Anti-ulcer activity of methanol extract of the leaves of Hannoa klaineana in rats

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ABSTRACT

Ulcer is a common gastrointestinal disease affecting 5% of the world’s population. Hannoa klaineana is used locally in the management of many gastrointestinal disorders and fevers. The study was conducted to evaluate toxicity profile and anti-ulcer effect of methanol extract of the leaves of Hannoa klaineana (Simaroubaceae). Acute toxicity test was conducted according to OECD guideline 423 using the limit test dose (5000 mg/kg) for 14 days. Sub-chronic toxicity study was carryout according to OECD guideline 407 by daily oral administration of the extract (500 and 1000 mg/kg) for 28 days. Anti-ulcer effect of the extract (100, 200 and 400 mg/kg b.wt) was evaluated using ethanol and indomethacin induced gastric ulcer models. In acute toxicity test, 5000 mg/kg dose of the extract does not caused mortality nor any sign of toxicity observed in the rats, thus, the LD50 value of the extract was above 5000 mg/kg. While sub-chronic toxicity test, the extract demonstrated significant (p<0.01) increase in body weight and weight of the liver, spleen and kidneys of the rats. The result also showed significant (p<0.05) dose-dependent decrease in serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, uric acid and creatinine. Ethanol-induced ulcer model, the extract demonstrated significant (p<0.05) dose-dependent decrease in mean ulcer index, with percentage inhibition (62.84%) of gastric damage at 400 mg/kg. While indomethacin model, the exhibit exhibited significant (p<0.001) dose-dependent decrease in mean ulcer index with percentage gastroprotection (99.20%) at 400 mg/kg. This study suggested that methanol extract of the leaves of Hannoa klaineana is safe for oral administration and exhibited strong anti-ulcer effect, thus validating the traditional use of the plant leaves in ulcer treatment.

Keywords: Ethanol, Gastric ulcer, Hannoa klaineana, Indomethacin, Toxicity, NSAIDs.

INTRODUCTION

Ulcer is defined as a break (open wound) in the epithelial lining of the stomach, duodenum, or lower esophagus that results from the excess secretion of gastric acid (hyperacidity), or possibly bacterial and mechanical action [1]. Based on the locus of occurrence in the gastro-intestinal tract (GI), ulcer is classified into four types; gastric, duodenal, esophageal, and meckel’s diverticulum ulcer [2]. Gastric ulcer is the most common gastrointestinal disease characterized by damage in the gastric mucosa associated with hemorrhage and perforation [3]. The cause of gastric ulcer is multi-factorial, and is mostly attributed to the disequilibrium between aggressive luminal factors such as hydrochloric acid, pepsin secretion, H. pylori infection and bile salts and defensive mucosa-protective factors such as mucin, bicarbonate secretion, prostaglandins, nitric oxide and growth factors [4, 5].

Ulcer remains a major public health problem whose complications are invariably associated with increase in morbidity and mortality worldwide [6, 7]. The prevalence of ulcer was estimated at 40% in developed countries and 80% in developing countries [8]. Current knowledge shows that about four million people are affected by peptic ulcer worldwide [9]. Studies showed that men are more affected by peptic ulcer disease (PUD) than women, with high prevalence of duodenal ulcer in young people and gastric ulcer in aged people [10, 11]. It was estimated that almost 5-15% of adult population are suffering from peptic ulcer disease and its consequences worldwide [12]. The prevalence rate of gastric ulcer is associated with age and sex, as well as lifestyle [13]. The incidence rate varies between 3~10%, with estimated mortality of about 15 out of every 15,000 complications worldwide [8].

Hannoa klaineana is belonging to the family Simaroubaceae and the genus is Hannoa [13]. In most of the African countries, the plant is locally used for the management of various gastro-intestinal diseases and fevers including malaria [13, 14]. The plant is found in different regions in Nigeria and is called “Takardar giwa” (in Hausa) and “Ofor” (in Igbo) by the Northerners and Southerners, respectively. Due to it's high
content of crude protein and crude fat, the plant is used traditionally in producing fodder banks which when properly located minimizes the time animals use to search for adequate fodder [15]. Hannoa klaineana has been described for its anti-microbial and anti-tumor activities (16, 17). Study showed that Hannoa klaineana exhibited antioxidant properties by virtue of its free-radical scavenging activity and significant lactate dehydrogenase (LDH) inhibitory effect [18].

Conventional anti-ulcer drugs are generally expensive, and therefore not affordable to the majority of patients. The drugs are associated with side effects such as nausea, dizziness, headaches, diarrhoea, abdominal pain, and constipation [19]. Several studies have been conducted on the anti-ulcer effect of local medicinal plants [20, 21, 22, 23]. To the best of our knowledge, there is paucity of information on the anti-ulcer effect of Hannoa klaineana. Local herbalists claimed success in curing ulcer by using different parts of the plant without scientific justification. This study was conducted to evaluate toxicity profile and gastroprotective effect of methanol extract of the leaves of Hannoa klaineana (Simaroubaceae).

MATERIALS AND METHODS

Experimental animals

Wistar rats weighing between 180-200g (n=90), housed in the experimental animal laboratory of Biochemistry Department, Usmanu Danfodiyo University, Sokoto were used in this study. The animals were maintained in polycarbonate cages (5 rats per cage) at standard temperature 23±2°C, relative humidity 30-70%, and 14 hours light per 10 hours dark cycle. The rats were fed with standard diet (normal rat’s pellets) and had free access to tap water.

Collection and authentication of plant material

Fresh leaves of the Hannoa klaineana were obtained from Anka and Shinkafi local government areas of Zamfara state by assistance of local herbalists in Sokoto Old Market. The plant material was identified and authenticated (UDUH/ANS/0335) by Abdulazeez Salihu a Herbarium Officer, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto.

Preparation of extract

The plant leaves were dried in a clean ventilated room for seven days. The dried leaves were pulverized to coarse powder using pestle and mortar. The leaves powder (1000g) was extracted in 80% methanol for 3 days with intermittent stirring at 1-hour interval. The extract was filtered through Whatman filter paper and concentrated by vacuum rotary evaporator at 40°C under reduced pressure for 3 hours. The weight and percentage yield of the extract was recorded and then stored in desiccators until further used. The required dose was reconstituted in distilled water for administration.

Preliminary phytochemical screening

The extracts of different solvents were screened for the presence of various phytoconstituents according the standard methods [24, 25, 26]. The best extract was used in this study.

Acute Toxicity Study

Acute toxicity study was conducted according to Organization for Economic Cooperation and Development (OECD) 423 Guidelines for Testing of Chemicals [27] using the limit test dose (5000 mg/kg b.wt). The animals (180-200 g, n = 6) were randomly divided into two groups of 3 rats each; control and test group. The animals were fasted overnight for 24 hours before administration of the extract. The test group received a single dose of 5000mg/kg body weight while the control group received 10ml/kg/day of normal saline. The animals were closely observed in the first 30 minutes after administration of the extract and continuously for the next 24 hours, and then daily for 14 days for a mortality and detection of clinical sign of toxicity. LD50 was determined in accordance with the Globally Harmonized System of Classification and Labelling of Chemicals [27].

Sub-chronic toxicity study

Repeat-dose oral toxicity test was performed according to Organization for Economic Cooperation and Development (OECD) 407 guideline for Testing of Chemicals [28]. Twenty-four albino rats (180-200g) were randomly distributed into three groups; 8 rats per group. Group A administered with 10ml/kg b.wt/day of normal saline and served as control while groups B and C received the extract at a dose of 500 and 1000 mg/kg b.wt/day, respectively. The extract was administered (2ml/200g b.wt) by oral gavage daily for 28 days. Clinical signs of toxicity and mortality were observed, and body weight of the animals was evaluated weekly. After 28 days of the administration, the rats were fasted for 12 hours, anaesthetized and then sacrificed. Whole blood was immediately collected by cardiac puncture; aliquoted into plain vacutainer tubes, and kept for 1 hour at room temperature (RT) to coagulate. The sample was centrifuged at 10,000 x g for 10 minutes at 4°C and the serum was separated for the analysis of biochemical parameters; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total proteins, albumin, creatinine, urea, uric acid, and glucose using standardized diagnostic kits.

Anti-Ulcer Activity Test

Ethanol-induced ulcer model

Gastric ulcer was induced according to the method described by Astudillo et al. [29]. Animals (180-200g, n=30) were grouped into six groups each of 5 rats. Group I and II received normal saline (10ml/kg b.wt/day) and served as normal control and negative control groups, respectively. Group III was treated as positive control administered orally reference drug, omeprazole (20mg/kg b.wt/day) [30]. Group IV, V and VI were respectively pre-treated with 100, 200 and 400 mg/kg dose of the extract. The rats were pre-treated with the varying doses of the extract and the standard drug for 14 days. On the 15th day, the animals were allowed for 24 hours starvation after which the last administration was given to the rats. Ulcer was induced thirty minutes after the last administration by oral administration of absolute ethanol (99%, 1ml/200g) [31] to all the groups except the normal control group. One hour after the induction of ulcer, the animals were anaesthetized and then sacrificed by cervical dislocation.

Indomethacin-induced ulcer model

Gastric ulcer was induced using the method described by Kakub and Gulfr (32). The rats (180-200g, n=30) distributed into six groups each comprising 5 rats. Group I and II were administered normal saline (10ml/kg bw./day) and served as normal control and negative control, respectively. Group III was treated as positive control administered reference drug, omeprazole (20mg/kg b.wt/day) [30]. Group IV, V and VI were pre-treated with various doses of the extract (100, 200 and 400 mg/kg). The treatment lasted for 14 days with the varying doses of the extract and the standard drug by gavage. On the 15th day, the rats were starved for 24 hours after which the last administration was given to the rats. Thirty minutes after the last administration, gastric ulcer was induced by single intra-peritoneal administration of indomethacin (60mg/kg, i.p.) [32] to all the groups except the normal control. Four hours after administration of indomethacin, animals were anaesthetized and then sacrificed by cervical dislocation.

Gross and microscopic examination of ulcers

The abdomen of the rats was incised and the stomachs were dissected out, opened along the greater curvature and washed with normal saline to remove gastric contents and blood contaminants. The stomachs were put on a glass slide and examined for damage in the gastric mucosa.
using 10 × magnification lenses and dissecting microscope. The number and the length of gastric lesions were evaluated.

Ulcer rating

Ulcers were scored based on their intensity according to the rating system adopted by Kulkarni [33].

| Severity                                  | Score |
|-------------------------------------------|-------|
| Normal appearance of stomach              | 0     |
| Red appearance of stomach                 | 0.5   |
| Spot ulcer                                | 1     |
| Hemorrhagic streaks                       | 1.5   |
| Ulcer ≥ 3 mm² but ≤ 5 mm²                 | 2     |
| Ulcer >5 mm²                              | 3     |

Calculation of ulcer index and percentage gastro-protection

Ulcer index was calculated using the formula below:

\[ UI = UN + US + UP \times 10^{-1} \] [34]

Where; \( UI \) = Ulcer Index; \( UN \) = Average of number of ulcer per animal; \( US \) = Average of severity score; \( UP \) = Percentage of animals with ulcer

The percentage gastro-protection was calculated using the formula below:

\[ \% \text{ gastro protection} = \frac{UIC - UIT}{UIC} \times 100 \] [34]

Where; \( UIC \) = Ulcer Index of Control; \( UIT \) = Ulcer Index of Test

Determination of gastric mucus content

The gastric mucus content was estimated using the method described by Perera et al. [35]. The glandular section of the stomach was gently scrapped on a glass slide, the gastric mucus was collected and homogenized in 4ml of normal saline. The weight of the distilled water and the homogenate was measured and the weight of the gastric mucus was obtained as follows:

\[ \text{Gastric Mucus Content (g)} = \text{Weight of Homogenate} - \text{Weight of Distilled Water} \]

Statistical analysis

Statistical Package for Social Sciences (SPSS) Statistics version 22 software (IBM Corp., Armonk, NY, USA) was used for data analysis. Comparison between the treated groups and the controls were performed by Mann–Whitney non-parametric test. Significant differences between the groups were computed by One-way analysis of variance (ANOVA) confidence level (95%) and Tukey-Kramer multiple comparisons test and two-tailed (\( p<0.05 \)) were considered significant.

RESULTS

Preliminary phytochemical screening

Table 1: Preliminary Phytochemical Screening of the Leaves Extracts of Hannoa klaineana

| GROUPS          | METHANOL | ETHANOLIC | AQUEOUS |
|-----------------|----------|-----------|---------|
| Alkaloids       | +++      | ++        | ND      |
| Flavonoids      | +++      | ++        | ++      |
| Tannins         | +++      | ++        | +       |

Results of the phytochemical screening of aqueous, ethanol and methanol extracts of the leaves of Hannoa klaineana showed the presence of alkaloids, flavonoids, saponins, glycosides, tannins, terpenoids, steroids, cardiac glycosides, glycoside saponins and balsams (Table 1). The methanol extract demonstrated more of the bioactive compounds than the aqueous and ethanol extracts and was used in this study.

Acute toxicity test

In acute toxicity study, the methanol extract (5000 mg/kg) of the leaves of Hannoa klaineana did not caused mortality or any sign of toxicity in rats in the first 24 hours and during the 14-days observation period. Thus, the LD\(_{50}\) value of the methanol extract of the leaves of Hannoa klaineana was greater than 5000 mg/kg.

Sub-chronic toxicity test

The effect of methanol extract of the leaves of Hannoa klaineana on body weight of rats is shown in Fig. 1. The extract at the dose 1000 mg/kg demonstrated significant (\( p<0.01 \)) increase in the body weight of rats in week 4 as compared to the control group and the initial (week 0) weight of the rats.

**Figure 1**: Effect of Methanol Extract of the Leaves of Hannoa klaineana on the Body Weight of Rats

Fig. 2 shows the effect of methanol extract of the leaves of Hannoa klaineana on the weight of organs of rats. The extract at the dose 500 mg/kg demonstrated significant increase in the weight of the liver (\( p<0.01 \)) and spleen (\( p<0.05 \)) as compared with the control group. However, at the dose 1000 mg/kg, the result showed significant increase in the weight of the liver (\( p<0.001 \)), spleen (\( p<0.001 \)) and kidneys (\( p<0.01 \)) of the rats as compared to the control group.
The effect of methanol extract of the leaves of Hannoa klaineana on biochemical parameters in rats is shown in Fig. 3. The extract at the doses 500 and 1000 mg/kg demonstrated significant decrease in serum levels of AST (p<0.05, p<0.01), ALP (p<0.001), urea (p<0.001) and uric acid (p<0.001) as compared with the control. The result also showed significant (p<0.01) decrease in serum levels of creatinine at the dose 1000 mg/kg of the extract. However, the result showed no significant differences in serum levels of ALT, total protein, albumin, and glucose.

**Figure 2: Effect of Methanol Extract of the Leaves of Hannoa klaineana on the Organs Weight**

The percentage gastro-protection of methanol extract of the leaves of Hannoa klaineana against the ethanol-induced gastric damage is shown in Fig. 6. The extract demonstrated gastro-protective effect in a dose-dependent manner. At the dose 400 mg/kg the extract exhibited the maximum protective effect (62.84%) against the gastric lesions and is comparable to that of the omeprazole (80.51%), the reference standard drug (Fig. 6).
Effects of methanol extract of the leaves of *Hannoa klaineana* on gastric mucus content

Fig. 7 shows the effect of methanol extract of the leaves of *Hannoa klaineana* on gastric mucus content in ethanol-induced gastric ulcer model. The extract demonstrated significant dose-dependent increase in gastric mucus content of the rats. The extract (100, 200 and 400 mg/kg) exhibited significant (*p<0.05, p<0.01, p<0.001*) increase in gastric mucus content of the rats in comparison to the negative control group, respectively (Fig. 7).

Effects of methanol extract of the leaves of *Hannoa klaineana* on indomethacin-induced ulcer in rats

The morphological appearance of stomach of the rats in indomethacin-induced ulcer model is shown in Fig. 8. Administration of indomethacin produced damage with extensive hemorrhages in negative control rats (Fig. 8 II). The mucosa the rats pre-treated with 100 and 200 mg/kg doses of the extract showed spot damage and red appearance (Fig. 8 IV, V) while the rats pre-treated with the extract (400 mg/kg) and the reference drug showed normal appearance of the mucosa (Fig. 8 VI, III).
gastric lesions in a dose-dependent manner. The maximum gastroprotective effect (99.20%) was found at 400 mg/kg dose of the extract and was almost similar to that of the omeprazole (99.84%), the reference standard drug (Fig. 10).

DISCUSSION

In the present study, LD50 value of methanol extract of the leaves of *Hannoa klaineana* is greater 5000 mg/kg. Limit test dose is mainly used in situations where a researcher had information indicating that test substance is likely to be non-toxic or has less toxic effect [27]. According to the OECD [27] guidelines, substances with an LD50 value of greater than 5000mg/kg through the oral route are regarded as being safe. This suggests that the methanol leaves extract of the *Hannoa klaineana* at the limit test dose 5000 mg/kg is relatively non-toxic and safe in oral formulation.

The result of the present study showed significant increase in body weight and weight of the liver, kidney, and spleen. Exposure to the possible toxic materials causes reduction in body weight and weight of organs which would be attributed to toxic effect of a chemical [36]. Alterations in body weight are indicator of adverse effects of a drugs and chemical substances [36, 37]. Relative organ weight is fundamental in assessing the exposure of organ to injury or damage. Change in organ weight is important indicator of physiological and biochemical status of animals. Heart, liver, kidney, spleen and lungs are the primary organs that are affected by a biochemical reaction of toxicants [38]. In the present study, the significant increase in the body weight and weight of the liver, kidney, and spleen of the animals signified that the methanol extract of the leaves of *Hannoa klaineana* has potential benefits in physiological and biochemical status of the animals.

The activity of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are good markers of liver functions and used as biomarkers to conclude probable toxic effect of a chemical substance [39]. Destruction to the liver parenchymal cells results in elevated level of AST and ALT in the blood (Wolf et al., 1972). In the present study, the methanol leaves extract of the *Hannoa klaineana* demonstrated a significant decrease in serum levels of AST, ALT and ALP. This suggests that repeat-dose oral administration of the extract did not cause changes in the liver cell functions and the normal metabolism of the rats. Urea, creatinine and uric acids levels are good indicators of kidney functions [39]. The extract demonstrated significant decrease in serum levels of urea, creatinine and uric acid suggesting that sub-chronic administration of the extract did not cause renal impairment.

Ethanol-induced gastric ulcer is a common animal model of peptic ulcer disease [40] to investigate anti-ulcer effect of a substance [41]. The model causes peptic ulcers in animals that mimic acute peptic ulcers in humans [40]. The model is used to evaluate cytoprotective effect of a chemical substance and/or antioxidant activity of a test agent [42]. On this basis, ethanol induced gastric ulcer was used in this research. Ethanol is a risk factor for developing peptic ulcer diseases (PUDs) [43]. Ethanol has ability to solubilizes mucosal protective layer penetrating gastric mucosa and expose the mucosa to proteolytic and hydrolytic activities of pepsin and hydrochloric acid causing damage to the membrane [43, 44]. In the present study, oral administration of the absolute ethanol caused severe damages in the gastric mucosa of negative control rats. The methanol extract of the leaves of *Hannoa klaineana* suppressed ulcerogenic tendencies of ethanol in the pretreated rats suggesting its effectiveness in preventing ethanol-induced gastric damages.

Mucus is a layer on the surface of gastric mucosa of stomach that plays a vital protective role [45]. At the normal physiological conditions, mucus and bicarbonate defense system is powerful to protect gastric mucosa against proteolytic and hydrolytic actions [45]. A number of chemicals such as ethanol and HCl can decrease gastric mucus content leading to gastric mucosal damage [46, 47]. In this study, the methanol extract of the leaves of *Hannoa klaineana* demonstrated significant increase in gastric mucus content of stomach of the rats.

Non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin are important anti-inflammatory groups which have been used widely to establish animal models of gastric ulcer [48]. The biochemical mechanism involves secretion of gastric acid and synthesis of mucosal prostaglandin [49]. The model is use to investigate anti-secretory and cytoprotective activities of a chemical substances [49]. The mechanism of the NSAIDs involves non-selectively inhibition of cyclooxygenase activity, resulting to a decrease in production of prostaglandins [49]. The inhibitory action of indomethacin on prostaglandin synthesis has been opined as critical biochemical events in the pathogenesis of gastric ulceration [50, 51, 52].

In the present study, the methanol extract of the leaves of *Hannoa klaineana* exhibited significant gastro-protective effect against indomethacin-induced gastric ulcer in rats. The anti-ulcerogenic effect of the extract could be attributed to its ability to enhances prostaglandins synthesis in the gastric mucosa or possess prostaglandins-like substances.

CONCLUSION

This study has provided explanation to support the local use of *Hannoa klaineana* for the treatment of gastric ulcer and other diseases among rural dwellers in different parts of Nigeria. Our findings suggest that methanol extract of the leaves of *Hannoa klaineana* is relatively non-toxic and provide the justification for further studies to investigate other beneficial pharmacological effects in preclinical studies. Further studies are also needed to further evaluate the gastroprotective effect of the solvent fractions of the extract and to isolate the specific compounds and elucidate their mechanisms of action.

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