Cihan University- Erbil
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In Vivo Study of the Effects of Centella Asiatica Aqueous Extract on Ethanol-Induced Stomach Ulcers in Male Albino Rats.

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Outline

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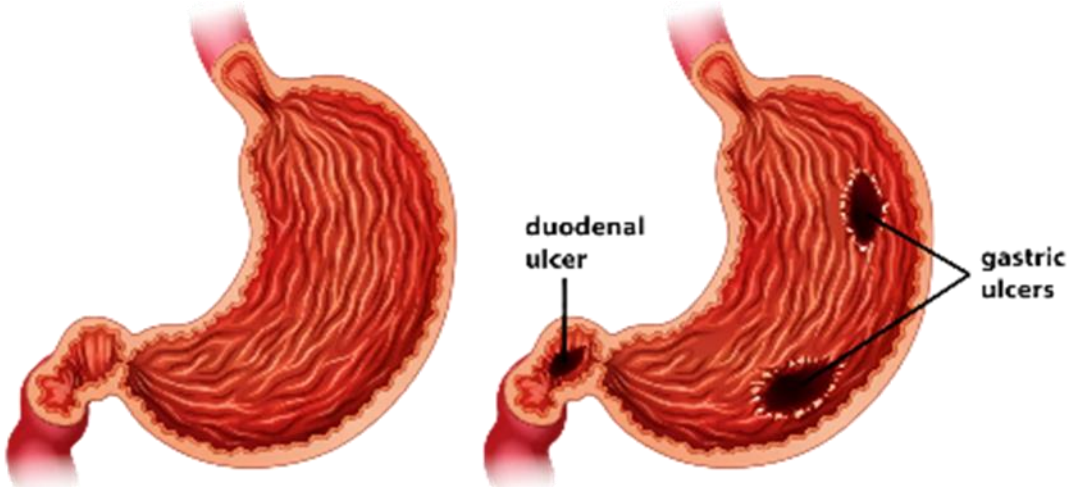
- Aim of study
- Materials and method
- Results
- Conclusion
- Recommendation

Introduction



- Persistent H.pylori infection & NSAIDs usage are the most common etiologies

- Usually diagnosed with: Medical history, urea breath test and endoscopy (EGD)

- Symptoms include: .abdominal pain, bloating, loss of appetite, weight loss, hematemesis , melena and gastric perforation leading to peritonitis

- Treatment with PPI and Histamin-2 receptor antagonists, have demonstrated adverse effects, relapses, and various drug interactions

Centella asiatica

- Gotu kola
- Indian pennywort
- Brahmi
- Pegaga

- Asiaticoside
- Madecassoside
- Asiatic acid
- Madecassic acid from the triterpene class

- Antioxidant
- Antimicrobial
- Neuroprotective
- Anti-inflammatory
- Thrombolytic

- **Antibacterial**

(Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio mimicus)

Reference (4)

- **Antifungal**

Aspergillus Species (reference 5)

- Omeprazole is considered the top/ first line treatment option for patients with stomach ulcers.

Aim of The Current Study

Comparing effectiveness of Centella asiatica aqueous extract against omeprazole as pretreatment for gastric ulcer, using male Sprague Dawley rats as the disease model

Materials and Methods

Extract Preparation

D.W Boiled and let to cool till 85 C°

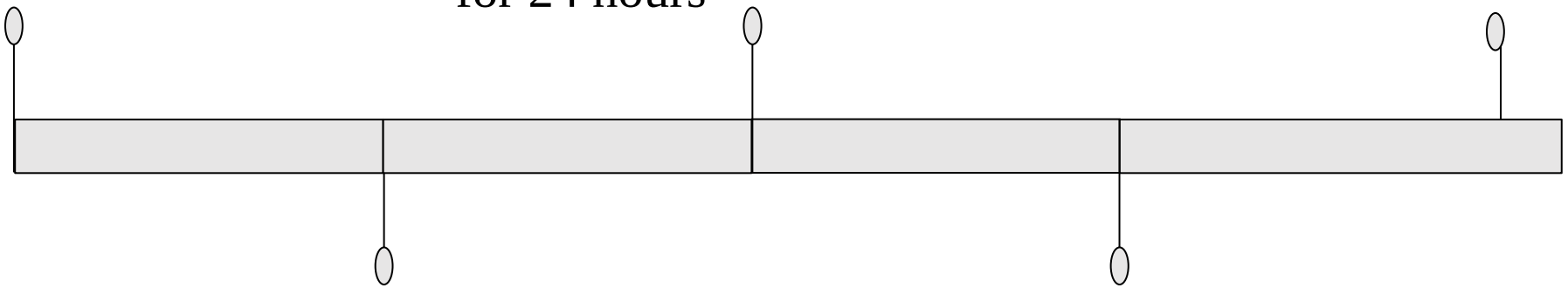
The flask was covered with aluminum foil and stored in dark condition for 24 hours

Administration to dose groups:

H dose= 600mg/kg in 6 ml D.W

M dose= 300 mg/kg in 6 ml D.W

L dose: 150 mg/kg in 6 ml D.W



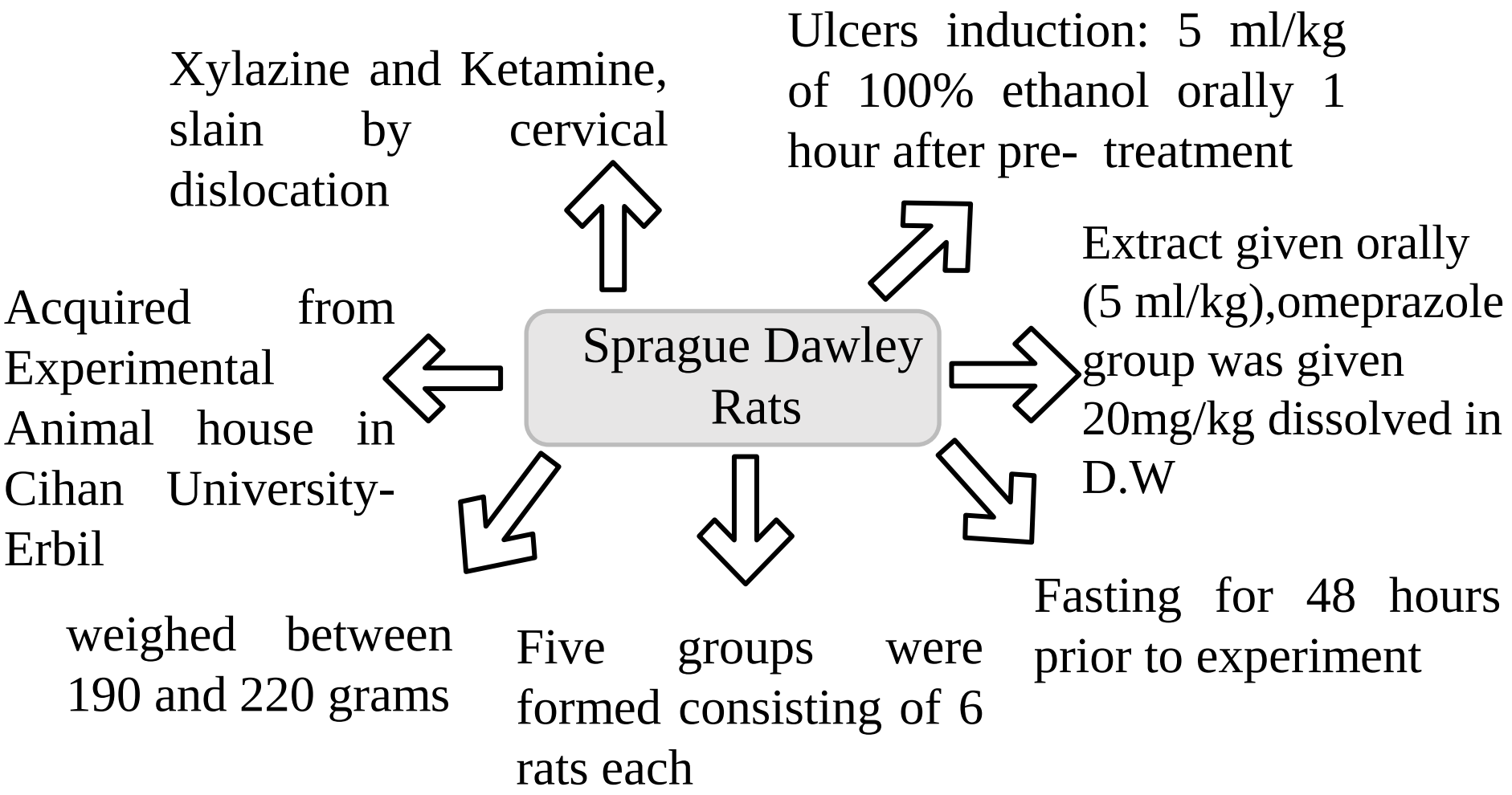
Plant powder was mixed with D.W until it was fully combined

Filtration was done after 24 hours using filter paper

Omeprazole Preparation

- Acquired from (happy) pharmacy in Erbil city

- Single omeprazole capsule with a dosage of 20 mg was dissolved in 5 cc of distilled water.


Xylazine and Ketamine, slain by cervical dislocation
Ulcers induction: 5 ml/kg of 100% ethanol orally 1 hour after pre- treatment
Extract given orally (5 ml/kg),omeprazole group was given 20mg/kg dissolved in D.W
Acquired from Experimental Animal house in Cihan University-Erbil
Sprague Dawley Rats
weighed between 190 and 220 grams
Five groups were formed consisting of 6 rats each
Fasting for 48 hours prior to experiment

Gross gastric lesions evaluation

- The planimeter was used to measure the length and width of the ulcers in mm by multiplying the total number of small squares by 4 and then by 1.8, resulting in the UA measurement in (mm2).

- Stomach wall tissues were processed using a paraffin tissue processing machine and stained with Hematoxylin and Eosin stain.

- Mucous weight was measured by collecting stomach contents and weighing it in an electronic scale.
- Gastric PH measurement was done after collecting the stomach contents using PH meter device.

- ANOVA test was used to test for statistical significance with P value <0.05 being considered significant.

Results

Acute toxicity study

- Each dose (150 mg/kg, 300 mg/kg, and 600 mg/kg) was studied for acute toxicity.
- The study was done for 14 days, no abnormal behaviour or any signs of toxicological effects.
- Triglycerides, creatinine, urea, haemoglobin, AST, ALT, and ALP levels were normal.

Table 1 Data presents PH, Mucus weight , UA, inhibition rate results for all rat groups

Animal Group	Pre-treatment (5 ml/kg dose)	pH (acidity)	Mucus weight (g)	Ulcer area (mm)2	Inhibition (%)
1	(Ulcer control)	2.62	0.77	822	-
2	*Omeprazole* (20 mg/kg)	4.97	2.47	125	84.79%
3	*C. asiatica* (150 mg/kg)	4.45	1.97	76	90.75%
4	*C. asiatica* (300 mg/kg)	5.42	2.24	61	92.57%
5	*C. asiatica* (600 mg/kg)	6.42	2.34	9.2	98.88%

Gross evaluation of gastric lesions

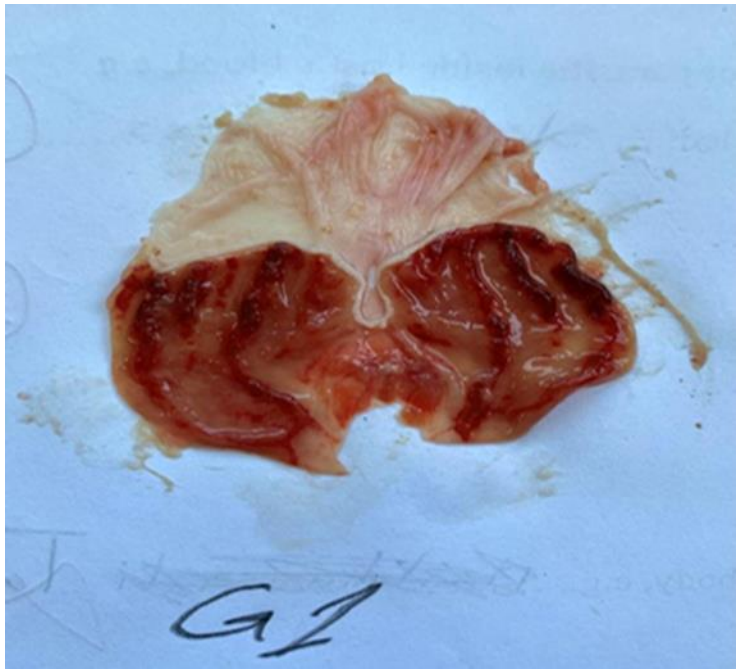


Fig1. Shows positive control rat shows significant damage to its gastric mucosa

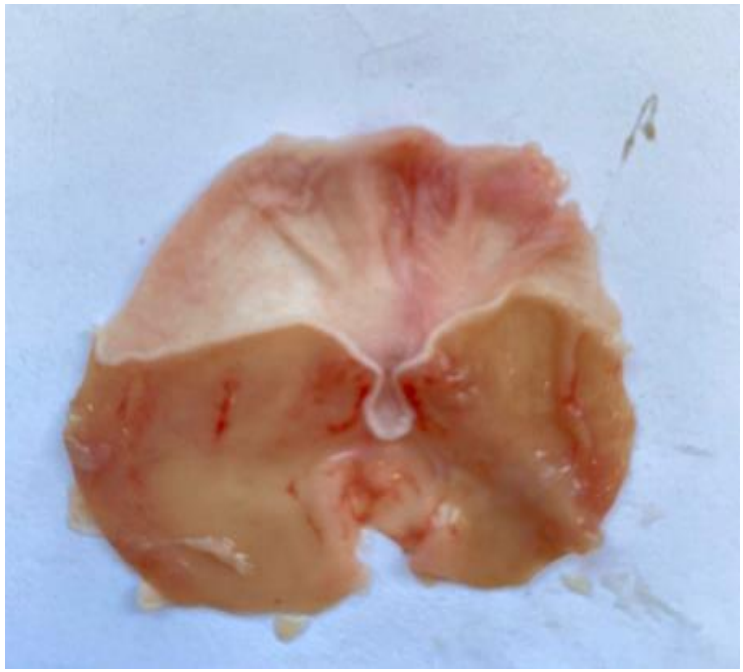


Fig2. Presents Pre- treatment with Omeprazole (20 mg/kg) exhibited milder damage compared to positive control

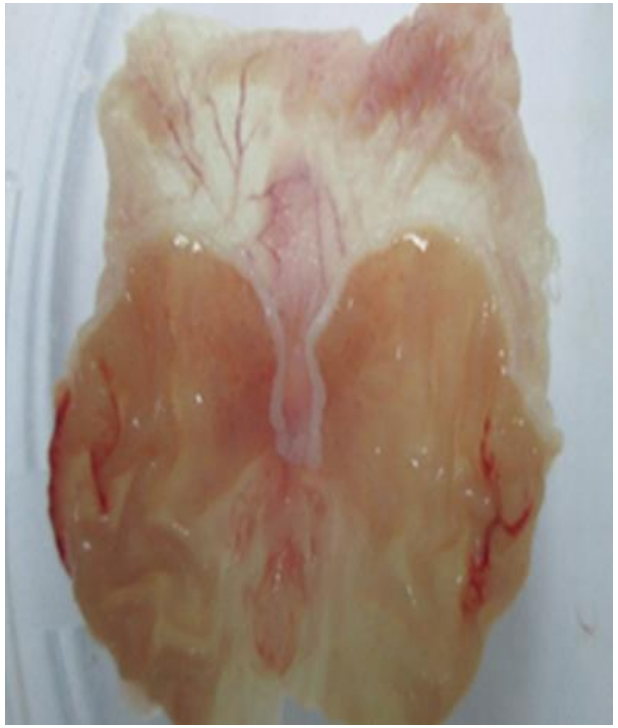


Fig3. Pre-treatment with (150 mg/kg) dose exhibited very slight macroscopic necrosis

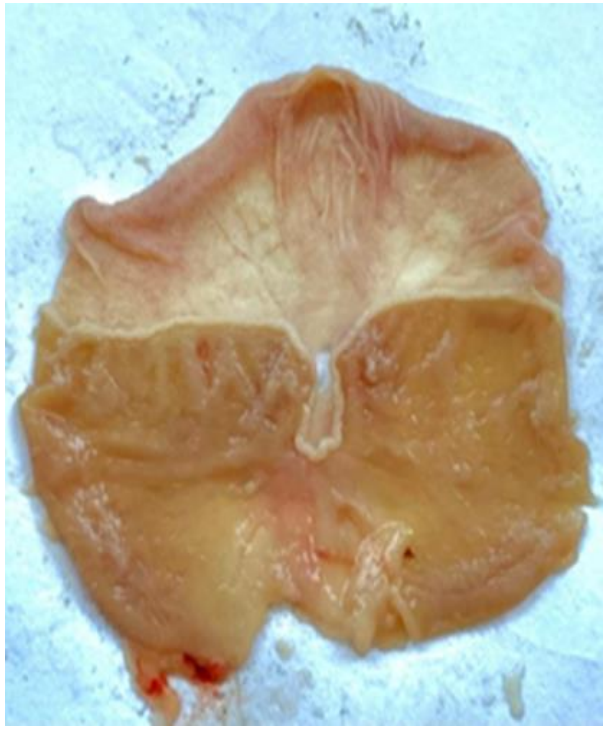


Fig4. (300 mg/kg) extract group showed weak indications of mucosa necrosis

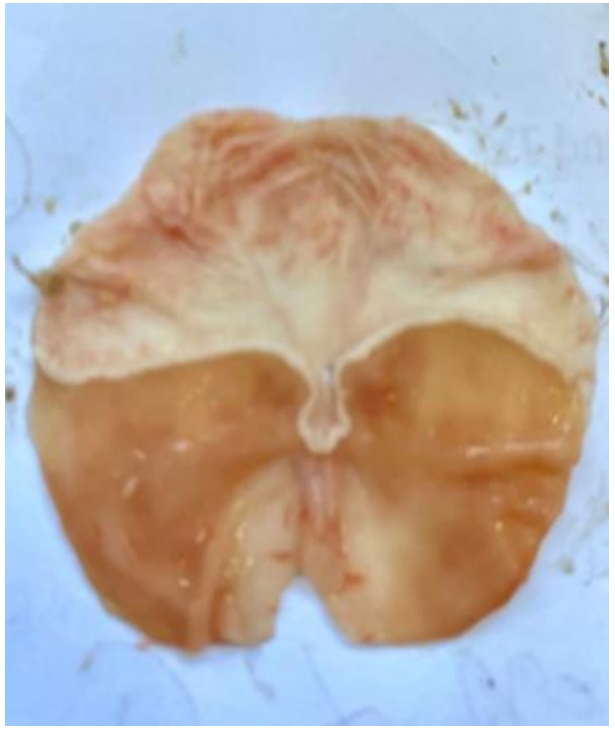


Fig5. C. asiatica extract (600 mg/kg) group presented with no observable tissue damage

Histological Evaluation of Gastric Lesions

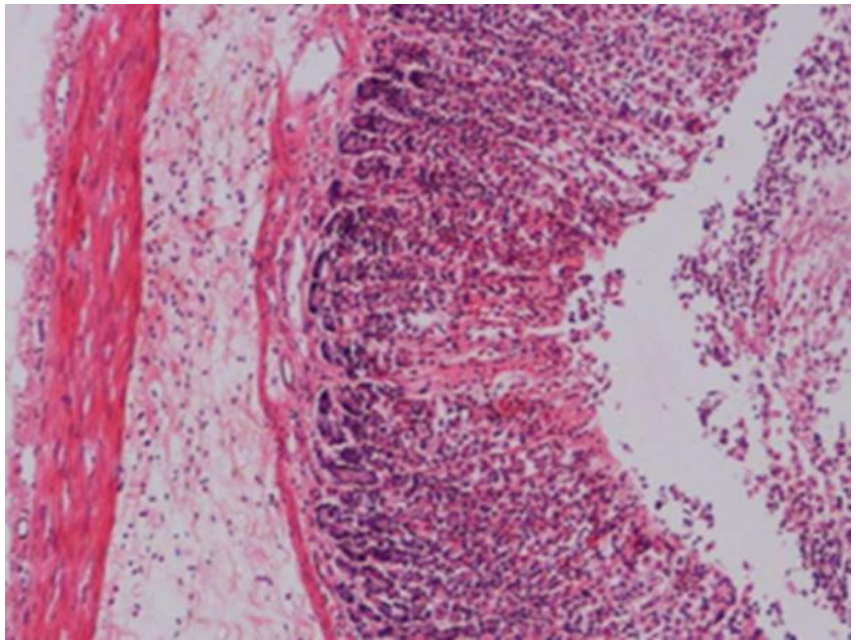


Fig6. Positive control group showed significant damage to surface epithelia, swelling of the submucosal layer, and the presence of WBC infiltrating

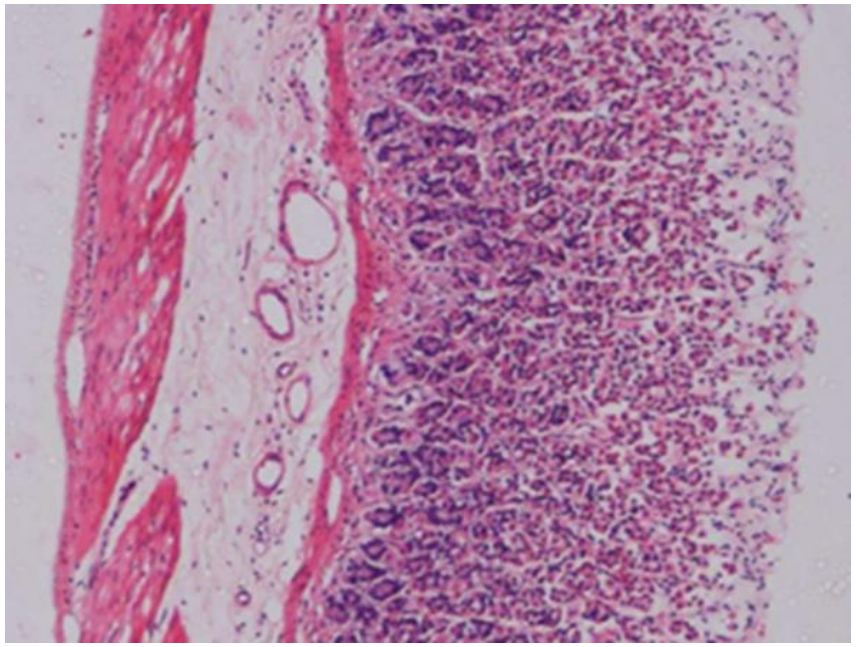


Fig7. Omeprazole group had slight disturbance to the outer layer of cells, as well as a slight swelling and the presence of WBCs

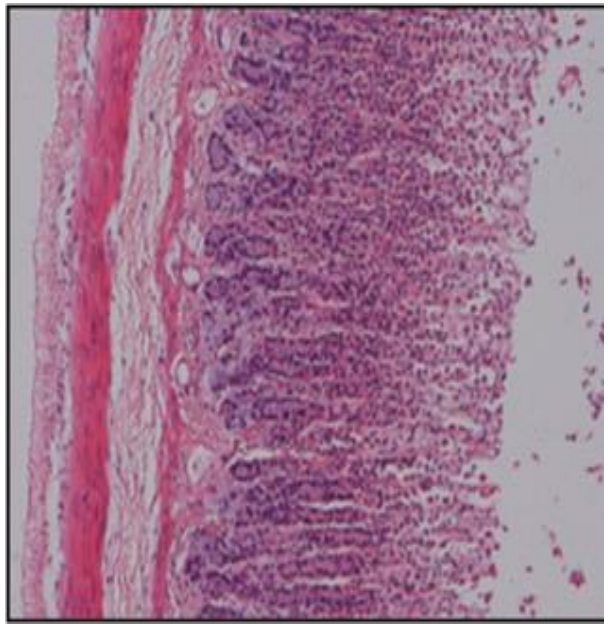
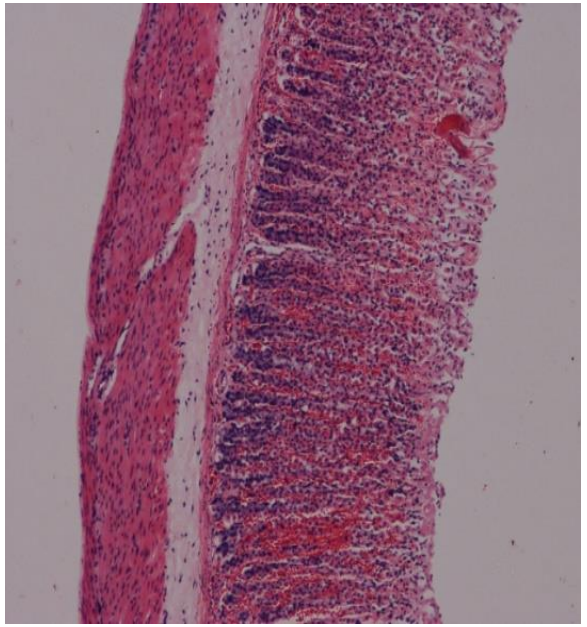
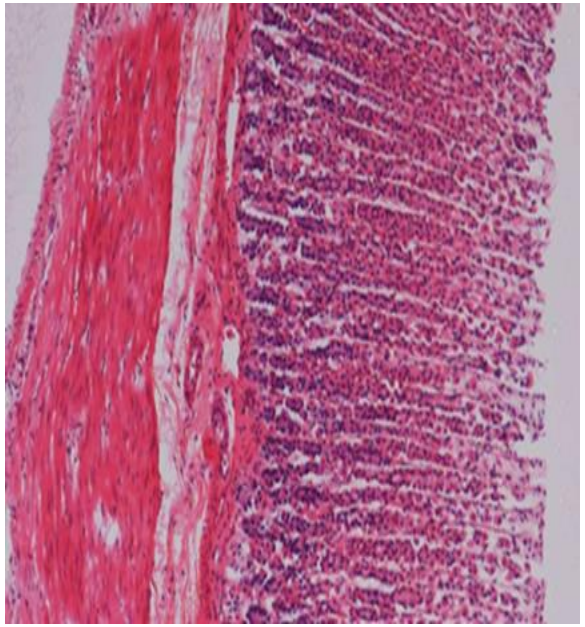
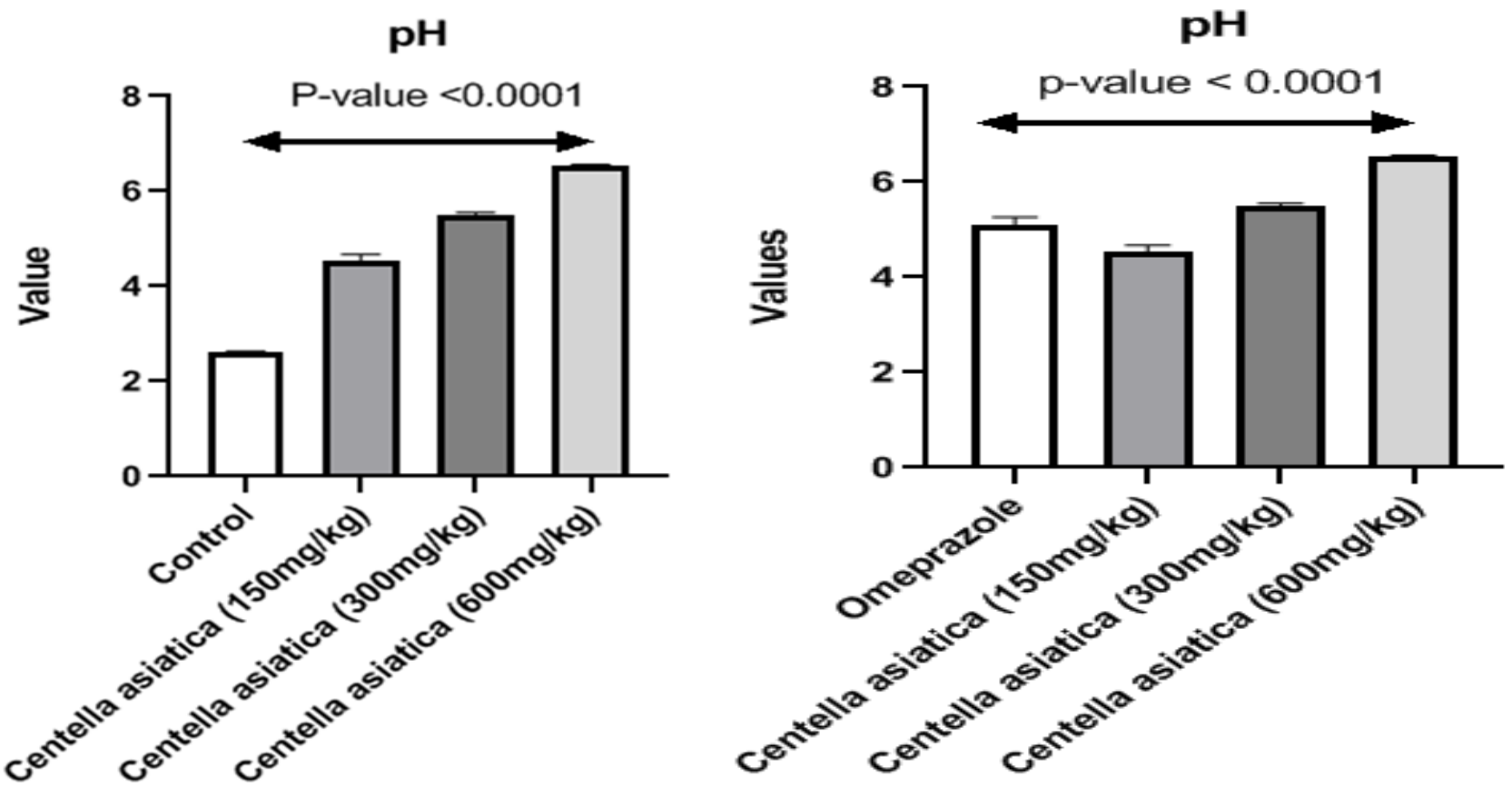
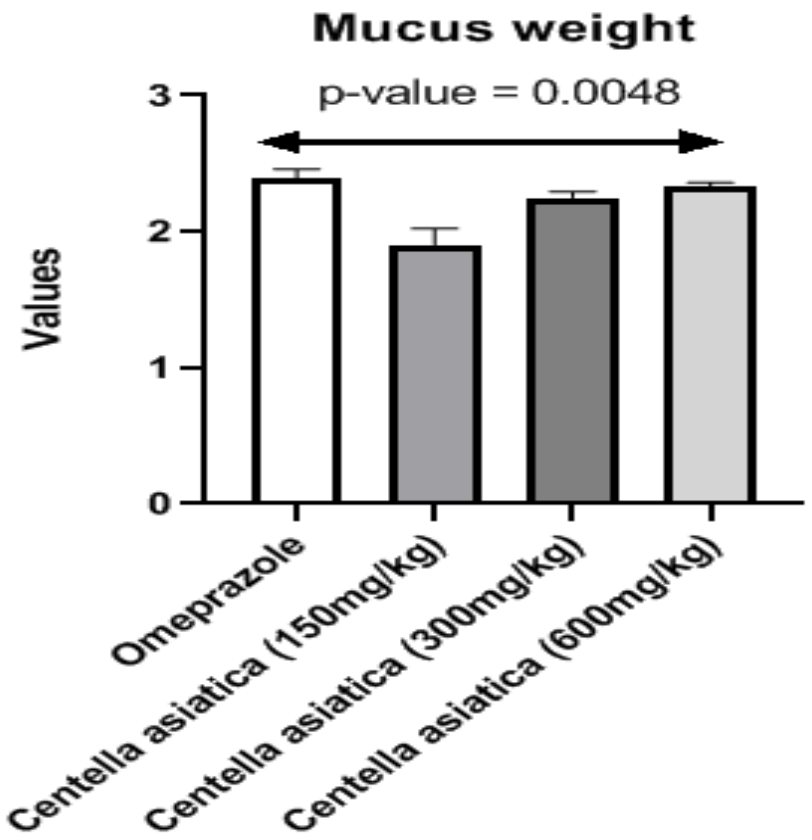
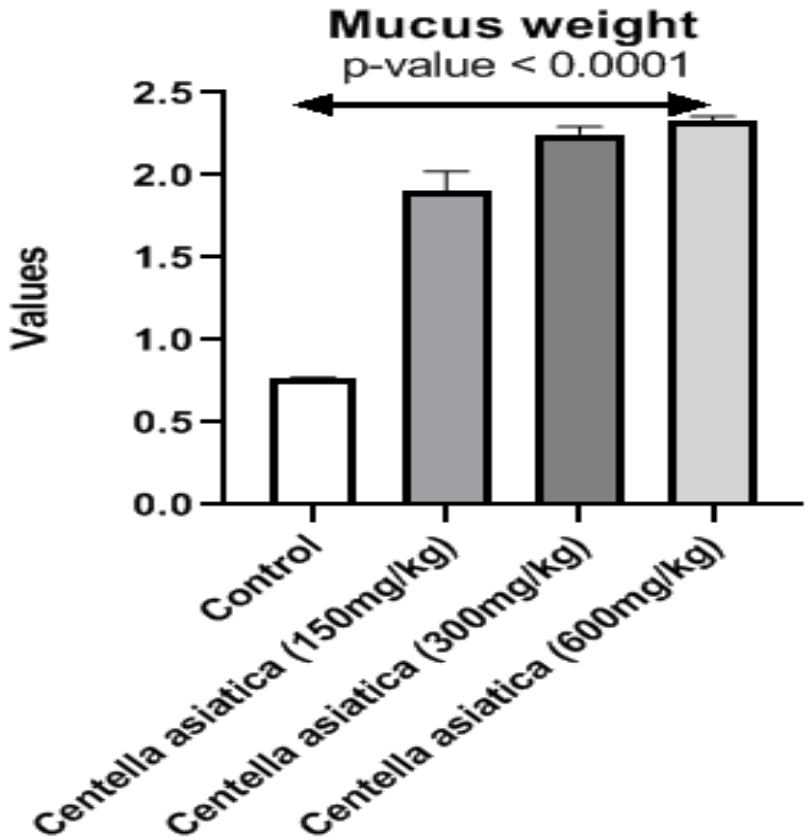
		

Fig8. (150 mg/kg) group shows mild damage to the outer layer of cells, along with slight swelling	**Fig9.** (300 mg/kg) extract group presented with a minimal and slight disturbance to the surface epithelium	**Fig10.** (600 mg/kg) extract group showed no damage to the surface epithelium ,no presence of swelling or WBC infiltration in the submucosal layer

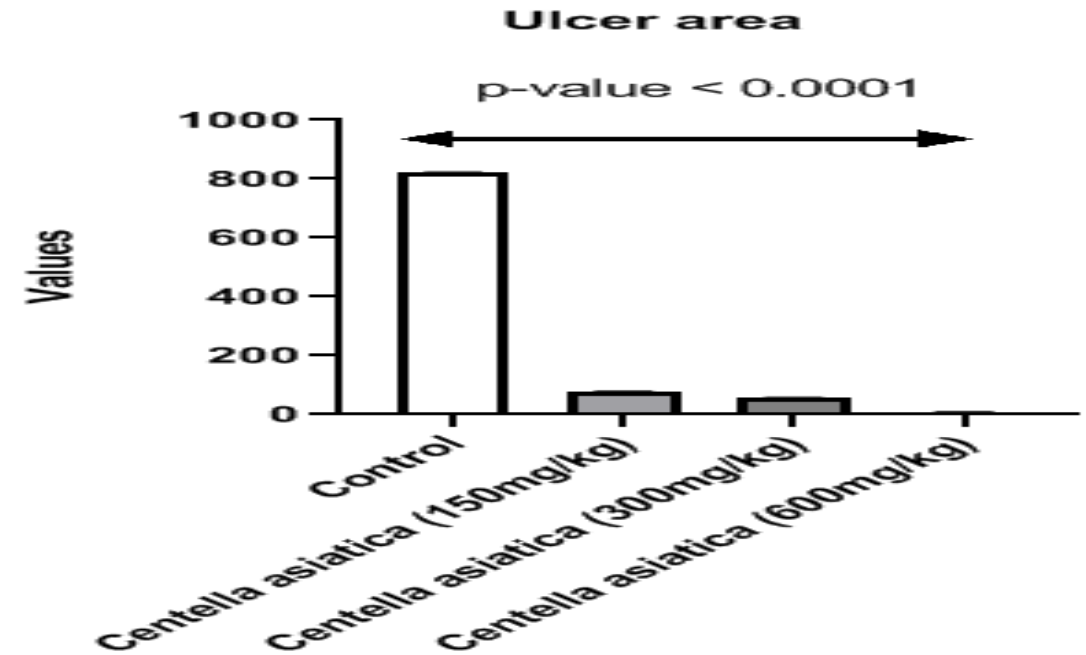


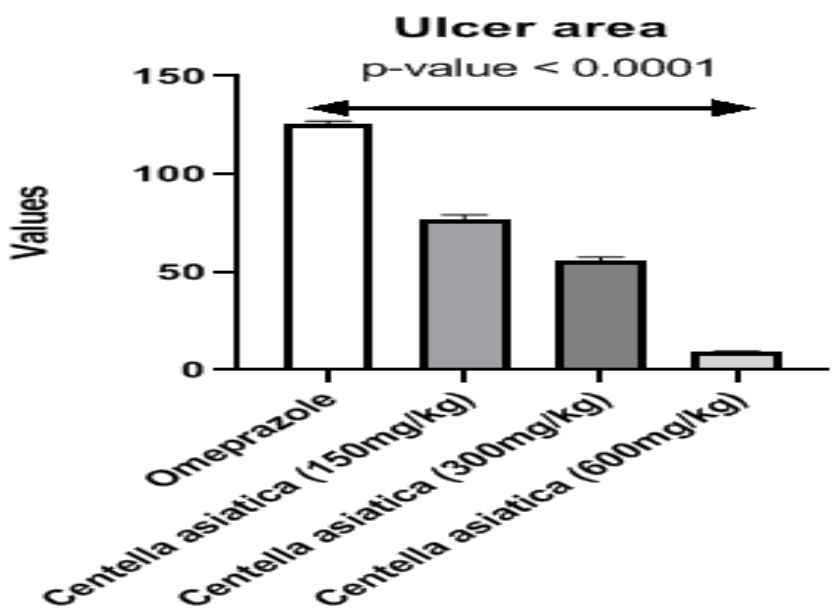
Figures11&12 . Show the comparison of pH value between control/ omeprazole group and rats pre-treated with three different doses of C. asiatica aqueous extract.

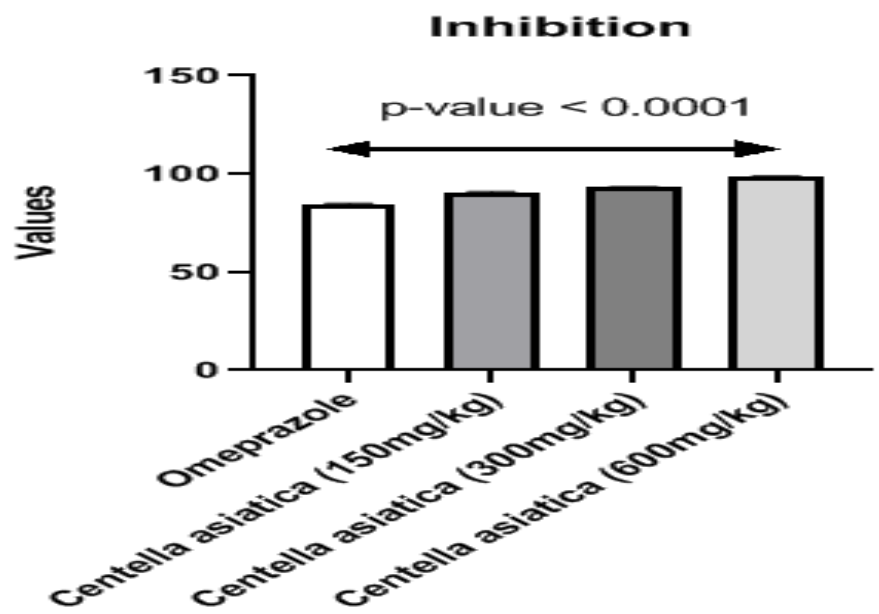




Figures 13& 14. Data presents of mucus weight of control/ omeprazole group and rats pre-treated with three different doses of C. asiatica aqueous extract.


Ulcer area
p-value < 0.0001
Values
1000
800
600
400
200
0
Control
Centella asiatica (150mg/kg)
Centella asiatica (300mg/kg)
Centella asiatica (600mg/kg)


Ulcer area
p-value < 0.0001
Values
150
100
50
0
Omeprazole
Centella asiatica (150mg/kg)
Centella asiatica (300mg/kg)
Centella asiatica (600mg/kg)


Inhibition
p-value < 0.0001
Values
150
100
50
0
Omeprazole
Centella asiatica (150mg/kg)
Centella asiatica (300mg/kg)
Centella asiatica (600mg/kg)

Conclusion

- Centella asiatica proved to hold an anti-ulcerogenic effect
- Centella asiatica appears to have gastroprotective effects based on increased mucus production at doses 150 mg/kg, 300 mg/kg, and 600 mg/Kg
- Histopathology demonstrated to be significant at doses adminstrated, mostly in 600 mg/Kg dose group

Recommendation

- **Optimal Dosage Determination:** Further investigation into the dosage of Centella asiatica aqueous extract is warranted to establish the most effective concentration for mitigating ethanol-induced stomach mucosal injury. This could involve exploring a broader range of doses or conducting dose-response studies to ascertain the optimal dosage that balances efficacy and safety.
- **Mechanistic Insights:** Future research should focus on elucidating the underlying mechanisms through which Centella asiatica exerts its protective effects against stomach mucosal injury induced by ethanol. Understanding the molecular pathways involved could provide valuable insights into the potential therapeutic targets and facilitate the development of novel treatments for gastric ulcers.

Recommendation

- **Comparative Studies:** Comparative studies with other natural compounds or conventional medications commonly used to treat gastric ulcers, such as proton pump inhibitors like omeprazole, would be valuable. This could help assess the relative efficacy and safety of Centella asiatica compared to existing treatments and provide clinicians with additional options for managing gastric ulcers.

- **Clinical Translation:** Translation of these findings into clinical trials involving human subjects is essential to validate the efficacy and safety of Centella asiatica aqueous extract in the management of gastric ulcers. Conducting well-designed clinical studies would provide robust evidence to support its use as a therapeutic agent and guide its integration into clinical practice.

References

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Thank You