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Original Research Article

Investigating the Protective Role of Luteolin against Aspirin-Induced Gastric Ulcer: Novel Pharmacological Approach

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Abstract:

Background: Gastric ulcer is a communal gastrointestinal disorder induced by an imbalance between protective as well as detrimental factors.

Methods: This experimental study used 30 Wister albino rats categorized into five groups (n=6/group): Group A:- Normal, given (1% CMC in water), Group B:- Control, given aspirin, 24 hrs before pylorus ligation, Group C:- Standard drug, given ranitidine, 30 mins before pylorus ligation, Group D:- luteolin low dose, given luteolin, 30 min before the pylorus ligation and Group E:- luteolin high dose, given luteolin, 30 min before the pylorus ligation. At the end of the study, rats underwent gastric acidity and gastric lesions measurement. Similarly, the stomach was subjected to histological evaluation.

Results: The study revealed that administering aspirin lead to the progression of gastric ulcers, as evidenced by a notable reduced in the Superoxide Dismutase (SOD), Glutathione (GSH), Catalase (CAT) and Lipid Peroxidation (LPO) in Pyloric Ligation Induced Ulcer. Additionally, it induced a substantial elevation in gastric acidity and decrease in PGE2 and SOD levels. ($p<0.05$) Luteolin supplementation reversed these changes induced by aspirin.

Conclusion: Luteolin has the potential to be a valuable antiulcer agent**,** where it could relieve the gastric ulcer caused using aspirin in rats via antiapoptotic, anti-inflammatory, antioxidant, and upregulated PI3K/Akt. Pathway mechanisms.

Keywords: Antioxidant, Anti-inflammatory, Anticancer, Anti-allergic, Neuroprotective, and Cardioprotective.

Introduction

Peptic ulcer disease (PUD) is a chronic condition that results from a precarious equilibrium between the protective mechanisms of the gastric mucosa and the aggressive factors that can cause its erosion.[1] Mucus and bicarbonate secretion, adequate blood flow, prostaglandin E2, nitric oxide, sulfhydryl compounds, and antioxidant enzymes are among the endogenous protective agents that are involved in this complex interplay.

Peptic ulcers is also significantly influenced by behavioral and environmental factors.[2] The use of non-steroidal anti-inflammatory medications (NSAIDs), excessive alcohol consumption, poor dietary practices, and smoking are all recognized risk factors for PUD. Furthermore, the development of gastric ulcers has been significantly associated with the colonization of the stomach mucosa by Helicobacter pylori (H. pylori).[3] Flavonoids are a diverse class of polyphenolic compounds widely found in fruits, vegetables, herbs, and other plant-derived foods. Renowned for their potent antioxidant, anti-inflammatory, and bioactive properties, these compounds have garnered significant attention for their role in preventing and managing various diseases.[4] By modulating critical biochemical pathways, protecting cells from oxidative damage, and promoting overall health, flavonoids offer promising therapeutic potential.[5] Here is an overview of their importance across different disease conditions.

Drug Profile

Luteolin is a flavonoid characterized by its chemical structure, which includes a flavone backbone featuring a 3',4',5'-trihydroxyphenyl group and a 4-keto group. It is widely found in various natural sources, particularly in fruits, vegetables, and herbs.

Luteolin exhibits gastro protective effects by enhancing the secretion of gastric mucus and bicarbonate, which are crucial for maintaining the mucosal barrier.[6]

Chemistry:

Molecular Formula – C15H10O6

Molecular Weight - 286.24g/mol

Melting Point – 329.5 °C

Solubility – Aq. Alkaline solution, Ethanol, NaOH, DMSO and Methanol.

Absorption – Luteolin is absorbed by intestinal mucosa.

Distribution – After oral administration, Luteolin and its metabolites are mainly distributed in Liver, Kidney, Lung and GI tract.

Half-life – The half-life of luteolin is 8.94 hrs for free luteolin and 4.98 hrs for conjugated luteolin.

Plasma Concentrations – Luteolin plasma concentration decreases rapidly at first, then more slowly after 90 minutes.[7]

Methodology:

Collection and authentication of drug: The sample is collected from Sundeep Scientific Instrument and chemicals, Chandigarh.

Animal ethical approval: Approved by the Institutional animal ethical committee (IAEC) Venus Medicine Research Centre, Baddi Himachal Pradesh.

Total 30 Wistar rats having 6-8 weeks of age and weight lies between 200-240 Gms. (Divided into 5 groups)

Table 1: Represents the animal grouping

S.no	Group	Treatment
1	Normal	1% w/v CMC
2	Control	Aspirin 400mg/kg p.o. followed by pylorus ligation after 24 hours
3	Standard	Ranitidine 50mg/kg p.o. 30 min before the pylorus ligation
4	luteolin (low dose test)	50mg/kg p.o. 30 min before the pylorus ligation
5	luteolin (high dose test)	100mg/kg p.o. 30 min before the pylorus ligation

Anti-Ulcer activity

Preparation of Reagents

- Preparation of 0.01N NaOH 40 g of NaOH was dissolved in 1 liter of distilled water.
- Preparation of phenolphthalein
- Phenolphthalein 1g was dissolved in 100 ml of 95% ethyl alcohol
- Preparation of Methyl orange indicator
- Methyl orange 1g was dissolved in 1 liter of distilled water.

Procedure: Pipetted 1ml of filtered gastric contents into a small beaker, then 2-3 drops of methyl orange were added and titrated with 0.01 N NaOH until all trace of the red color disappeared and yellowish orange color appeared. The volume of alkali added was noted and this indicated the free acidity. Then 2-3 drops of phenolphthalein were added and titration was continued until definite red tinge reappeared.

Percentage protection of ulcer - The percentage of ulcer protection was determined from mean of ulcer index. The formula Percentage protection is as follows: - % Protection = Disease Control mean ulcer index- Treatment group mean ulcer index / Disease Control mean ulcer index * 100.

Determination of gastric content - After sacrifice, the abdomen was opened and a small cut to the pyloric region of the stomach was given just above the knot & the contents of the stomach were collected in a graduated centrifuge tube and measured.

Determination of free and total acidity - The supernatant of the gastric contents was subjected to analysis for free and total acidity.139 Free acidity and total acidity were determined using 0.01N NaOH using phenolphthalein and methyl orange as an indicator.

Biochemical Estimation: Preparation of Tissue Homogenate – The tissue homogenate was prepared from the dissected part of the stomach. The clear supernatant was recovered and used for various biochemical estimations.

Estimation of Protein by Biuret Method - . In the presence of alkaline media cupric ion (Cu2+) in the biuret reagent forms complex with the groups involved in the peptide bond to form a violet colored chelate.

Estimation of Lipid Peroxidation (LPO) - Lipid peroxidation (MDA) assay provides a convenient tool for sensitive detection of the malondialdehyde (MDA) present in tissue samples. The MDA in the sample reacts with thiobarbituric acid (TBA) to generate an MDA-TBA adduct.

Estimation of Reduced Glutathione (GSH) - Glutathione reductase reduces oxidized glutathione (GSSG) to reduced glutathione (GSH). The sulfhydryl group of GSH reacts with DTNB (5, 5'-dithiobis-2-nitrobenzoic acid, Ellman's reagent) to produce a yellow colored 5-thio-2- nitrobenzoic acid (TNB) that absorbsat 405 or 414 nm.

Statistical Analysis: All the results were expressed as mean ± standard error mean (SEM). The data of experimental results and biochemical estimations was statistically analyzed by one-way ANOVA followed by Tukey's multiple range tests using Graph pad prism version 6.0 software. The p-value < 0.05 was considered to be statistically significant.

Result and discussion: Effect of Luteolin on Gastric Content in Pyloric Ligation Induced Ulcer

After the pylorus ligation, gastric content was significantly increased (*p<0.001) in control group as compared to normal control.

After administration of luteolin at the dose of 50 mg/kg and 100 mg/kg, gastric content was significantly (@p<0.001) reduced in a dose dependent manner as compared to control.

The administration of ranitidine (50 mg/kg), also significantly (#p<0.001) decreased the gastric content with respect to control.

Table 2: Effect of Luteolin on Gastric Content in Pyloric Ligation Induced Ulcer

S. No.	Experimental Groups	Gastric Content (ml)
1	Normal	1.23±0.22
2	Control	3.98±0.24
3	Standard	1.74±0.18
4	luteolin low dose test	2.62±0.17
5	luteolin high dose test	2.18±0.21

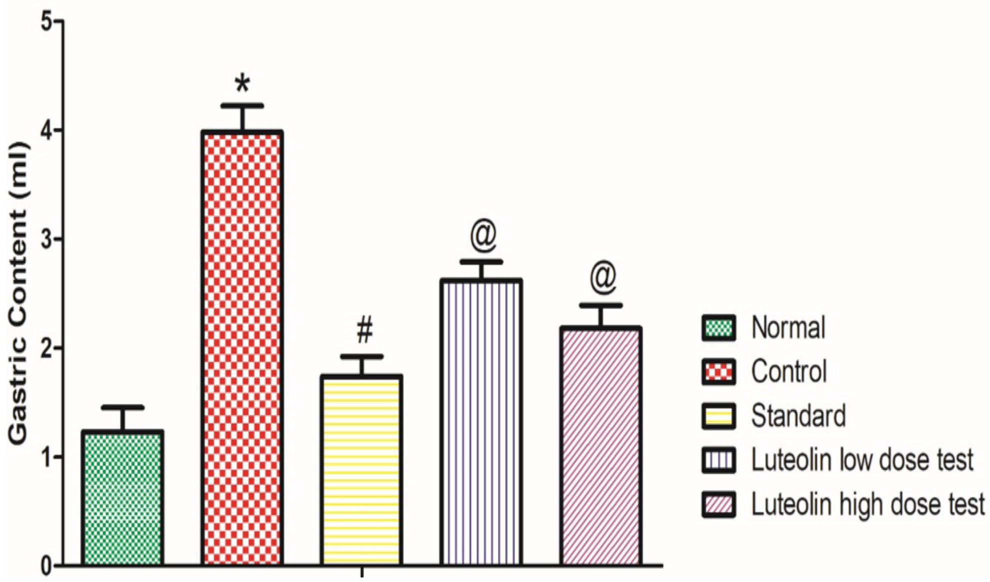

Figure 1: Effect of Luteolin on Gastric Content in Pyloric Ligation Induced Ulcer

Effect of Luteolin on pH in Pyloric Ligation Induced Ulcer: After the pylorus ligation, pH was significantly decreased (*p<0.001) in control group as compared to Normal which indicated perforation in gastric lining causes acidity. After administration of luteolin at the dose of 50 mg/kg and 100 mg/kg, pH was significantly (@p<0.001) increased (near to normal pH) in a dose dependent manner as compared to control. The administration of ranitidine (50 mg/kg), also significantly (#p<0.001) improves the pH with respect to control.

Table 2: Effect of Luteolin on pH in Pyloric Ligation Induced Ulcer

S. no.	Experimental Groups	Ph
1	Normal	6.53±0.32
2	Control	2.24±0.24
3	Standard	5.47±0.26
4	luteolin low dose test	3.82±0.29
5	luteolin high dose test	4.96±0.30

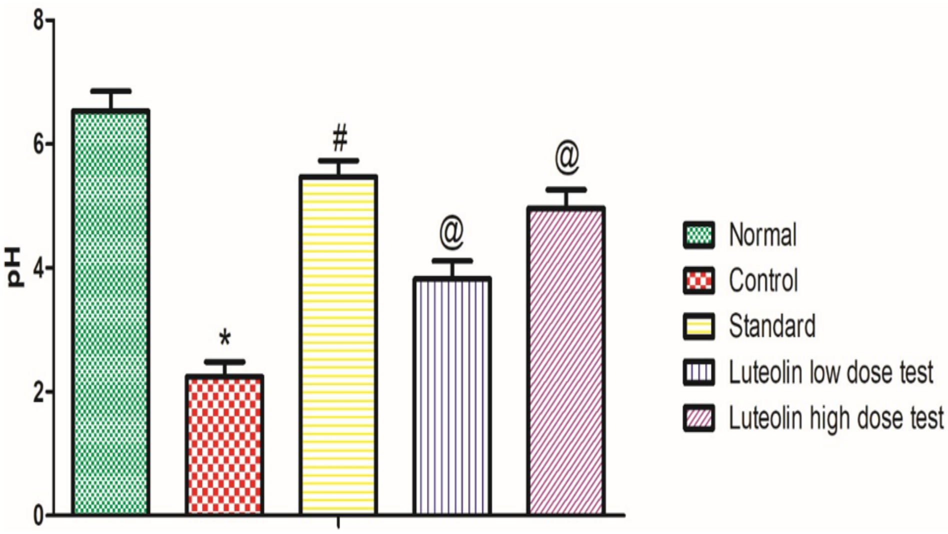

Figure 2: Effect of Luteolin on pH in Pyloric Ligation Induced Ulcer

Effect of Luteolin on Free Acid in Pyloric Ligation Induced Ulcer: After the pylorus ligation, free acid content was significantly increased (*p<0.001) in control group as compared to normal group. After administration of luteolin at the dose of 50 mg/kg and 100 mg/kg, free acid content was significantly (@p<0.01) reduced in a dose dependent manner as compared to control.

The administration of ranitidine (50 mg/kg), also significantly (#p<0.001) decreased the free acid content with respect to control.

Table 3: Effect of Luteolin on Free acidity in Pyloric Ligation Induced Ulcer

S. no.	Experimental Groups	Free acidity(mEq/l)
1	Normal	9.86±0.86
2	Control	30.8±2.74
3	Standard	11.2±1.2
4	luteolin low dose test	26.41±2.3
5	luteolin high dose test	17.37±1.6

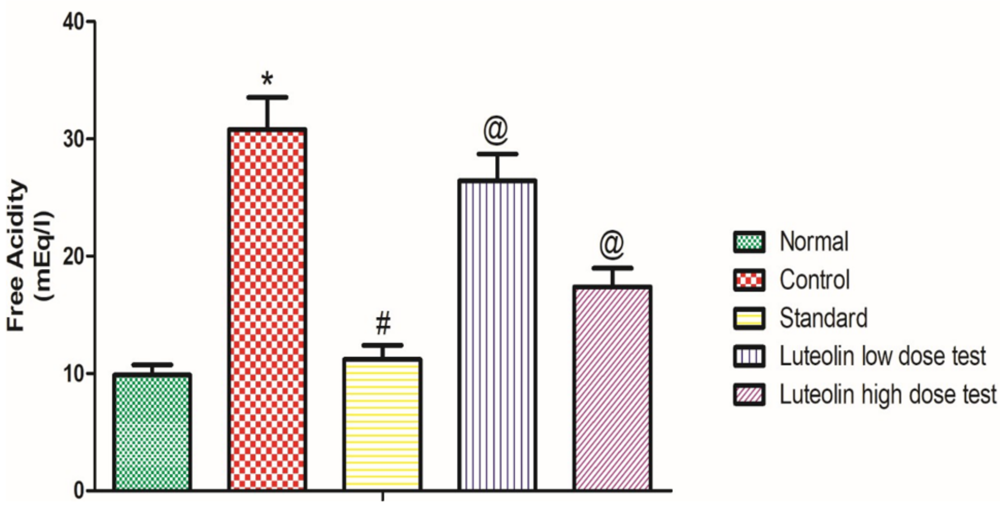

Figure 3: Effect of Luteolin on Free Acid Level in Pyloric Ligation Induced Ulcer

Effect of Luteolin on Ulcer Index in Pyloric Ligation Induced Ulcer: After the pylorus ligation, ulcer index was significantly increased (*p<0.001) in control group as compared to normal group. After administration of luteolin at the dose of 50 mg/kg and 100 mg/kg, ulcer index was significantly (@p<0.01) reduced in a dose dependent manner as compared to control. The administration of ranitidine (50 mg/kg), also significantly (#p<0.001) decreased the ulcer index with respect to control.

Table 4: Effect of Luteolin on Ulcer Index in Pyloric Ligation Induced Ulcer

S. no.	Experimental Groups	Ulcer Index
1	Normal	00±00
2	Control	6.92±0.68
3	Standard	1.96±0.42
4	luteolin low dose test	5.89±0.38
5	luteolin high dose test	3.63±0.24

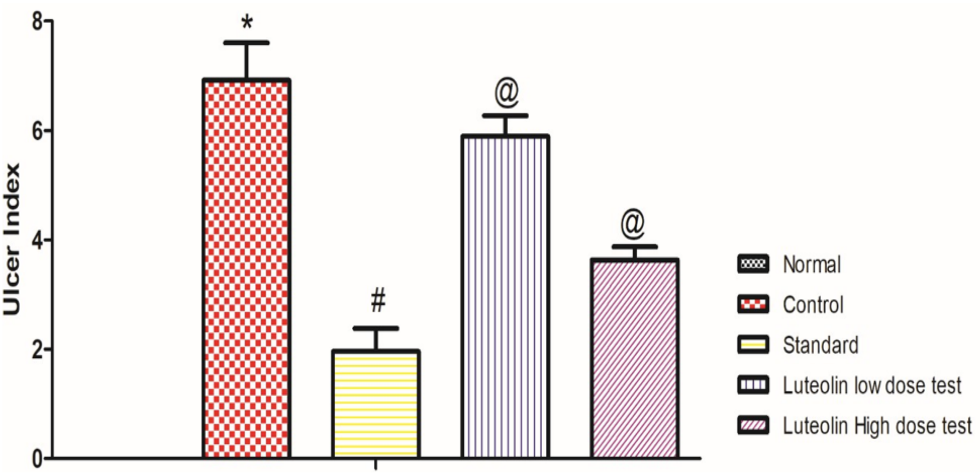

Figure 4: Effect of Luteolin on Ulcer Index in Pyloric Ligation Induced Ulcer

Biochemical Estimations:

Effect of Luteolin on Lipid Peroxidation (LPO) in Pyloric Ligation Induced Ulcer: After the pylorus ligation, LPO level was significantly increased (*p<0.001) in control group as compared to normal group. After administration of luteolin at the dose of 50 mg/kg and 100 mg/kg, LPO level was significantly (@p<0.01) reduced in a dose dependent manner as compared to control.

The administration of ranitidine (50 mg/kg), also significantly (#p<0.001) decreased the LPO level with respect to control.

Table 6: Effect of Luteolin on LPO in Pyloric Ligation Induced Ulcer

S. no.	Experimental Groups	LPO (nmol/mgprotein)
1	Normal	22.43±1.86
2	Control	47.38±3.43
3	Standard	27.52±1.94
4	luteolin low dose test	41.32±3.53
5	luteolin high dose test	33.74±2.73

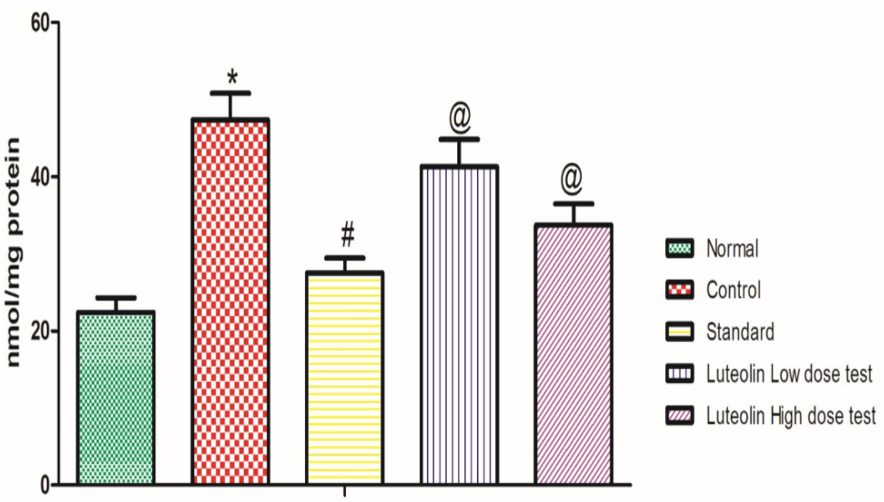

Figure 5: Effect of Luteolin on LPO in Pyloric Ligation Induced Ulcer

Effect of Luteolin on Reduced Glutathione (GSH) in Pyloric Ligation Induced Ulcer: After the pylorus ligation, GSH level was significantly reduced (*p<0.001) in control group as compared to normal group. After administration of luteolin at the dose of 50 mg/kg and 100 mg/kg, GSH level was significantly (@p<0.01) increased in a dose dependent manner as compared to control.

The administration of ranitidine (50 mg/kg), also significantly (#p<0.001) increased the GSH level with respect to control.

Table 6: Effect of Luteolin on GSH in Pyloric Ligation Induced Ulcer

S. no.	Experimental Groups	GSH (µmol/mg protein)
1	Normal	1.93±0.12
2	Control	0.72±0.10
3	Standard	1.78±0.21
4	luteolin low dose test	1.15±0.18
5	luteolin high dose test	1.37±0.26

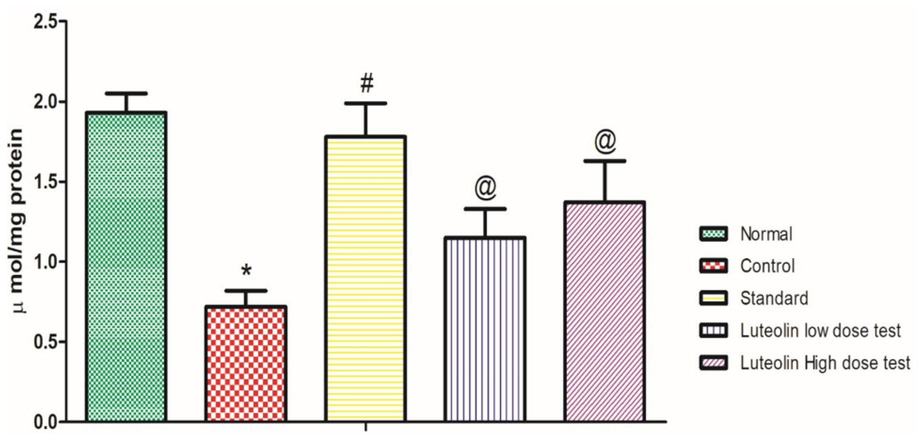

Figure 6: Effect of Luteolin on GSH in Pyloric Ligation Induced Ulcer

Effect of Luteolin on Superoxide Dismutase (SOD) in Pyloric Ligation Induced Ulcer: After the pylorus ligation, SOD level was significantly reduced (*p<0.001) in control group as compared to normal group. After administration of luteolin at the dose of 50 mg/kg and 100 mg/kg, SOD level was significantly (@p<0.05) increased in a dose dependent manner as compared to control.

The administration of ranitidine (50 mg/kg), also significantly (#p<0.001) increased the SOD level with respect to control.

Table 7: Effect of Luteolin on SOD in Pyloric Ligation Induced Ulcer

S. no.	Experimental Groups	SOD (U/mg protein)
1	Normal	4.45±0.19
2	Control	2.15±0.17
3	Standard	4.18±0.38
4	luteolin low dose test	2.58±0.18
5	luteolin high dose test	3.43±0.21

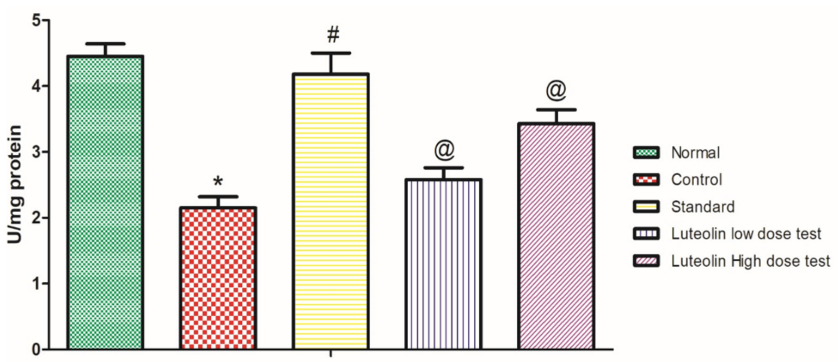

Figure 7: Effect of Luteolin on SOD in Pyloric Ligation Induced Ulcer

Effect of Luteolin on Catalase (CAT) in Pyloric Ligation Induced Ulcer: After the pylorus ligation, CAT level was significantly reduced (*p<0.001) in control group as compared to normal group. After administration of luteolin at the dose of 50 mg/kg and 100 mg/kg, CAT level was significantly (@p<0.01) increased in a dose dependent manner as compared to control.

The administration of ranitidine (50 mg/kg), also significantly (#p<0.001) increased the CAT level with respect to control.

Table 11: Effect of Luteolin on CAT in Pyloric Ligation Induced Ulcer

S. no.	Experimental Groups	CAT (U/mg protein)
1	Normal	35.79±2.7
2	Control	12.34±1.78
3	Standard	32.49±2.35
4	luteolin low dose test	16.74±1.29
5	luteolin high dose test	25.83±1.96

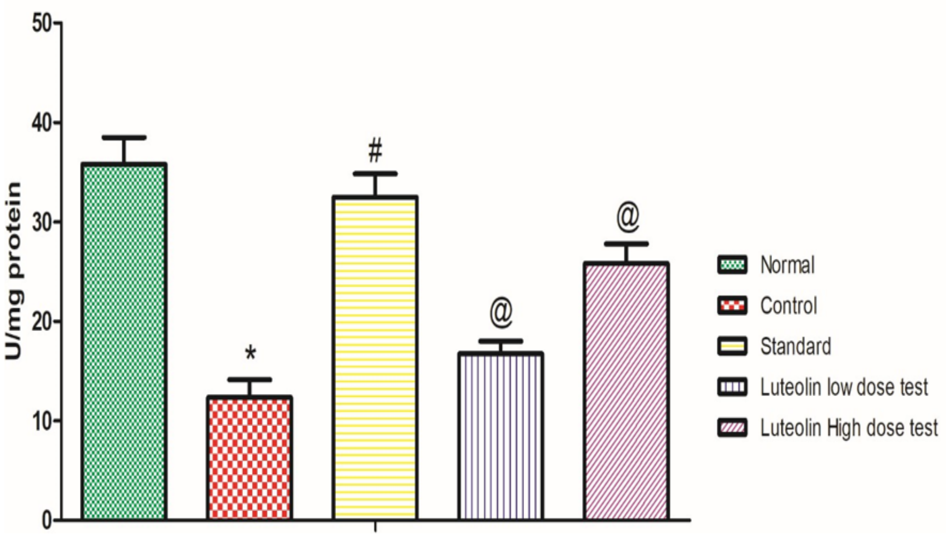

Figure 8: Effect of Luteolin on CAT in Pyloric Ligation Induced Ulcer

Conclusion

The present thesis entitled as Investigating the Protective Potential of Luteolin against Aspirin-Induced Gastric Ulcers: This study seeks to evaluate the protective efficacy of luteolin in experimental models of aspirin- induced gastric ulcers.

By investigating its antioxidant, anti-inflammatory, and mucosal protective effects, this research could establish luteolin as a promising natural therapeutic agent for the prevention and management of NSAID-induced gastric ulcers.

As an adjunct therapy, luteolin may offer a safer option for patients requiring prolonged aspirin use, effectively reducing gastric side effects while preserving the drug's therapeutic efficacy.

In conclusion, this research holds the potential to pave the way for natural, safe, and well-tolerated interventions for gastric ulcers, offering substantial clinical benefits, particularly for individuals reliant on aspirin for cardiovascular health but susceptible to gastric mucosal injury.

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