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Research Article

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Gastro-Protective Effects of N-Hexane Extract of Lawsonia Inermis Leaves on Indomethacin-Induced Peptic Ulcers in Adults Wistar Rats

Ogundare SO[1,2], Showunmi AO[1], Ogundare EI[3], Ehiremen SE[4], Idehen IC[5], Echekwube M[6], Asibor E[6], Obohwemu KO[7], Airhomwanbor KO[8], Iyevhobu KO[9*] and Augustine EE[10]

[1]Morbid Anatomy and Histopathology Department, Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State, Nigeria
[2]Beacon Hospital, Dublin, Ireland
[3]Tissue Tek Specialist Diagnostic Centre, Ijebu-Ode, Ogun State, Nigeria
[4]Anatomy Department, Faculty of Basic Medical Sciences, Olabisi Onabanjo University Ogun State, Nigeria
[5]Department of Medical Laboratory Science, School of Allied Health Sciences, Kampala International University, Western Campus, Ishaka, Uganda
[6]Department of Histopathology and Cytopathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[7]Faculty of Health, Wellbeing and Social Care, Oxford Brookes University, Birmingham Campus, Birmingham, United Kingdom
[8]Department of Chemical Pathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[9]Department of Medical Microbiology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[10]Mishem Medicals Limited, Venego Plaza, Lokotiye, Nasarawa State, Nigeria

[*]Corresponding author
Iyevhobu Kenneth Oshiokhayamhe, Department of Medical Microbiology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

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ABSTRACT

The stomach is an important organ and the most dilated portion of the digestive system. The oesophagus precedes it, and the small intestine follows. Indomethacin is a potent nonsteroidal anti-inflammatory drug (NSAID) that is very versatile. Its utility lies primarily in its ability to function as an antipyretic, anti-inflammatory, analgesic agent as a commonly used drug, researchers have conducted numerous studies on the side effects of indomethacin. Lawsonia inermis commonly called henna is used all over the world, and is abundantly available in tropical and subtropical areas. This study aims to investigate the effects of Lawsonia inermis leaves in Indomethacin-induced peptic ulcers in adult wistar rats. Thirty female wistar rats of weight between 150-200 g were purchased from a licensed private breeder in Ibadan, Oyo State of Nigeria. No mortality was observed throughout the experiment. The animals across the groups showed similar behaviour, except when 80mg/kg Indomethacin was administered. Group (therapeutic group) exhibited sleepiness, drowsiness, less mobility for up to 18 hours after administration of Indomethacin. Group receiving the aqueous extract before Indomethacin was administered, showed similar signs, and returned to normal behaviour faster than Group IV. Results obtained from this study showed that the n-hexane extract of the fresh leaves of L. inermis had dose-dependent (with high dose being the most effective) histo-gastroprotective effects against Indomethacin-induced peptic ulcerations. N-hexane extract of the fresh leaves of Lawsonia inermis could be used as a therapy for peptic ulceration. Further research on the effect of the other tissue part of Lawsonia inermis on peptic ulceration should be investigated. Further studies on Lawsonia intermis and its effects on other tissue parts or organs should be done.

Keywords: N-Hexane, Lawsonia Inermis, Indomethacin, Peptic, Ulcers, Gastro-Protective

Introduction

The stomach is an important organ and the most dilated portion of the digestive system. The oesophagus precedes it, and the small intestine follows. It is a large, muscular, and hollow organ allowing for a capacity to hold food. It is comprised of 4 main regions, the cardia, fundus, body, and pylorus. The primary functions of the stomach include the temporary storage of food and the partial chemical and mechanical digestion of food. The upper portions of the stomach (cardia, body, and fundus) relax as food enters to allow for the stomach to hold increasing quantities of food. The lower portion of the stomach contracts in a rhythmic

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fashion (mechanical digestion) to aid with the breaking down of food and mixes it with stomach juices (chemical digestion) which also serve to break food down and prepare the mixture, termed chyme at this point of digestion, for further digestion. Stomach stores food, possesses antibacterial action, and secretes gastric juices [1]. Ulcers are most common on the skin of the lower extremities and in the gastrointestinal tract, although they may be encountered at almost any site. There are many types of ulcers such as mouth ulcer, esophagus ulcer, peptic ulcer, and genital ulcer. Of this peptic ulcer is seen among many people. The peptic ulcers are erosion of lining of stomach or the duodenum. The two most common types of peptic ulcer are called "gastric ulcer" and "duodenal ulcer." The name refers to the site of ulceration while gastric ulcer is a rupture in the normal gastric mucosa that extends throughout the muscularis mucosa. Peptic ulcer diseases comprise heterogeneous disorders, which manifest as a break in the lining of the gastrointestinal mucosa bathed by acid and pepsin. It is the most predominant of the gastrointestinal diseases with a worldwide prevalence of about 40% in the developed countries and 80% in the developing countries [2,3]. It is generally recognized that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors [4]. The aetiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermal growth factors) [5]. According to Peckenpaugh and Poleman (1997), some other factors, such as bad dietary habits, excessive intake of nonsteroidal anti-inflammatory agents, stress, hereditary predisposition and Helicobacter pylori infection, which is reported to account more than 70% of cases, are responsible for the development of peptic ulcer diseases [6]. Helicobacter pylori, non-steroidal anti-inflammatory drugs (NSAIDs), pepsins, hydrochloric acid (HCl) and bile acid are the aggressive factors [6].

One powerful and multipurpose nonsteroidal anti-inflammatory medication (NSAID) is indomethacin. As a frequently used medication, indomethacin's main usefulness is its capacity to act as an antipyretic, anti-inflammatory, and analgesic agent. Researchers have studied its negative effects in great detail. Hypersensitivity reactions have been noted to occur because of indomethacin- these reactions can have a plethora of symptoms, including but not limited to anaphylaxis, urticarial, angioedema. Indomethacin (and most other NSAIDs) can impact most organ systems of the body, including the gastrointestinal, neurological, renal, hematologic, and cardiopulmonary systems. As previously mentioned, indomethacin is a non-selective COX inhibitor, and COX-1 is responsible for producing prostaglandins involved in the maintenance of the gastric mucosa. Inhibition of this process can result in dyspepsia (indigestion), nausea, constipation, and diarrhoea. However, the most severe gastric side effect of indomethacin involves the formation of peptic ulcers. NSAIDs weaken the protective mucous layer of the stomach wall and increase the secretion of HCl. Several orthodox pharmaceutical drugs such as anticholinergic drugs, histamine H2-receptor antagonists, antacids, and more recently, proton-pump inhibitors have been employed in the management of peptic ulcers, but they provoke many adverse effects. In recent years, there has been growing interest in alternative therapies especially from plant sources due to their perceived lower side effects, ease of accessibility and affordability [7].

Medicinal plants are some of the most attractive sources of new drugs, and some have been shown to have promise for the treatment of gastroduodenal ulcer with minimum side effects [8,9]. They are a part of human society to combat diseases, from the dawn of civilization. Among the estimated 2,500,000 higher plant species on earth, only 35,000 to 70,000 species (less than 1%) have been used for medicinal purpose [10]. Medicinal plants have contributed immensely to health care in Nigeria [11]. This is due in part to the recognition of the value of traditional medical systems, and the identification of medicinal plants from indigenous pharmacopoeias, which have significant healing power [11]. Plants provide an alternative strategy in the search for new drugs. There is a rich abundance of plants reputed in traditional medicine to possess protective and therapeutic properties. It is likely that plants will continue to be a valuable source of new molecules which may, after possible chemical manipulation, provide new and improved drugs [12]. There are plenty of chances to find out a new compound of plant origin, including those used as ornamentals, amongst which is Lawsonia inermis [13,14].

L. inermis commonly called henna is used all over the world, and is abundantly available in tropical and subtropical areas [15,16]. Henna is a flowering plant, two to six meters in height. It's the sole specie in the genus Lawsonia in the family Lythraceae [17]. Some common names of L. inermis in different languages are: Henna (English), lalle (Hausa), lali (Yoruba), mehndi/heena (Urdu), mehndi (Hindi). Ancient history of India describes diverse uses and appreciable role of henna in Ayurvedic or natural health medicines [1]. It has been used both cosmetically and medicinally for over 9,000 years. Traditionally in India, it is applied to hands and feet as a symbol of fertility [18]. With the remarkable attributes of Lawsonia inermis in alleviating stomach ache related disorders and wound healing enhancement, the present study compared its therapeutic efficacy to a reference drug (esomepra-zole) on indomethacin-induced gastric ulceration in rats. This study aims to investigate the effects of Lawsonia inermis leaves in Indomethacin-induced peptic ulcers in adult wistar rats.

Materials and Methods

Equipment/instruments

Laboratory mortar and pestle, separating funnel, filter paper, water bath, evaporating dish, spatula, weighing scale, plastic tubes, syringes, cannula, timer, chloroform chamber, scalpels, scissors, forceps, blades, icebox, ice, centrifuge (HERMLE MR-2 BY Lab net), Pasteur pipettes, Apetman Micropipettes, test tubes, cuvettes, spectrophotometer, beakers, measuring cylinders, Eppendorf micro centrifuge tubes, plastic homogenizer, incubator (Thermolyne Type 17600 Dry-bath), microwave oven (Binatone MWO2519EG), gel electrophoresis machine, gel documenting machine, coupling jars, funnel, rotary microtome (RM2125RTS, Leica Bio systems, Germany), glass slides, cover slips, drying racks, light microscope (Olympus BX43), Fluorescence microscope (Olympus BX51), Arm scope MD900 and Scoptek 2.0 M digital camera.

Procurement, identification and authentication of adult wistar rats

Thirty female Wistar rats of weight between 150-200 g were purchased from a licensed private breeder in Ibadan, Oyo state of Nigeria. Animal identification was also done at the Department of Zoology, Olabisi Onabanjo University, Ago-iwoye and confirmed as adult Wistar rats (Rattus norvegicus). These rats were transferred to the animal house facility of the Basic Medical Sciences, Sajama Campus under hygiene condition. The animals were fed during the experiment with Growers Feed from Joyful Feed and Flour Mill Ltd., Nigeria. The animals were caged under standard condition in a well-ventilated animal house of the department of anatomy, Olabisi Onabanjo University, and Ogun State, Nigeria at room temperature 250C with water supplied ad libitum to the rats.

Plant material: Collection, identification and authentication of the fresh leaves of L. inermis

The fresh wet leaves of Lawsonia inermis were collected from Ikenne, Ogun state, Nigeria. These leaves were plucked from the plant, pounded, blended then soaked in normal hexane (solvent), sieved or filtered thereby forming a solution and finally concentrated to form the extract. This was done between 25th of October to 29th of October in the year of 2021. These samples were identified and authenticated at the Department of Botany, Olabisi Onabanjo University, Ago Iwoye, Ogun state, Nigeria.

Extract preparation: The fresh L. inermis leaves were washed with tap water, pounded and then blended using a mechanical blender to a very thick paste. 750g of this pasty sample was added into 1.5L of normal hexane using a glass funnel and spatula for 24 hours with occasional shaking. The solution was then filtered. The filtrate was allowed to settle and then decanted. The decant was placed in a rotatory evaporator (evaporating dish) and then put in a water bath at 60 °C for excess water to evaporate gently after which the dried residue was scraped out and stored in a refrigerator.

Acclimatization and feeding of animals: The rats were acclimatized for three weeks before the commencement of the experiment. Their environment was cleaned daily to prevent infection of any form upon the animals. During the acclimatization, pelletized feeds from Joyful Feed and concentrates, Nig. Ltd were given to the rats as well as ad libitum with their bedding changed daily.

Determination of Body weight and relative stomach weight: The body weight of the rat was monitored closely with the aid of a digital weighing balance. The record was used to analyze the state of health as well as monitor the effect of induction of peptic ulcer with Indomethacin and treatment with administered oral solutions (fresh leaves of Lawsonia inermis) on the body weight of the treated group as well as the untreated group. Each rat in the group was measured using the digital weighing balance.

The mean weight value was calculated as:

$$\text{Mean Body Weight} = \frac{\text{Total body weight of rats in a group}}{\text{Total number of rats in that group}}$$

Relative stomach weight = Stomach weight/ animal body weight

Experimental design: Animals were selected at the end of the acclimatization, weighed and physically accessed. The animals were randomly selected and kept in a well-ventilated plastic cage of 40×60×20cm with bedding cleaned wood shavings. A total number of thirty female (30) rats were assigned into six (6) groups with five (5) animals each. During the experimental period, all the animals were given feed and water ad libitum.

Group I: Normal Control (NC): these are normal rats that were given feed and water for (9) weeks without extract.
Group II: Ulcerated Control: This constitutes the Negative control. These rats were given 80mg/kg of Indomethacin orally.
Group III: Therapeutic group: they were given indomethacin after pretreatment with Omeprazole (40mg/kg b.w.).
Groups IV: Prophylactic group: comprised ulcerated rats pretreated with low dose of n-hexane extract of Lawsonia inermis (100 mg/kg b.w.)
Group V: Prophylactic group; comprised ulcerated rats pretreated with medium dose of n-hexane extract of Lawsonia inermis (200 mg/kg b.w.)
Group VI: Prophylactic group; comprised ulcerated rats pretreated with high dose of n-hexane extract of Lawsonia inermis (400 mg/kg b.w.) respectively.

Treatments with the reference drug and extracts lasted for 14 days prior to indomethacin administration. These were orally administered once daily using oral intubator with ad libitum provision of food and water throughout the experimental period.

Ulcer induction: Gastric ulceration was induced in the animals. Briefly, rats were administered with a single oral dose of indomethacin (80 mg/kg body weight). They were deprived of food but had free access to water 24 h prior to ulcer induction. Various degrees of ulceration have manifested 4 hrs after indomethacin administration.

Sacrifice of animals: The animals were humanely sacrificed on the fifteenth (15) day, eighteen (18) hours after peptic ulcer was induced, by cervical dislocation. The stomach was harvested, through a longitudinal incision made in the abdominal cavity, and processed respectively for the analysis.

Morphological studies: The rats were weighed in grams using a weighing scale, before the start of the experiment and before their sacrifice. After sacrifice, the stomach organs were weighed using a sensitive weighing balance in order to determine the stomach weight. The organs were fixed immediately into 10% formal saline solution and were kept in universal bottles for histological sectioning.

Preparation of Tissues for Histological Examination

The stomach tissues were prepared and processed for histological and histochemical technique at the Histological Laboratory of the Department of Anatomy, Olabisi Onabanjo University, and Sagamu Campus.

Fixation: The tissues of the stomach were fixed in formal saline solution (from 0.85g of NaCl, 90ml of water, 10ml formaldehyde) for about 24hours after which the process, dehydration, was embarked upon

Dehydration: The tissues were dehydrated in the following solution at different stages; 60% alcohol, 70% alcohol, 80% alcohol, 90% alcohol, 95% alcohol, first absolute alcohol and finally second absolute alcohol (all at one (1) hour interval each).

Clearing: Clearing was done by using xylene (a hydrophilic clearing agent) to remove the alcohol from the stomach tissue which was changed at one (1) hour interval in first xylene initially and finally, second xylene.

Infiltration: The tissues were then infiltrated with paraffin wax at a temperature between the ranges of 50 - 60°C for an hour. The tissues were then embedded in paraffin wax with the proper orientation.

Embedding: The tissues were then embedded in paraffin wax with proper orientation. The embedding took place in a LUKAT embedding mold coated with glycerol. The paraffin wax was allowed to solidify forming a visible scum before cooling at a temperature of about 10 - 15°C.

Sectioning: The cassette containing the embedded tissue was mounted on the microtome. The microtome was set to 5um thickness. During sectioning, cubes of ice block were placed on the block so as to reduce the heat generated. The first set of sections was discarded due to the trimming of the blocks. While sectioning, both thick and thin sections were gotten but only the thin sections were used. The thin sections were placed immediately in 5% alcohol for five (5) minutes and we're later transferred into a warm bath for five (5) minutes ensuring that the sections were well spread out in order for them to be visible when viewed under the microscope. Fresh slides were smeared with egg albumin. They were layer dipped in the warm water to pick the sectioned tissues. The tissues having glued on the slides were then dried on the hot plate.

Hematoxylin and Eosin Staining Method and Protocol

Preparation of Harris alum hematoxylin: The alum was dissolved in hot water, the hematoxylin (1g) powder was also dissolved in absolute alcohol (10ml) and was added to the alum (20g) solution. This was brought quickly to boil and mercury oxide (0.5g) was added to the mixture. It was added to the mixture. It was cooled rapidly under tap water. The addition of about 8.0ml of glacial acetic acid to the stain was recommended to sharpen nuclear staining and filter before use. Staining time is usually 5 - 10 minutes.

Preparation of Eosin: 2% Solution: About 350ml of 70% Acid alcohol was added to 150ml of distilled water and 10g of Eosin powder was added, shaken and the stock solution was filtered before use.

Preparation of Acid Alcohol: About 99ml of 70% alcohol was mixed with 1ml of concentrated hydrochloric acid (HCL)

Hematoxylin and Eosin Staining

The labelled slides were dewaxed in xylene for fifteen (15) minutes. Hydration was done in descending grades of alcohol (from 100% to 90% to 80% to 70% to 60% and finally to 50%). Slides were stained in Harris Hematoxylin solution for five (5) minutes. Slides were rinsed in running tap water for few minutes. Differentiation in 1% alcohol (differentiation solution) for one to two dips. Checking done under the microscope for a satisfactory effect of the stain. Slides were rinsed in running tap water. Differentiation was repeated on some which were checked microscopically for best results. Slides were then immersed in bluing solution for one (1) minute. Rinsing done in running tap water. Immersion of slides in 96% alcohol for 30 seconds. Sections were stained with Eosin solution for thirty (40) seconds to three (3) minutes. Stained sections were then dehydrated in 80% alcohol and 95% alcohol for one minute each and changed to 100% alcohol for three minutes. Clearing was done in two changes of Xylene for five minutes. Mounting done with mounting medium (DPX) and then air dried. RESULTS: Nuclei stained blue black and cytoplasm, pink.

Photomicrography

Image Acquisition and Analysis:

- A bright light microscope (10 - 40× magnification objective) used.
- Digital Camera - OMAX Toup View 3.7 attached to P.C - HP used.
- Application Software (image J Software) used.

All of these stated above were used to analyze:

- The gastric gland
- Epithelial layer

Statistical Analysis

For statistical analysis, one - way Analysis of Variance (ANOVA) using Graph Pad Prism software to analyze the data. The results were expressed as mean ± standard deviation and statistical significance was considered at a 96% confidence interval ($P< 0.05$).

Results

General Observation

No mortality was observed throughout the experiment. The animals across the groups showed similar behavior, except when 80mg/kg Indomethacin was administered. Group (therapeutic group) exhibited sleepiness, drowsiness, less mobility for up to 18 hours after administration of Indomethacin. Group receiving the aqueous extract before Indomethacin was administered, showed similar signs, and returned to normal behaviour faster than Group IV.

Table 1: Body Weight at Different Weeks

	Group I	Group II	Group III	Group IV	Group V	Group VI
Week 1 - 3	180.20 ± 7.88	172.40 ± 12.10	195.60 ± 25.89	169.20 ± 9.20	180.40 ± 13.80	179.00 ± 5.80
Week 4 - 6	192.40 ± 5.03	180 ± 10.00	182.8 ± 15.23	203.20 ± 21.10	198.80 ± 23.23	193.20 ± 7.46
Week 7 - 9	199.4 ± 19.07	188.0 ± 12.59	182.80 ± 16.63	211.00 ± 23.05	195.40 ± 10.95	196.40 ± 10.95

Where; Week 1 - 3 = Pre induction
Week 4 - 6 = Induction/ Pre treatment
Week 6 - 9 = Post treatment
Values are recorded as Mean ± Standard deviation (SD).

Body Weight of Rats at Different Weeks

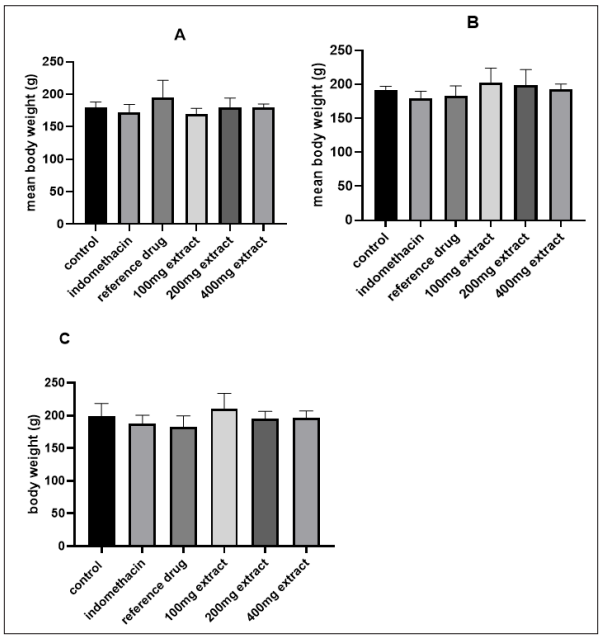

Figure 1: Graph showing body weights at different weeks across all group. A;Body weight of animals before induction. B; body weight of animals during induction. C; Body weight of animals after the experiment. Data analyzed by One Way ANOVA followed by Post Hoc Tukey test. There is no significant difference (P > 0.05) in the body weight of the animal in all group.

Table 2: Stomach-body weight ratio

	Control	Ulcer group	Reference drug	100mg extract	200mg extract	400mg extract
Value	3.0 ± 0.00	1.87 ± 0.13	2.48 ± 0.48	2.24 ± 0.43	2.36 ± 0.42	2.98 ± 0.45

Values recorded as Mean ± Standard deviation.

Table 3: Relative Organ weight

	Control	Ulcer group	Reference drug	100mg extract	200mg extract	400mg extract
Value	0.0150 ±0.0014	0.0103 ±0.001	0.014 ±0.03	0.011 ±0.002	0.012 ±0.003	0.015 ±0.0001

Values recorded as Mean ± Standard deviation.

Stomach Weight of Rats Across the Groups

Histological Analysis

Histological evaluations were carried out on the stomach of each rat using routine laboratory haematoxylin and eosin method (Kiernan, 1990) to evaluate the histo-gastroprotective effects of L. inermis on Indomethacin - induced peptic ulcerated mucosa of wistar rats. None of the rats used in this study died while the experimental procedures lasted. Thus, all rats were histologically examined to evaluate the degree of gastro-protection and other intrinsic histopathological alterations on the mucosal lining of the stomach.

Photomicrograph of histo-pathological examinations of the stomach sections of rats of Groups I - VI are presented in Plates 1 - 6.

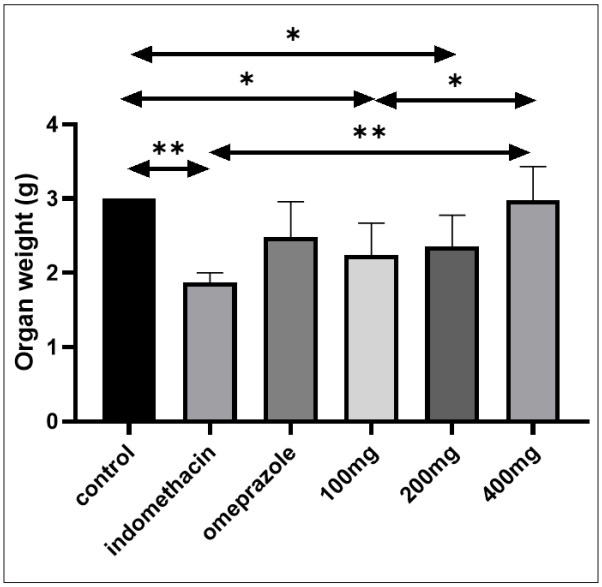

Figure 2: Stomach weight of animals after the experiment. Data analyzed by One Way ANOVA followed by Post Hoc Tukey test * = P > 0.05, # = P > 0.05. The stomach weight is significantly higher in indomethacin group when compared with control group and 400mg extract group. (** indicate highly significant)

Histological analyses of the stomach body wall in rats of Groups II and IV that received 80 mg/kg/bw of Indomethacin only and 100 mg/kg/bwt of L. inermis show erosion of the mucus-secreting cells, gastric pit, upper and middle parts of gastric glands and the parietal cells.

Histological observations of the stomach body wall in rats of Group V that received 200 mg/kg/body weight of L. inermis show mild erosion of few mucus-secreting cells, gastric pit and the parietal cells.

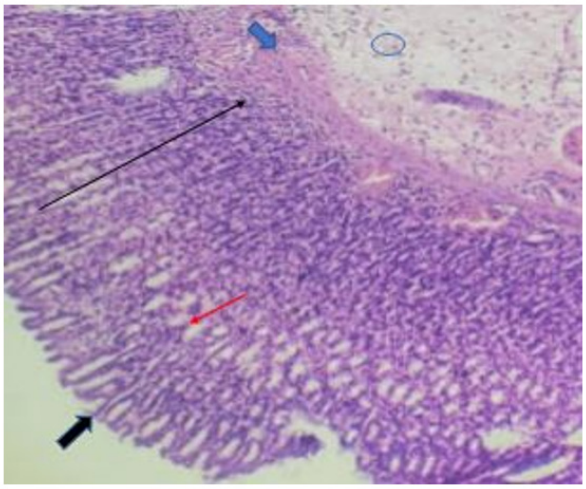

Plate 1: Photomicrograph sample of the stomach body wall in rats of Group I which is the normal control. H&E X400. The black thin and thick arrow shows the mucosa and the gastric pit, the blue thick arrow is muscularis mucosa and the circle is submucosa, the red thin arrow is the gastric gland. The histological analysis of the stomach body wall show typical gastric histoarchitecture with intact epithelium and glands.

Histological observations of the stomach body wall in rats of Groups I, III and VI that received feed and water, 40 mg/kg/body

weight of Omeprazole, 400 mg/kg/body weight of L. Inermis and respectively show normal morphological appearances of the different components of the mucosa layer. Therefore, the n-hexane extract of the fresh leaves of L. Inermis had dose - dependent histo-gastroprotective effects against Indomethacin-induced peptic ulceration.

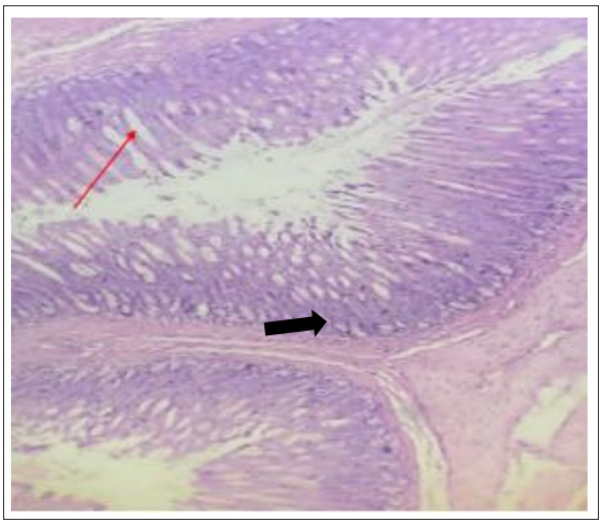

Plate 2: Photomicrograph sample of the stomach body wall in rats of Group II which received oral administration of 80mg/kg/bw of Indomethacin only. H&E X400. The red thin arrow is the gastric gland. The histological analysis of the stomach body wall displayed several changes in the integrity of gastric mucosa such as erosion of mucus secreting cells, dilated gastric gland and loss of surface epithelial. The stomach mucosa is ulcerated.

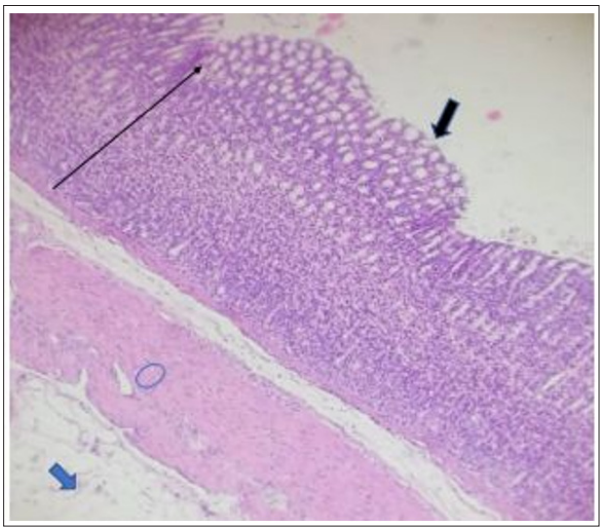

Plate 3: Photomicrograph sample of the stomach body wall in rats of Group III which received oral administration of 40mg/kg/bw of Omeprazole prior to receiving further oral administration of 80mg/kg/bw of Indomethacin. The circle indicate the normal muscularis mucosa, the black thick and thin arrow shows the normal gastric pits and mucosa. H&E X400. The histological analysis of the stomach body wall displayed normal morphological appearance of different component of mucosa layer.

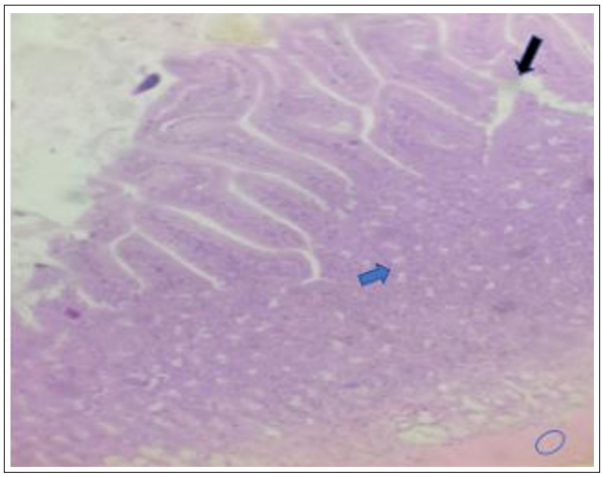

Plate 4: Photomicrograph sample of the stomach body wall in rats of Group IV which received oral administration of 100mg/kg/bw of L. Inermis prior to receiving further oral administration of 80mg/kg/bw of Indomethacin. The circle indicate the muscularis mucosa, the black thick arrow shows the gastric gland, the blue thick arrow is the parietal cells. H&E X400. The histological analysis of the stomach body wall shows erosion of mucus secreting cells, gastric pits and parietal cells. The stomach mucosa is ulcerated.

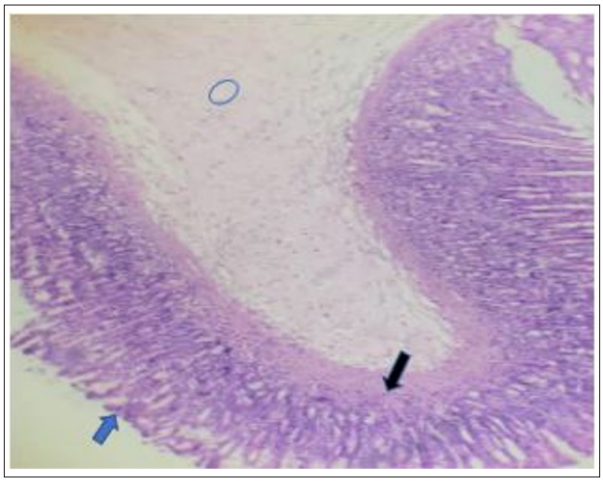

Plate 5: Photomicrograph sample of the stomach body wall in rats of Group V which received oral administration of 200mg/kg/bw of L. inermis prior to receiving further oral administration of 80mg/kg/bw of Indomethacin. The circle indicate the submucosa, the black thick arrow shows the muscularis mucosa, the blue thick arrow is the gastric pits H&E X400. The histological analysis of the stomach body wall shows mild erosion of mucus secreting cells, gastric pits and parietal cells.

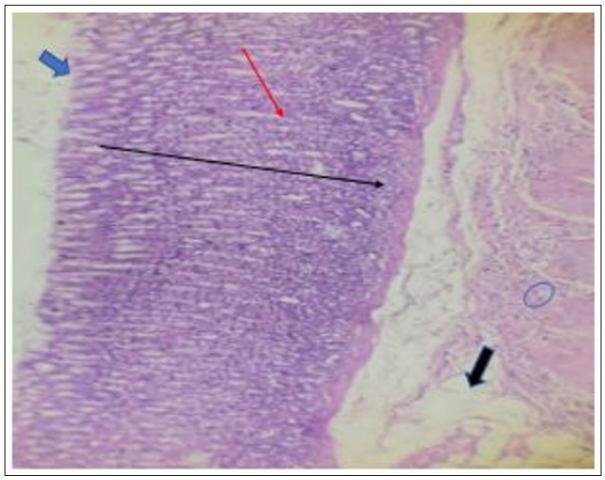

Plate 6: Photomicrograph sample of the stomach body wall in rats of Group VI which received oral administration of 400mg/kg/bw of L. inermis prior to receiving further oral administration of 80mg/kg/bw of Indomethacin. The circle indicate the muscularis mucosa the black thick and thin arrow shows the submucosa and mucosa layer, the blue thick arrow is the gastric pits, the red arrow is the gastric gland. H&E X400. The histological analysis of the stomach body wall shows normal morphological appearance of different component of mucosa layer.

Discussion

Indomethacin is an established ulcerogen, especially in an empty stomach [19,20]. The incidence of Indomethacin induced ulceration is mostly predominant in the glandular (mucosal) part of the stomach [20]. Although, the mechanisms underlying the ulcerogenicity of indomethacin are not completely understood; it has been known that indomethacin induces gastric mucosa ulceration through inhibition of prostaglandins synthesis [20,21]. This view is supported by the fact that several prostaglandins and prostaglandin analogues are potent antisecretory and anti-ulcer agents. Prostaglandins normally serve protective functions in the stomach by maintaining gastric micro circulation via mucus and bicarbonate stimulation [21,22]. Indomethacin stimulates catecholamines release from adrenal medulla, resulting in mucosal vasoconstriction [21,22]. It has also been established to uncouple mitochondrial respiration resulting in depletion of Adenosine Triphosphate and a reduced potential of gastric epithelial cells to coordinate normal cellular functions [21,22]. Hence, Indomethacin reduces both the quality as well as amount of mucus secretion and changes in ionic permeability characteristics of gastric mucosa [20,21]

Lawsonia inermis is a well-known ethnomedicinal plant used medicinally for over 9000 years. In various studies of Lawsonia inermis, it has been reported to have antibacterial, antifungal, hepatoprotective, antiparasitic, antiviral, anticancer, antidiabetic, tuberculostatic, anti-inflammatory, antifertility and wound healing properties. Although, the content of its medicinal properties has not been ascertained and is still under study. Pretreatment of rats with Lawsonia inermis extracts produced a dose dependent protection in the indomethacin induced ulceration as compared to control group. However, the protection significantly reduced the severity of the ulcer at high dose dependent (400mg/kg/bw). The reference drug(omeprazole) also produced gastric ulcer protection as compared to control group. Pretreatment with n-hexane extract of Lawsonia inermis delivered a remarkable anti-ulcer effect which was observed by the effect on gastric secretion in indomethacin induced peptic ulcer.

Histological analyses of the stomach body wall in rats of Groups II and IV that received 80 mg/kg/bw of Indomethacin only and 100 mg/kg/bw of L. inermis show erosion of the mucus-secreting cells, gastric pit, upper and middle parts of gastric glands and the parietal cells (Plates 2 and 4). Erosion of few mucus-secreting cells, gastric pit and the parietal cells were observed in rats of Group V that received 200 mg/kg/bw of L. inermis (Plate 5). Normal morphological appearances of the different components of the mucosa layer were observed in rats of Groups I, III and VI that received feed and water, 40 mg/kg/bw of Omeprazole and 400 mg/kg/bw of L. inermis respectively (Plates 1, 3 and 6).

Conclusion

Result obtained from this study showed that the n-hexane extract of the fresh leaves of L. inermis had dose-dependent (with high dose being the most effective) histo-gastroprotective effects against Indomethacin-induced peptic ulcerations. N-hexane extract of the fresh leaves of Lawsonia inermis could be used as a therapy for peptic ulceration. Further research on the effect of the other tissue part of Lawsonia inermis on peptic ulceration should be investigated.

Conflict of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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Authors' Contributions

The entire study procedure was conducted with the involvement of all writers.

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