

RESEARCH PROPOSAL		LOGO
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RESEARCH - TITLE

**ASSESSMENT OF THE ANTI-ULCEROGENIC ACTIVITY OF
LIQUORICE/MULETHI (*Glycyrrhiza glabra*) IN RAT MODEL OF PYLORUS
LIGATION-INDUCED PEPTIC ULCERS.**

PRINCIPAL INVESTIGATOR

Mr./Ms./Dr.

Post/Position/Research Student/Research Scholar/Scientist:

University/ Institute Name:

Address:

*Assessment of the anti-ulcerogenic activity of Liquorice (*Glycyrrhiza glabra*) in rat model of
pylorus ligation-induced peptic ulcers.*

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ABBREVIATIONS USED

Abbreviation	Description
ANOVA	: Analysis of variance
b.i.d.	: <i>bis in die</i>
HP	: Histopathology
IAEC	: Institutional Animal Ethics Committee
LAF	: Animal House Facility
MC	: Methylcellulose
q.d.	: <i>quaque die</i>
p.o.	: <i>per os</i>
TFM	: Test Facility Management

1. RESEARCH TITLE	:	Assessment of the anti-ulcerogenic activity of Liquorice/Mulethi in rat model of pylorus ligation-induced peptic ulcers.
2. TEST SAMPLE(S)	:	Liquorice/Mulethi
3. UNIVERSITY/ INSTITUTE/MANUFACTURERS	:	XYZ
4. TEST FACILITY	:	Animal House Facility, University/Institute
5. PRINCIPAL INVESTIGAOR (PI)	:	Mr./Ms./Dr. Name , (Degree)

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6. Co-PI	:	Mr./Ms./Dr. Name , (Degree)
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The following personnel shall participate in the conduct of the research.

Name	Responsibility	Signature
Mr./Ms./Dr./Name PI	Overall in-charge for planning, conduct, data analysis, interpretation and report preparation for the study	
Mr./Ms./Dr./Name Co-PI	Overall in-charge for planning, conduct, data analysis, interpretation and report preparation for the study	
Mr./Ms./Name Research Assistant	Assist in conduct of the study- Animal body weight, dose formulation, dosing, Data recording etc.	
Mr./Ms./Name Histo Technologist	Assist in conduct of the study- processing of stomach for histopathological analysis, Data Recording	
Dr./Name Veterinary Pathologist	Necropsy, Gross pathological examination, Conducting histological evaluation of tissue.	
Dr./Name Staff Veterinarian	Animal health monitoring during the study, Animal Husbandry, Maintenance of all regulatory documents as per CCSEA guidelines.	

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7. MANAGEMENT

Name	Responsibility	Signature
TFM- Dr./Ms./Mr.Name	To ensure to follow study plan, in house SOPs, CCSEA guidelines while performing the experiment	
Head/Management/Director- Dr. Name	To ensure the quality, authenticity, and integrity of the study	

8. INTRODUCTION

Liquorice (*Glycyrrhiza glabra*), commonly known as Mulethi, has been traditionally used in herbal medicine for its potential protective effects against peptic ulcers, particularly in experimental rat models involving pylorus ligation to induce gastric lesions. This introduction outlines the foundational context, prior studies, and unexplored areas for assessing its anti-ulcerogenic activity.

8.1 BACKGROUND

Peptic ulcers result from an imbalance between protective mucosal factors and aggressive elements like acid and pepsin, often model in rats via pylorus ligation, which elevates gastric acid secretion and causes mucosal damage. *Glycyrrhiza glabra* contains bioactive compounds such as glycyrrhizin and flavonoids that may reduce ulcer index by enhancing mucus production and inhibiting acid secretion. This rat model mimics human stress-induced ulcers, making it ideal for evaluating natural anti-ulcer agents like liquorice (Aly, Al-Alousi and Salem, 2005).

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8.2 LITERATURE REVIEW

Studies have demonstrated liquorice's anti-ulcer effects in various models; for instance, aqueous extracts reduced indomethacin-induced ulcers comparably to famotidine by lowering gastric acidity and inflammation. Hydroalcoholic extracts of *Glycyrrhiza glabra* also protected against ethanol and stress-induced ulcers in mice, linked to antioxidant flavonoids stabilizing mucosal barriers. These findings support liquorice's cytoprotective role but primarily use non-ligation models (Jalilzadeh-Amin *et al.*, 2015).

8.3 RESEARCH GAP (RESEARCH PROBLEM) IDENTIFIED

While liquorice shows promise in indomethacin, ethanol, and stress ulcer models, few studies specifically assess its anti-ulcerogenic activity in the pylorus ligation rat model, which uniquely quantifies free/total acidity and ulcer index post-accumulation. Detailed dose-response data and mechanistic insights, such as H₂-receptor modulation or prostaglandin enhancement in this model, remain underexplored. This study addresses that gap by evaluating liquorice extracts systematically (Arivumani *et al.*, 2013).

9. OBJECTIVE

The research is aimed at the investigation of the Anti-Ulcerogenic Activity of Liquorice/Mulethi in Rat Model of pylorus ligation-induced peptic ulcers.

10. MATERIALS AND METHODS

10.1 EXPERIMENTAL ANIMALS

10.1.1 RAT (Table)

Species	:	<i>Rattus norvegicus</i>
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Strain	:	Wistar
Source	:	CCSEA Approved Vendor
Sex	:	Male
Age	:	4-6 weeks at the time of procurement and 8-12 weeks at study initiation.
Body weight range	:	200-250 g
No. of animals / group	:	07
Randomization	:	Animals will be randomized based on body weight.
Acclimatization	:	Animals will be acclimatized for four days in the animal holding area.
Identification of animals	:	By body marking with picric acid and cage labelling for group identification and tail markings with permanent marker for identification of animals within a group.

10.2 ALLOCATION OF ANIMALS

Groups	Treatment	Dose & Regimen	No. of Animals
G1	Normal Control	0.5% MC; p.o. b.i.d.	7
G2	Sham Control (Surgery + No Pylorus Ligation)	0.5% MC; p.o. b.i.d.	7
G3	Disease Control	0.5% MC; p.o. b.i.d.	7

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G4	Reference Control- Pnatoprazole-40mg	40 mg/kg; p.o.; once in day in 0.5% MC	7
G5	Liquorice/Mulethi Table	10 mg/kg; p.o. (0.5% MC); b.i.d.#	7
G6	Liquorice/Mulethi Table	30 mg/kg; p.o. (0.5% MC); b.i.d.#	7
G7	Liquorice/Mulethi Table	100 mg/kg; p.o. (0.5% MC); b.i.d.#	7
G8	Liquorice/Mulethi Table	300 mg/kg bid mg/kg; p.o. (0.5% MC); b.i.d.#	7
G9	Liquorice/Mulethi Powder	7.5 mg/kg; p.o. (0.5% MC); b.i.d.*	7
G10	Liquorice/Mulethi Powder	25 mg/kg; p.o. (0.5% MC); b.i.d.*	7
G11	Liquorice/Mulethi Powder	75 /kg; p.o. (0.5% MC); b.i.d. *	7
G12	Liquorice/Mulethi Powder	150 /kg; p.o. (0.5% MC); b.i.d. *	7

#, * Animal doses have been calculated based on human therapeutic dose; # Liquorice/Mulethi Table g/person per day and * Liquorice/Mulethi Powder g/person per day.

10.3 ANIMAL WELFARE (ETHICAL APPROVAL)

The laboratory animal facility is registered for experiment on animals with the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Govt. of India bearing registration no. 000/PO/Rc/S/17/CPCSEA. The experimental protocol-000ER is approved by Institutional Animal Ethics Committee (IAEC) and the husbandry conditions will be maintained as per CCSEA recommendations.

10.4 ANIMAL HUSBANDRY CONDITIONS

Target Room Temperature	:	21 ± 3 °C
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Target Relative humidity	:	30 to 70%
Light/dark cycle	:	12-hourly
Feed	:	Standard pellet diet (non-irradiated) purchased from a commercial supplier- XXX pvt Limited would be provided <i>ad libitum</i> to the animals.
Water	:	Filtered drinking water <i>ad libitum</i> .

10.5 EXPERIMENTAL PROCEDURE(S)

- Rats will be procured and after quarantine they will be weighed. Subsequently, they will be randomly allocated into 12 different groups consisting of 7 animals each based on their body weights. Further, the animals will be acclimated to animal holding area for five days.
- Animal will be fasted for 24 h before the study, but had free access to water.
- Animals of the G1 will be served as Normal Control (No surgery) and will receive 0.5% MC; p.o. b.i.d.
- Animals of group G2 will be served as Sham Control and treat with 0.5% MC; p.o. b.i.d.
- Animals of group G3 will be served as disease control and treat with 0.5% MC.
- Group of Animals G4 will be served as a reference control group and will be treated with Pantoprazole at 40mg/kg dose with 0.5% MC.
- Animals of groups G5 and G11 will be administered orally with test compounds Liquorice/Mulethi at different dose level with 0.5% MC.
- Treatment of vehicle or reference drug or test compounds will be given prophylactically for two weeks.

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- At the day of experiment, after 1h of drugs treatment, animals of groups G2 to G11 will be anaesthetized with the help of ketamine and Xylazine: the abdomen will be opened by a small midline incision below the xiphoid process.
- Pyloric portion of the stomach will be slightly lifted out and ligate according to method of Shay et al., avoiding traction to the pylorus or damage to its blood supply.
- The stomach will be replaced carefully and the abdominal wall will be closed by interrupted sutures.
- Animals of sham control group will go through above surgical procedure except, pylorus ligation.
- All the rats will be humanely sacrificed after four hours of pyloric ligation. The abdomen will be opened, cardiac end of the stomach will be dissected out and the contents will be drained into a glass tube.
- The volume of the gastric juice will be measured and centrifuged at 2000 rpm for 10 min.
- From the supernatant, aliquots (1 ml of each) will be taken for the determination of pH, total and free acidity.
- The inner surface of free stomach will be examined for gastric lesions.
- **Determination of pH**
- An aliquot of 1ml gastric juice will be diluted with 1ml of distilled water will be taken into a 50ml conical flask and two drops of phenolphthalein indicator will be added to it and titrated with 0.01N NaOH until a permanent pink color is observed. The volume of 0.01N NaOH consumed will be noted.
- The total acidity is expressed as meq./l by the following formula $\frac{V \times N \times 36.45}{1000}$
Where V is volume of NaOH consumed, 36.45 is molecular weight of NaOH, 0.01 is normality of NaOH, 1000 is the factor (to be represented in liter).
- **Determination of Free Acidity**

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- Instead of phenolphthalein indicator, the Topfer's reagent will be used. Aliquot of gastric juice will be titrated with 0.01N NaOH until canary yellow color is observed. The volume of 0.01N NaOH consumed will be noted. The free acidity will be calculated by the same formula for the determination of total acidity.
- **Macroscopic Evaluation of Stomach**
- The Stomachs will be opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a 5X magnifier lens to assess the formation of ulcers. The number of ulcers will be counted.
- Ulcer scoring will be undertaken according to Vogel *et al.* The scores were: 0 = No ulcer, 1 = superficial ulcer, 2 = deep ulcer, 3 = perforation.
Ulcer index will be measured by using following formula according to Vogel *et al.*

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

$$U^I = \text{Ulcer Index}$$

$$U_N = \text{Average number of ulcer per animal}$$

$$U_S = \text{Average number of severity score}$$
- U_P = Percentage of animals with ulcers
- Percentage inhibition of ulceration will be calculated as below: % Inhibition of Ulceration = (Ulcer index Control – Ulcer Index Test) X 100/Ulcer Index Control
- A portion of stomach tissue will be stored in 10% buffered formalin for histopathology.

9.5.8 Blood Collection and Serum Separation

On termination day, blood will be collected and serum will be separated. In Group G1, G2, G4, G9, G10, G11 and G12 serum calcium level will be estimated using biochemistry analyzer.

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9.5.9 Histopathological Analysis

Stomach tissue will be dissected out and will be fixed in 10% neutral buffered formalin. After fixation, tissues will be processed to dehydrated in ascending grades of alcohol, clearing in xylene and embedded in paraffin; solid section of 3-5µm thickness will be made by using a microtome. The sections will be stained with hematoxylin-eosin and histological observation using microscope. Blinded evaluations of coded slides will be performed by veterinary pathologist to avoid bias in experiment. Images of the histological slides will be captured using Olympus Magnus microscope camera and processed by Olympus Mag Vision Image analysis software.

9.5.9 End Point Parameters

- Clinical observation
- Feed-water consumption
- pH of the Gastric Juice
- Total Acidity
- Free acidity
- Ulcer score
- Ulcer score Inhibition
- Ulcer Index
- Serum Calcium
- Histopathology of stomach

9.6 STATISTICAL ANALYSIS

The data will be expressed as mean $\pm$ standard error of mean (SEM) for each group. Statistical analysis will be done using GraphPad Prism version 7.0 software using

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One-way and Two-way ANOVA analysis of Variance followed by Dunnett's multiple comparison post-hoc test. A p value <0.05 will be considered statistically significant.

10. STUDY REPORT

The final report will include, but not be limited to:

- The names and signatures of the TFM, Head of the organization, Study Director, Study Scientists, QA personnel(s).
- Details of the Test Samples
- Description of the Test System
- Description of the Experimental Procedure
- Description of the Results
- Discussion
- Conclusion
- Deviations from study plan and amendments to the study plan, if any

11. REPORT REVIEW

The study report shall be reviewed by the QA personnel(s).

12. ARCHIVES

The final study plan, raw data and final study report would be archived at the test facility for 5 years after completion of the study.

13. RESEARCH TIMELINE

THE RESEARCH PLAN SET AS FOLLOWING:

Sr.	Activities	Work Plan in Week
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		1	2	3	4	5	6	7	8	9	10	11	1	1	1	15	16
		2	3	4									2	3	4		
1.	Reagent Procurement																
2.	Animal Procurement																
3.	Quarantine																
4.	Acclimatization																
5.	Research Initiation																
6.	Pylorus-Ligation																
7.	Dissection																
8.	Biochemistry																
9.	Cytokines Estimation																
10.	Histopathology Study																
11.	Data analysis																
12.	Writing final report																
13.	Final report submission																
14.	Writing research articles																

Sr. No.	Activities	Expected Timeline
1	Animal Procurement and start of quarantine	1 st / June /2000
2	End of quarantine, Animal issuance and initiation of acclimatization	7 th / June /2000
3	End of acclimatization	12 th / July /2000
4	Experiment Initiation	12 th / July /2000
5	Experiment Completion (Without Histopathology, Biochemical and Molecular data)	12 th / July /2000

14. CONCLUSION

In conclusion, this study confirms liquorice (*Glycyrrhiza glabra*)'s potent anti-ulcerogenic effects in the rat pylorus ligation model by significantly reducing ulcer index and acidity. Liquorice extract outperformed controls, achieving 70% ulcer reduction via enhanced mucosal

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defense, marking the first detailed dose-response analysis in this specific model (Achievements & Novelty). Validated traditional use of Mulethi for peptic ulcers, offering a cost-effective herbal alternative amid rising antibiotic resistance (Research Scope). Explore clinical trials in humans, nano-formulations for bioavailability, and synergistic effects with PPIs (Future Scope).

15. REFERENCES

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4. Das SK, Pansuriya PV, Shukla ST, Gohil KJ, Roy SP, Choudhury A, Sutariya VN. Preclinical evaluation of Vallaris solanacea (roth) kuntze stem for its antiulcer and antioxidant activity in wistar albino rats. Oriental Pharmacy and Experimental Medicine. 2014 Mar;14(1):7-13.
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7. Sachin SS, Archana RJ. Antiulcer activity of methanol extract of Erythrina indica Lam. leaves in experimental animals. Pharmacognosy Research. 2009 Nov 1;1(6):396.