Full Length Research Paper

Anti-emetic activity of *Grewia lasiodiscus* root extract and fractions

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Preparation of *Grewia lasiodiscus* (K Schum) root is used in African traditional medicine to treat fever, pains and emesis. A 70% aqueous methanolic extract of *G. lasiodiscus* root (Extract) was obtained and fractionated on column chromatography using solvents of increasing polarities to yield three fractions designated F1 to F3. The effect of the extract on pentobarbitone-induced hypnosis was evaluated in mice while the anti-emetic activities of extract and its fractions were studied on anhydrous copper sulphate-induced emesis in one-day-old chicks. The oral median lethal dose (LD_{50}) of extract was studied and estimated to be 774 mg/kg. Extract at 25, 50 and 100 mg/kg produced a significant ($p<0.05$) prolongation of sleeping time when compared with the control groups. Extract at 50, 100 and 200 mg/kg produced a significant ($p<0.05$) decrease in copper sulphate-induced emesis in a dose-dependent manner. Fraction F1, at 50 mg/kg produced a 61.51% increase in emesis, while fractions F2 and F3 produced a 39.0 and 56.50% reduction in frequency of emesis, respectively. Our results suggest that the aqueous methanolic extract of *G. lasiodiscus* root and its fractions F2 and F3 have anti-emetic properties, which provide for the first time the rationale for its application in traditional medicine especially in the management of emesis.

Key words: *Grewia lasiodiscus*, methanol extract, root, fractions, pentobarbitone, sleep, anti-emetic.

INTRODUCTION

Nausea and vomiting may be manifestation of a wide variety of conditions, including pregnancy, obstruction, peptic ulcer, drug toxicity, myocardial infarction, renal failure, and hepatitis. Vomiting can follow the administration of many drugs particularly cancer chemotherapeutic agents. Symptoms may occur upon emergence from general anaesthesia and often accompany infectious and non infectious gastrointestinal disorders (Kovac, 2000). In cancer chemotherapy, drug induced nausea and vomiting may occur so regularly that anticipatory vomiting occurs when patients return for treatment before the chemotherapeutic agent is given. If not controlled, the discomfort associated with drug- induced emesis does not only affect the quality of life but can lead to rejection of potentially curative anti-neoplastic treatment (Marty et al, 1990). In addition uncontrolled vomiting can produce dehydration, profound metabolic imbalances, and nutrient depletion.

Chemotherapeutic agents or their metabolites can directly activate the medullary chemoreceptor trigger zone or vomiting center or act peripherally by causing cell damage in the gastro-intestinal tract and releasing serotonin from enterochromaffin cells of the small intestinal mucosa. The released serotonin activates $5-HT_3$ receptors on vagal and splanchnic afferent fibres, which then carry sensory signals to the medulla, leading to the emetic response. Current anti-emetic agents are costly and possess serious side effects which include sedation, extral-pyramidal symptoms, severe headache, hypergly-

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cemia in patients with diabetes mellitus, hence the need to search for safe, effective and affordable alternative anti-emetic agent.

Grewia lasiodiscus K Schum (family Tiliaceae), synonym *Grewia kerstingii* (Burret.) is a shrub that grows up to 3 m high. The leaves are pale green with prominent yellowish nerves beneath and closely toothed margins. The flowers are yellowish in colour with petals appearing greenish outside and pale yellow within, 2-4 in each inflorescence. The fruits are entire, round with both long and short pubescence (Irvine, 1961). The plant is widely distributed in savannah regions of Senegal, Guinea, Sudan and northern part of Nigeria (Irvine, 1948). The branches and the pounded stem bark are cooked with food by the Bassari and Purlaar of Senegal to prepare a sauce for stomach-upsets. The stem bark is reported to contain some active principles including farnesol which has spasmolytic effect on the smooth muscle fibre of the intestines and calming effect in anxiety conditions (Oliver-Bever, 1983). In northern part of Nigeria, the root is used in folkloric medicine for the management of fever, pains and emesis. The aim of this study was to investigate the anti-emetic activity of the root of *G. lasiodiscus* against anhydrous copper sulphate-induced emesis in one-day-old chicks.

MATERIALS AND METHODS

Plant material

G. lasiodiscus (K Schum.) root was purchased from Bodija market (Ibadan, Nigeria). The plant was identified by the Forestry Research Institute of Nigeria, Ibadan, Nigeria, where a voucher specimen has been deposited.

Reagents

Anhydrous copper sulphate and metoclopramide were obtained from Sigma Chemical Company (USA). Hexane, ethyl acetate, and methanol were purchased from Sigma-Aldrich Chemie GmbH (Switzerland).

Extraction

Air-dried, powdered root of *G. lasiodiscus* (200 g) was macerated with 70% methanol in distilled water at room temperature for 24 h shaking continuously for the first 6 h using a GFL shaker (No. 3017 Gesell Schaftfur Labortechnik Mbh, Burgwedel, Germany) and thereafter allowed to stand for the remaining 18 h. The mixture was filtered using whatman No.1 filter paper and the filtrate was concentrated using rotary evaporator (Bibby Sterilin Ltd. UK.) at reduced pressure and 40^oC and freeze-dried, yielding 18.0 g (9.0%) of chocolate coloured powder.

Fractionation

2.5 g of extract was column chromatographed on silica gel, 60-120 mesh (Aldrich chemical Co. USA) 150 g, eluted with 500 ml each of hexane (100%), ethyl acetate (100%), methanol (100%), and 70% methanol in distilled water to obtain three fractions. The three fractions were ethyl acetate fraction (F1), methanol fraction (F2) and

aqueous methanolic fraction (F3). The fractions were concentrated to dryness using rotary evaporator (Bibby Sterilin Ltd. UK.) at reduced pressure and 40^oC. The yields of the fractions F1-F3 were 0.1201 g (4.8%), 1.4436 g (57.7%) and 0.4653g (18.8%), respectively.

Animals

One-day-old chicks of either sex (30 - 40 g), and male albino mice (20 - 25 g) were used in the study. The chicks were obtained from Echo Poultry in Kubwa, Abuja, while the mice were obtained from the Animal Facility Centre of the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. They were fed with starter's mash and standard mouse feed and given water *ad libitum.* All experiments performed on laboratory animals in this study followed the "Principle of Laboratory Animal Care" (NIH Publication No. 85, rev. 1985).

Phytochemical screening

Qualitative phytochemical analysis was carried out on extract and the most bioactive fraction F3 for secondary metabolites (Trease and Evans, 1996). The secondary metabolites tested for were saponins, tannins, glycosides, anthraquinones, phenols, flavonoids, terpenes, sterols, alkaloids and carbohydrates.

Acute toxicity (LD$_{50}$) study

The median lethal dose (LD$_{50}$) of the extract was determined in mice orally according to the method described by Lorke (1983). The study was carried out in two phases. In the first phase, nine mice were randomized into three groups of three mice each given 10, 100 and 1000 mg extract/kg body weight orally. Another mouse was given normal saline to serve as the control and all the mice were kept under the same conditions and observed for signs of toxicity and mortality for 24 h. The result of the first phase of the study informed the choice and administration of 140, 225, 370 and 600 mg extract/kg body weight orally to another four groups of three mice each in the second phase of the study. These mice were also observed for signs of toxicity and mortality for 24 h.

Pentobarbitone-induced hypnosis in mice

This test was performed in 6 groups of 5 mice each. Three groups received extract at the doses of 25, 50 and 100 mg/kg orally, while distilled water (10 ml/kg) was administered (orally) to the fourth group, which served as control. The last two groups received diazepam (1 mg/kg) and apomorphine (2 mg/kg). Thirty min after this treatment, pentobarbitone sodium (25 mg/kg i. p.) was administered to each mouse. Each mouse was observed for the on-set and duration of sleep in minutes, with the criterion for sleep being loss of righting reflex, indicated by the animals inability to resume or return to its up right position on all four limbs after being gently rolled side ways (Miya et al., 1973; Roland et al., 1991) and the interval between loss and recovery of righting reflex will be tested as the index of hypnotic effect.

Bioassay for antiemetic activity

The one day old chicks were divided into five groups of five chicks each and each chick was kept in a large beaker at 25^oC for 20 min. Group 1 served as the control and was treated with 5 ml normal saline/kg. Groups 2, 3, and 4 were treated with 50, 100, and 200

Table 1. Effect of extract on pentobarbitone- induced hypnosis in mice.

Treatments/dose	On-set of sleep (minutes)	Duration of sleep (min)
30 mg Pentobarbitone /kg+ normal saline	7.0±0.98	99.8±6.02
2 mg Diazepam/kg + 30 mg Pentobarbitone/kg	3.0±0.63*	155.2±9.82*
100 mg extract/kg + 30 mg Pentobarbitone/kg	2.5±0.63*	122±1.87*
50 mg extract/kg + 30 mg Pentobarbitone/kg	3.2±0.91*	94.6±1.08
25 mg extract/kg+ 30 mg Pentobarbitone/kg	4.8±.72*	72.6±1.91
2 mg apomorphine/kg + 30 mg pentobarbitone/kg	15.2±0.18*	36.2±3.08

*Significantly different from control at p<0.05.

mg extract/kg body weight orally, while group 5 was treated with 50 mg metoclopramide/kg body weight intraperitoneally. One hour later 50 mg anhydrous copper sulphate/kg body weight was administered orally to each chick, and then the number of retches (an emetic action without vomiting gastric material) was counted for 10 min. The antiemetic effect was assessed as the decrease in number of retches in the treated group in contrast to the control. The inhibition (%) was calculated as follows:

Inhibition (%) = [(A-B)/A] x 100

Where A is the control frequency of retching and B is the frequency of retching of the treated group.

Antiemetic activity of fractions

The three column chromatographic fractions F1, F2 and F3 were individually evaluated for antiemetic activity as described above.

Statistical analysis

All numerical data are expressed as the mean ± standard error of mean (SEM). Statistical analysis was carried out using student's t-test and differences between means were considered to be significant when p < 0.05.

RESULTS

Phytochemical tests

Qualitative phytochemical analysis showed the presence of saponins, tannins, anthraquinones, glycosides, phenols, terpenes, sterols and carbohydrates in extract while saponins, tannins and sterols were present in F3.

Acute toxicity tests

The behavioral signs of toxicity exhibited by mice that received 100 mg extract/kg and above are decreased respiratory rate, inactivity, increased abdominal contractions. There was however mortality at 1000 mg extract/kg body weight within 24 h post-treatment. The oral LD_{50} of extract in mice was estimated to be 774 mg/kg body weight.

Pentobarbitone- induced hypnosis in mice

Extract produced significant (P<0.05) dose dependent shortening of on-set of sleep, while significant (P<0.05) prolongation of duration of pentobarbitone-induced was observed at 100 mg/kg body weight (Table 1).

Anti-emetic activity

Extract significantly (p < 0.05) and dose-dependently suppressed the frequency of copper sulphate-induced emesis in the day-old chicks when compared with the control. Metoclopramide was observed to be more potent than the extract in inhibiting copper- sulphate-induced emesis. The methanolic fractions F2 and F3 significantly (P<0.05) reduced the frequency of emesis in one day chicks when compared to control. Fraction F3 was observed to possess more anti-emetic activity. Metoclopramide highly significantly (P<0.01) reduced the frequency of emesis in one day old chicks when compared to both control and fractions F2 and F3 respectively as shown in Figures 1a and 1b below while Fraction F1 significantly (P< 0.05) increases the frequency of emesis when compared to the control (Figures 2a and 2b below).

DISCUSSION

The qualitative phytochemical test revealed the presence of saponins, tannins, anthraquinones, glycosides, phenols, terpenes, sterols and carbohydrates in extract of root of *G. lasiodiscus*. In this study, the extract was found to be lethal to mice in doses of 1000 mg/kg and above and the oral LD_{50} was determined to be 774 mg/kg. This shows that the extract is not toxic acutely (OECD, 1996). The extract significantly reduced on-set of sleep and prolonged duration of sleep in pentobarbitone-induced hypnosis in mice. This prolongation of pentobarbitone-induced hypnosis observed in this study strongly suggests that the extract possess central depressant activity (Perez et al., 1998) and supports the suggestion that the extract may be centrally acting anti-emetic. The
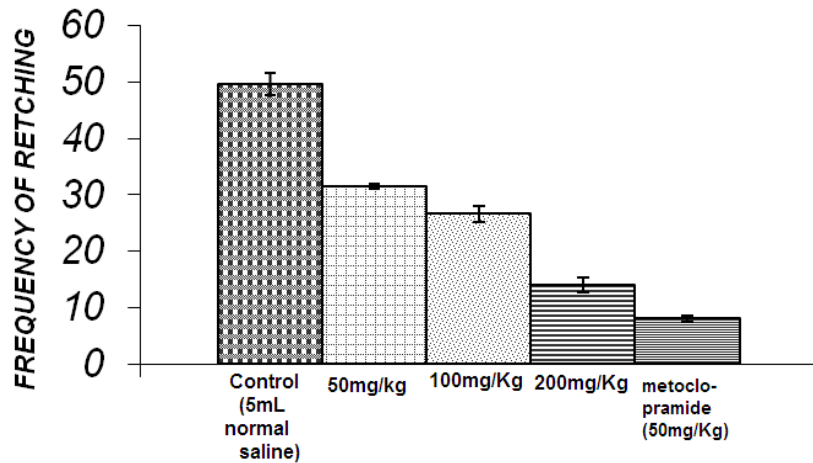
Figure 1a. Anti-emetic activity of *Grewia lasiodiscus* root extract on copper-sulphate-induced emesis one-day-old chicks.
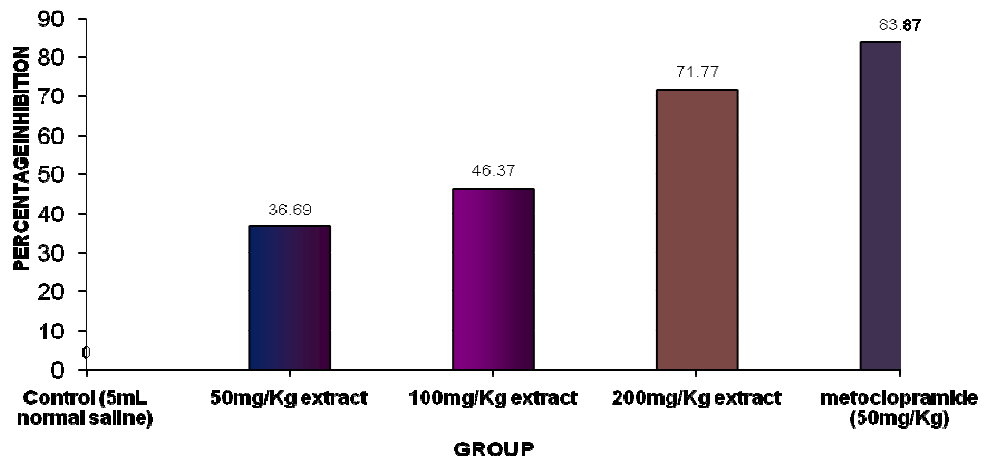

Figure 1b. Anti-emetic activity of *Grewia lasiodiscus* root extract on copper-sulphate-induced emesis one-day-old chicks.
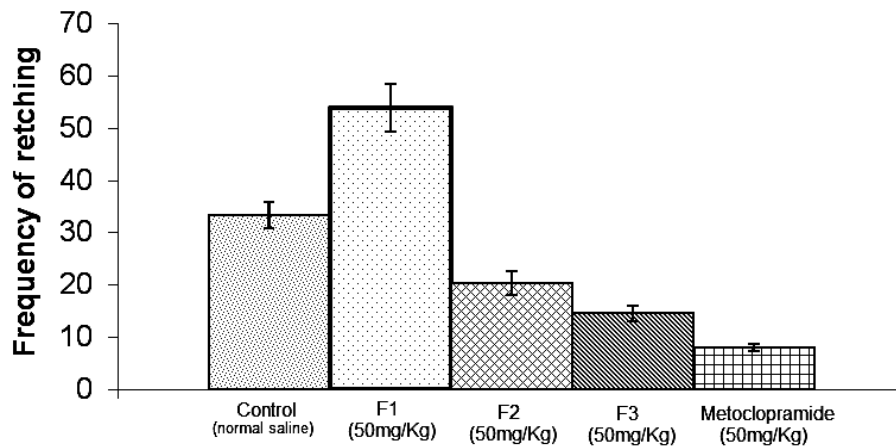

Figure 2a. Anti-emetic activity of fractions f1, f2, and f3 of *Grewia lasiodiscus* root extract on copper-sulphate-induced emesis one-day-old chicks.
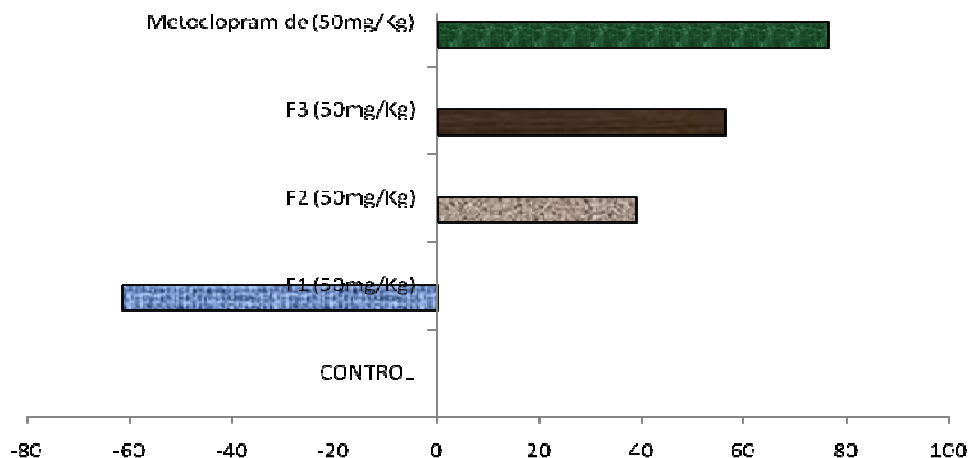
Figure 2b. Antiemetic activity of Fractions F1, F2, and F3 of *Grewia lasiodiscus* on copper-sulphate-induced emesis on one-day-old chicks.

increase in pentobarbitone sleeping time could be due to inhibition of pentobarbitone metabolism (Kaul and Kulkarni, 1978) or an action related to the central mechanism involved in regulation of sleep. For example, endogenous neurotransmitters particularly dopamine and GABA, have been implicated in sleep mechanisms (Osuide and Wanbebe, 1980; Gottesmann, 2002). It is therefore possible that the extract of *G. lasiodiscus* root produced this effect through augmentation of the central inhibitory effect of GABA or through dopaminergic pathways.

The extract of *G. lasiodiscus* root also caused a significant decrease in the frequency of vomiting as shown by the result of copper sulphate-induced emesis in one- day-old chicks test. The partially purified fractions F2 and F3 obtained from the crude extract, however, significantly reduced frequency of emesis in one day old chicks at a much lower dose compared to the crude. However, fraction F1 significantly increased the frequency of emesis in one-day-old chicks. Copper sulphate-induced retching in one-day-old chicks is a useful animal model used in screening for anti-emetic effects of compounds and was adopted for this anti-emetic study because this model mimic acute emesis seen in man and serves as a useful model for evaluating the involvement of the brain in the observed anti-emetic effects of the extracts. Extract and its bioactive fractions F2 and f3 may be acting as agonist at the inhibitory neurotransmitter receptors in the gastrointestinal tract and the brain particularly, dopamine and GABA as suggested by the result of pentobarbitone-induced hypnosis in mice. Nausea and vomiting are caused by interaction of gastrointestinal (GI) system, the vestibular system, and the various parts of the brain (Gan et al., 2003). The various areas in the brain and GI tract involved in the process of nausea and vomiting are the chemoreceptor trigger zone (CTZ) in the brain, the vestibular system, visceral afferents from the GI tract and the cerebral

cortex (ASHP, 1999). Stimuli may reach the vomiting centre in the dorsal part of the lateral reticular formation in the medulla oblongata from chemoreceptor trigger zone along the vagal or sympathetic fibres from the gastrointestinal tract. Copper sulphate induces vomiting through excitation of visceral afferent nerve fibres of the GIT. It has also been established that the peripheral 5-HT4 play an important role in copper sulphate induced emesis (Bhandar and Andrews, 1991; Fukui et al., 1994).

Metoclopramide, which has already been known to elicit antiemetic activity through acceleration of gastrointestinal tract movement (Akita et al., 1998), was found to be more effective than extract and its fractions. The ability of extract and its fractions to significantly attenuate the effect of copper sulphate-induced emesis in one-day-old chicks may in part be due to its effect on the visceral afferent sympathetic fibre in the GIT and possibly through agonistic effect at the inhibitory receptor sites such as dopamine and GABA in central nervous system. The observed antiemetic activity of *G. lasiodiscus* root extract and its active fractions may be attributed to its saponins, tannins and terpenes constituents. This is the first result to show the antiemetic activity of *G. lasiodiscus* root extract and thus provides scientific basis for its use in folk medicine for the management of vomiting.

ACKNOWLEDGEMENTS

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