shimadzu Corporation, Kyoto Japan); column, VP-ODS 5µm and dimensions (150 x 4.6 mm). The chromatographic conditions included mobile phase: solvent A: 0.2% v/v formic acid; solvent B: acetonitrile; mode: linear gradient; flow rate 0.6 ml/min; injection volume 10 µl of 250 µg/ml solution of extract in methanol; detection UV 254 nm; reference standard, Rutin (Fluka, Germany) 50 µg/ml in methanol was used as internal standard. The HPLC operating conditions were programmed to give the following: at 0.01 min, solvent B: 10%; at 5 min, solvent B: 20%; at 15 min, solvent B: 30%. Column oven temperature was 40°C while the total run time was 15 minutes.

Acute toxicity study

The oral acute toxicity study of *Acanthospermum hispidum* was carried out in chicks and mice using the up-and-down method (15). A start dose of 300 mg/kg was used and was repeated in a fresh set of chicks and mice after 48 hours. In the absence of mortality the dose was scaled up to 2000 mg/kg in new set of chicks and mice. The dose of 2000 mg/kg body weight was repeated in a fresh set of chicks and mice after 48 hours. The chicks and mice were individually observed for the appearance of clinical signs of toxicity for four hours after extract administration, and the pattern of mortality within 48-hour if any was recorded. Thereafter all the surviving chicks and mice were monitored for 14 days.

Antiemetic assay

Antiemetic study of the extract was carried out as reported previously (16). Four day old male chicks were divided into five groups of ten chicks each and each chick was kept in a large beaker at 25°C for 20 min to stabilize. Group 1 served as the control and was treated with normal saline (5 ml/kg). Groups 2, 3 and 4 were treated with extract (50, 100 and 200 mg/kg) orally, while group 5 chicks received metoclopramide (5.0 mg/kg) into the leg muscle. After one hour of extract and 30 minutes of metoclopramide administration, anhydrous copper sulphate (50 mg/kg) was administered orally to each chick and the number of retches (an emetic action without vomiting gastric material) was counted for 10 min. The index of antiemetic activity was taken as

the decrease in number of retches in the treated group.

Apomorphine-induced pecking assay in chicks

The effect of the extract on apomorphine –induced pecking in chicks was assayed according to method described by (17) with slight modification in the dimension of the observation cage and duration of observation. Young (4 day old) male chicks were divided into five groups of ten chicks each and each chick was kept in a large propylene cage at 25°C for 30 min to stabilize. Group 1 served as the control and was treated with normal saline (5 ml/kg). Groups 2, 3 and 4 were treated with extract (50, 100 and 200 mg/kg) orally, while group 5 received chlopromazine (2.0 mg/kg) into the thigh muscle. Thirty minutes later 0.5 mg apomorphine/kg was injected into the upper leg muscle of the chicks. Each chick was placed in the testing cage (45×45×45) cm with three lateral walls covered with black paper sheets with dots because dots elicit a strong pecking response to apomorphine injection (18).

Observation started within one minute of apomorphine administration to each chick through a one way mirror by a single experimenter stationed in the room, thus minimizing disturbance of the chicks. The number of pecking was counted cumulatively by direct observation for 30 min after apomorphine injection.

Pentobarbitone - induced sleep test mice

The method described by (19) was used. Swiss albino mice were randomized into five groups of six mice each. Group I mice received 5 ml normal saline/kg body weight orally. Groups 2, 3 and 4 were given 50, 100 and 200 mg extract/kg body weight respectively orally. Mice in group 5 received 25 mg diazepam/kg body weight intraperitoneally. At one hour of extract administration respectively, 25 mg pentobarbitone sodium /kg body weight was administered to each mouse intraperitoneally. The mice were placed in a transparent cage and then observed for onset and duration of sleep, with the criterion for sleep being loss of righting reflex on all four limbs after being gently rolled sideways (20). The interval between administration of pentobarbitone and the loss of righting reflex was used to mark the onset of sleep. Similarly the period between loss and recovery of righting reflex was used as the index of hypnotic effect (duration of sleep) (21).

Test for motor co-ordination (Rotarod Test)

The study was carried out according to the method described by (22). Rota rod treadmill device (Ugo Basile no. 7680, Italy) was used for this experiment. Mice trained to remain on slowly moving (16rpm) rods of 5cm diameter for 180 seconds were selected and randomised into 4 groups of six mice each. Group I mice received 5 ml distilled water/ kg orally. Groups 2, 3, and 4 received 50, 100 and 200 mg extract/kg orally respectively. At one hour of extract administration, each mouse was placed on the rod for 3 minutes, at 30 minutes intervals for 3 h. Inability of each mouse to remain on the rod for 3 minutes indicated lack of motor co-ordination.

Hole-board test for exploratory behaviour in mice

The method described by (23) was adopted for the study. The apparatus consisted of wooden box 60 cm by 30cm with 16 evenly spaced holes (1cm diameter, 2cm depth). The apparatus was elevated to the height of 50 cm, in a dimly illuminated room. Swiss albino mice of either sex were randomly divided into five groups of six mice each. Three groups received graded doses of the extract (50, 100 and 200 mg/kg, orally) while the control group received 5 ml normal saline/kg body weight orally. The positive control group received diazepam (2.5 mg/kg, intraperitoneally). Thirty minutes later, the cumulative number of head dips into the holes was counted for each animal for 5 min (24). An agent that decreases this parameter reveals a sedative

behaviour (25, 26) while anxiolytics have been shown to increase the number of head dips (27).

Statistical analysis

All data were expressed as the mean ±standard error of mean (SEM). Statistical analysis was carried out using one-way analysis of variance (ANOVA). Any significant difference between means was assessed by student's t-test at 95% level of significance

Results

Yield of the extract

The yield obtained was 8.66% on dry weight basis

Phytochemical screening

The phytochemical tests carried out on the *A. hispidum* aqueous extract indicated the presence of saponins, alkaloids, tannins and flavonoids. Anthraquinones and cardiac glycosides were not detected.

HPLC analysis

The HPLC chromatogram of the aqueous extract of *A. hispidum* revealed four major peaks as shown (Fig. 1). The first peak had retention time of 2.95 minutes. The second peak eluted at 3.45 minutes. The third and fourth peaks eluted at 3.69 and 3.94 minutes respectively while Rutin eluted at 4.77 minutes.

Acute toxicity study

The oral administration of aqueous whole plant extract of *Acanthospermum hispidum* at the dose of 2000 mg/kg did not produce clinical symptom of toxicity and mortality in the chicks and mice. The oral median lethal dose (LD_{50}) of the extract was estimated to be greater than 2000 mg/kg in both chicks and mice.

Antiemetic study

Acanthospermum hispidum whole plant extract as well as metoclopramide significantly ($F_{4, 49}$=222, *p<0.0001*) inhibited copper sulphate-induced retching when compared to the normal saline treated control in a dose-dependent manner. There was no difference in the effect of the extract at 200 mg/kg and 5 mg/kg body weight of metoclopramide (Fig. 2).

Effect of extract on apomorphine-induced perking in chicks

Apomorphine produced remarkable increase in frequency of pecking by chicks treated with 5 ml normal saline/kg body weight. The extract as well as chlorpromazine significantly ($F_{4, 49}$=331, p<0.0001) reduced the frequency of pecking in chicks when compared to normal saline treated control (Fig.3).

Pentobarbitone-induced sleep test

The extract significantly ($F_{4, 29}$=18, *p<0.0001*) shortened onset and prolonged ($F_{4, 29}$=637, *p<0.0001*) duration of sleep in pentobarbitone –induced sleep test (Fig. 4 and 5). Diazepam also significantly shortened onset and prolonged duration of sleep.

Effect on exploratory behaviour

Acanthospermum hispidum whole plant extract significantly ($F_{4, 49}$=98, *p<0.0001*) reduced frequency of head dip on hole board apparatus when compared to the normal saline treated control (Fig. 6).

Effect on rota-rod

The extract had no effect on the motor function of the mice as shown by the stability of the mice on the tread mill (Fig.7)

Discussion

The results obtained from the studies showed

that aqueous whole plant extract of *Acanthospermum hispidum* possesses anti-emetic and sedative properties. The extraction method used in this study closely resembles the traditional dosage form in which the plant material is boiled in water. The extract obtained was freeze-dried to produce a lyophilized form which was then standardized against Rutin using high performance liquid chromatography. The retention time at which the three eluates appear is distinct and characteristic of the freeze-dried aqueous extract of *A. hispidum*. Therefore the chromatogram gives reliable qualitative indices for quality assurance. The phytochemical tests carried out on the *A. hispidum* aqueous extract indicated the presence of saponins, alkaloids, tannins and flavonoids. Anthraquinones and cardiac glycosides were not detected. The presence of saponins and polyphenolic constituents (flavones, caffeic acid and acanthospermol galactoside) in *A. hispidum* has been reported for methanolic extract of *A. hispidum* (28).

The acute toxicity study indicated that *Acanthospermum hispidum* aqueous extract at a dose of 2000 mg/kg caused neither visible signs of toxicity nor mortality and it is therefore safe acutely in chicks and mice. *Acanthospermum hispidum* toxicity was classified based on the Globally Harmonized System of Classification and Labelling of chemicals, thus providing relevant index for protecting human and animal health (29). In the anti-emetic study, administration of copper sulphate produced retches in all the treated chicks. However, *Acanthospermum hispidum* produced significant decrease in the bout of retches in a dose-dependent manner. Metoclopramide is a dopamine D_2- receptor antagonist, its blockade of this receptor within the gastrointestinal tract is responsible for its primary prokinetic mechanism of action (stimulation/activation of dopamine receptor within the gastrointestinal tract inhibits cholinergic smooth muscle stimulation and peristalsis is halted). Its additional blockade of dopamine D_2-receptor at the chemoreceptor trigger zone of the medulla (area post rema) results in potent anti-nausea and anti-emetic effects (30, 31). The retch produced by copper sulphate may have been mediated via excitation of visceral afferent nerve fibres of the gastrointestinal tract (16). The serotonergic neurotransmission pathway in the stomach has been implicated in the copper sulphate-induced emesis (32). Therefore the most effective treatments for the control of emesis are drugs that block the serotonin (5-hydroxytryptamine [5-HT]) type 3 receptor antagonists (32, 33, 34). Since the extract effectively reduced frequency of copper sulphate –induced retches, it may therefore be suggested that its action may have been mediated via interaction with peripheral serotonergic receptors as well as dopaminergic receptors located within the gastrointestinal tract as well as in the chemoreceptor trigger zone centrally. The observed antiemetic effect of *Acanthospermum hispidum* may be attributed to its saponins, alkaloids or flavonoid contents (phytoconstituents) *Crinum zeylanicum* bulb as earlier reported for *Grewia lasiodiscus* leaves extracts (16, 26). The apomorphine-induced pecking is a reliable and an established method of evaluating drugs with antipsychotic/neuroleptic potential (Apomorphine acts directly on the post- synaptic dopamine D_2-receptors to induce hyperactivity and stereotypic behaviour. The study was carried out to ascertain the potential antipsychotic effect of *A. hispidum* in chicks.

In the study on apomorphine-induced pecking in young chicks, pecking was produced by apomorphine in all the chicks used. The extract of *A. hispidum* produced significantly reduced the frequency of apomorphine-induced pecking. The reference drug, chlopromazine (2 mg/kg), completely attenuated pecking behaviour produced by apomorphine in chicks. The behavioural responses observed in chicks after administration of apomorphine, are attributed to activation of D1 and D2 receptors (35, 36). The reduction in apomorphine –induced pecking behaviour in chicks may have been mediated by antagonism of D1 and D2 receptors. Mesolimbic and nigrostriatal dopaminergic pathways play key roles in the mediation of locomotor activity and stereotyped behaviour such as pecking observed in apomorphine treated chicks. The mesolimbic and

nigrostriatal dopaminergic pathways are significantly involved in the mediation of locomotor and stereotyped behavioural activities. Stereotyped behaviour is differentially associated with the caudate striatum area of the brain (37). Animal models used for screening antipsychotic drugs are based on the neurochemical hypothesis of schizophrenia, involving mainly the neurotransmitters dopamine and glutamate (38). Most clinically effective antipsychotic drugs against hallucinations and delusions act via antagonism of dopamine D2 receptors (39). The dopamine-based model of schizophrenia usually employs apomorphine, a direct agonist, or amphetamine, a drug that increases the release of this neurotransmitter and blocks its re-uptake. The reduction in frequency of apomorphine-induced pecking behaviour in chicks in this study is suggestive of possible interference with central dopaminergic neurotransmission. This observation indicates potential of *Acanthospermum hispidum* in managing neuropsychiatric disorders associated with psychosis. It has been demonstrated by (40) that substances which reduce apomorphine –induced behaviour in laboratory animals possess neuroleptic effect that may be useful for managing schizophrenia. The present study results are consistent with these findings and revealed that *Acanthospermum hispidum* might have dopamine D1 and/or D2 receptors antagonistic phytoconstituents.

Pentobarbitone sleep time in laboratory animals is an accepted method for evaluation of sedative effect of drugs (41, 42). *Acanthospermum hispidum* strongly increased the total sleep time induced by pentobarbitone in mice and shortened the onset of sleep. These effects suggest the presence of sedative principles in aqueous extract of *Acanthospermum hispidum.* The extract prolongation of pentobarbitone-induced sleep is in agreement with the findings of (43, 44) that prolongation of barbital-induced hypnosis is a valid indicator of central nervous system depressant activity.

The central depressant effect of the extract was further confirmed by the reduction of exploratory behaviour of mice as indicated by decrease in number of head dips in the head dip tset on hole board Suppression of exploratory behaviours has been reported to indicate a central nervous system depressant activity (45, 46) Substances like diazepam which reduce the onset of sleep, increase duration of barbiturate-induced sleep, and reduce exploratory activity possess potentials as sedatives (45). The sedative effects of *A. hispidum* could be related to the presence of phytochemical constituents in the extract activating the benzodiazepine and / GABA sites in the GABA receptor complex (47).

The lack of inhibitory effect of the extract on motor co-ordination as observed in the treadmill suggests that the extract may not be acting via peripheral neuromuscular blockade but rather centrally thus confirming its central sedative property (48). Flavonoids and tannins found in the extract could be the active components of *A. hispidum* since these phytochemicals have been reported to possess anticonvulsant and sedative properties (49, 50). The aqueous extract of *A. hispidum* possesses antiemetic, antipsychotic and calming/sedative effects. These properties could explain the use of this plant in traditional medicine in Africa, particularly in Northern Nigeria in the treatment of behavioural alterations associated with cerebral malaria. Therefore, it can be concluded that *Acanthospermum hispidum* when administered as a single dose is non-toxic and can be used safely for oral formulation of antiemetic agent with sedative effects for management of malaria.

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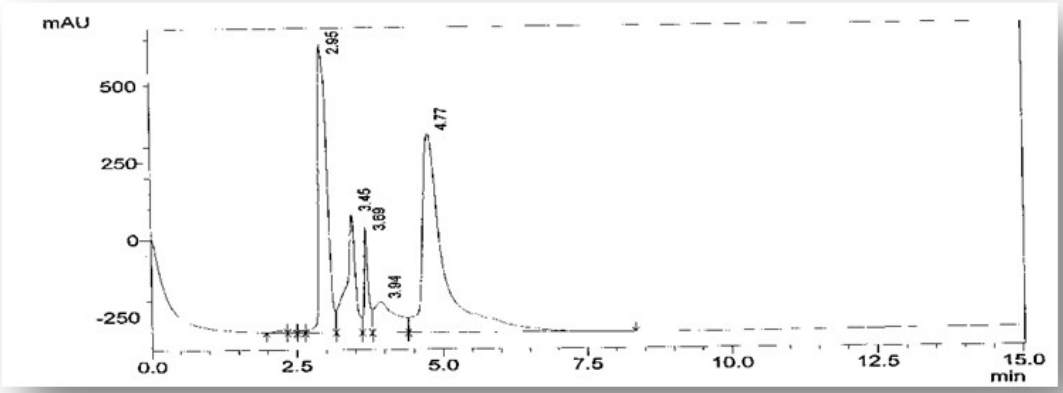

Fig 1: HPLC chromatogram of aqueous whole plant extract of *Acanthospermum hispidum*

Phytochemicals	Test/Reagents
Alkaloids	Dragendorff's reagent and Meyer's reagent
Anthraquinones	Borntrager's test
Cardiac glycosides	- Lieberman's test
	- Keller-Killiani test
Saponins	Frothing test
Tannins	Ferric chloride reagent
Flavonoids	Sodium hydroxide reagent

Table 1: Phytochemical screening of *A. hispidum* aqueous extract

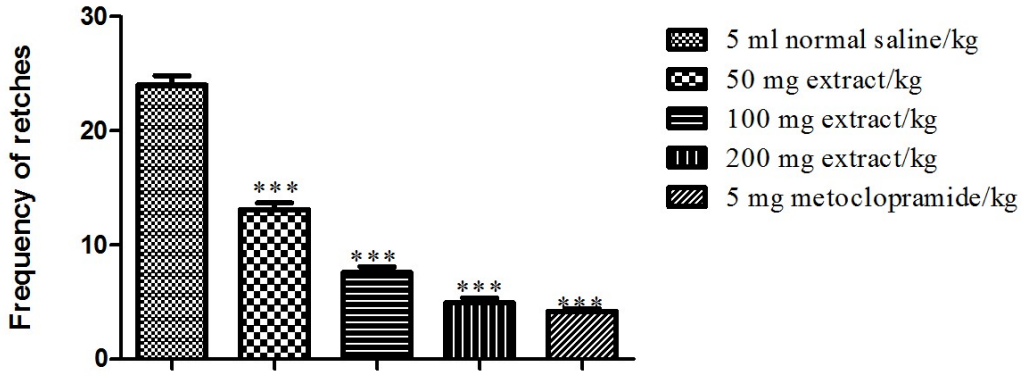

Fig 2: Effect of aqueous whole plant extract of *Acanthospermum hispidum* on frequency of copper sulphate-induced retches in young chicks.
*** significantly different from the control at $F_{4, 49}$=222, p<0.0001, N=10

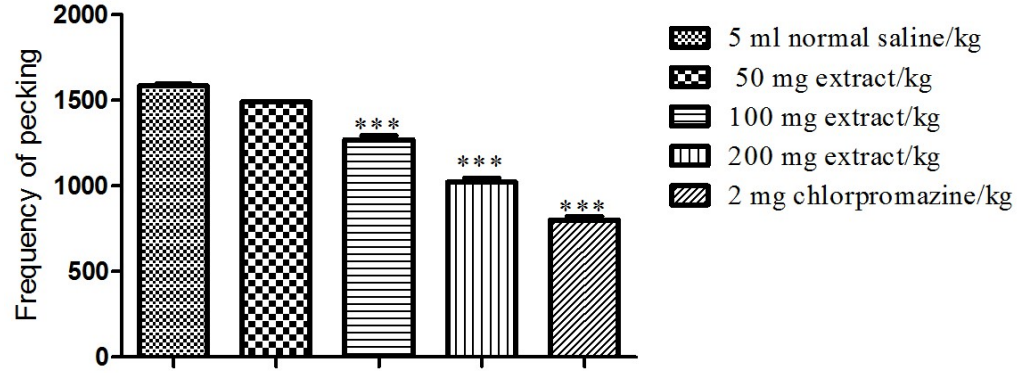

Fig 3: Effect of aqueous whole plant extract of *Acanthospermum hispidum* on apomorphine-induced pecking in young chicks
*** significantly different from the control at $F_{4, 49}$=331, p<0.0001 when compared to the normal saline treated control, n=10

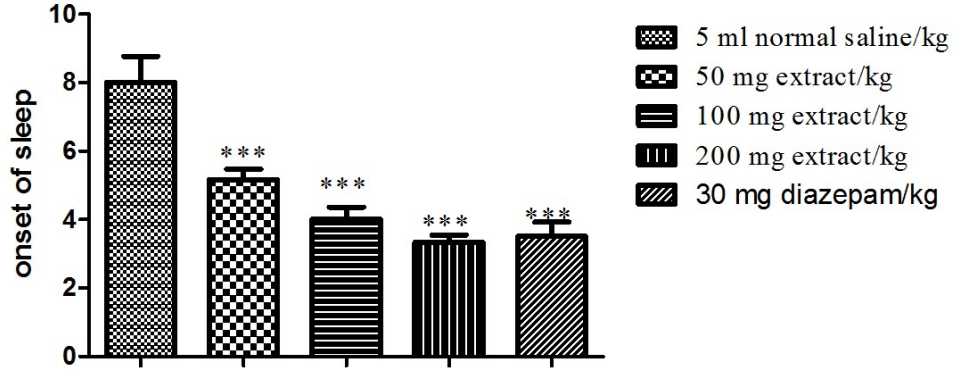

Fig 4: Effect of aqueous whole plant extract of *Acanthospermum hispidum* on sleep onset in pentobarbitone- induced sleep test in mice.*** significantly different from control at F4, 29=18, N=6

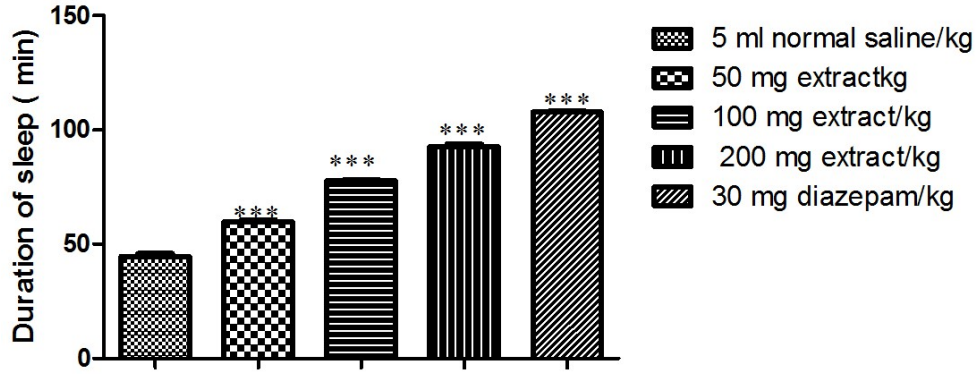

Fig 5: Effect of aqueous whole plant extract of *Acanthospermum hispidum* on duration of sleep in pentobarbitone- induced sleep test in mice. *** significantly different from the control at $F_{4, 29}$=637, p<0.0001, N=6

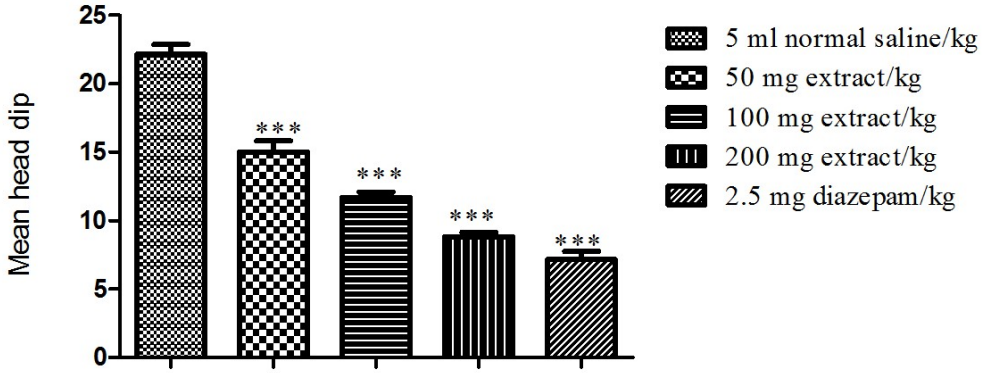

Fig 6: Effect of aqueous whole plant extract of *Acanthospermum hispidum* on frequency of head dip in mice on hole board apparatus. *** significantly different from the control at $F_{4, 29}$=98, p<0.0001, N=6

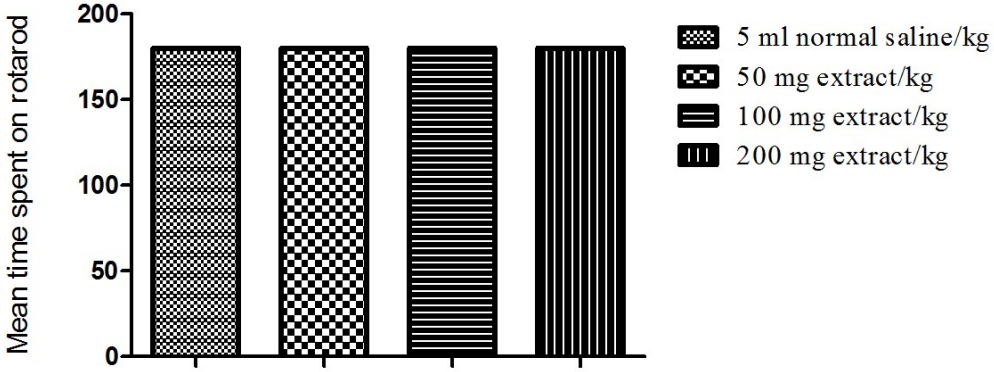

Fig 7: Effect of aqueous whole plant extract of *Acanthospermum hispidum* on mean time spent by mice on rotarod apparatus. N=6