INTRODUCTION

Ulceration refers to any break in the skin or mucus membrane and is classified according to the part of the digestive system in which it occurs [1,2]. Gastric ulcer occurs in the stomach while peptic ulcer occurs in sections of the gastrointestinal tract exposed to gastric acid and pepsin such as the stomach and duodenum [1,2]. The etiology of gastric ulceration is not clearly known. It results probably from an imbalance between aggressive (acid, pepsin and Helicobacter pylori infection) and defensive (gastric mucus and bicarbonate secretion, prostaglandins (PGs), cyclooxygenases, nitric oxide, innate resistance of the mucosal cells) factors; as well as factors such as genetic, psychosomatic, humoral and vascular derangements [1]. Pathological examination of gastric ulcer could be evaluated in any part of the stomach, but is most commonly obtained from the lesser curvature. Histologically, there is a break in the superficial epithelium penetrating down to the muscularis mucosa with a fibrous base accompanied with increase in inflammatory cells [1].

Croton zambesicus is a tree native to West and Central Africa. It grows up to 16 m in height and is traditionally used to treat fever, dysentery, hypertension, convulsions and bacterial infections [3-5]. Amongst the Yorubas of South West, Nigeria; it is locally called “ajekobale” which means “witches do not dare to perch on it” and, therefore, believed to possess spiritual properties that can be used to counter the forces of witchcrafts [3]. Scientific studies have observed antimicrobial properties of the bark of C. zambesicus [6], antiplasmodial [7] and anti-ulcer potentials of the ethanolic extracts of its roots [8] and anti-coagulant properties of dichloro methane and aqueous extracts of C. zambesicus leaves [9]. This study, therefore, compared the anti-ulcer properties of doses of methanolic extract and essential oils of the leaves of C. zambesicus to determine which of the extraction products would better improve the status of alanine aminotransferase (ALT) concentrations in the liver; gastric acidity and histopathological restoration of the stomach of adult Wistar rats in indomethacin-induced gastric mucosa ulceration.

MATERIALS AND METHODS

Collection, Authentication and Preparation of Plant Extract

Methanolic extraction of C. zambesicus leaves

C. zambesicus leaves were purchased from local traders at Oja-Tuntun market of Ilorin, Kwara State, Nigeria. The collected
samples were identified at the Department of Plant Biology of the University of Ilorin, Kwara State, Nigeria and deposited at the herbarium. Air-dried samples of *C. zambesicus* leaves were pulverized and 200 g of the plant material was extracted with 70% methanol for 24 h. The plant materials were re-soaked in 70% methanol for 2 weeks for maximum extraction. The extract was filtered, concentrated with rotary evaporator and further dried on a water bath to yield 5.4 g of the extract.

**Extraction of essential oils of *C. zambesicus* leaves**

*C. zambesicus* leaves weighing about 0.75 kg were placed in a Clevenger (distillation apparatus) overlying water. As the water got heated, the steam passed through the plant material, vaporizing the volatile compounds. The vapors flowed through a coil where they condensed back to liquid, which was then collected in the receiving vessel. This procedure yielded 1 ml of oil which was dissolved in 2.5 ml of dimethylsulphoxide and normal saline up to 100 ml.

**Ethical Approval, Care and Feeding of Animals**

Thirty-five adult female wistar rats weighing between 100 and 170 g were obtained from the colony bred of the Physiology Department of University of Ibadan, Ibadan, Oyo State, Nigeria. Animals were fed during the experiment with growers feed from Bendel Feed and Flour Mill Ltd., Nigeria. The animals were caged under standard condition in the well ventilated animal house of the Faculty of Basic Medical Sciences of University of Ilorin, Ilorin, Kwara State, Nigeria at room temperature of 25°C. Water was supplied *ad libitum* to the rats. Ethical approval was sought and received from the ethical committee of the Faculty of Basic Medical Sciences of University of Ilorin, Ilorin, Kwara State, Nigeria on the usage of animals for experimental studies.

**Chemicals, Reagents and Laboratory Equipments**

Indomethacin (Hovid, Nigeria), Omeprazole (Epazole, China), sodium dihydrogen phosphate (NaH₂PO₄), disodium hydrogen phosphate (Na₂HPO₄), hydrogen peroxide (H₂O₂) and trichloroacetic acid were products of Aldrich Chemicals; sulphuric (VI) acid (H₂SO₄) and hydrochloric acid were products of BDH Chemical Limited, Poole, England; Tris buffers, phosphoric acid and pyrogallol were products of Sigma Chemicals, St. Louis USA and ALT assay kits of Randox Laboratories, United Kingdom. Spectrophotometer (Jenway Model 6405; UV/visible), centrifuge, pH meter (Rex model pH 25), Norm-jet needles and syringes (Norm-jet Inc. Tuttingler, Germany) and anti-coagulant tubes (Sterling products, England).

**Administrations of Doses of Drugs/Extracts to Animals**

Doses of drugs (Normal Saline, Indomethacin and Omeprazole), methanolic extract and essential oils were administered orally to Wistar rats according to the earlier described protocol on animal models of gastric ulceration experimentation [10,11]. Oral administrations of drugs were carried out using 3-ml syringe with a 16-G, 3-inches needle attached. The thirty-five rats were divided into seven groups with five rats per group. Rats of Control Group I received physiological saline daily for 5 days (days 1-5). On day 1, a single oral dose of 80 mg/kg bodyweight of indomethacin administered to rats of Group II-VII that have been deprived of food for the previous 18-24 h, produced erosive lesions in the gastric mucosa within 4-6 h. Rats of Group II were euthanized after induction of gastric ulceration on day 1 for scoring of ulceration, gastric acidity assay, histological and biochemical evaluations.

Treatments of gastric ulceration was started on day 1 with a single oral administrations of 40 mg/kg bodyweight of omeprazole, 5 and 10 mg/kg bodyweight of essential oils of *C. zambesicus* leaves, 250 and 500 mg/kg bodyweight of methanolic extract of *C. zambesicus* leaves to rats of Groups III-VII respectively, 4 h after administration of 80 mg/kg bodyweight of indomethacin. The treatment procedures were continued daily for another 3 days (days 2-4). Administered drugs or extract doses were freshly prepared daily. Upon completion of treatment procedures on day 4, the animals were left without food but provided with water *ad libitum* 14 h prior to euthanasia. 30 min prior to euthanasia, 1 ml of 1% Evan’s blue in saline was injected intravenously into the tail vein of rats of Group II (on day 1); Groups I and III-VII (on day 5) using a 1 ml 25-G, 5/8-inch needle to aid identification and evaluation of lesions and ulcerations.

**Scoring System for Gastrointestinal Lesions in the Rats**

| Score | Characteristics                                      |
|-------|-----------------------------------------------------|
| 0     | No ulcerations or mucosal damage                     |
| 1     | Up to 15 small mucosal ulcerations (<1 mm in diameter) |
|       | observable only as slight depressions in reflected light |
| 2     | Small and medium mucosal ulcerations (1-4 mm in diameter); no ulcerations >4 mm in diameter |
| 3     | Predominantly medium and large ulcerations; ulcerations >4 mm in diameter; no intestinal adhesions |
| 4     | Large ulcerations; exhibit signs of perforations and adhesions which make it difficult to remove the intestinal tracts |
| 5     | Necropsy of dead or euthanized animals reveals evidence of massive peritonitis resulting from intestinal perforations |

**Evaluations of Gastric Acidity in Stomach Tissues**

The stomach contents were collected into a centrifuge bottle, mixed properly with Normal Saline and the mixture centrifuged at 2000 revolutions/min for 10 min. One drop of phenolphthalein indicator was then added to the supernatant. 1 ml of the supernatant (volume of acid-Vₐ) was pipetted and titrated against 0.01M NaOH (concentration of the base-Cₐ). The color change was noted (end point) and the volume of base (Vₐ) used was recorded. Gastric acidity was calculated using CₐVₐ = CₐVₐ.

**Evaluations of ALT Levels in Liver Tissues of Rats of Groups I-VII**

The liver was excised and removed from each rat of Groups I-VII, cut into small pieces, placed in a mortar to which 0.1M phosphate buffer (extracting solution) of at least four times the
volume of the organ was added. The organ was homogenized into fine solution with the use of mortar and pestle. The homogenate was poured into a test tube and centrifuged at 5000 revolutions/min for 5 min. The supernatant was carefully removed and the residue was discarded. The supernatant served as the sample for the estimation of ALT levels which were determined in liver samples of rats of Groups IV-VII based on the protocols described in assay kits of Randox Laboratories, United Kingdom.

**Histological Analyses**

The stomach samples of rats of Groups I-VII were excised and removed for histopathological evaluations as earlier described [11].

**Statistical Analyses**

The mean ± standard error of mean value of each of the measured parameters of gastric acidity and ALT assays in rats of Control Group I (which received physiological saline) and Group II (which received indomethacin only) were compared with those of Groups III-VII (indomethacin plus omeprazole, Indomethacin plus methanolic extract doses or indomethacin plus essential oils of *C. zambesicus* leaves) for any significant difference using the Student’s t-test for unpaired samples. *P* = 0.05 (or less) were taken as statistically significant.

**RESULTS**

Analyses of gastric acid assays and histopathological examinations showed dose-dependent statistically significant higher levels (*P* ≤ 0.05) of gastric acidity and non-restorations of the gastric mucosa layer to pre-ulceration states in rats of Groups IV-VII treated with extract doses of *C. zambesicus* leaves when compared to rats of Group III treated with 40 mg/kg bodyweight omeprazole [Table 1 and Figures 1-7]. Specifically, the cytoarchitectural components of the stomach of rats of Control Group I appeared normal while it appeared disrupted in rats of Group II which received only 80 mg/kg bodyweight Indomethacin without further treatment. In rats of Group II, the gastric mucosa components were eroded with multi-focal cellular necrosis, total degeneration of mucus secreting cells and excessive hemorrhage of the mucosa. However, the cytoarchitectural components of the stomach of rats of Group III treated with 40 mg/kg bodyweight omeprazole appeared normal though with mild hemorrhage of the gastric mucosa and few ulcerated sites. There was gradual regeneration of disrupted mucosa following indomethacin-induced gastric ulceration.

The cytoarchitectural components of the stomach of rats of Group IV treated with 5 mg/kg bodyweight of essential oils of *C. Zambesicus* leaves appeared disrupted. Large parts of the mucosa were eroded with multi-focal cellular necrosis, degeneration of mucus secreting cells and excessive hemorrhage of the mucosa. The cytoarchitectural components of the stomach of rats of Group V treated with 10 mg/kg bodyweight of essential oils of *C. Zambesicus* leaves appeared disrupted. The mucosa was eroded with multi-focal cellular necrosis, degeneration of mucus secreting cells and excessive hemorrhage of the mucosa. The cytoarchitectural components of the stomach of rats of Group VI treated with 250 mg/kg bodyweight of methanolic extracts of *C. Zambesicus* leaves appeared disrupted. The mucosa was eroded with multi-focal cellular necrosis, degeneration of mucus secreting cells and excessive hemorrhage of the mucosa. However, some parts of the gastric mucosa showed normal cytoarchitectural components indicating possible gradual restoration of the gastric mucosa to pre-ulceration state.

![Figure 1](image_url)

**Table 1:** Analyses of the effects of extracts of *C. zambesicus* leaves on ulcer index and gastric acidity

| Groups of rats | Dose of drugs/extract | Ulcer index±S.E.M. | Gastric acidity (meq/l) | Statistical significance at *P*≤0.05 (Groups IV-VII vs. III) |
|----------------|-----------------------|-------------------|------------------------|---------------------------------------------------------------|
| II             | 80 mg/kg b.w. indomethacin | 3.25±0.25         | 0.06                   | Significant increase                                          |
| III            | 40 mg/kg b.w. omeprazole | 0.36±0.23         | 0.009                  | Nil                                                          |
| IV             | 5 mg/kg b.w. essential oils of *C. zambesicus* leaves | 3.25±0.48         | 0.02                   | Significant increase                                          |
| V              | 10 mg/kg b.w. essential oils of *C. zambesicus* leaves | 3.75±0.25         | 0.02                   | Significant increase                                          |
| VI             | 250 mg/kg b.w. methanolic extract of *C. zambesicus* leaves | 1.5±0.29          | 0.003                  | Significant increase                                          |
| VII            | 500 mg/kg b.w. methanolic extract of *C. zambesicus* leaves | 2.75±0.48         | 0.02                   | Significant increase                                          |

b.w.: Bodyweight, *C. zambesicus*: Croton zambesicus, S.E.M.: Standard error of mean of five determinations, *P*≤0.05
Statistically non-significant (Group IV) or significant (Groups V-VII) higher ALT levels ($P \leq 0.05$) were observed in liver samples of rats treated with doses of essential oils and methanolic extract of *C. zambesicus* leaves when compared to Group II [Table 2].

**DISCUSSIONS**

Indomethacin is a potent ulcerogen, especially in an empty stomach [10,11]. It induces ulceration mostly in the glandular (mucosal) part of the stomach [1,12] possibly through the inhibition of the release of protective factors like cyclooxygenases, PGE2, bicarbonate, mucus and antioxidants; while aiding vasoconstriction [13] and the increase of aggressive factors such as acid and oxidants [2,12,14]. PGs are potent anti-secretory and anti-ulcer agents which serve protective functions in the stomach by maintaining gastric micro circulation via mucus and bi carbonate stimulation [1,2,12].

Analyses of gastric acid assays and histopathological examinations showed statistically significant higher levels ($P \leq 0.05$) of...
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Table 2: Statistical analyses ALT levels in liver samples of rats of Groups II and I-VII

| Groups of rats | Doses of drugs/extract | ALT concentrations (mg/dl) ± S.E.M. | Statistical significance at P ≤ 0.05 (Groups IV-VII vs. II) |
|----------------|------------------------|-------------------------------------|-------------------------------------------------------------|
| II             | 40 mg/kg b.w. indomethacin | 46 ± 14.04                          | NIL                                                         |
| IV             | 5 mg/kg b.w. essential oils of C. zambesicus leaves | 48.5 ± 11.53                         | Non-significant increase                                     |
| V              | 10 mg/kg b.w. essential oils of C. zambesicus leaves | 91.5 ± 11.53                         | Significant increase                                         |
| VI             | 250 mg/kg b.w. methanolic extract of C. zambesicus leaves | 70.0 ± 5.015                         | Significant increase                                         |
| VII            | 250 mg/kg b.w. methanolic extract of C. zambesicus leaves | 77.5 ± 2.51                          | Significant increase                                         |

ALT: Alanine aminotransferase, b.w.: Bodyweight, C. zambesicus: Croton zambesicus, S.E.M.: Standard error of mean of five determinations, P ≤ 0.05

of the stomach of rats of Group II showed that the gastric mucosa components were eroded with multi-focal cellular necrosis, total degeneration of mucus secreting cells and excessive hemorrhage of the mucosa [Table 1 and Figures 1-3]. This implied that the administration of indomethacin possibly induced the generations of acids and reactive oxygen species which resulted in the disruption of the cytoarchitectural components of the gastric mucosa of rats of Group II. The observed pathological changes were further made possible with the inhibition of increased production of endogenous antioxidants and prostanglandins by Indomethacin actions; and in the absence of the administration of treatment drugs which could aid the production of protective factors such as PGs, mucus and antioxidants.

Evaluations of gastric acid assays and histopathological examinations showed dose-dependent statistically significant higher levels (P ≤ 0.05) of gastric acidity and non-restorations of the gastric mucosa layer to pre-ulceration states in rats of Groups IV-VII treated with extract doses of C. zambesicus leaves when compared to Group III [Table 1 and Figures 1-7]. The gastric mucosae of rats of Groups IV-VII were eroded with multi-focal cellular necrosis, total degeneration of mucus secreting cells and excessive hemorrhage of the mucosa. This implied that the treatments of indomethacin-induced gastric ulceration in rats with extracts doses were not able to significantly reverse the adverse effects of indomethacin administration in treated rats. Furthermore, extracts doses of C. zambesicus leaves could possibly not induce the generation of adequate cyclooxygenases, PGs, bicarbonate, mucus and antioxidants in treated rats. However, some parts of the gastric mucosa in rats treated with 500 mg/kg bodyweight of the methanolic extract of C. zambesicus leaves (Group VII) showed normal cytoarchitectural components indicating possible gradual restoration of the gastric mucosa to pre-ulceration state.
Statistically non-significant (Group IV) or significant (Groups V-VII) higher ALT levels ($P \leq 0.05$) were observed in liver samples of rats treated with doses of essential oils and methanolic extract of *C. zambesicus* leaves when compared to Group II [Table 2]. ALT is the enzyme produced within the cells of the liver and is the most sensitive marker for liver cell damage [16]. Increased ALT levels occur in conditions where cells of the liver have been inflamed or undergone cell death. As the cells are damaged, the ALT leaks into the bloodstream leading to a rise in the serum levels. However, ALT levels may or may not correlate with the degree of cell death or inflammation [16]. The significantly increased elevated levels of ALT in liver samples of rats of Groups II and VII could possibly indicate decreased functional capacity and cellular damage of the liver consequent to the adverse effects of indomethacin-induced generations of reactive oxygen species.

Phytochemical analyses of different parts of *C. zambesicus* showed the presence of flavonoids in its ethanolic root extract [5], diterpenes [17,18], triterpenes and trihydroxyflavone [18], cardiac glycosides and steroids in its stem bark [19]. Flavonoids such as flavones and glycosides promote mucosa PG content, decrease histamine production by mast cells, scavenge free radicals and are natural anti-ulcer agents [20]. Similarly, di-and tri-terpenes have been reported to possess gastoprotective effects in gastric ulceration [2].

The observed low anti-ulcerogenic potentials of administrations of 5 and 10 mg/kg bodyweight of essential oils and 250 mg/kg bodyweight of methanolic extract of *C. zambesicus* leaves in this study could possibly be due to the presence of flavonoids, di-and tri-terpenes in reduced non-potential quantities in *C. zambesicus* leaves. These observed anti-ulcerogenic potential of administration of 500 mg/kg bodyweight of methanolic extract of *C. zambesicus* leaves was lower than that of the standard drug (40 mg/kg bodyweight of omeprazole). This is in agreement with a previous study which observed lower anti-ulcerogenic potentials of Ethanolic root extract of *C. zambesicus* when compared to the standard drug (Cimetidine) [8].

The observed low anti-ulcerogenic potentials of administrations of 5 and 10 mg/kg bodyweight of essential oils, 250 and 500 mg/kg bodyweight of methanolic extract of *C. zambesicus* leaves in this study could possibly be due to the presence of flavonoids, di-and tri-terpenes in reduced non-potential quantities in *C. zambesicus* leaves.

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