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Effects of ethanol extract of *Cissus quadrangularis* on induced gastric ulcer in rats

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Antiulcer activities of the ethanol extract of *Cissus quadrangularis* roots on indomethacin and ethanol-induced gastric ulcers were investigated. The results obtained show that the ulceration in gastric linings of the stomach of rats pre-treated with the *C. quadrangularis* extract before induction with ethanol and indomethacin decreased significantly when compared to the control. The protective effect of the extract increased in a dose-dependent manner in both ulcer models. There were significant decreases ($p < 0.05$) in the number of ulcer lesions when rats were administered with the graded doses of the extract and ranitidine (100 mg/kg body weights) compared with the control groups in both models. Results from this study suggest that the extract of *C, quadrangularis* roots possesses antiulcer activities.

Key words: *Cissus quadrangularis,* ranitidine, indomethacin, ethanol and ulcer lesions.

INTRODUCTION

The use of plants and its extracts have aided mankind in the treatment of different ailments including infectious and non-infectious diseases for many years. Over the decades, researchers have been exploring the biodiversity of the plant kingdom to find new and better drugs that could cure many diseases that are afflicting human population (Abdul et al*.,* 2009 ; Omale and Okafor, 2008; Shanthi et al., 2010; Anoop and Jagdeesan, 2006; Jainu and Devis, 2004; Jainu and Devis, 2003).

Several natural products, mostly of plant origin have been shown to possess promising activities that could assist in the prevention and/or amelioration of diseases such as human immunodeficiency virus / acquired immunodeficiency syndrome (HIV/AIDS), malaria, tuberculosis,among others. Many of these agents have other medicinal values as well, which afford them further prospective as novel, which leads to the development of new drugs that could deal with both viruses and other diseases (Adebayo and Kretti, 2011).

According to the World Health Organization (2004), about three-quarters of the world population rely on plants for the treatment of many illnesses and useful drugs have been developed from plants used in traditional medicine which aspects of toxicity and efficacy may be known from the long history of usage.

In the traditional system of medicine, various plants parts such as stem back, root back, aerial root, vegetative bud, leaves, fruits and latex are used in the treatment of variety of ailments (Davies and Evans, 2008).

Most of the clinical drugs that are currently in use were derived from plants and developed because of their usage in traditional medicine. Aspirin (anti-pyretic), atropine, digoxin, morphine (pain killer), quinine and so on, were discovered through the study of ethno-botany.

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Medicinal plants are of great importance to the health of individuals and communities.

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes, and some of these medicinal plants used in herbal medicine in South Eastern Nigeria include Cleome *rutidosperma, Emilia coccinea, Euphrobia heterophylla, Physcalis bransilensis, Sida acuta, Spigelia anthelmia, Stachytarpheta caynnensis and Tridxa procumbens* (Okwu, 2001).

Cissus quadrangularis is a low growing shrub with a characteristic, four sided stem. It is a climbing plant, often found growing over the lower growing vegetation. *C. quadrangularis* has a slender stem with varying length. It is dichotomously branched sub-angular, glabrous, brown fleshy, fibrous, smooth with four-winged internodes constricted at nodes. *C. quadrangularis* reaches a height of 15 cm and has quadrangular-sectioned branches with internodes of 8 to 10 cm long and 1.2 to 1.5 cm wide. Along each angle is a leathery edge, toothed leaves 2 to 5 cm wide appear at the nodes.

Each has a tendril emerging from the opposite side of the node (Attawish et al., 2002; Jainu and Devi, 2004; Jainu et al., 2006; Shanthi et al., 2010; Anoop and Jagdeesan, 2006; Jainu and Devis, 2003). Extracts of *C. quadrangularis* roots have been used traditionally in the treatment of inflammation and pain in which the plant functions as an anti-inflammatory agent and analgesic (Shanthi et al., 2010; Anoop and Jagdeesan, 2006; Jainu and Devis, 2004; Jainu and Chennam, 2006; Jainu and Chennam, 2005; Jainu and Devis, 2003; Evans, 2001; Attawish et al., 2002). The aim of this study was to investigate the therapeutic effects of ethanol root extract of *C. quadrangularis* against indomethacin and ethanol-induced ulcer in rats.

MATERIALS AND METHODS

Animals

Adult albino rats (120-200 g) of either sex were used for the experiment. They were procured from the animal house of the Faculty of Agricultural Sciences, University of Nigeria, Nsukka. The animals were acclimatized in metal cages for one week in the animal house of Department of Biochemistry, University of Nigeria, Nsukka, prior to analysis.

Plants

Roots of *C. quadrangularis* were obtained from Mr. A. Ani of BDCP (Bioresource Development and Conservation Program) Nsukka. The leaves were identified and authenticated by the officer in-charge of the *hebarium* in the Botany Department, University of Nigeria, Nsukka. The roots were dried under the sun for two weeks until they were completely dried and then milled to a coarse powder with an electric milling machine.

Instruments/apparatus

The following instruments/apparatus were used in the current study: Chemical balance (Gallenkamp England), plastic container (Gallenkamp England), filter paper (Whatman), electric blender machine, (Gunagztion, China), measuring cylinder (Pyrex, England), beakers (Pyrex, England), refrigerator (Kelvinator, Germany), metal cages, Petri-dish, dissecting kit (Stainless, Japan), cotton wool (Neomedic Ltd, Thailand), hand gloves (DANA JET, Nigeria), syringes, rotary evaporator, razor (Czech Republic), scissors (steel, China), magnifying glass and plastic funnel

Analytical chemicals/reagents

Analytical chemicals/reagents used included: ethanol (absolute) (Sigma-Aldrich, Switzerland), distilled water (Energy Research Center, UNN), ethanol (analytical) (Sigma-Aldrich, Switzerland), chloroform (Sigma-Aldrich, Switzerland), sodium chloride (BDH Chemicals, England), inodmethacin (Emzor Pharmaceuticals, Nigeria) and ranitidine (Emzor Pharmaceuticals, Nigeria).

Extraction procedure

The fresh roots of *C. quadrangularis* were dried under the sun for two weeks and the dried roots were reduced to powder with an electric blending machine. 1.25 kg quantity of the pulverized roots was obtained and then this stock quantity was macerated with ethanol and allowed to stay for 24 h after which filtration was done using Whatman filter paper. The filtrate was concentrated in beakers with the aid of a rotary evaporator and water bath at reduced temperature. After concentration, a crude brownish semi-solid substance weighing 52.6 g was obtained. The substance was then preserved in a small container (film container), covered in a water-proof and then kept inside a cupboard at stable room temperature until when needed.

Determination of the weight of the extract

After concentration, the weight of the beaker with the extracted material inside it was measured using a sensitive weighting balance. The actual weight of the extract was obtained by subtracting the original weight of the beaker from that of the weight of beaker plus extracted material.

Determination of the percentage yield of the extract

The percentage yield of the extract is calculated as follows:

$$Percentage\ (\%)\ yield\ of\ the\ extract = \frac{Weight\ of\ extract\ X\ 100}{Weight\ of\ pulverized\ leaf}$$

Determination of the acute toxicity of ethanol extract of *C. quadrangularis*

The acute toxicity studies were investigated in mice following Pihan et al. (1987) method with slight modification. Sixteen (16) albino mice of either sex were used. They were divided into four groups of

four mice each. Group one received 100 mg/kg body weight of the extract. Group two mice were treated with 1500 mg/kg body weight of the extract. Group three received 2500 mg/kg body weight of the extract and Group four mice were treated with 5000 mg/kg body weight of the extract using the dosage control formula given as:

$$Dosage\ control\ formula = \frac{mg\ of\ drugs/kg\ B.W}{1000}\ X\ \frac{Weight\ of\ animal\ (g)}{conc.\ of\ drug\ (mg/ml)}$$

After the administration, the animals were left for 24 h after which the number of death were observed and recorded in each group.

Determination of the effect of the extract on indomethacin-induced ulcer in rat stomach

Twenty (20) albino rats of either sex were fasted for 24 h with access to water following the method of Agrawal and Dajani (1993) with slight modification. At the end of the fasting period, the animals were weighed using weighing balance (triple beam) and their weights (120-194 g) determined. They were grouped into five (5) main groups with four rats each and were treated orally as follows: Group 1 (control) were administered with 0.9% normal saline (50 ml/kg) and indomethacin (50 m/kg). Group 2 were treated with 100 mg/kg body weight of the extract and indomethacin (50 mg/kg). Group 3 received 200 mg/kg body weight of the extract followed by inodmethacin (50 mg/kg). Group 4 received 400 mg/kg body weight followed by indomethacin (50 mg/kg). Group 5 received standard drug (ranitidine) 100 mg/kg and indomethacin (50 mg/kg) using the dosage control formula described above.

After the administration, the animals were left for 8 h after which they were sacrificed by chloroform anesthesia in an air-eight plastic container and then dissected. There stomach were removed by cutting from the oesophagoal and pyrolic ends and were operated by cutting along the greater curvature. The contents were removed and the stomach washed with distilled water. The stomach was spread over a dissecting board and the ulcer lesions in the glandular potion were determined with the aid of a hand lens (x10). The lesions were coded as n_1, n_2 and n_3 representing n≤1mm, 1<n≤2mm and 2<n≤3 mm as described by Trease and Evans(1983).

Ulcer indices (mm)

Individual ulcer index (mm)

This can be defined as the total number of ulcerations found in each rat. It is denoted by $N=n_1, + n_2 + n_3 + n_4$.

Group ulcer index (GUI) (mm)

This is the sum of all the individual ulcer indices in a group. It is denoted by $TN = N_1 + N_2 + N_3$. Where, N_1, All n_1 in the group; N_2, all n_2 in the group; N_3, all n_3 in the group; N_4, all n_4 in the group.

Mean ulcer index (MUI) (mm)

This is simply the average of the ulcerations found in a group. It is obtained by dividing the total number of individual ulcerations in a group (GUI) with number of rats in that group.

Percentage inhibition (PI)

With respect to this study, the percentage inhibition can be calculated using the formula:

% Ulcer inhibition (%UI) = (1-Ut/Uc) X 100

Where, Ut represents the ulcer index of the treated group and Uc represents the ulcer index of the control group.

Effect of the extract on ethanol-induced ulcer in rats stomach

This determination was carried out using the method of Pihan et al. (1987). 15 adult rats were randomly divided into five groups of three rats each. The rats were deprived of food for 2 h and treated orally with normal saline and varying doses of the plant extracts. The extracts and drugs used were freshly prepared as a suspension in normal saline and administered orally (PO) to the animals in 5 ml/kg doses. Group I (normal control) was administered normal saline (5 ml/kg). Group II, II and IV were treated with 100, 200 and 400 mg/kg of the plant extracts, respectively. Group 5 (positive control) was administered 100 mg/kg of ranitidine, a standard anti-ulcer drug. Each animal received 1 ml of absolute ethanol orally 30 min later, the animals were sacrificed 4 h after administration of the ethanol, and their stomach removed and opened along the greater curvature. The stomach was rinsed with water, pinned flat on a board, examined with a hand lens (X10) and scored for ulcer. The total ulcer scores and ulcer indices for the groups were obtained and used to calculate the percentage ulcer inhibition as shown above using Trease and Evans (1983) method.

RESULTS

Extraction

After the extraction and concentration, the weight of the extract was 52.6 g from the 1.25 kg of the powdered roots of *C. quadrangularis* and the percentage yield was calculated using the formula shown below:

$$Percentage\ (\%)\ yield = \frac{Weight\ of\ extract\ X\ 100}{Weight\ of\ pulverized\ leaf}\quad \frac{52.6g}{1025g} = 5.13\%$$

Acute toxicity of the extract

From the results shown in Table 1, no mortality was observed in all the groups of mice that were given *C. quadrangularis* orally after 24 h of treatment. Therefore, the LD_{50} value of *C. quadrangularis* was estimated to be above 5000 mg/kg body weight. This results show that ethanol extract of *C. quadrangularis* is relatively safe.

Protective effect of the extract on indomethacin-induced gastric ulceration in rats

The results presented in the Table 2 and Figure 1 below indicate that pretreatments with test extracts reduced the ulceration markedly. The percentage inhibition of ulceration by the text extracts were 70.15, 82.00 and 83.10% at 100, 200 and 400 mg/kg doses, respectively which is comparable to that of standard anti-ulcer

Table 1. The acute toxicity (LD_{50}) of the extract.

Group	Mice used	Dose (mg/kg)	Dead (%)
1	4	100	0
2	4	1500	0
3	4	2500	0
4	4	5000	0

Table 2. The protective effect of the extract on indomethacin-induced ulcer.

Group	Number of rats	Mean ulcer index (MUI) (mm)	Percentage inhibition (%)
1 (Normal control)	4	2.50±0.5439	-
2 (100 mg/kg)	4	0.75±0.3862	70
3 (200 mg/kg)	4	0.45±0.0577	82
4 (400 mg/kg)	4	0.4±0.0816	85

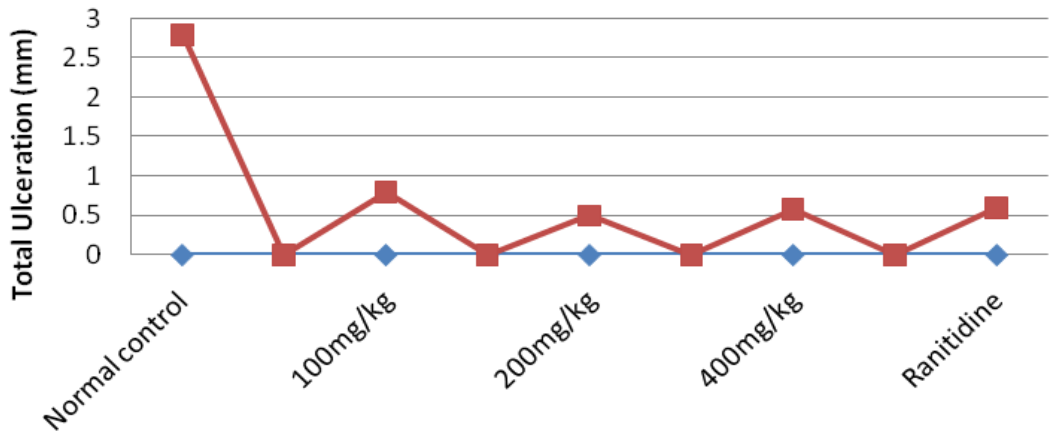


Figure 1. The effect of extract on indomethacin induced ulcer in rats.

drug ranitidine (100 mg/kg) as shown in Table 2. Results in Figure 1 and Table 2 show a significant increase ($p < 0.05$) in the ulceration level of the control group administered with normal saline as compared with the ulceration level of groups [3(200 mg/kg), 4(400 mg/kg) and 5 (ranitidine)]. But there was no significant difference in the ulceration level of group 2 (100 mg/kg) when compared with groups [3(200 mg/kg), 4(400 mg/kg) and 5 (ranitidine)].

Protective effect of the extract on ethanol induced gastric ulceration in rats

The results obtained in ethanol induced gastric ulcer as shown were comparable to that obtained in indomethacin-induced ulcer. The extract proved to be more efficient on indomethacin-induced ulcer than in the ethanol-induced ulcer. This was observed in ulcer lesions inside the rat stomach where the dark spot-like lesions induced by indomethacin reduced more readily than the reddish lesions that were induced by ethanol. From Table 3, the percentage (%) inhibition of the ulceration induced by ethanol by the test extracts are 30, 53, and 57% for 100, 200 and 400 mg/kg B.W., respectively in dose dependent manner. Also, from Figure 2 and Table 3, the result obtained shows that ulceration in groups 3 (200 mg/kg), 4 (400 mg/kg), and 5 (ranitidine) significantly decreased ($p < 0.05$) compared to group 1 (normal control). There was no significant difference ($p < 0.05$) in the level of ulceration in group 2 when compared with group 1 (normal control).

DISCUSSION

Ulceration of body tissue lining such as the gastric lining of the stomach by the non-steroidal anti-inflammatory

Table 3. The protective effect of the extract on ethanol-induced gastric ulcer in rat.

Group	Number of rats	Mean ulcer index (MUI) (mm)	Percentage inhibition (%)
1 (Normal control)	3	2.86±2.76666	-
2 (100mg/kg)	3	2.33±2.3333	30
3 (200mg/kg)	3	1.33±1.3333	53
4 (400mg/kg)	3	1.20±1.20000	57
5 (ranitidine)	3	1.23±1.3333	60

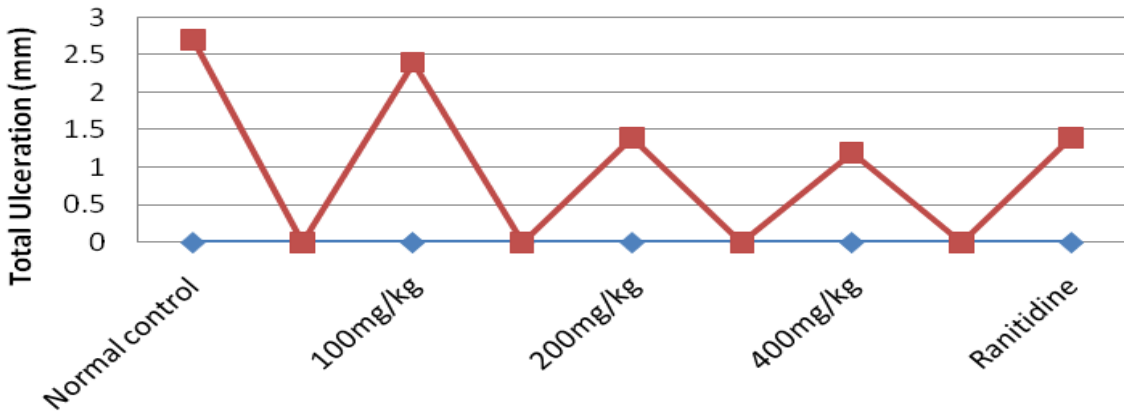


Figure 2. The effect of extract on ethanol-induced ulcer in rats.

drugs (NSAID) which includes indomethacin, ibuprofen could be as a result of inhibition of the cyclooxygenase I (COX-I) enzyme by their metabolic action in order to suppress inflammatory diseases (Agrawal and Dajani, 1993), but the concern over severe side effects of these drugs has led to the search for new antiulcerogenic agents from plants with low toxicity and minimal side effects (Curtis and Griffin, 1991). Previous study on *C. quadrangularis* root extracts showed that the root contains carbohydrates and minerals like sodium, potassium, iron and calcium and also some bioactive components like saponin, flavonoids, glycosides, tannins and phenolic compounds (Enechi and Odonwodo, 2003) and these bioactive compounds could be responsible for its anti-ulcer healing.

The present research has provided first hand information on acute toxicity and the protective effects of the plant extract on indomethacin and ethanol-induced gastric ulceration in rats. The acute toxicity study revealed that the plant is relatively not toxic to the experimental animals and could be used in medical treatments. The results from Table 2 and Figure 1, show that indomethacin (50 mg/kg) that induced ulcer in the stomach of the rats was qualitatively antagonized in a dose-dependent fashion as observed in groups 2, 3 and 5 that were administered with 100, 200 and 400 mg/kg body weight of the extract, respectively.

Also, the results show that the ulceration rate was significantly higher ($p < 0.05$) in the group administered with normal saline (control) compared with the positive control group and test groups. This indicates that the level of ulceration in negative control group is high due to the absence of antiulcerogenic agents and this agrees with the findings of Kabe and Kutimu (1994), who observed the same effect on the negative control group. Also, from Table 3 and Figure 2, the level of ulceration induced by ethanol was also antagonized dose-dependently by the extracts but the level of inhibition was higher in indomethacin induced ulceration compared to that of the ethanol induced ulcer, though both are comparable.

Conclusion

The results shown in this work suggest that ethanol root extract of *C. quadrangularis* has the potential efficacy of protecting the stomach linings against ulceration induced by both indomethacin and ethanol in rats.

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