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Evaluation of Antiulcer activity of Tamanu oil by using pylorus ligation induced model in Wistar rats

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ABSTRACT:
The present study was undertaken to evaluate the antiulcer potential of Tamanu oil obtained from *Calophyllum inophyllum* using in vitro antioxidant assays and pylorus ligation-induced gastric ulcer model in Wistar rats. Gastric ulcer is a common gastrointestinal disorder caused by an imbalance between aggressive factors such as gastric acid, pepsin, and oxidative stress and protective mechanisms including mucus secretion and antioxidant defense systems. Tamanu oil was extracted by maceration method and subjected to physicochemical characterization, phytochemical screening, and GC-MS analysis for identification of bioactive constituents. Preliminary phytochemical analysis confirmed the presence of flavonoids, phenols, terpenoids, glycosides, and coumarins, which are known for antioxidant and gastroprotective activities. In vitro antioxidant activity was evaluated using DPPH free radical scavenging assay, where Tamanu oil showed significant dose-dependent antioxidant potential. In vivo antiulcer activity was assessed by measuring ulcer index, gastric volume, gastric pH, free acidity, and total acidity in pylorus ligation-induced ulcerated rats. Treatment with Tamanu oil significantly reduced ulcer index, gastric secretion, free acidity, and total acidity while increasing gastric pH compared to ulcer control animals. Histopathological examination revealed marked protection of gastric mucosa with reduced epithelial erosion, edema, necrosis, and inflammatory cell infiltration. The observed gastroprotective effect may be attributed to the antioxidant, antisecretory, anti-inflammatory, and cytoprotective properties of the phytoconstituents present in the oil. The findings of the study conclude that Tamanu oil possesses significant antiulcer activity and may serve as a promising natural therapeutic agent for the management of gastric ulcers.

INTRODUCTION:
Gastric ulcer is one of the most common gastrointestinal disorders affecting millions of people worldwide. It is characterized by the formation of lesions or sores in the gastric mucosal lining due to an imbalance between aggressive factors such as gastric acid, pepsin, Helicobacter pylori infection, reactive oxygen species, and protective mechanisms including mucus secretion, bicarbonate production, prostaglandin synthesis, and adequate

mucosal blood flow. The disruption of this balance leads to inflammation, oxidative stress, erosion of the gastric mucosa, and eventually ulcer formation. Gastric ulcer disease continues to be a major public health problem because of its high prevalence, recurrence rate, and associated complications such as bleeding, perforation, penetration, and gastric obstruction.

In recent years, the prevalence of gastric ulcer has increased considerably due to modern lifestyle practices such as irregular dietary habits, stress, smoking, alcohol consumption, and excessive use of non-steroidal anti-inflammatory drugs (NSAIDs). Infection caused by Helicobacter pylori is considered one of the most important etiological factors responsible for gastric ulcer formation. The bacterium colonizes the gastric mucosa and induces chronic inflammation, leading to impairment of the protective barrier of the stomach. Along with H. pylori infection, prolonged administration of NSAIDs causes inhibition of cyclooxygenase enzymes, reducing prostaglandin synthesis and compromising gastric mucosal defense. The resulting decrease in mucus and bicarbonate secretion enhances susceptibility of the stomach lining to acid-induced injury.

Conventional therapy for gastric ulcer mainly includes proton pump inhibitors, H2 receptor antagonists, antacids, cytoprotective agents, and antibiotics for eradication of H. pylori infection. Although these medications are effective, prolonged use is often associated with adverse effects such as nausea, diarrhea, headache, vitamin deficiency, gastric rebound acidity, and development of antibiotic resistance. Moreover, recurrence of ulcers after discontinuation of therapy remains a significant clinical concern. These limitations have created interest among researchers in exploring safer and more effective therapeutic alternatives from natural sources, particularly medicinal plants and phytoconstituents.

Medicinal plants have been used since ancient times for the prevention and treatment of various diseases. Traditional systems of medicine such as Ayurveda, Siddha, and Unani extensively describe the therapeutic potential of herbal drugs in gastrointestinal disorders. Herbal medicines are considered comparatively safer, economical, culturally acceptable, and readily available. Plant-derived products contain a wide variety of bioactive compounds such as flavonoids, tannins, alkaloids, terpenoids, saponins, polyphenols, and glycosides that exhibit antioxidant, anti-inflammatory, antimicrobial, and cytoprotective activities. These phytochemicals help in protecting the gastric mucosa from oxidative stress and inflammation, thereby promoting ulcer healing and mucosal regeneration.

Among various medicinal plants, *Calophyllum inophyllum* has attracted significant attention because of its therapeutic importance. The oil obtained from the seeds of *Calophyllum inophyllum*, commonly known as Tamanu oil, has been traditionally used for wound healing, skin infections, inflammation, burns, and tissue regeneration. Tamanu oil possesses a unique composition of fatty acids, flavonoids, coumarins, xanthones, triterpenoids, and polyphenolic compounds, which contribute to its pharmacological activities. The presence of these bioactive constituents suggests its possible gastroprotective and antiulcer potential.

Flavonoids are among the most important phytoconstituents present in Tamanu oil and related extracts. Compounds such as quercetin, rutin, kaempferol, luteolin, and epicatechin are known for their strong antioxidant and anti-inflammatory properties. These compounds scavenge free radicals, reduce lipid peroxidation, inhibit inflammatory mediators, and strengthen the gastric mucosal barrier. Flavonoids also improve microcirculation and stimulate mucus production, thereby enhancing mucosal defense against ulcerogenic agents.

Coumarins present in Tamanu oil, particularly calophyllolide and inophyllums, possess potent anti-inflammatory and wound-healing activities. Calophyllolide has been reported to modulate inflammatory cytokines such as tumor necrosis factor-alpha and interleukins, thereby reducing tissue inflammation and promoting repair of damaged mucosa. Xanthones and polyphenols further contribute to antioxidant protection and antimicrobial effects, which may help in controlling H. pylori-associated gastric injury.

Fatty acids present in Tamanu oil also play an important role in maintaining cellular membrane integrity and promoting tissue regeneration. Essential fatty acids help in reducing inflammation, improving epithelial repair, and restoring gastric mucosal architecture. In addition, tannins and phenolic acids exhibit astringent and antioxidant properties that help form a protective layer over the ulcerated mucosa, thereby accelerating healing.
The pathophysiology of gastric ulcer involves complex interactions between aggressive and defensive factors. Gastric acid secretion is regulated by neural, hormonal, and paracrine mechanisms involving acetylcholine,

gastrin, and histamine. Excessive acid secretion or impaired mucosal defense leads to erosion of the gastric lining. Reactive oxygen species generated during inflammation further aggravate tissue damage by inducing lipid peroxidation, protein oxidation, and DNA damage. Therefore, agents possessing antioxidant and anti-inflammatory activities are considered beneficial in the management of gastric ulcers.

Experimental studies have demonstrated that medicinal plant extracts rich in flavonoids and polyphenols exhibit significant antiulcer activity in various animal models. These natural compounds reduce gastric acidity, increase mucus secretion, inhibit oxidative stress, and promote regeneration of gastric tissues. Furthermore, herbal medicines generally produce fewer side effects compared to synthetic drugs and may provide long-term protection against ulcer recurrence.

Standardization and quality control of herbal medicines are essential for ensuring their safety, efficacy, and reproducibility. Scientific validation of medicinal plants through phytochemical screening, pharmacological evaluation, and toxicological studies is necessary to establish their therapeutic potential. Bioanalytical techniques play an important role in identifying active constituents and evaluating their pharmacological effects. Standardized herbal preparations with proven efficacy can serve as promising alternatives or adjuncts to conventional antiulcer therapy.

The economic importance of medicinal plants has also increased considerably due to the growing global demand for herbal products. In developing countries, medicinal plants provide affordable healthcare solutions and support livelihood opportunities for rural populations involved in cultivation and processing. Sustainable utilization and scientific exploration of medicinal plants are therefore essential for promoting healthcare and biodiversity conservation.

Despite the availability of several antiulcer drugs, the search for novel therapeutic agents with better safety profiles and enhanced efficacy continues. Plant-derived bioactive compounds offer a valuable source for development of new gastroprotective agents. Considering the pharmacological properties of Tamanu oil and its phytoconstituents, it may provide beneficial effects in the prevention and treatment of gastric ulcers through antioxidant, anti-inflammatory, antimicrobial, and cytoprotective mechanisms.

Therefore, the present study is designed to evaluate the antiulcer potential of Tamanu oil and investigate its possible mechanisms of gastroprotection. The study aims to scientifically validate the traditional medicinal use of Tamanu oil and explore its effectiveness as a natural therapeutic agent for gastric ulcer management. The findings of this research may contribute toward the development of safer and more effective plant-based antiulcer formulations for future clinical applications.

MATERIALS AND METHODS:

Materials

Chemicals and Reagents

All chemicals and reagents used in the present study were of analytical grade. Methanol, sulfuric acid (H_2SO_4), ferric chloride, iodine, ethyl acetate, chloroform, and petroleum ether were procured from Solanki Enterprise, Pune. Additional chemicals used for phytochemical screening, antioxidant assays, and antiulcer studies included sodium phosphate buffer, α-amylase, α-glucosidase, starch, DPPH, potassium ferricyanide, ferric chloride, trichloroacetic acid, thiobarbituric acid, ATP, magnesium chloride, potassium chloride, ammonium molybdate, perchloric acid, and other laboratory-grade reagents.

Methods

Standardization of Plant Material

Standardization of the authenticated plant material was carried out using physicochemical parameters according to Indian Pharmacopoeial procedures. Standardization ensures the identity, purity, and quality of crude drugs before their use in pharmacological investigations.

I. Determination of Total Ash Value

Ash value determination was performed to estimate the inorganic constituents present in the crude drug. About 2 g of accurately weighed air-dried powdered drug was taken in a silica crucible and incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed. The percentage ash value was

calculated with reference to the air-dried sample.

II. Determination of Water-Soluble Ash
The ash obtained from total ash determination was boiled with 25 mL distilled water for 5 minutes. The insoluble matter was collected on ashless filter paper, washed with hot water, ignited, cooled, and weighed. The difference in weight between total ash and insoluble matter represented water-soluble ash.

III. Determination of Acid-Insoluble Ash
The obtained ash was boiled with 25 mL of 2M hydrochloric acid for 5 minutes. The insoluble matter was filtered, washed, ignited, cooled in a desiccator, and weighed. Acid-insoluble ash was calculated as a percentage with reference to the air-dried sample.

IV. Determination of Moisture Content
Moisture content was determined using the loss on drying method. Samples were dried in a hot air oven at a suitable temperature until constant weight was achieved. The moisture percentage was calculated from the difference between initial and final weights.

Extraction of Tamanu Oil
Tamanu oil was extracted from the seeds of *Calophyllum inophyllum* using the maceration extraction technique. Different solvents including hexane, acetone, and hexane-acetone mixture (3:2 v/v) were used during extraction.

Seed particles of appropriate size were immersed in solvent in a 250 mL glass beaker. The mixture was heated in a water bath at 50°C for 4 hours. After extraction, the mixture was filtered through filter paper and washed with fresh solvent. The filtrate containing tamanu oil and solvent was subjected to rotary evaporation to remove the solvent and obtain pure tamanu oil.

Optimization studies were performed to determine the effect of moisture content, material size, solvent type, extraction time, temperature, and stirring on oil yield. Maximum oil recovery was achieved using material size of 2 mm, moisture content of 4%, extraction temperature of 50°C, extraction time of 4 hours, and acetone solvent system with material-to-solvent ratio of 1:30 (w/v).

Physicochemical Characterization of Tamanu Oil
The extracted tamanu oil was subjected to physicochemical evaluation to determine quality and purity. Parameters evaluated included density, acid value, saponification value, peroxide value, and iodine value using standard analytical procedures.

Physicochemical Parameters
- Density: 0.932 ± 0.007 g/mL
- Acid value: 42.61 ± 0.04 mg KOH/g
- Saponification value: 190.65 ± 1.39 mg KOH/g
- Peroxide value: 0.338 ± 0.04 meq/kg
- Iodine value: 93 gI_2/100 g

GC-MS Analysis of Tamanu Oil
Chemical composition of tamanu oil was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS). Thirteen major compounds representing approximately 92.935% of total oil composition were identified.
Major compounds identified included:
- Glycidyl oleate
- Palmitic acid
- Glycidyl palmitate
- β-monostearin
- Arachic acid
- Oleamide
- Coumarin derivatives
- Pregnan-12-one

These bioactive constituents are responsible for antioxidant, anti-inflammatory, and gastroprotective activities of

tamanu oil.

Preliminary Phytochemical Screening
Preliminary phytochemical analysis was carried out to identify the presence of various secondary metabolites such as flavonoids, alkaloids, glycosides, phenols, terpenoids, steroids, tannins, and resins.

Phytochemical Tests
1. Sulfuric Acid Test
One milliliter of extract was treated with concentrated sulfuric acid. Development of orange color indicated the presence of phytoconstituents.
2. Iodine Test
Extract was treated with iodine solution. Appearance of reddish coloration indicated positive reaction.
3. Ferric Chloride Test
Extract was mixed with ferric chloride solution. Green, blue, or violet coloration indicated the presence of phenolic compounds.
4. Test for Terpenoids
Extract was mixed with chloroform followed by concentrated sulfuric acid. Formation of reddish-brown coloration confirmed terpenoids.
5. Borntrager's Test
Development of pink or red color indicated the presence of glycosides.

Thin Layer Chromatography (TLC)
Thin Layer Chromatography was carried out for separation and identification of phytoconstituents.

Principle
TLC is based on differential migration of compounds between stationary and mobile phases. Components with greater affinity for stationary phase migrate slowly, while those with greater affinity for mobile phase move faster.

Procedure
Silica gel-G slurry was prepared and coated on TLC plates. Plates were activated at 105°C for 30 minutes. Samples were applied as spots approximately 2 cm from the lower edge.
The chromatographic chamber was saturated with mobile phase before development. Plates were developed until solvent front migrated 10–15 cm. Developed plates were visualized under UV light and with spraying reagents such as vanillin-sulfuric acid and methanolic ferric chloride.

Solvent Systems
- Alkaloids: Ethyl acetate : Chloroform : Water (6:3:1)
- Phenols: Methanol : Water (8:2)

Retention factor (Rf) values were calculated using:
Rf = Distance traveled by sample / Distance traveled by solvent front

6.2.7 In Vitro Antiulcer Activity
Alpha-Amylase Inhibition Assay
Alpha-amylase inhibitory activity was determined spectrophotometrically. Preparation of α-Amylase Solution Twenty-five milligrams of α-amylase was dissolved in distilled water and volume adjusted to 50 mL to obtain 0.5 mg/mL solution. Preparation of Starch Solution One gram starch was dissolved in 100 mL distilled water.

Procedure
Different concentrations of extract (100–500 µg/mL) were prepared. Extract solution was mixed with α-amylase and phosphate buffer and incubated at 37°C. Starch solution was added and reaction was terminated using DNS reagent. Absorbance was measured at 540 nm.
Acarbose was used as standard drug.
Percentage inhibition was calculated using:
% Inhibition = (A − C / C) × 100
Where:
- A = Absorbance of sample

- C = Absorbance of control

Alpha-Glucosidase Inhibition Assay
Alpha-glucosidase inhibitory activity was evaluated using p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate.

Procedure
Different concentrations of plant extract (100–500 µg/mL) were mixed with α-glucosidase enzyme and phosphate buffer in a 96-well plate and incubated at 37°C for 20 minutes.
After incubation, pNPG substrate was added and further incubated for 30 minutes. Reaction was stopped using sodium carbonate solution and absorbance measured at 405 nm.
Acarbose served as positive control.
Percentage inhibition was calculated as:
% Inhibition = (ODblank − ODsample / ODblank) × 100
IC50 values were determined by plotting inhibition percentage against concentration.

Determination of Antiulcer Activity In Vitro
H+, K+-ATPase Inhibition Assay
H+, K+-ATPase enzyme inhibition assay was performed to evaluate proton pump inhibitory activity of tamanu oil.

Fresh sheep stomach obtained from slaughterhouse was used for isolation of parietal cells. Gastric mucosa was homogenized in Tris buffer containing Triton X-100 and centrifuged. Supernatant served as enzyme extract.
The reaction mixture contained enzyme extract, Tris buffer, ATP, $MgCl_2$, and KCl. Extract samples were incubated with reaction mixture at 37°C for 30 minutes. Reaction was terminated using ammonium molybdate and perchloric acid.
Inorganic phosphate released was measured spectrophotometrically at 400 nm. Lansoprazole was used as standard proton pump inhibitor.

Agar Diffusion Assay Against Helicobacter pylori
Anti-*Helicobacter pylori* activity was evaluated using agar diffusion method.
Petri plates containing Ham's F-12 agar medium supplemented with 5% FBS were inoculated with *H. pylori* culture. Sterile discs impregnated with extract concentrations (5, 10, and 15 µg/disc) were placed on agar plates.
Amoxicillin served as positive control while saline was used as negative control. Plates were incubated under microaerophilic conditions and inhibition zones measured.
Minimum Inhibitory Concentration (MIC)
MIC was determined using broth dilution method. Serial dilutions of extract were prepared and inoculated with *H. pylori* suspension.
After incubation for 24 hours at 37°C, bacterial growth was measured spectrophotometrically at 625 nm. MIC was defined as the lowest concentration preventing visible growth.
Antioxidant Activity

DPPH Radical Scavenging Assay
DPPH assay was performed to determine antioxidant activity.
Different concentrations of extract were mixed with DPPH solution and incubated in dark for 30 minutes. Absorbance was measured at 517 nm.
Percentage scavenging activity was calculated using:
% Scavenging = [(Acontrol − Asample) / Acontrol] × 100

Reducing Power Assay
Extracts were mixed with phosphate buffer and potassium ferricyanide and incubated at 50°C. Trichloroacetic acid was added followed by ferric chloride.
Absorbance was measured at 700 nm. Increased absorbance indicated greater reducing power.
Lipid Peroxidation Inhibition Assay
Rat liver homogenate was used for estimation of lipid peroxidation inhibition.
Extracts were incubated with liver homogenate followed by ferric chloride and ascorbic acid. Reaction was terminated using thiobarbituric acid reagent and absorbance measured at 532 nm.

Results were expressed as percentage inhibition of lipid peroxidation.

In Vivo Antiulcer Activity
Healthy Wistar rats weighing 180–220 g and aged 6–8 weeks were used. Animals were maintained under standard laboratory conditions with controlled temperature, humidity, and 12-hour light-dark cycle.
Animals had free access to food and water and were acclimatized for one week before experimentation.

Experimental Design
Animals were divided into six groups:
- Group I: Normal control
- Group II: Ulcer control
- Group III: Standard drug-treated group
- Group IV: Tamanu oil 100 mg/kg
- Group V: Tamanu oil 200 mg/kg
- Group VI: Tamanu oil 400 mg/kg

Induction of Gastric Ulcer
Gastric ulcer was induced using pylorus ligation method.
Animals were fasted for 24 hours before surgery. Under anesthesia, pyloric end of stomach was ligated carefully without disturbing blood supply. Abdomen was sutured and animals allowed to recover.
After 4–6 hours, animals were sacrificed and stomachs removed for evaluation.

Parameters Evaluated
Following parameters were studied:
- Ulcer index
- Gastric juice volume
- Gastric pH
- Free acidity
- Total acidity
- Mucus content

Histopathological Analysis
Stomach tissues were fixed in 10% neutral buffered formalin for 24–48 hours. Tissues were dehydrated, cleared, and embedded in paraffin wax.
Sections of 4–5 µm thickness were prepared using microtome and stained with hematoxylin and eosin (H&E).
Slides were examined under light microscope to evaluate mucosal damage, necrosis, edema, inflammatory infiltration, and ulcer healing.
Statistical Analysis
Experimental data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.
Differences between groups were considered statistically significant at $P < 0.05$. Statistical analysis was carried out using GraphPad Prism software.

RESSULT:

7.1 Collections and Drying

7.1.1 Collection of *Tamanu oil*

The plant material of Tamanu oil is collected from its natural habitat or cultivated sources, ensuring that only healthy and mature parts are selected. The collected material is carefully inspected to remove any unwanted debris, dirt, or infected parts. It is then thoroughly washed with distilled water to eliminate dust, microbes, and other contaminants, ensuring the purity of the sample before further processing.

7.1.2 Drying of *Tamanu oil*

After collection and cleaning, the plant material is dried using either shade drying or oven drying. Shade drying is done at room temperature to preserve heat-sensitive bioactive compounds, while oven drying at a controlled temperature of 40–50°C can be used to speed up the process without degrading essential phytoconstituents. Once dried completely, the plant material is ground into a coarse powder and stored in an

airtight container for future extraction and analysis, ensuring the retention of its medicinal properties.

7.1.3 Authentication

The plant was authenticated department of ..... by comparing morphological features.

Standardization of plants materials:

Table 7.1: Physicochemical Evaluation of *Tamanu oil* Extract

Sr No.	Evaluation parameters	Values
		***Tamanu oil* extract**
1	Total ash value	3%
2	Acid insoluble ash	1%
3	Water soluble ash	2%
4	Moisture content	8.3%

Conclusion

The physicochemical evaluation of Tamanu oil extract revealed a total ash value of 3%, indicating the total mineral content. The acid-insoluble ash (1%) suggests a low presence of non- soluble inorganic matter, while the water-soluble ash (2%) reflects the presence of water-soluble minerals. The moisture content was found to be 8.3%, indicating the level of water retention in the extract. These parameters help assess the purity, quality, and suitability of the extract for further pharmaceutical or nutraceutical applications.

7.1.4 Extraction

The Tamanu oil powder, extracted with various 80% methanol and 20% water.. The appearance and percent yield of extracts are reported in below table.

Table 7.2: Yield of various extracts obtained from the whole plant powder

Sr. No.	Evaluation Parameters	Color	Nature	Percentage Yield (% W/W)
1.	Methanol (80%) and water (20%)	Dark Green	Semisolid and Sticky	7

7.2 Preliminary Phytochemical Screening:

Extracts were tested for the presence of active principles such as Triterpenoids, Glycosides, Alkaloids, Phenol.

Table 7.3: Phytochemical test of Tamanu oil powder

Sr. No.	Phytochemical test	Test Extracts
1.	**Test for alkaloids**	
A	H2SO4 Test	+
B	Iodine test	+
2.	**Test for Phenols present**	
	Ferric Chloride test	+
3	**Test for Terpenoids**	
	Sulfuric Acid-Chloroform Test	-
4	**Test for Glycosides**	
	Borntrager's Test	-

7.3 TLC for Phytoconstituents:

TLC chromatographic study were carried out to separate the active constituents which may having pharmacological active constituents.

Table 7.4: TLC for Phytoconstituents

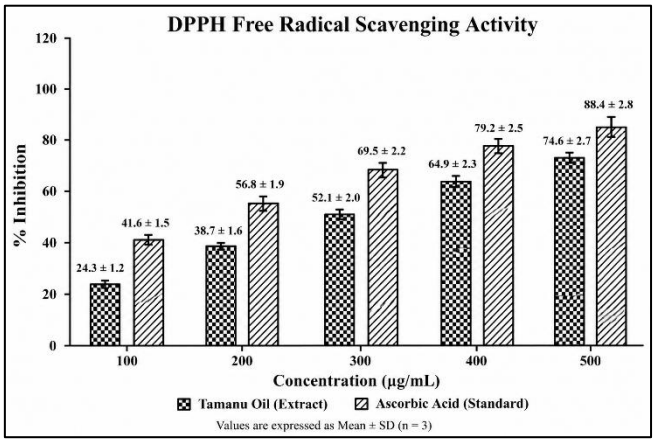
	Mobile phase	RF value found
Alkaloids	EA: Chloroform: water 6: 3: 1	0.67
Phenols	Methanol: water 8:2	0.81

7.4 DPPH Free Radical Scavenging Activity

Table 7.5: DPPH Free Radical Scavenging Activity of Tamanu Oil

Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Ascorbic Acid)
100	24.3 ± 1.2	41.6 ± 1.5
200	38.7 ± 1.6	56.8 ± 1.9

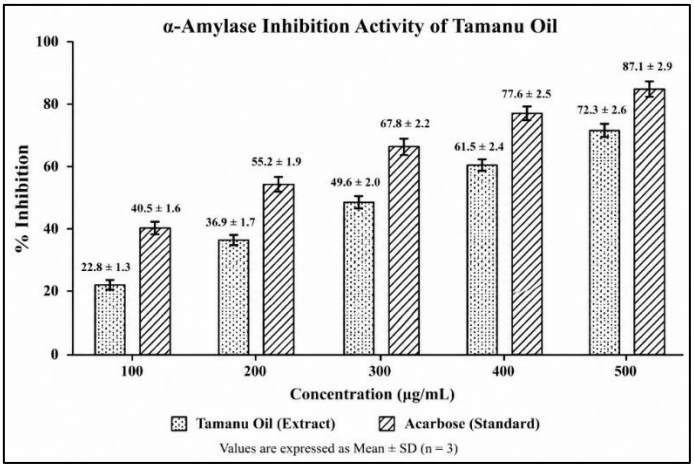
300	52.1 ± 2.0	69.5 ± 2.2
400	64.9 ± 2.3	79.2 ± 2.5
500	74.6 ± 2.7	88.4 ± 2.8



Graph 7.1: DPPH Free Radical Scavenging Activity of Tamanu Oil

Table 7.8: α-Amylase Inhibition Activity of Tamanu Oil

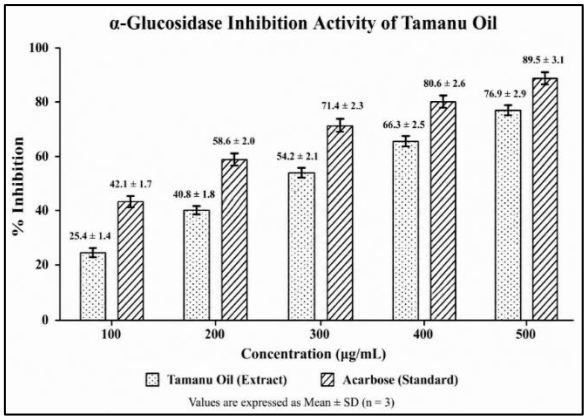
Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Acarbose)
100	22.8 ± 1.3	40.5 ± 1.6
200	36.9 ± 1.7	55.2 ± 1.9
300	49.6 ± 2.0	67.8 ± 2.2
400	61.5 ± 2.4	77.6 ± 2.5
500	72.3 ± 2.6	87.1 ± 2.9



Graph 7.2: α-Amylase Inhibition Activity of Tamanu Oil

Table 7.9: α-Glucosidase Inhibition Activity of Tamanu Oil

Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Acarbose)
100	25.4 ± 1.4	42.1 ± 1.7
200	40.8 ± 1.8	58.6 ± 2.0
300	54.2 ± 2.1	71.4 ± 2.3
400	66.3 ± 2.5	80.6 ± 2.6
500	76.9 ± 2.9	89.5 ± 3.1

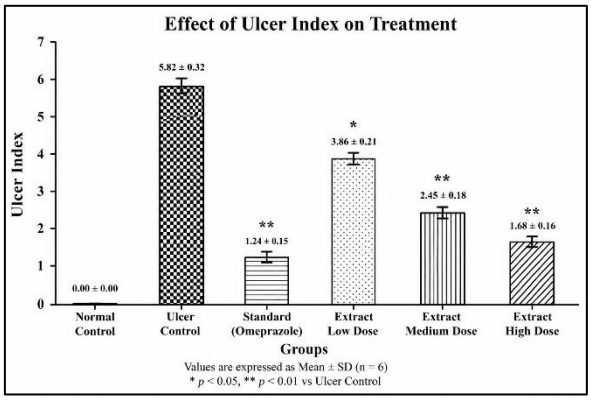


Graph 7.3: α-Glucosidase Inhibition Activity of Tamanu Oil

7.5 *In-vivo* Studies

Table 7.10: Effect of Ulcer Index on treatment

Group	Ulcer Index
Normal Control	0.00 ± 0.00
Ulcer Control	5.82 ± 0.32
Standard (Omeprazole)	1.24 ± 0.15**
Extract Low Dose	3.86 ± 0.21*
Extract Medium Dose	2.45 ± 0.18**
Extract High Dose	1.68 ± 0.16**



Graph 7.4: Effect of Ulcer Index on treatment

Table 7.11: Effect of treatment on Gastric Secretion Parameters

Group	Gastric Volume (mL)	pH	Free Acidity (mEq/L)	Total Acidity (mEq/L)
Normal	1.42 ± 0.11	3.8 ± 0.2	18.5 ± 1.2	32.6 ± 1.5
Ulcer Control	4.85 ± 0.25	1.6 ± 0.1	62.4 ± 2.1	92.3 ± 2.8
Standard	2.02 ± 0.15**	3.5 ± 0.2**	28.6 ± 1.5**	45.8 ± 2.1**
Low Dose	3.68 ± 0.18*	2.2 ± 0.1*	48.5 ± 1.8*	72.6 ± 2.3*
Medium Dose	2.85 ± 0.16**	2.8 ± 0.2**	36.7 ± 1.6**	58.4 ± 2.0**
High Dose	2.25 ± 0.14**	3.2 ± 0.2**	30.2 ± 1.4**	49.5 ± 1.8**

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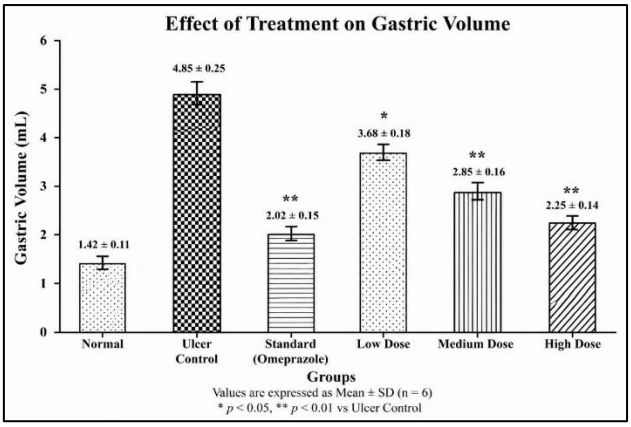
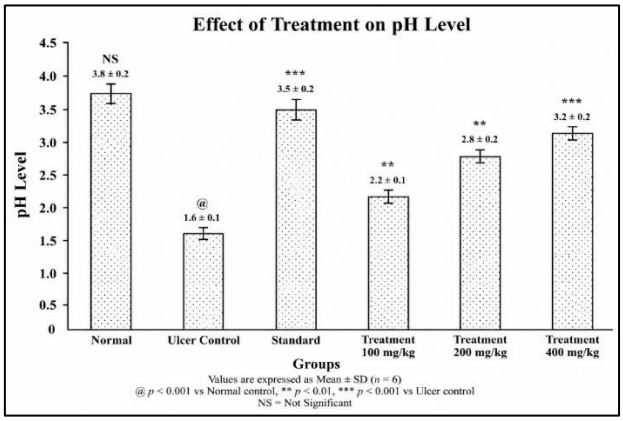
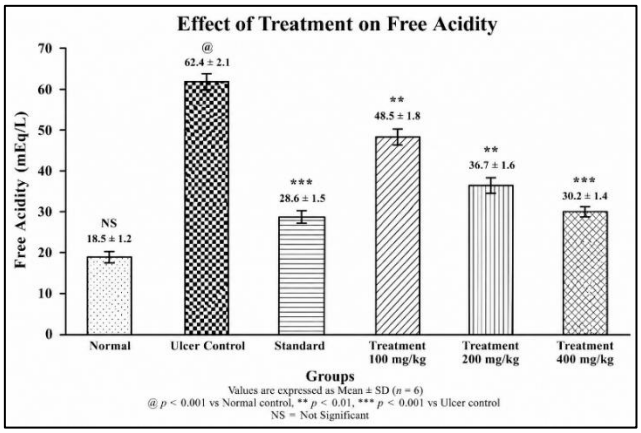


Graph 7.5: Effect of treatment on Volume



Graph 7.6: Effect of treatment on pH Level



Graph 7.7: Effect of treatment on Free Acidity

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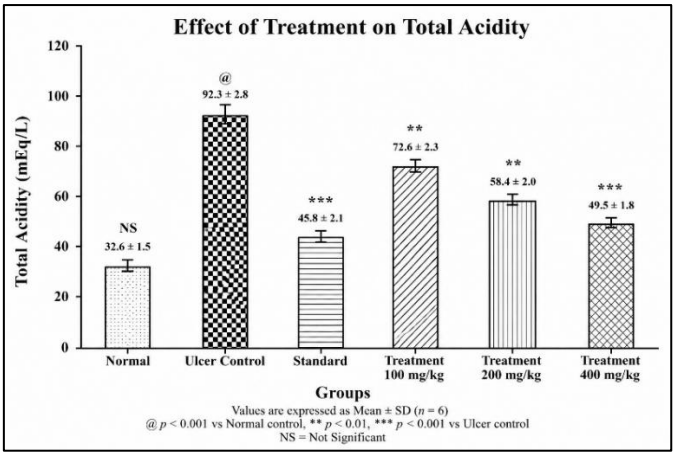
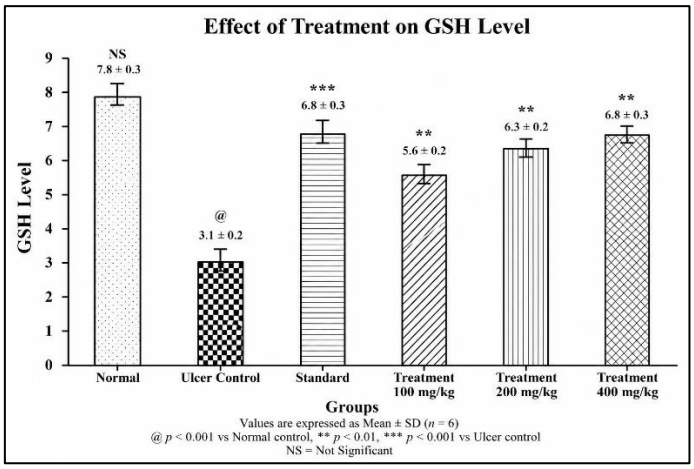


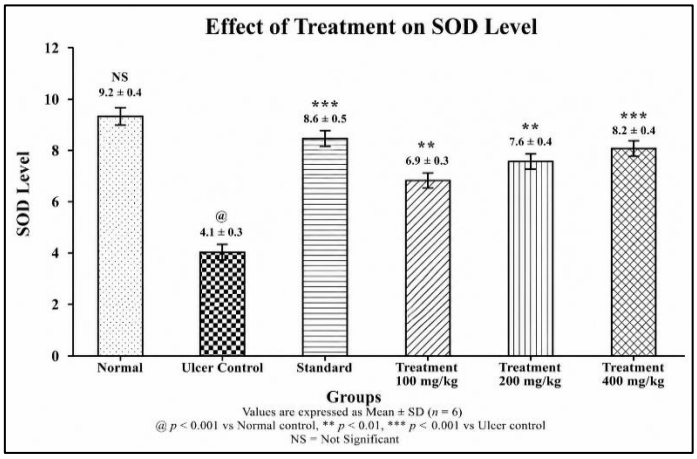
Graph 7.8: Effect of treatment on Total Acidity

Table7.12: Effect of treatment on Oxidative Stress Parameters

Group	GSH	SOD	CAT	MDA
Normal	7.82 ± 0.21	9.25 ± 0.32	58.4 ± 1.2	1.35 ± 0.05
Ulcer Control	3.12 ± 0.12	4.08 ± 0.18	27.6 ± 1.0	4.76 ± 0.12
Standard	6.95 ± 0.18**	8.64 ± 0.21**	53.8 ± 1.1**	1.62 ± 0.06**
Low Dose	5.62 ± 0.16*	6.92 ± 0.20*	44.2 ± 1.0*	2.58 ± 0.09*
Medium Dose	6.34 ± 0.17**	7.65 ± 0.19**	48.6 ± 1.2**	2.12 ± 0.08**
High Dose	6.88 ± 0.15**	8.21 ± 0.18**	52.3 ± 1.1**	1.84 ± 0.07**



Graph 7.9: Effect of treatment on GSH Level



Graph 7.10: Effect of treatment on SOD Level

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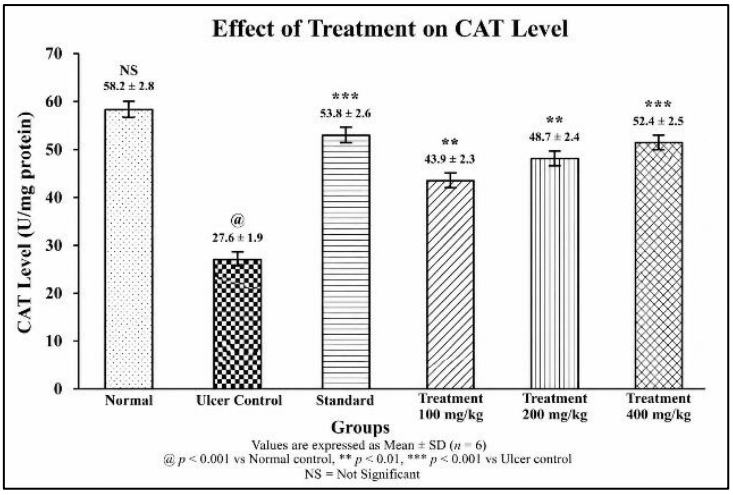
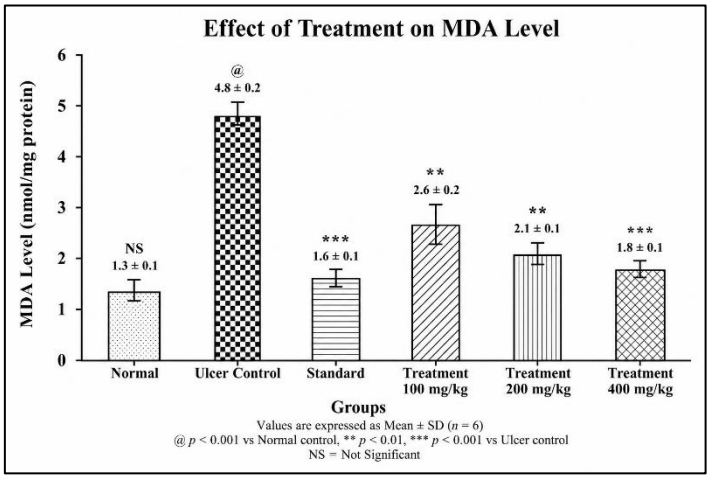
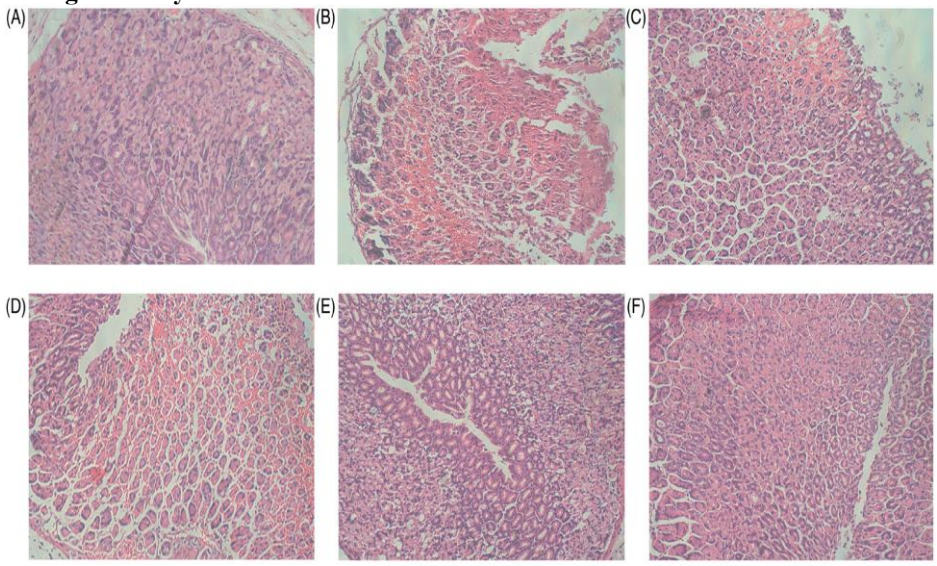


Graph 7.11: Effect of treatment on CAT Level



Graph 7.12: Effect of treatment on MDA Level

7.6: Histopathological analysis



Histopathological examination of gastric tissues was carried out using hematoxylin and eosin (H&E) staining to evaluate the extent of mucosal damage and the protective effect of the test extract.

The normal control group showed intact gastric mucosa with a well-preserved epithelial lining, normal glandular structure, and absence of edema, hemorrhage, or inflammatory cell infiltration, indicating healthy gastric architecture. In contrast, the ulcer control group exhibited severe gastric mucosal damage characterized by extensive epithelial erosion, necrosis, disruption of the mucosal layer, submucosal edema, and marked inflammatory cell infiltration. These findings confirm successful induction of gastric ulcers due to accumulation of gastric secretions and increased acid-pepsin activity.

The standard drug-treated group showed significant protection of the gastric mucosa, with only mild epithelial disruption and minimal inflammatory changes. The mucosal architecture was largely preserved, indicating effective gastroprotective activity. The extract-treated groups demonstrated a dose-dependent protective effect. The low-dose group showed moderate mucosal damage with partial preservation of epithelial integrity. The medium-dose group exhibited reduced ulceration, mild inflammation, and improved mucosal structure. The high-dose group (400 mg/kg) showed marked protection, with near-normal mucosal architecture, minimal epithelial damage, and very mild inflammatory infiltration.

Overall, the histopathological findings clearly indicate that the extract significantly protects the gastric mucosa against ulcer-induced damage and promotes healing in a dose-dependent manner.

DISCUSSION:

The present study was carried out to evaluate the antiulcer potential of Tamanu oil (*Calophyllum inophyllum*) using pylorus ligation-induced gastric ulcer model in Wistar rats along with in vitro antioxidant evaluation. Gastric ulcer is a multifactorial gastrointestinal disorder that develops due to an imbalance between aggressive factors such as hydrochloric acid, pepsin, reactive oxygen species, and protective factors including mucus secretion, bicarbonate production, prostaglandin synthesis, and mucosal blood flow. Disturbance of this balance results in erosion of the gastric mucosa and subsequent ulcer formation. The pylorus ligation model is one of the most commonly used experimental methods for evaluating antiulcer activity because it promotes accumulation of gastric secretions, resulting in increased acidity and autodigestion of gastric mucosal tissues.

In the present investigation, the ulcer control group showed a significant increase in ulcer index, gastric volume, free acidity, and total acidity, along with a reduction in gastric pH. These observations confirm successful induction of gastric ulcer and are in agreement with earlier findings reported in pylorus ligation studies. Retention of gastric secretions following pyloric obstruction exposes the gastric mucosa to excessive acid and pepsin activity, leading to tissue injury and mucosal erosion. Increased gastric volume indicates accumulation of gastric secretions, whereas elevated free and total acidity reflect excessive hydrogen ion concentration within the stomach. Reduction in gastric pH creates a highly acidic environment that further aggravates mucosal injury. Excess acid damages epithelial cells, disrupts membrane integrity, and increases mucosal permeability, allowing back diffusion of hydrogen ions into deeper tissues. This process leads to edema, inflammation, and progressive destruction of gastric mucosa.

Treatment with Tamanu oil produced a marked and dose-dependent reduction in ulcer index, suggesting significant gastroprotective activity. The highest dose demonstrated maximum protection and showed results comparable to the standard antiulcer drug. Reduction in ulcer index indicates decreased severity and number of gastric lesions, suggesting preservation of gastric mucosal integrity. This protective action may be attributed to enhancement of defensive mechanisms such as mucus secretion, stabilization of epithelial cells, and prevention of direct contact between gastric acid and mucosal surface. Such effects are characteristic of cytoprotective compounds that not only prevent tissue damage but also support mucosal healing and regeneration. Similar dose-dependent reductions in ulcer index have been reported for various plant-derived antiulcer agents, supporting the findings of the present investigation.

Gastric secretion parameters play a central role in ulcer development and progression. In the ulcer control group, gastric volume, free acidity, and total acidity were significantly elevated, indicating excessive gastric secretion and accumulation of acidic contents. Elevated acid levels contribute directly to mucosal erosion by promoting protein denaturation and epithelial damage. Administration of Tamanu oil significantly reduced gastric volume and acidity while increasing gastric pH. These findings indicate a strong antisecretory effect of the oil. Increase in gastric pH reflects reduction in acidity and establishment of conditions favorable for mucosal healing. Reduction in gastric secretion decreases exposure of the gastric lining to harmful acidic conditions, thereby

preventing further mucosal damage and facilitating tissue repair. The antisecretory effect may be associated with inhibition of H^+/K^+ ATPase activity, modulation of histamine release, or interference with gastrin-mediated stimulation of acid secretion. Regulation of gastric acid secretion is considered one of the primary mechanisms in ulcer management, and the observed effects indicate that Tamanu oil effectively controls ulcerogenic factors.

Oxidative stress is recognized as an important contributor to gastric ulcer pathogenesis. Excessive generation of reactive oxygen species causes lipid peroxidation, protein oxidation, DNA damage, and cellular apoptosis. Lipid peroxidation damages cellular membranes and produces toxic compounds such as malondialdehyde (MDA), which further aggravate tissue injury. In the present study, the ulcer control group showed elevated MDA levels along with reduction in endogenous antioxidant enzymes including superoxide dismutase, catalase, and reduced glutathione. These findings indicate impaired antioxidant defense mechanisms and increased susceptibility of gastric tissues to oxidative damage. Reactive oxygen species disrupt cellular integrity and promote inflammation, leading to progressive deterioration of gastric mucosa. Depletion of glutathione further weakens cellular defense mechanisms and contributes to accumulation of free radicals.

Treatment with Tamanu oil significantly reduced lipid peroxidation and restored antioxidant enzyme levels toward normal values, indicating potent antioxidant activity. Reduction in MDA levels suggests stabilization of cellular membranes and prevention of oxidative degradation, whereas restoration of antioxidant enzymes reflects improved ability of tissues to neutralize reactive oxygen species. The antioxidant effect of Tamanu oil may be attributed to the presence of flavonoids, polyphenols, coumarins, xanthones, and phenolic compounds. These phytoconstituents act as free radical scavengers by donating electrons or hydrogen atoms, thereby neutralizing reactive oxygen species and interrupting oxidative chain reactions. Flavonoids are known to inhibit lipid peroxidation, enhance antioxidant enzyme activity, and improve tissue resistance against oxidative stress. Polyphenols stabilize cellular membranes and reduce inflammation, while coumarins and xanthones exhibit strong antioxidant and anti-inflammatory effects. The combined action of these compounds contributes significantly to preservation of gastric mucosal integrity.

The in vitro antioxidant activity evaluated by DPPH radical scavenging assay further supported the in vivo findings. Tamanu oil demonstrated dose-dependent free radical scavenging activity, indicating strong antioxidant potential. The DPPH assay is based on the ability of antioxidants to donate hydrogen atoms or electrons to stabilize free radicals. The observed scavenging activity confirms the presence of bioactive compounds capable of neutralizing reactive oxygen species. Since oxidative stress plays a major role in gastric ulcer development, the antioxidant property of Tamanu oil contributes significantly to its gastroprotective effect. By reducing oxidative damage, the oil preserves membrane integrity, protects epithelial cells, and maintains normal physiological function of gastric tissues.

Histopathological examination provided clear morphological evidence supporting the biochemical and pharmacological observations. Gastric tissues from the ulcer control group showed severe epithelial erosion, necrosis, edema, and inflammatory cell infiltration, confirming extensive mucosal damage caused by accumulated gastric acid and oxidative stress. In contrast, Tamanu oil-treated groups showed considerable improvement in gastric architecture. The low-dose group exhibited moderate mucosal preservation, whereas the medium-dose group demonstrated reduced inflammation and tissue injury. The high-dose group showed near-normal gastric mucosal structure with minimal epithelial disruption and inflammatory infiltration. These observations indicate enhanced healing and regeneration of gastric epithelial cells. Preservation of tissue integrity and reduction in inflammatory changes suggest that Tamanu oil possesses both cytoprotective and healing properties. The close correlation between histopathological findings and biochemical parameters further strengthens the evidence for its antiulcer activity.

The gastroprotective activity of Tamanu oil can be explained by its rich phytochemical composition. The oil contains flavonoids, tannins, phenolic acids, coumarins, xanthones, and fatty acids, which collectively contribute to its therapeutic effects through multiple mechanisms. Flavonoids stimulate prostaglandin synthesis, which enhances secretion of mucus and bicarbonate, forming a protective barrier over gastric mucosa. Tannins form a proteinaceous layer on the epithelial surface, reducing permeability and protecting tissues from chemical irritation. Phenolic compounds exhibit strong antioxidant activity, whereas coumarins and xanthones possess anti-inflammatory and free radical scavenging properties. Fatty acids present in the oil help maintain membrane fluidity and reduce inflammatory responses. The synergistic action of these constituents contributes significantly

to the antiulcer activity of Tamanu oil.

Maintenance of adequate gastric mucosal blood flow is another important factor involved in mucosal protection and healing. Proper circulation supplies oxygen and nutrients necessary for cellular metabolism and tissue regeneration while removing toxic metabolites from damaged tissues. The observed gastroprotective effect of Tamanu oil may also involve preservation of mucosal blood flow, thereby supporting rapid recovery and regeneration of gastric epithelium. In addition, the anti-inflammatory effect of the oil reduces inflammatory cell infiltration and minimizes tissue damage. By controlling oxidative stress, inflammation, and gastric secretion simultaneously, Tamanu oil effectively protects gastric mucosa and promotes healing.

The findings of the present study are consistent with previously reported investigations demonstrating significant antiulcer activity of plant extracts rich in phenolic compounds and flavonoids. Herbal medicines are increasingly gaining attention because of their multitargeted mechanisms, reduced toxicity, and minimal side effects compared to synthetic drugs. Conventional antiulcer agents such as proton pump inhibitors and H_2 receptor antagonists are effective but may cause adverse effects including nutrient malabsorption, infections, and ulcer recurrence after discontinuation. In this context, Tamanu oil represents a promising natural alternative with antioxidant, antisecretory, anti-inflammatory, and cytoprotective properties.

Overall, the present study clearly demonstrates that Tamanu oil possesses significant antiulcer activity through multiple complementary mechanisms. Its ability to reduce gastric acidity, scavenge free radicals, strengthen mucosal defense, and promote tissue regeneration contributes to effective gastroprotection. The combined antioxidant, antisecretory, cytoprotective, and anti-inflammatory properties of Tamanu oil make it a promising candidate for the development of safer and more effective herbal therapies for gastric ulcer management. Further studies involving isolation of active constituents and clinical evaluation may help establish its therapeutic potential in human subjects.

CONCLUSION:

The present study successfully demonstrated the significant antiulcer potential of Tamanu oil (*Calophyllum inophyllum*) using both in vitro and in vivo experimental approaches. Gastric ulcer remains a major gastrointestinal disorder caused by an imbalance between aggressive factors such as gastric acid, pepsin, oxidative stress, and weakened mucosal defense mechanisms. The findings of the study clearly indicate that Tamanu oil possesses remarkable gastroprotective activity through multiple complementary mechanisms.

In the pylorus ligation-induced gastric ulcer model, Tamanu oil produced a dose-dependent reduction in ulcer index, gastric volume, free acidity, and total acidity while significantly increasing gastric pH. These observations suggest strong antisecretory and cytoprotective effects of the oil. Reduction in gastric acidity minimizes mucosal irritation and protects the stomach lining from acid-pepsin mediated injury. The improvement in gastric secretion parameters further indicates restoration of mucosal defense mechanisms and preservation of gastric integrity.

The antioxidant studies revealed that Tamanu oil possesses potent free radical scavenging activity. The oil significantly reduced oxidative stress and lipid peroxidation while restoring endogenous antioxidant enzymes such as superoxide dismutase, catalase, and reduced glutathione. These findings confirm that the gastroprotective activity of Tamanu oil is closely associated with its antioxidant properties. The presence of bioactive phytoconstituents including flavonoids, phenolic compounds, coumarins, xanthones, and fatty acids may be responsible for its strong antioxidant, anti-inflammatory, and mucosal protective effects.

Histopathological examination further supported the biochemical findings by demonstrating marked protection of gastric mucosa in treated groups. Tamanu oil reduced epithelial erosion, necrosis, edema, and inflammatory cell infiltration while promoting regeneration of gastric tissues. The highest dose showed near-normal gastric architecture comparable to the standard drug-treated group.

Overall, the results of the present investigation conclude that Tamanu oil exhibits significant antiulcer activity through antisecretory, antioxidant, anti-inflammatory, and cytoprotective mechanisms. The study scientifically validates the traditional medicinal use of *Calophyllum inophyllum* and highlights its potential as a promising natural therapeutic agent for gastric ulcer management. Further studies involving isolation of active constituents, detailed mechanistic investigations, and clinical evaluation are recommended to establish its safety and therapeutic

efficacy for future pharmaceutical applications.

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