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Phytochemical Investigation and Antiulcer Potential of *Strychnos Nux vomica* Seed Extract in Adult Wistar Rats

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Abstract: The current work sought to assess the possible anti-ulcer efficacy of *Strychnos nux-vomica* methanolic seed extracts in rats by conducting a thorough phytochemical analysis. This study aims to indicate and discover beneficial molecules present in the seeds with the potential for its medicinal use. This work aimed to determine the primary phytochemicals that have antiulcer properties while maintaining safe profiles at dosages that work. Strychnine is the main phytoconstituent, and it is present in 0.65 % of Nux vomica seeds and is responsible for gastric ulcer activity. When given in 0.05%, it shows therapeutic action. The methanolic seed extract's phytochemical screening identified alkaloids, flavonoids, tannins, and glycosides. Wistar rat models of ulceration produced by methanol were used to evaluate the anti-ulcer efficacy. Four groups of rats were used: control, ulcer-induced (indomethacin induced ulcer), conventional therapy (20 mg/kg omeprazole), and test treatment (50 mg/kg and 100 mg/kg of *S. nux-vomica* methanolic extract). The outcomes showed that rats treated with *S. nux-vomica* extract had a significantly lower ulcer index than the control group ($p < 0.01$). In particular, the ulcer index dropped by 63% in the 100 mg/kg group, which was similar to the omeprazole therapy as a whole ($p > 0.05$). Reduced stomach mucosal injury was further confirmed by histopathological investigation in the groups who received extract treatment. The originality of this work consists in the safe therapeutic application of *S. nux-vomica* seeds, usually recognized for their poisonous qualities, in the treatment of stomach ulcers. The findings imply that the *S. nux-vomica* seed methanolic extract has strong antiulcer properties, providing a unique therapeutic option for ulcer treatment.

Introduction

An open sore is called "ulcer." The term "peptic" implies that acid is the primary source of the issue. When a gastroenterologist refers to "ulcer," they often mean a pressure ulcer. "Duodenal ulcers" and "gastric ulcers" are the two most prevalent kinds of peptic ulcers. The stomach is the location of gastric ulcers. The duodenum, the opening of the small intestine, is where duodenal ulcers are discovered (also known as the small bowel). It is possible for stomach and duodenal ulcers to form simultaneously. Erosive gastric or duodenal mucosa is a common gastrointestinal ailment known as peptic ulcer disease (Konturek et al., 2020). Treatment of peptic ulcers remains difficult despite advances in traditional medicine because of problems such medication resistance, recurrence, and adverse effects. As a result, there is increasing interest in looking for new antiulcer drugs from unconventional sources, including medicinal plants known medicinally (Lanas and Chan, 2008). Because of its medicinal qualities, it has long been employed in a number of indigenous medicine systems. A great deal of attention is paid to its possible antiulcer properties. The pharmacological actions of *Strychnos nux-vomica* are linked to a wide range of phytochemicals,

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including phenolic compounds, alkaloids, flavonoids, and terpenoids. Among them, the pharmacological effects, particularly antiulcer capabilities, of alkaloids like strychnine and brucine have been the subject of substantial research (Vikhe and Kunkulol, 2020). Using an animal model, this work intends to examine the phytochemical content of the methanolic extracts of *Strychnos nux-vomica* seeds and assess any possible anti-ulcer efficacy. Because the Wistar rat model may generate ulceration through a variety of experimental techniques and has a physiological resemblance to humans, it is frequently used to evaluate antiulcer activity (Malfertheiner et al., 2009). The deadly alkaloid strychnine is often found in the seeds of the Strychnos nux-vomica tree, indigenous to Australia, India, and Southeast Asia (Sung et al., 2009; Jain et al., 2020). The purpose of the present study is to evaluate anti-ulcer potential of *Strychnos Nux vomica* seed extract in adult Wistar rats, along with an assessment of phytochemicals responsible for the activity.

Materials and methods

The seeds of *Strychnos Nux vomica* were obtained from Sahyadri Pvt. Ltd., Rahata, Maharashtra. It was authenticated by Dr. Wabale Anil Sopanrao, Head of Department of Botany, PVP College of Arts, Science, and Commerce, Pravaranagar, Maharashtra, India, via letter number PVPC/Bot/2023-24/238 dated 25/10/2023. The food and animals were purchased from Lacsmi Biofarms Pvt. Ltd., Pune. The test protocol was approved by the institutional animal ethical committee (1942/PO/Re/S/17/CPCSEA/2023/09).

Chemicals: Indomethacin, Pepsin, Dimethyl sulfoxide (DMSO), Methanol, Diethyl ether, sodium hydroxide, omeprazole, acetonitrile, Distilled water.

Detoxification of Strychnous Nux vomica

The seeds of *Strychnos nux vomica* are used medicinally and soaked in kanji (fermented, acidic water) for three days. After that time, the outer layer is removed with a knife and the seeds are left to cure in the sun. Once the seeds are completely dry, process them and store them in an airtight container. In goghrtia (cow's ghee), Strychnos *Nux vomica* seeds are cooked at low heat. Their skin splits after frying ends (Sudha and Suneetha, 2020). Seeds devoid of skins are preserved and powdered. Soaking cow milk seeds (*Nux vomica*) in dolayantra for three hours is the third way. When the swedana procedure is complete, the outer layer is scraped off and the region is dried in the sun. A fine powder is made by grinding dried seeds (Chopra et al., 2010).

Preparation of plant extract

After being meticulously cleaned with water to eliminate any debris, the recently harvested *Strychnos Nux vomica* seeds were allowed to air dry at room temperature in the shade. Following thorough drying, the plant was ground into a powder using a mechanical grinder (Chowdhury, 2023). The dried powdered *Nux vomica* seeds were then extracted using a cold maceration technique using 80% methanol (hydromethanol). Approximately 1 kg of the powder was mixed with 8000 ml of 80% methanol and left on a platform shaker at room temperature for 72 hours at 120 rpm (Zhu et al., 2002). The extract was first filtered through gauze (muslin cloth), then through Whatman No. 1 filter paper. To maximize yield, the residue was twice separately re-soaked in identical solvents and under comparable conditions. The filtrate was then concentrated again using a rotary evaporator at 40°C and 60 rpm. The concentrate filter was frozen overnight utilizing an electric refrigerator and freeze drying with lyophilised at -50°C and vaccum pressure (200 millibar) to eliminate water, finally yield of extract was determined, labeled and maintained in refrigerator at -4^0 till usage (Sunayana Vikhe et al., 2024).

In vitro method

The Potential of Plant Extracts to Diminish Gastric Acidity

Dissolve 2 grams of sodium chloride and 3 grams of pepsin powder in 500 milliliters of distilled water to generate fake stomach acid juice. Make sure you have adequate water and an HCl solution ready. The freshly made solution's pH was adjusted to 1.2, which is very acidic. Extreme acidity destroys the mucosal layer and leads to the development of stomach ulcers. If this pH is not controlled, more serious stomach ulcers will form. Both medications were introduced separately to create gastric juice with a low pH value of 1 to 2.4 in order to evaluate the test pharmaceuticals and reference medication for their neutralizing action on gastric acidity. The neutralization impact was evaluated using artificial gastric juice. Impacts of Neutralization on Synthetic Gastric Acid. Artificial gastric juice is mixed with plant extracts, distilled water, or reference medication at a pH of 1.2. The pH levels are determined to investigate the neutralizing effects of artificial gastric juice (Alam and Gomes, 2020).

Determination of Consistent Neutralization on Artificial Gastric Acid

The length of time for consistent neutralization on artificial gastric acid is measured using a modified version of Vatier's artificial stomach. The stomach is

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composed of three components: the gastric emptying flux, the secretary flux, and the reservoir. The time the pH value returns to its starting point determines how long the neutralizing effect will last. Every natural plant extract, distilled water, or prescription drug is combined with synthetic stomach juice to a pH of 1.2. The artificial stomach was swirled continuously (30 rpm) at 37°C using 2.5 cm magnetic stirring apparatus. The fake stomach fluid content is kept in the reservoir chamber at the same rate as it is pushed out, and artificial gastric juice with a pH of 1.2 is pumped directly there. A pH metre is fastened to the reservoir of an artificial stomach in order to measure pH fluctuations (Wang et al., 2024).

In vitro Neutralizing Capacity

Heat each freshly made plant extract, distilled water, or reference medication to 37^0C in a 250 mL beaker. To mimic stomach motions, a magnetic stirrer is continually spun at 30 rpm. Artificial gastric juice is used to titrate each extract to the end point of pH 4, which is the pH of Wistar rat gastric juice. For titration, the test drug from the plant is freshly extracted, and the reference drugs are put into the beaker & heated up in a water bath to 37ºC with a magnetic mixer running continuously at 30 revolutions per minute to mimic stomach activities. Plant extract and reference medicine are individually titrated using laboratory-prepared stomach juice until the endpoint pH value reaches 3. Finally, following titration, the total volume of laboratory stomach juice consumed is evaluated, the total hydrogen ion consumption is determined, and the neutralized capacity is assessed (Rajeshwari et al., 2020).

H+ K+-ATPase Activity Evaluation

Toxic chemicals, such gastric acid or HCl secreted into the stomach lumen, might weaken defense systems and cause damage to the gastrointestinal mucosa. The enzyme H+, K+-ATP'ase, which catalyzes the exchange of one H+ for one K+ at the expense of an ATP molecule, is responsible for acid secretion. The oxyntic cell contains the ion channel hydrogen-potassium ATPase, which is crucial for controlling acid secretion. Most people just call it a proton pump. The exchange of internal H+ for external K+ occurs in the canaliculi of parietal cells when defense components are compromised by toxic materials such as hydrochloric acid and stomach acid discharge toward the gastric cavity. This leads to damage to the gastric mucosa. Anti-ulcer drugs that contain proton pump inhibitors are being utilized increasingly often (Wadkar et al., 2019).

In vivo activity of indomethacin-induced gastric ulcer

Medicinal herbs like *Strychnos nux-vomica* are among the medicines whose anti-ulcer potential is assessed in vivo using indomethacin (40mg/kg) as a model. In the indomethacin-induced gastric ulcer animal model, non-steroidal anti-inflammatory medication (NSAID) indomethacin is used to generate stomach ulcers in experimental animals, most often rodents like rats or mice. Due to its inhibition of prostaglandin production and increase in gastric acid output, indomethacin damages the mucosa of the stomach, causing erosion and the development of ulcers. A control group, a positive control group, and one or more treatment groups are among the groups into which the animals are split. The animals are put to death after a predetermined amount of time, and their stomachs are checked for ulcers. Parameters such as ulcer index, stomach mucosal damage, histological alterations, and biochemical indicators of ulceration are evaluated to measure the degree of ulcer development and mucosal injury. In order to screen and assess novel medications, natural items, and therapeutic treatments for the treatment and prevention of stomach ulcers, researchers frequently utilize the indomethacin-induced gastric ulcer model (Bhatt and Patel, 2012). Using this model, scientists may study how possible antiulcer drugs work by examining how they affect stomach acid secretion, mucosal integrity, prostaglandin production, oxidative stress, and inflammatory pathways, among other aspects of ulcer development. The results of these investigations provide important insights into the safety, effectiveness, and mechanisms of action of novel antiulcer medications and treatment approaches in preclinical settings (Woretaw and Tezera, 2020). The final findings were reported as mean ± standard error mean (SEM) or standard deviation (SD).

Total Ash Content

Including the seed powder, the overall ash content of *Nux vomica* seeds is often between 5 and 7%. However, this can change a little based on things like the seeds' place of origin and the processing techniques applied. An exact analysis of a particular material would need to be performed in a lab (Ajiboye and Okesola, 2017).

Acid insoluble Ash

Nux vomica seeds, including the powder, have an acid-insoluble ash concentration of usually 0.5–1.5%. Once more, this can change based on things like the seeds' origin and the processing techniques used. This would require laboratory testing of a particular sample in order to obtain reliable information (Sunayana Vikhe et al., 2022).

Water soluble Ash content

Nux vomica seeds, including the powder, have an approximate water-soluble ash concentration of 2–4%.

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Table 1. Preliminary phytochemical screening of *strychnos Nux vomica* extract.

Phytochemicals	Test /Reagent	Metahnolic extract of *Strychnos Nux vomica*
Alkaloides	Dragandroff test	+
	Mayers test	+
	Hagers test	+
	Wagners test	+
Glycosides	Buret test	+
	Legal test	+
	Borntrager test	+
Strychnine	Sulfuric acid test	**+**
	Potassium dichromate test	**+**
Brucine	Nitric acid test	+
Terpenoide	Salkwoski test	+
Phenol	Sodium hydroxide test	+
+ indicate presence of compounds		

As with other ash content measures, this one has some variation depending on the processing techniques and source. Laboratory examination of a particular sample would be required for accurate information (Vikhe et al., 2022).

LD50 of *Nux vomica*

Various factors, including the animal species studied, the delivery technique, and the exact preparation, can affect the *Nux vomica* lethal dosage (LD_{50}, 50%). Nonetheless, *Nux vomica* is regarded as exceedingly hazardous and perhaps fatal even at comparatively moderate dosages because of its high toxicity. When, strychnine is given orally, the LD_{50} values for different animal models described in the literature range from 0.15 to 0.5 milligrams per kilogram of body weight (Thaberew and Arawwawala, 2016).

Statistical analysis

The results of all the experiments were statistically analyzed using Graph Pad Prism and Microsoft Office Excel 2016. A one-way analysis of variance (ANOVA) test was used to calculate the mean difference at a significance threshold of 0.05, and the standard deviation was used to calculate all of the mean values from different instances compared (Nahla E. et al., 2016).

Results and Discussion

HPLC -DAD interpretation report (RP-HPLC)

The phenolic /poly phenolic compounds were present in Strychnos *Nux vomica* seeds in 2.85%. Alkaloids such as strychnine and brucine are mainly present in 65.0% of cases. The flavonoids present in Strychnos *Nux vomica* were 0.35%. The glycosides were present in 3.85%. The terpenoids were present in 0.37%.

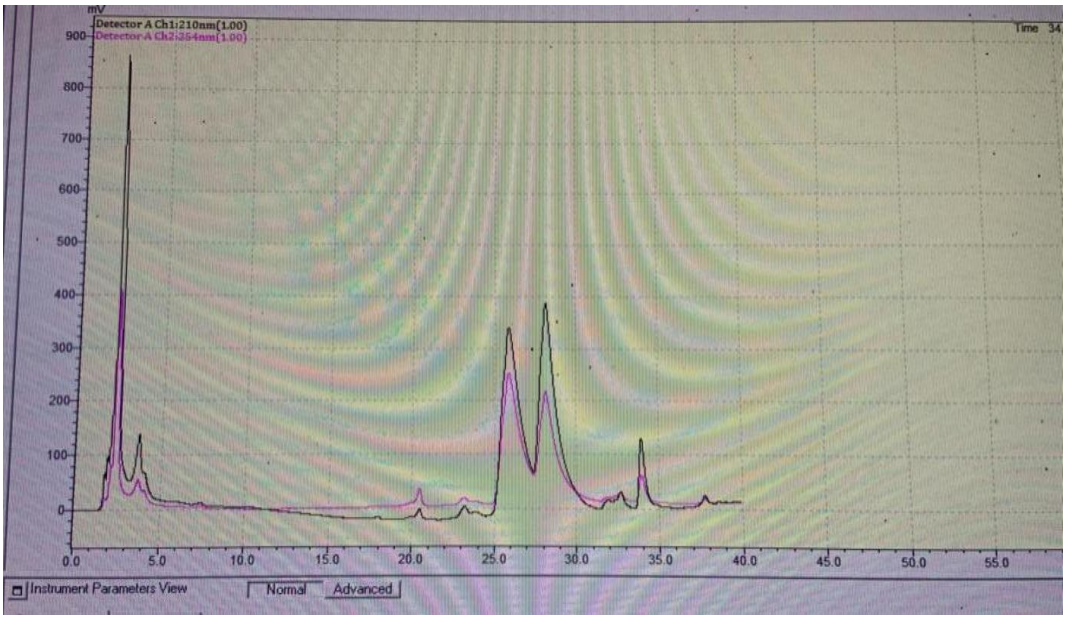

Figure 1. Detection of HPLC.

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HPLC chromatogram interpretation report

Phenolic acids like Gallic acids were found. Major components were alkaloids, which include Strychnine, brucine and vomicine. Few polyphenolic compounds like flavonoids have been detected. Importantly, Loganin, as sugar sugar-conjugated glycoside was detected. Tocopherol derivatives had been identified.

Table 2. Evaluation parameters of *Strychnos Nux vomica.*

Parameters	Value (% w/w) (Mean±SD)
Total ash	5.17 ±_0.25
Acid insoluble ash	3.06±0.05
Water soluble ash	0.93±0.05
Loss on drying	7.83±0.28
Swelling index	53.33±11.54
Extractive value in water	2.2±1.55
Extractive value in methanol	8.4±0.36

Table 3. Detection of HPLC 210 nm.

Peak	Rate time	Area	Area %	height	Height %
1	1.870	265728	0.304	43691	2.161
2	2.250	14788919	16.917	812930	40.202
3	3.670	2495858	2.855	104672	5.176
4	4.029	699527	0.800	39126	1.935
5	20.457	269430	0.308	15541	0.769
6	28.104	397150	0.454	15762	0.779
7	25.840	27279867	31.205	351423	17.379
8	28.104	29233058	33.439	394954	19.532
9	32.671	319142	0.365	18023	0.891
10	33.939	3371120	3.856	128893	6.374
11	37.675	422950	0.484	18039	0.892
12	46.853	7553937	8.641	54633	2.702
13	65.492	204613	0.234	15868	0.785
14	67.824	121331	0.139	8573	0.424
Total		87422629	100.000	2022128	100.000

Detector A ch 210 nm; Peak table

Table 4. Detection of HPLC 254 nm

Peak #	Ret. time	Area	Area %	Height	Height %
1	1.747	211288	0.440	21498	1.849
2	2.046	793819	1.654	81458	7.006
3	2.252	7772528	16.193	410114	35.272
4	3.671	1712052	3.577	52293	4.4972
5	4.025	850594	1.772	31269	2.689
6	7.362	86504	0.180	3089	0.266
7	20.454	926649	1.931	32522	2.797
8	23.143	238260	0.496	9338	0.803
9	25.843	18337004	38.202	243079	20.906
10	28.098	15224281	31.717	206417	17.753
11	32.664	319987	0.667	12573	1.081
12	33.939	1323371	2.757	49199	4.231
13	37.761	199010	0.415	9879	0.850
Total		48000348	100.000	1162729	100.000

Detector A Ch2 254 nm ; Peak table

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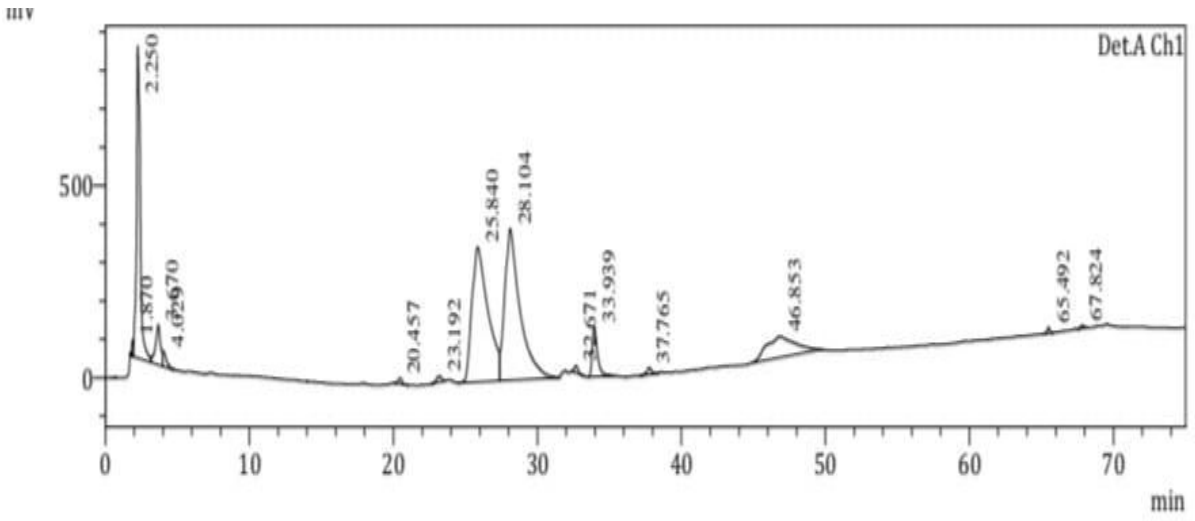

Figure 2. Detector A ch 210 nm.

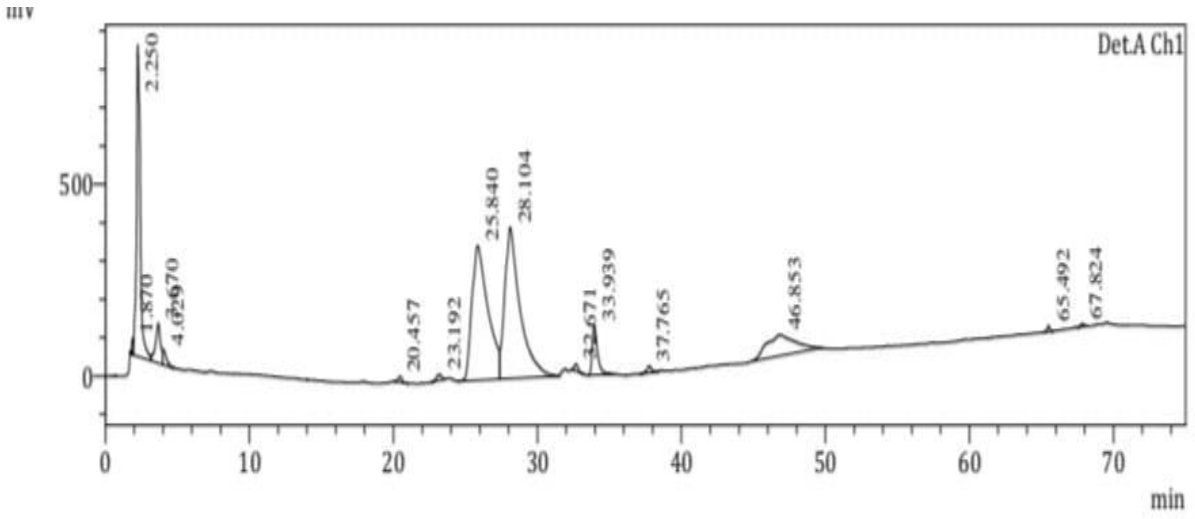

Figure 3. Detector A ch 254 nm.

Table 5. Preliminary studies in HPLC.

Sample weight /volume	200 mg sample
Solvents selected for extraction	Water : ACN: MeOH (1:2:2v/v)5ml
Sample concentration	20 mg/ml ;20,000 ppm
Temperature (℃)	29℃
PH (100% Distilled water)	NA
Wavelength (nm)	210,254 nm

In vitro Results

Table 6. In vitro results.

In vitro activity	Results
Plant Extracts' Ability to Reduce Gastric Acidity.	pH 1.2 (very acidic)
Determination of Consistent Neutralization on Artificial Gastric Acid	pH 2 (acidic)
Neutralizing Capacity in Vitro.	pH 4 (gastric juice pH of Wistar rats was 4).

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In Vivo results:

Effects of *Strychnos Nux vomica* extracts on Wistar animal groups

Table 7. Grouping for in vivo activity.

Group 1	Treatment
Group 1	Normal control
Group 2	Indomethacin (40 mg/kg)
Group 3	Indomethacin + Omeprazole (50 mg/kg)
Group 4	Indomethacin + *Strychnos Nux vomica* methanolic extract (100 mg/kg).
Group 5	Indomethacin + *Strychnos Nux vomica* methanolic extract (200 mg/kg).
Group 6	Indomethacin + *Strychnos Nux vomica* methanolic extract (400 mg/kg).

Table 8. The impact of *Nux vomica* extracts on gastric volume, total acidity, pH and stomach mucin level.

Group	Gastric volume (ml)	Ph	Acidity in MEq/L	Mucus content (ug/g)
Normal control	2.37±0.48	6.00±0.07	30.65±1.45	61.65±1.07
Indomethacin 40	3.14±0.15	4.02±0.9	53.20±2.33	81.85±1.37
Omeprazole 50	1.25±0.15	5.98±0.10	32.82±0.46	67.77±2.10
SNVE 100	2.84±0.45	4.42±0.09	50.20±2.16	80.85±1.11
SNVE 200	2.85±0.25	4.74±0.22	48.53±0.77	80.45±1.00
SNVE 400	1.35±0.12	4.75±0.07	35.28±0.64	70.50±1.75

Table 9. The effects of *Nux vomica* crude extracts on ulcer number, ulcer score, ulcer index and percentage protection.

Group	Mean ulcer number (UN)	Mean ulcer score (US)	Mean ulcer index (UI)	% Protection
Normal control	1.03±0.11	0.75±0.22	7.45±0.12	40.9
Indomethacin 40	30.84±1.27	50.00±1.18	18.99±0.25	10.4
Indo + Omeprazole50	1.45±0.23	0.90±0.18	9.90±0.06	47.9
Indo+ SNVE 100	27.85±1.80	43.15±3.80	17.90±0.58	15.9
Indo + SNVE 200	12.85±1.20	16.78±1.90	12.98±0.40	29.6
Indo+ SNVE 400	1.55±0.25	1.69±0.70	10.40±0.10	45.6
SNVE- *Strychnos Nux vomica* methanolic extract				

Histopathology study

Normal rats showed predominantly revealed colonic tissue, which showed mucosa, submucosa, muscularis propria, and serosa mucosa. It is lined by columnar epithelial with global cells, and lamina propria shows prorate mixed inflammatory comprising the eosinophils and plasma cells extending into the submucosa (Sander and Bloeme, 2008). In Indomethacin-induced ulcer rats**,** **t**he chronic inflammatory cells and granulation tissue endarteritis obliterans and epithelial proliferation, gastric ulcers have prototypical zones, superficially located neutrophils, bacteria, and narcotic areas (Panda et al., 2021). Ulcer is treated with Omeprazole, in which the esophagus shows mucosa, submucosa, muscularis propria, and serosa. Mucosa is lined by keratinized stratified squamous epithelial lining without atypia, muscularis mucosa appears thickened, subepithelial minimal to mild lymphoma mononuclear in filtrate noted submucosa and muscularis propria appears unremarkable. Ulcer treated with *Nux vomica*, esophagus shows mucosa, submucosa, muscularis propria and serosa mucosa shows hyperkeratotic stratified squamous epithelial lining, superficial corneal layer shows mucosa lined by faveolar epithelial gastric glands.

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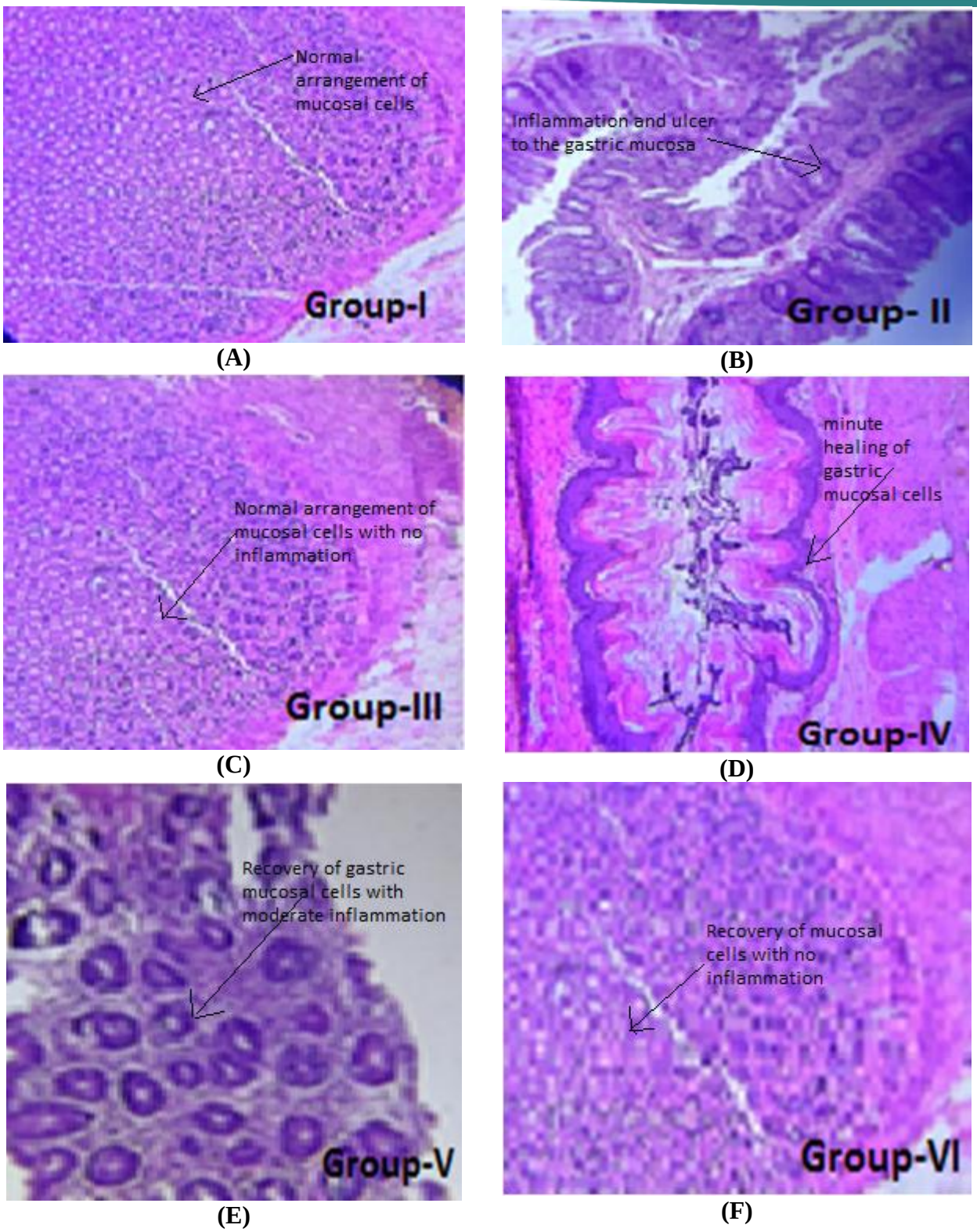

Figure 4. Histopathological findings of gastric tissue samples. (A) Normal control (Haematoxylin and Eosin stain, 100x), (B) Indomethacin (40 mg/kg) (Haematoxylin and Eosin stain, 400x), (C) Indomethacin (40 mg/kg) +Omeprazole50 mg/kg (Haematoxylin and Eosin stain, 100x), (D) Indomethacin (40 mg/kg) + *Strychnos Nux vomica* methanolic extract (100 mg/kg) (Haematoxylin and Eosin stain, 400x), (E) Indomethacin (40 mg/kg) + *Strychnos Nux vomica* methanolic extract (200 mg/kg) (Haematoxylin and Eosin stain, 400x), (F) Indomethacin (40 mg/kg) + *Strychnos Nux vomica* methanolic extract (400 mg/kg) (Haematoxylin and Eosin stain, 100x).

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Discussion

The majority of medications used to treat peptic ulcer disease may be broadly classified into two groups: those that work to prevent the effects of gastric acid and those that protect the mucosa of the stomach and duodenum from damage (Bisgin et al., 2023). At one point in the disease, gastric hypersecretion seems to be the main cause, but the mucosal membrane also becomes crucial since it shields the body from harmful substances and may even prevent damage from occurring (Sunayana Vikhe and Sunil Nirmal, 2018). The putative anti-ulcer effects of *Strychnos nux-vomica* seed methanolic extract have drawn attention. Studies on phytochemistry have uncovered a wide range of bioactive substances, including as tannins, flavonoids, and alkaloids, which are thought to be involved in the plant's medicinal properties. This talk intends to address experimental design constraints, investigate potential mechanisms of action for the anti-ulcer activity, and compare our results with those of other research on *Strychnos nux-vomica* and related plants (Sharma and Chaudhary 2021). Additional factors contributing to peptic ulcer disease include nutrition, psychological stress, NSAID usage, H. pylori infections, and cigarette smoking. The presence of alkaloids, which are recognized for their pharmacological actions, including gastro-protective and neuroprotective properties, was discovered during the phytochemical screening of Strychnos nux-vomica seeds. Research like that conducted by Panda et al. (2021) has shown that brucine and strychnine are present and may be involved in regulating the release of stomach acid and shielding the mucosa in the stomach. Comparatively, flavonoids and other polyphenolic chemicals with strong antiulcer action are also present in other plants with comparable medicinal uses, such as Curcuma longa (turmeric) and *Glycyrrhiza glabra* (licorice). For example, research has demonstrated that flavonoids contain antioxidant qualities that scavenge free radicals, lowering oxidative stress and protecting the stomach lining (Vikhe S et al., 2022). The methanolic seed extract showed strong anti-ulcer effectiveness in our investigation, which is consistent with results from prior investigations on Strychnos nux-vomica. According to a study, S. nux-vomica extracts significantly improved mucosal healing and decreased ulcer index in rats with stomach ulcers caused by indomethacin. Comparable results were observed with Glycyrrhiza glabra, where the extract enhanced mucin production, offering a buffer against gastrointestinal irritants. Due to indole alkaloids, which has enormous biological potential, methanolic seed extracts of *Strychnos Nux vomica*, like the one under investigation, are widely marketed and used worldwide. Additionally, *Nux vomica* seeds are commonly used in traditional medicine to treat gastrointestinal diseases, and there is already experimental evidence of their potential benefits in treating ulcerative colitis and gastric ulcers. Given the information presented here, which represents fresh discoveries on the extract's potential for preventing ulcers, it can be concluded that the extract's anti-ulcer properties are due to its ability to boost stomach acid secretion, decrease it, enhance digestion, manage stress, encourage healing, and have an impact on H. pylori. Our research group was among those who traditionally used the indomethacin-induced ulcer model to search for products or preparations with antiulcer potentials. This model was chosen due to its ulcerogenic potentials, which cause damage to the gastric mucosa that is readily visible to the unaided eye as hemorrhagic streaks in the gastric mucosa of animals exposed to indomethacin and pretreated with vehicle. On the other hand, when compared to vehicles, it was shown that the extracts were given orally at 100, 200 and 400 mg/kg but not by subcutaneous and intraperitoneal route at 30 mg/kg decreased the ulcer area (Chukwuma et al., 2017). The potential mechanisms of action for the antiulcer activity of *Strychnos nux-vomica* may involve several pathways:

Inhibition of Gastric Acid Secretion: Alkaloids present in the extract could inhibit gastric acid secretion through muscarinic receptor antagonism, as suggested by similar studies involving *S. nux-vomica*.

Promotion of Mucosal Defense: The extract may enhance the synthesis of mucin and other protective factors in the gastric mucosa. Compounds like flavonoids found in the extract could promote mucosal healing by increasing antioxidant defense mechanisms.

Anti-inflammatory Activity: The presence of phenolic compounds may contribute to anti-inflammatory effects, reducing the levels of pro-inflammatory cytokines in the gastric mucosa and promoting healing (Kumar et al., 2015). The results of this study suggest that the topical effects on the gastric mucosa or modification dependent on gastric acidity may be necessary for the gastroprotective actions of the extracts. The dose of *strychnos Nux vomica* administered intraperitoneally was found to be ten times lower than that administered orally. This is likely due to the fact that the intraperitoneal route does not exhibit the same first-pass effects as the oral route, which can impact bioavailability of the extracts after abortion. The fact that this approach has already been used further supports these findings. Oral administration of *strychnos Nux vomica* seed extract promotes the decrease in lesion area when compared to

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the vehicle group in ulcers caused by indomethacin. The strychnine and brucine content in *Nux vomica* may contribute to the extract's effects against NSAIDS-induced gastric mucosal aggregation. Indomethacin-induced intestinal ulcers and intestinal mucosa through antioxidant, anti-inflammatory and antiapoptotic mechanisms. The gastroprotective effects of strychnine in the indomethacin-induced ulcer model have been described in the literature. Regarding the quantity of strychnine in Nux vomica, the presence of indole alkaloids was determined to be present and approximated to be 0.25 mg/kg of *Nux vomica*. This indicates that the principal indole alkaloid compounds in commercial extracts of *Nux vomica* seeds are brucine and strychnine, with lesser compositions being present (Bhatt and Bhargav, 2016). Apart from the gastro-protection induced by strychnine, the presence of bracine in *Nux vomica* has already been documented. The extract's indole alkaloid components would facilitate the gastroprotection that *Nux vomica* evolved. Adequate rationale and attention must go into the selection of experimental models for the assessment of anti-ulcer medications. It is challenging to identify a single model for both researching the genesis of ulcers and comprehending the mechanism of pharmacological anti-ulcer action, given the intricate multifactorial process involved in the pathogenesis of gastric and duodenal ulcers.

The model should be chosen with simplicity, repeatability and moderate predictability in mind. Nevertheless, not all models respond to anti-ulcer medications now on the market. Because the ethanol-induced stomach ulcer model relies on the cyto-protection pathway, H2 antagonists have little to no impact. A study was conducted to investigate the in vitro activity of gastric ulcers on our extracts. First, we prepared an artificial gastric juice by adding pepsin and water. The extract had a pH of approximately 1.2, which was highly acidic. Next, we neutralized the pH by adding omeprazole, which made the extract's pH of 2 somewhat acidic. Finally, we performed a titration using our artificial gastric juice, which produced a pH of approximately 4, which was somewhat acidic, and 4 was the pH of the gastric juice of Wistar rats. Wistar rats have been employed as experimental animals in the current studies. There were five groups that we chose, and each group could contain up to six animals: the first was the normal group; the second was the positive control group, where rats were given indomethacin and treated with omeprazole; the third group received indomethacin and treated with 100 mg of a methanolic extract of *Nux vomica*; the fourth group received indomethacin and treated with 200 mg of extract; and the fifth group received indomethacin and treated with 400 mg of extract. Over the course of the 21-day activity, we compared our drug with omeprazole and the results we discovered.

The Shay model is an easy-to-use, dependable, and repeatable methodology for assessing anti-ulcer medications. It does not make use of exogenous ulcerogens and is unaffected by external interference. This technique has been used to evaluate the effectiveness of anti-ulcer drugs for application in human medicine and future research. *Nux vomica* may contain strychnine. this was determined by HPLC analysis, which revealed 0.65% of the drug to be present in the plant. Strychnine was found to be helpful in treating gastric ulcers in rats; a dose of 0.25 mg/kg was administered; however, if the dose of strychnine increased as the severity of the ulcer increased, it may result in death. Therefore, we chose to give rats doses of 100 mg, 200 mg, and 400 mg, and we saw results. The histology results have shown the number and location of ulcer production and the severity of ulcers discovered. The ulcers were treated with omeprazole for ulcer treatment and *Nux vomica* for normal ulcers. Reports demonstrated the outcomes, and based on these reports, we discussed how helpful our extracts were in treating ulcers and compared our medication to omeprazole. Among healthy rats. The mostly red colonic tissue is bordered by coloumnar epithelial cells with global cells. The lamina propria has proderate mixed inflammation with eosinophils and plasma cells extending into the submucosa. The mucosa, submucosa, muscularis propria, and serosa mucosa are all visible. Rats with indomethacin-induced ulcers had archetypal zones, superficially positioned neutrophils, bacteria, and narcotic regions in their stomach ulcers, as well as chronic inflammatory cells, granulation tissue endarteritis obliterans, and epithelial growth. Esophageal mucosa, submucosa, muscularis propria, and serosa are observed in an ulcer treated with omeprazole. The mucosa is lined by keratinized stratified squamous epithelial lining without atypia, the muscularis mucosa appears thickened, and the subepithelial minimal to mild lymphoma mononuclear in the filtrate is noted. Following *Nux vomica* treatment for an ulcer, the mucosa, submucosa, muscularis propria, and serosa mucosa exhibit hyper-keratin stratified squamous epithelial lining, while the mucosa lined with faveolar epithelial gastric glands is visible in the superficial corneal layer. In clinical settings, gastric ulcers are managed with gastric acid secretion inhibitors, particularly proton pump inhibitors. The

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antisecretory potential of *Nux vomica* was confirmed using indomethacin-induced models, and the results showed that *Nux vomica* administration did not alter volume, pH total acidity, or peptic activity. The limitations in the experimental design are dosage and length of therapy: Various doses and lengths of therapy may not yield the same results from the extract. Prospective research ought to examine an array of dosages in order to develop a dose-response correlation (Olatunji et al., 2018). Control Groups: Including a wider variety of control groups, such as those receiving treatment with other well-known antiulcer drugs (such as omeprazole), might help to clarify the extract's relative efficacy.

Mechanistic Research

More research into the precise mechanisms of action using molecular and biochemical tests is needed to further understand how the extract works. This may entail measuring mucin secretion levels, evaluating oxidative stress indicators, and histologically examining stomach tissues. Pharmacokinetics: It is essential to comprehend how the body absorbs the active ingredients in the methanolic extract. Research into ADME can be beneficial (Sunayana Vikhe et al., 2023).

Conclusion

Because *Strychnos nux-vomica* methanolic seed extracts include a variety of bioactive substances such as alkaloids, flavonoids, and tannins, the phytochemical analysis of these extracts shows promise antiulcer action in rat models. Despite the fact that the extract may have therapeutic advantages, it is imperative to address safety issues regarding the poisonous nature of *S. nux-vomica*. This is especially important because the plant contains strychnine and brucine, both of which can present serious health problems. Subsequent research endeavors ought to concentrate on refining extraction techniques to reduce toxicity while maintaining therapeutic effectiveness. Translating these discoveries into practical applications would also require developing acceptable dose recommendations and conducting thorough toxicological studies. To guarantee the safe application of *S. nux-vomica* extracts in the treatment of stomach ulcers and to fully realize their potential, these obstacles must be overcome.

Conflict of Interest

The authors declare no conflict of interest.

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