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Original Article

Protective Effects of Hibiscetin on Ethanol-Induced Ulcers in Rats through Inhibition of Prostaglandin Synthesis/ Oxidative Stress/Caspase-3 and 9 Pathways

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ABSTRACT

Aim/Background: Ethanol (EtOH)-induced gastric ulcers are a prevalent gastrointestinal disorder characterized by severe inflammation, oxidative stress, and apoptotic cell death. This study aimed to investigate the potential protective effects of hibiscetin against EtOH-induced ulcers in rats. **Materials and Methods:** Rats were randomly divided into five groups: Normal control (normal saline), EtOH (1.5 mL), hibiscetin 10 mg/kg + EtOH, hibiscetin 20 mg/kg + EtOH, and hibiscetin 20 mg/kg alone (control). Ulcer index, pH, gastric juice, pro-inflammatory cytokines i.e., Interleukin-6 (IL-6), IL-1β, Tumor Necrosis Factor-alpha (TNF-α), and Prostaglandin E-2 (PGE2), oxidative stress-Malondialdehyde (MDA), antioxidant enzymes-Catalase (CAT), Superoxide Dismutase (SOD), reduced Glutathione (GSH), oxidative enzyme-Myeloperoxidase (MPO), apoptosis markers-caspase-3 and caspase-9 were estimated. **Results:** Both doses of hibiscetin administration significantly ameliorated EtOH-induced ulceration, as evidenced by reduced ulcer areas and preserved mucosal architecture. Furthermore, hibiscetin treatment downregulated IL-6, IL-1β, TNF-α, and PGE2 synthesis, which correlated with attenuated inflammation. Hibiscetin also exhibited robust antioxidant potential, as indicated by decreased MDA levels and increased CAT, SOD, and GSH activities. Moreover, hibiscetin suppressed MPO activity, indicating reduced neutrophil infiltration. Caspase-3 and caspase-9 activation demonstrated apoptotic cell death in the gastric mucosa which was restored by hibiscetin. **Conclusion:** The findings suggested that hibiscetin has gastroprotective activity against EtOH-induced ulcers and this might be related to its positive influence on oxidative stress, inflammatory response, and apoptotic pathways. Further research is warranted to explore its full clinical potential and safety profile.

Keywords: Antioxidant enzymes, Ethanol, Hibiscetin, Ulcer, Pro-inflammatory cytokines.

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INTRODUCTION

Gastric ulcers are a prevalent gastrointestinal disorder characterized by the erosion of the gastric mucosal lining, leading to severe pain and discomfort. Ethanol (EtOH) consumption is known to be a significant risk factor for the development of gastric ulcers due to its ability to disrupt the delicate balance between protective and damaging factors in the gastric mucosa. The pathogenesis of EtOH-induced ulcers involves complex mechanisms, including excessive inflammation, oxidative stress, and apoptotic cell death. Consequently, there is a growing interest in identifying natural compounds with potent gastroprotective properties that can combat these underlying mechanisms and prevent ulcer formation.[1-3] As a result of the high rate of illnesses, deaths, and economic damages associated with ulcers as well as the prevalence of *Helicobacter pylori* infection, ulcers have become a global health concern, making them a typical gastrointestinal disorder that affects 15-20% of the world's population.[4-6] Despite their promise, antiulcer treatments can result in a variety of side effects, such as hypersensitivity, arrhythmia, impotence, gynecomastia, galactorrhea, hematological abnormalities, and kidney damage. It is difficult for many patients to deal with these adverse reactions. There is a lot of interest in finding new and efficient alternatives at the moment. Many long-term drug users turn to herbal medications because they are effective, inexpensive, and widely available. Increasing evidence indicates that natural remedies and plant extracts can treat peptic ulcers.[7-9]

Excessive alcohol use can harm the stomach by upsetting the balance between defensive and offensive components. Models based on EtOH-ingested ulcers have shown to be an efficient method of describing the underlying causes of gastroprotective disease and assessing their effectiveness.[10-13] High quantities of Reactive Oxygen Species (ROS) are present in EtOH-induced ulcers, which can impede the protective actions of Glutathione

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(GSH), Superoxide Dismutase (SOD), and Catalase (CAT). Since it stimulates the production of proinflammatory cytokines including Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1β (IL-1β), and Interleukin-6 (IL-6), all of which exacerbate inflammation, the generation of ROS is also recognized as a major offensive factor in inflammation.[14-16] Flavonoids are reported to be effective in controlling gastric ulcers. In addition to preventing acid secretion, flavonoids inhibit the activity of pepsin and increase the secretion of mucus and bicarbonate in the stomach. Furthermore, flavonoids act as mucosal cytoprotectants, antioxidants, anti-inflammatory agents, and antibacterial agents.[17-20] In recent years, there has been growing interest in exploring natural compounds with potential gastroprotective properties as alternative or adjunctive treatments for gastric ulcers. Hibiscetin, a flavonoid compound found in the *Hibiscus* plant, has emerged as a promising candidate due to its diverse pharmacological properties, including anti-inflammatory, antioxidant, and cytoprotective effects. Several studies have reported the beneficial effects of hibiscetin in various disease models in rats.[21-25] The proposed research will focus on evaluating the effects of hibiscetin treatment on the integrity of the gastric acid, Prostaglandin E-2 (PGE2) synthesis, pro-inflammatory cytokines, oxidative stress, and apoptotic pathways.

MATERIALS AND METHODS

Chemicals

Chemicals such as EtOH (Sigma-Aldrich, USA) were used in the current protocol. Interlukins-6 (IL-6), IL-1β, TNF-α, caspase-3, and caspase-9 were performed by using an enzyme-linked immunosorbent assay kit (ELISA, MSW Pharma, India). The rest of the materials used in the study were of analytical standards.

Animals

Male Wistar rats (180 ±20g, 10 weeks old) were maintained in standard conditions with 12/12 hr light/dark cycle components, at 30°C, 40–50%. Rodents were given regular pallets of food and water ad libitum. Rodents were acclimated for 7 days in accordance with standard laboratory practices. The research was permitted by the Institutional Animal Care and Use Committee (IAEC-LNCT/IAEC/2023/005), India, and was conducted as per the ARRIVE guidelines.[26] The study used animals that had never been treated before as per the guidelines.

Induction ulcers by EtOH

For the recent study 30 healthy adult male Wistar rats were randomly split up into clusters as follows (n=6).

Cluster I- Normal control (normal saline).

Cluster II- EtOH control (1.5 mL).

Cluster III- EtOH +10 mg/kg hibiscetin.

Cluster IV- EtOH +20 mg/kg hibiscetin.

Cluster V-20 mg/kg hibiscetin per se.

The animals were fasted for 24 hr prior to receiving EtOH. Except for Group I, all of the animals received EtOH by oral administration at a dose of 1.5 mL/animal. After 7 days, the animals received hibiscetin by oral administration in accordance with their respective groups.

Ulcer index

On day 8 animals were subjected to an ulcer study with the help of the following formula[11-13]

$$UI = 10/X.$$

Where,

UI= Ulcer index.

X= Total area under ulceration.

The following scale was used to evaluate the ulcer score.

3= Perforated ulcer, 2= Deep ulcer, 1= Superficial ulcer, 0= No ulcer.

Estimation of pH

A digital pH meter was used to test the pH after the gastric contents of several animals were collected, and centrifuged, and the gastric fluids were extracted as a supernatant. This supernatant was then subjected to examination in accordance with normal methods.[14-16]

Gastric volume

The fluid content from gastric mucosa was collected and centrifuged in a centrifuge at 1000 rpm for 15 min. Gastric volume was determined by measuring the separated supernatant.

Pepsin activity

Pepsin activity was determined by a stop-point bioassay using previously reported methods. To prepare a stock solution of the substrate, 10 mg of p-nitrophenyl sulfite is dissolved in 2 mL of acetonitrile. Ice was used to keep this solution stable for 3 hr. As a substrate concentration, 100 mL of the stock substrate solution was pipetted into 10 mL of 0.01 M glycine hydrochloride buffer at 25°C. The maximum rate of enzyme hydrolysis was achieved between pH values 1.8 and 2.0. Therefore, pH 1.9 was chosen. The substrate may be assayed if it is slightly turbid, indicating that it has not yet been hydrolyzed. It takes 8 min to completely hydrolyze this working substrate solution to complete the following operations as quickly as possible. A reference cuvette was filled with the working substrate solution and 3 mL of the same solution was added quickly to the sample cuvette. It is recommended that 1 to 10 IL of pepsin (5 to 100 ILl of the gastric specimen, depending upon the concentration of pepsin) be added to the sample cuvette before placing both cuvettes in

a UV spectrophotometer. An enzyme hydrolyzes the substrate, releasing p-nitrophenol, which can be measured by changing the absorbance at 320 nm during the reaction.[27,28]

Biochemical parameters

The biochemical parameters of all animals were evaluated based on previous studies. Based on recent research, stomach tissues were stored at -80°C for all experimental groups. Approximately 50 mg of powdered tissue was homogenized in phosphate buffer solution and centrifuged after preparation. Using a microplate reader and commercially available ELISA kits, stomach homogenates were subjected to various biochemical analyses after homogenization. Several analyses were performed, including estimations of GSH, MDA, CAT, and SOD.[18-20]

Estimation of MPO activity

We treated the gastric homogenate content in PBS (50 mM) with pH 6.0 and added hexadecyl trimethyl ammonium bromide (0.5%) for further analysis. In addition to freezing the mixture, a sonicator was used to sonicate it. After centrifuging the suspension solution for 15 min at 4°C at 15000 revolutions per minute, the final solution was filtered. To measure MPO activity, o-dianisidine dihydrochloride and 0.005% hydrogen peroxide were added to the supernatant.[29-31]

Determination of NO

Nitric Oxide (NO) was measured using nitrite as the result of oxidizing Nitric oxide. The assay follows previous studies on diazotization reactions, which measured the nitrite formed as a result of oxidizing Nitric oxide.[32]

Estimation of inflammatory markers

The estimation of PGE_2, IL-6, IL-1β, and TNF-α was performed by using ELISA kits as per the standard protocol.

Determination of Caspases 3 and 9 level

An ELISA kit was used to confirm Caspases 3 and 9 activities. The estimated approach is fully explained in the manuals provided with commercially available kits. The marker concentrations were determined using standard curves and then expressed in pg/mL.

Statistical analysis

A licensed version of Graph Pad Prism 8.0 was used to assess the data obtained from the present study. Results were expressed as mean ± SEM. Statistical significance levels among all variables were calculated by using a one-way Analysis of Variance (ANOVA), followed by a *post hoc* test Tukey's test, and except ulcer index by using Kruskal-Walli's test.

RESULTS

Hibiscetin effect on ulcer index

As depicted in Figure 1A ulcer index was high ($p<0.001$) in EtOH-induced rats in context to normal control rats. The data was analyzed by using a one-way ANOVA followed by the Kruskal-Walli's test revealed that the hibiscetin at both doses was able to lower the ulcer index substantially [$p<0.001$]. The hibiscetin per se group has no significant effect.

Hibiscetin effect on pH

The pH in EtOH-induced rats was found to be highly acidic ($p<0.01$) in contrast to normal control. The outcomes of the statistical analysis, employing one-way ANOVA followed by Tukey's post-hoc test showed that the flavonoid hibiscetin has significantly restored the pH to alkaline [$F(4, 25) = 10.62$; $p<0.001$]. The hibiscetin per se group did not exhibit any effect (Figure 1B).

Hibiscetin effect on gastric volume

Figure 1C indicates that EtOH-induced rats exhibited a high volume of gastric acid ($p<0.01$) in the context of normal control. The data was analyzed by using a one-way ANOVA followed by Tukey's *post hoc* test revealed that the gastric volume was dropped down to a significant level [$F(4, 25) = 43.50$; $p<0.0001$] by hibiscetin treatment. The effect on hibiscetin per se was non-significant.

Hibiscetin effect on pepsin activity

As depicted in Figure 1D, it was observed that the pepsin activity was remarkably increased in the EtOH-induced group ($p<0.001$) in association with the normal control group. The outcomes of statistical analysis, employing one-way ANOVA followed by Tukey's *post hoc* test exhibited that hibiscetin proved to be effective in bringing the pepsin activity to normal substantially [$F(4, 25) = 12.44$; $p<0.0001$]. The hibiscetin per group did not have any significant effect.

Estimation of GSH, MDA, CAT, and SOD levels

From Figure 2A-D it can be estimated that the EtOH-induced rats exhibited substantially ($p<0.001$) elevated amounts of MDA and declined levels of GSH, CAT, as well as SOD ($p<0.01$). The outcomes of statistical analysis, employing one-way ANOVA followed by Tukey's *post hoc* test. It was also depicted that the level of MDA was substantially declined [$F(4, 25) = 93.56$; $p<0.0001$] by hibiscetin as well as brought up the concentrations of GSH [$F(4, 25) = 49.18$; $p<0.0001$], CAT [$F(4, 25) = 52.22$; $p<0.0001$], and SOD [$F(4, 25) = 22.43$; $p<0.0001$]. The per se group has no effect.

MPO activity and NO levels

In the current observation and as per Figure 3A it was found that MPO activity was at higher levels ($p<0.01$) in the EtOH-induced

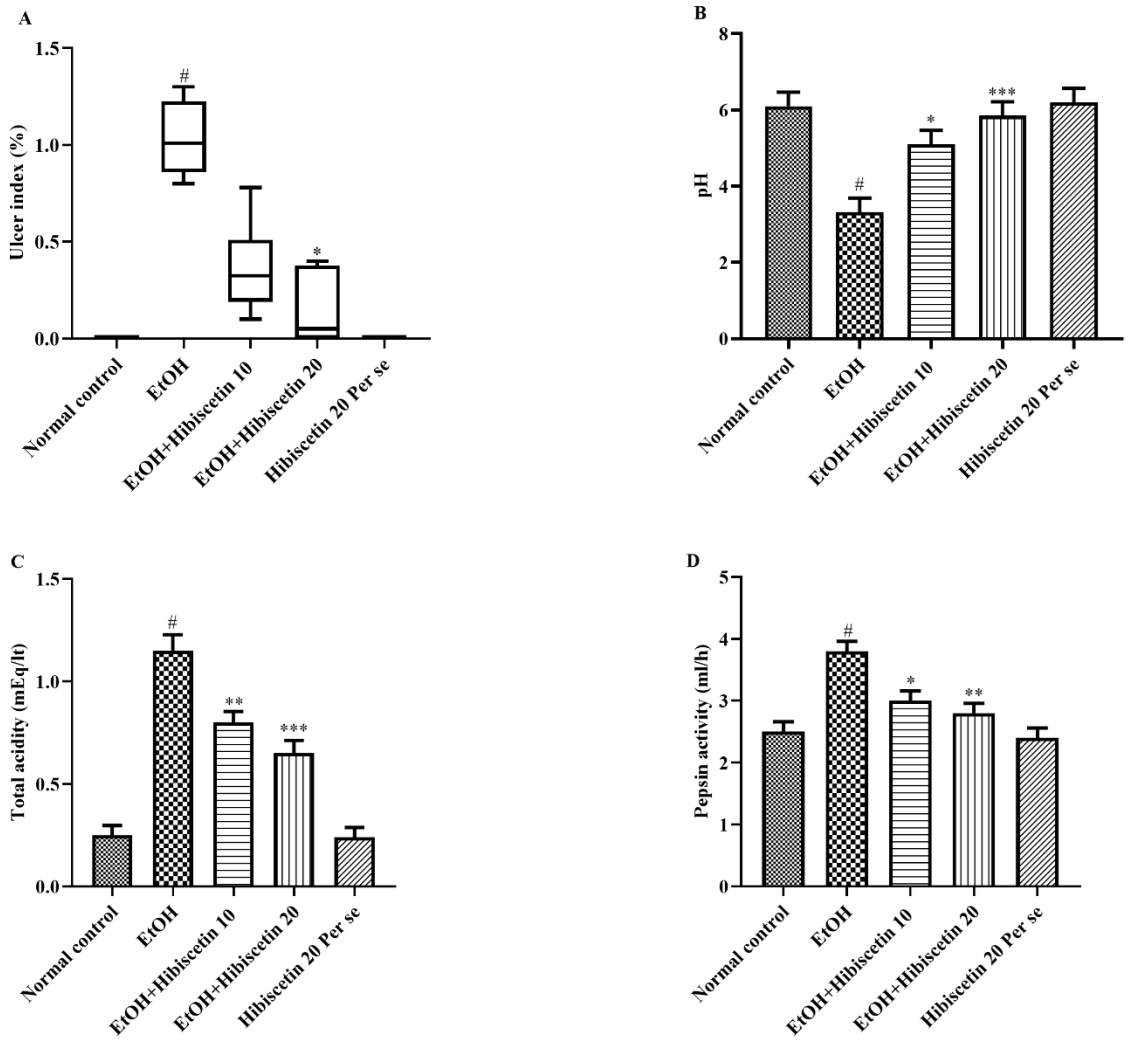

Figure 1A-D: Effect of hibiscetin on A] Ulcer index (#p<0.001 vs. normal control rats, *p<0.05 vs. EtOH control rats. One-way ANOVA followed by a non-parametric test (Kruskal-Wallis) B] pH C] Total acidity D] pepsin activity. Values are expressed in mean ± S.E.M. (n=6). #p<0.001 vs. normal control rats, *p<0.05, **p<0.001, ***p<0.0001 vs. EtOH control rats. One-way ANOVA followed by Tukey's test

group than in normal control. The outcomes of statistical analysis, employing one-way ANOVA followed by Tukey's *post hoc* test showed that hibiscetin declined the MPO activity significantly [F (4, 25) = 26.19; p<0.0001]. The hibiscetin per se group did not exhibit any significant effect.

As per Figure 3B, it can be observed that nitric oxide levels declined to a significant extent in the EtOH-induced group (p<0.001), The outcomes of statistical analysis, employing one-way ANOVA followed by Tukey's *post hoc* test revealed that the hibiscetin has brought the levels of NO to the normal significantly [F (4, 25) = 43.64; p<0.0001]. The per se group was not have any substantial effect.

Pro-inflammatory parameters

From Figure 4A it was evident that PGE_2 levels were lowered substantially (p<0.001) in the EtOH-instigated group in association with normal control, it was also observed that hibiscetin at both the doses elevated the level of PGE_2 to a substantial extent [F (4, 25) = 15.90; p<0.0001]. The hibiscetin per se group showed no effect.

EtOH-induction in the animal model also resulted in a significantly higher amount of IL-6, IL-1β, and TNF-α (p<0.01) in association with normal control. The outcomes of statistical analysis, employing one-way ANOVA followed by Tukey's *post hoc* test hibiscetin substantially reduced the proportions of these pro-inflammatory mediators [F (4, 25) = 53.05; p<0.0001], [F (4, 25) = 16.79; p<0.0001], and [F (4, 25) = 35.10; p<0.0001]. The hibiscetin per se group has no significant effect (Figure 4B-D).

Effect of hibiscetin on caspase-3 and caspase-9

It has been depicted in Figure 5A-B that the EtOH induction resulted in the activation of caspase-3 and caspase-9 in rats to a

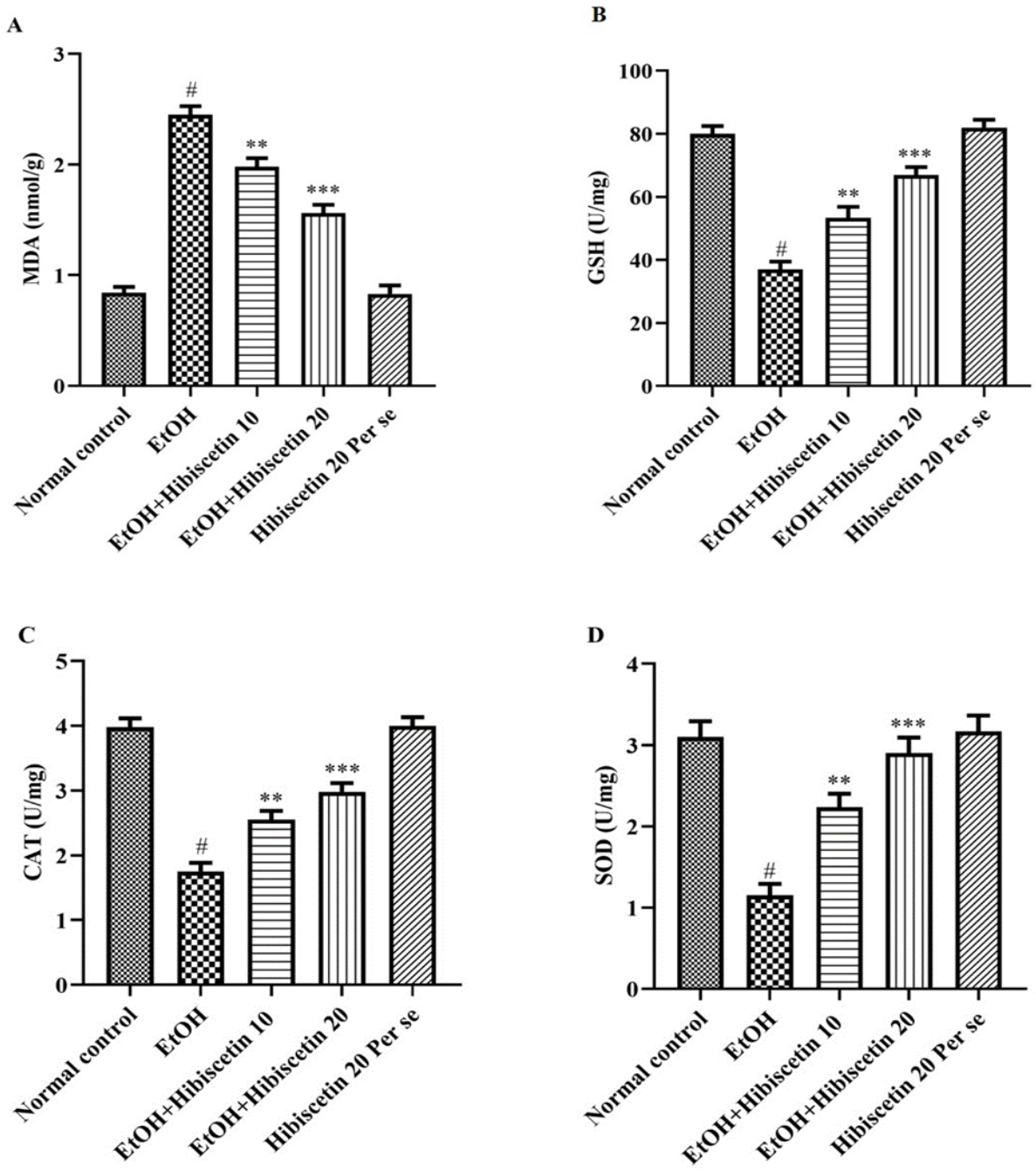

Figure 2A-D: Effect of hibiscetin on A] MDA] B] GSH C] CAT D] SOD. Values are expressed in mean ± S.E.M. (n=6). #p<0.001 vs. normal control rats, **p<0.001, ***p<0.0001 vs. EtOH control rats. One-way ANOVA followed by Tukey's test

substantial level (p<0.01) in association with which the hibiscetin (one-way ANOVA followed by Tukey's *post hoc* test) lowered down the caspase-3 [F (4, 25) = 5.225; p=0.0034] and caspase-9 [F (4, 25) = 29.97; p<0.0001] activities significantly. The hibiscetin per se group did not exert any effect.

DISCUSSION

Globally, chronic uncontrolled alcohol consumption is often associated with gastric ulcers. Additionally, some researchers suggested that these conditions could also be caused by food or beverage contamination or excessive alcohol consumption. It was recently proposed that plant-derived compounds could be used as novel therapeutic strategies to treat ulcers. An experiment was conducted recently to simulate the conditions under which humans may be exposed to EtOH by administering it[17, 18] orally to animals.[33-36]

When given orally to animals, absolute EtOH caused stomach tissue damage, since it penetrated well into the mucosa and caused gastric lesions. There is a role for the mucosa in the gastric cavity

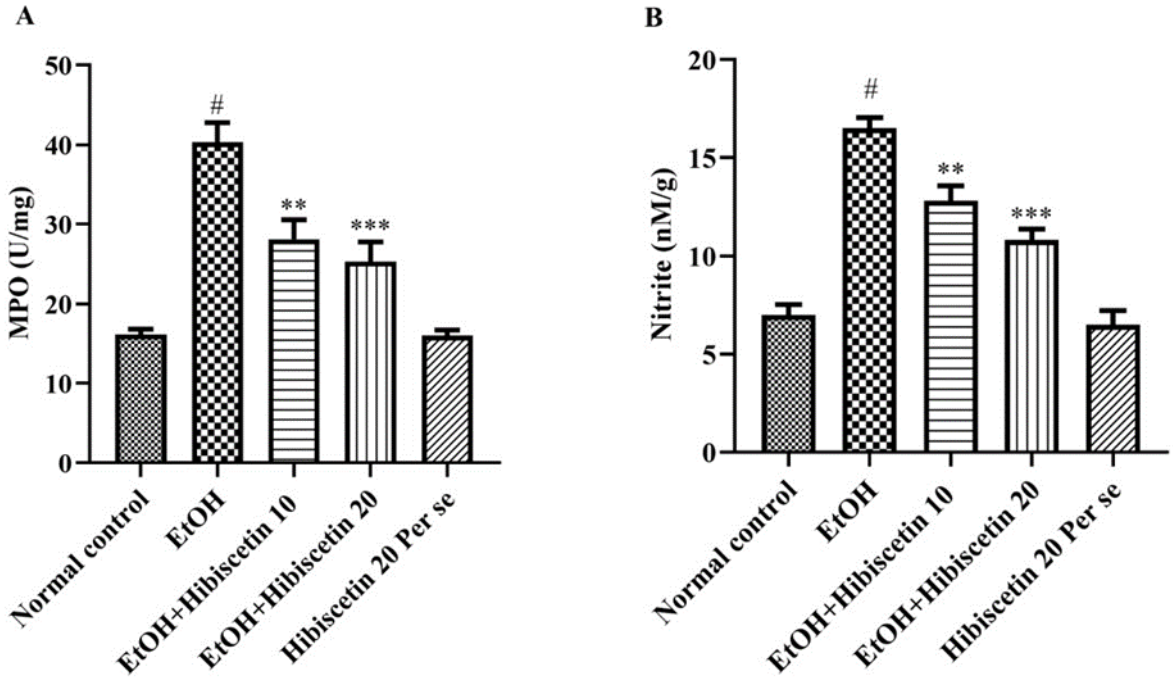

Figure 3A-B: Effect of hibiscetin on A] MPO B] NO level. Values are expressed in mean ± S.E.M. (n=6). #p<0.001 vs. normal control rats, **p<0.001; ***p<0.0001vs. EtOH control rats. One-way ANOVA followed by Tukey's test.

in cellular damage. Gastric lesions are characterized by blood loss, mucosal destruction, submucosal edema, and damaged gastric fibers.[1-3,37-40]

Natural compounds such as flavonoids are popular due to their multiple properties, including their ability to heal, suppress oxidation, and prevent the formation of ROS. The effectiveness of flavonoid hibiscetin in gastric ulcers induced by EtOH was evaluated in a recent study.[7-9,41] This study used rats as experimental animals and divided them into various groups: normal control, EtOH-induced, hibiscetin-treated, and per se group. Within a few days after EtOH was administered orally to rats, mucosal ulcers developed.[42-45] Gastric ulcers are caused by the activation of the stomach's defense mechanism, producing an inflammatory response that activates pro-inflammatory mediators like PGE2, IL-6, IL-1β, and TNF-α. Furthermore, ROS are formed as a result of this reaction. Additionally, oxidative stress leads to increased gastric volume and rises in pH, which may affect the liver and kidneys slowly as well as the surrounding areas. The flavonoid hibiscetin was administered orally at doses of 10 and 20 mg/kg in our study. Hibiscetin is thought to act on ulcer pathophysiology by inhibiting the production of ROS and through a decrease in the release of various inflammatory mediators.[1-3,37-40]

The hibiscetin was able to drop down this enhanced ulcer index score, which occurs due to disruption of the gastric mucosa caused by EtOH induction. It was therefore found to be a promising agent for the recovery of ulcer areas caused by EtOH. In addition to decreasing the pH of the stomach, EtOH was also responsible for ulcer development as it turned the surroundings into an acid that damaged the mucosa of the stomach. By raising the pH to the desired level of alkalinity, hibiscetin effectively protects the gastric system from acidity's damaging effects. It was also observed that this decrease in pH caused by EtOH also increased acid secretions or the volume of secretions, whereas hibiscetin decreased them.[46-48]

Pepsinogen converts into pepsin, which digests indigested protein, and it is most active at pH ranges between 1.8 and 3.[13,36,49,50] Pepsin is the key enzyme that converts pepsinogen into pepsin. Due to the increased acid secretion and low pH, the activity of pepsin is further increased, causing damage to the gastric mucosa by the digestion of useful proteins. As Hibiscetin restores the pH of the stomach and decreases gastric acid secretion, the pepsin activity is reduced, thereby supporting ulcer healing. Research has shown that salivary enzymes become significantly active when mucosal cells are inflamed or infected. Cell membrane damage caused by ROS is a major factor in ulcer development, as it decreases antioxidant enzyme activity.[33-35,51,52] When rats were induced with EtOH, free radicals formed, MDA activity increased, and CAT, SOD, and GSH levels decreased, further reducing antioxidant activity. EtOH-induced ulcers are associated with increased oxidative stress in the gastric mucosa. Hibiscetin acts as an antioxidant, which helps to scavenge and neutralize free radicals. It can reduce the levels of MDA, a marker of lipid peroxidation while enhancing the activity of antioxidant enzymes such as CAT, SOD, and GSH. These antioxidant effects mitigate oxidative damage and protect the gastric mucosa from EtOH-induced injury.[14-16,20,53-55]

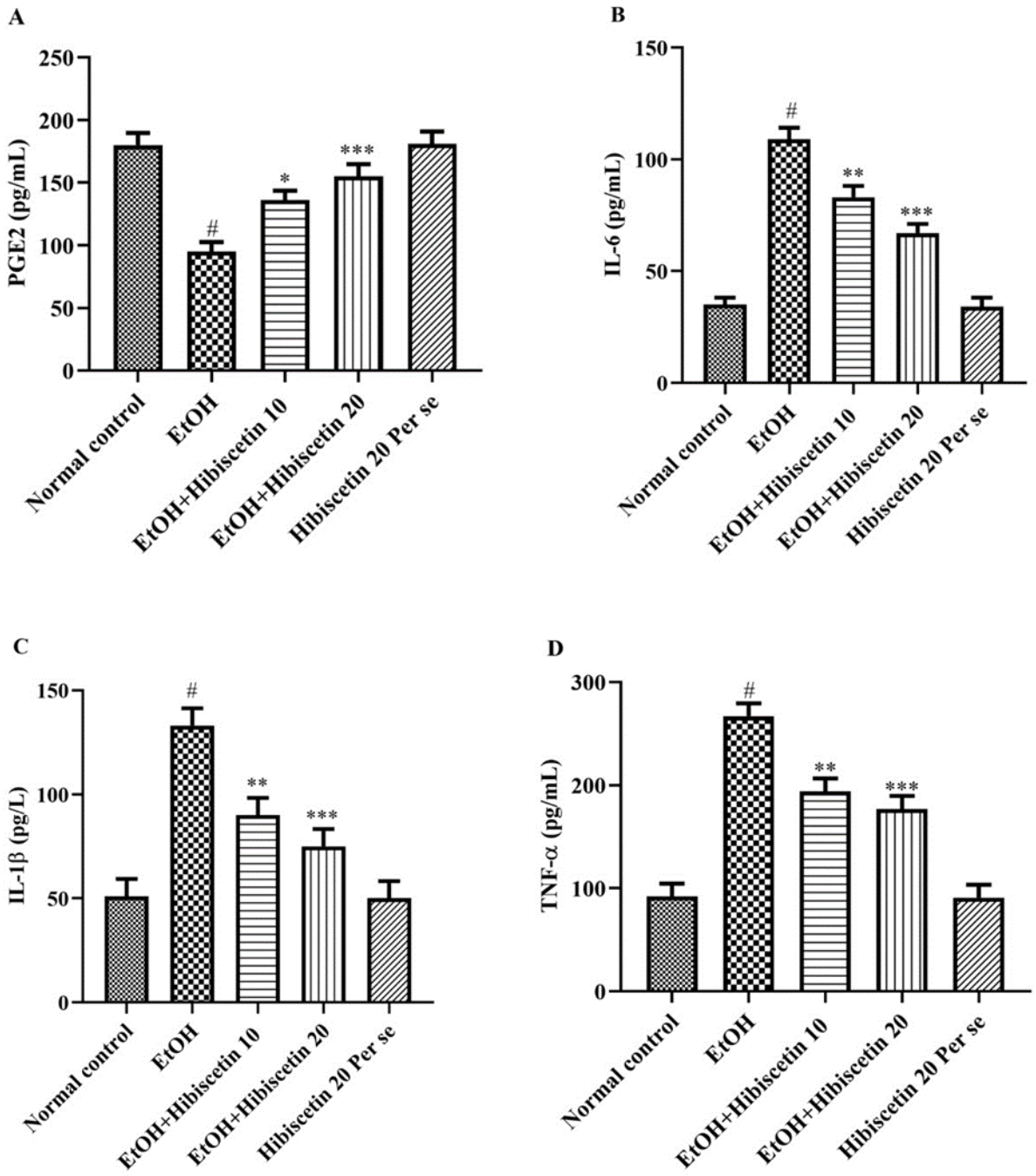

Figure 4A-D: Effect of hibiscetin on A] PGE2 B] IL-6 C] IL-1β D] TNF-α. Values are expressed in mean ± S.E.M. (n= 6). #p<0.001 vs. normal control rats, *p<0.05, **p<0.001, ***p<0.0001 vs. EtOH control rats. One-way ANOVA followed by Tukey's test.

The production of hydrochlorous acid and oxidative activity of MPO, as reported in previous research, may increase the severity of gastric ulcers, as the levels of MPO were observed to be high after EtOH administration, suggesting that this could lead to complications. MPO levels were reduced by hibiscetin at both doses to normal and the damaging effects were minimized.[29-31,56,57] MPO is an enzyme released by activated neutrophils during inflammation. It generates ROS that contributes to tissue damage. Myeloperoxidase (MPO) is an enzyme released by activated neutrophils during inflammation. It generates Reactive Oxygen Species (ROS) that contribute to tissue damage. Hibiscetin may inhibit MPO activity, thereby reducing the production of harmful ROS and preventing further damage to the gastric mucosa.

EtOH induction significantly reduced levels of NO, which possess anti-inflammatory properties. Hibiscetin increased NO levels.[58] The stomach has a protective layer of mucus that lines its inner surface and shields it from the corrosive effects of gastric acid. PGE2 promotes the production of this protective mucus, helping to maintain the integrity of the stomach lining and

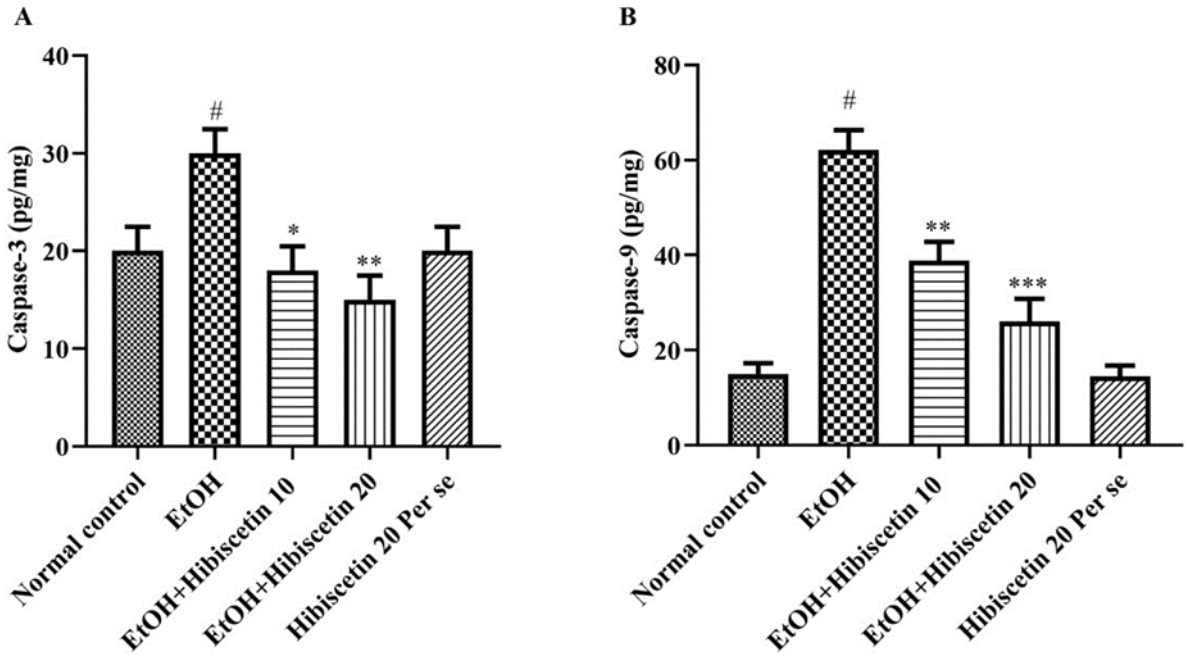

Figure 5A-B: Effect of hibiscetin on A] Caspase 3 B] Caspase 9. Values are expressed in mean ± S.E.M. (n=6). #p<0.001 vs. normal control rats, *p<0.05, **p<0.001, ***p<0.0001 vs. EtOH control rats. One-way ANOVA followed by Tukey's test

prevent ulcer formation. PGE2 inhibits the secretion of gastric acid, reducing the acidity.[27,59-65] PGE2 is involved in the regulation of inflammation. Inflammatory processes can contribute to ulcer development and prolong the healing process. PGE2 helps to modulate the immune response and limit excessive inflammation, aiding in ulcer healing. PGE2 is a type of prostaglandin that promotes inflammation and contributes to the development of ulcers. Hibiscetin may inhibit the enzyme cyclooxygenase, which is responsible for the synthesis of PGE2, thus reducing its levels and dampening the inflammatory response in the gastric mucosa. EtOH administration caused the reduction in PGE2 levels and increased in concentrations of IL-6, IL-1β, and TNF-α. These cytokines play a crucial role in initiating and propagating the inflammatory response. Hibiscetin can suppress the production of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α. By inhibiting their synthesis, hibiscetin helps to reduce inflammation in the gastric mucosa and protect it from damage caused by ethanol.[53,66]

Caspase-3 and caspase-9 are enzymes that mediate apoptosis or programmed cell death. Ethanol activates these enzymes in the gastric mucosa, leading to cell death and ulcer formation. Ethanol-induced ulcers can trigger apoptotic pathways in the gastric mucosa, leading to cell death.[29] Hibiscetin may have an inhibitory effect on the activation of caspase-3 and caspase-9, key enzymes involved in apoptosis, thereby reducing cell death and promoting tissue repair.

Hibiscetin exerts its protective effects against ethanol-induced ulcers in rats through multiple mechanisms, including inhibition of pro-inflammatory cytokines, suppression of PGE2 synthesis, antioxidant activity, and attenuation of apoptosis. By targeting these pathways, hibiscetin helps to maintain the integrity of the gastric mucosa and promote healing, thus offering a potential therapeutic approach for the prevention and treatment of ethanol-induced ulcers.

Furthermore, it was reported that the per se group did not exhibit any adverse effects in rats, indicating that the hibiscetin has no adverse interactions at its highest dose. In this study, only six animals were in each group, resulting in a very small number of animals. This can affect the validity of the analysis and the generalizability of the results. We have not extensively explored the molecular mechanisms underlying hibiscetin's effects, so further research is necessary to elucidate the intricate pathways involved in immunohistochemistry and molecular studies, such as western blotting, histopathology, RT-PCR, tissue immunohistochemistry, gene protein expression studies, and other genetic models.

CONCLUSION

Hibiscetin has the potential to reduce pro-inflammatory mediators, increase antioxidant enzyme activity, reduce gastric acid secretion, maintain pH, and reduce a variety of other complications that may result in ulceration in EtOH-induced rats. Hibiscetin administration at both 10 mg/kg and 20 mg/kg doses effectively protects against ethanol-induced ulcers in rats by inhibiting pro-inflammatory cytokines, PGE2 synthesis, oxidative stress markers, MPO activity, and the caspase-3 and caspase-9 apoptotic pathways. These findings highlight the potential of hibiscetin as a promising therapeutic agent for preventing and

treating gastric ulcers associated with inflammatory and oxidative stress conditions. Further research is warranted to explore its full clinical potential and safety profile.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ANOVA: Analysis of variance; **CAT:** Catalase; **ELISA:** Enzyme-linked immunosorbent assay; **EtOH:** Ethanol; **GSH:** Glutathione; **IL-1β:** Interleukin 1 beta; **IL-6:** Interleukin-6; **MDA:** Malondialdehyde MDA; **MPO:** Myeloperoxidase; **NO:** Nitric oxide; **PGE2:** Prostaglandin E-2, oxidative stress; **ROS:** Reactive oxygen species; **SOD:** Superoxide dismutase; **TNF-α:** Tumor necrosis factor-alpha; **UI:** Ulcer index.

SUMMARY

- The current research provided evidence that flavonoid hibiscetin produced a tremendous response in Ethanol instigated ulcers in rats.
- Hibiscetin reduced the excess gastric acid secretion restoration of pH and pepsin activities.
- A flavonoid hibiscetin activated the activity of PGE_2 required in the healing of ulcers.
- Hibiscetin significantly controlled the pro-inflammatory cytokines in EtOH-induced rats.
- The hibiscetin was effective in managing oxidative stress and enhancing the activities of antioxidant enzymes.

ETHICS APPROVAL

The research was approved by the Institutional Animal Ethical Committee, LNCT, India (IAEC/LNCT/2023/005) and was conducted as per the ARRIVE guidelines.

REFERENCES

1. Abdel-Kawi SH, Hashem KS, Saad MK, Fekry G, Abdel-Hameed EMM. The ameliorative effects of cinnamon oil against ethanol-induced gastric ulcer in rats by regulating oxidative stress and promoting angiogenesis. J Mol Histol. 2022;53(3):573-87. doi: 10.1007/s10735-022-10072-y, PMID 35290563.
2. Aman RM, Zaghloul RA, El-Dahhan MS. Formulation, optimization and characterization of allantoin-loaded chitosan nanoparticles to alleviate ethanol-induced gastric ulcer: *in vitro* and *in vivo* studies. Sci Rep. 2021;11(1):2216. doi: 10.1038/s41598-021-81183-x, PMID 33500454.
3. Ammar NM, Hassan HA, Ahmed RF, G El-Gendy AE, Abd-ElGawad AM, Farrag ARH, *et al.* Gastro-protective effect of Artemisia sieberi essential oil against ethanol-induced ulcer in rats as revealed via biochemical, histopathological and metabolomics analysis. Biomarkers. 2022;27(3):247-57. doi: 10.1080/1354750X.2021.2025428, PMID 34978233.
4. Da Silva DT, Rodrigues RF, Machado NM, Maurer LH, Ferreira LF, Somacal S, *et al.* Natural deep eutectic solvent (NADES)-based blueberry extracts protect against ethanol-induced gastric ulcer in rats. Food Res Int. 2020;138(A):109718. doi: 10.1016/j.foodres.2020.109718, PMID 33292963.
5. El-Din MIG, Youssef FS, Said RS, Ashour ML, Eldahshan OA, Singab ANB. Chemical constituents and gastro-protective potential of *Pachira glabra* leaves against ethanol-induced gastric ulcer in experimental rat model. Inflammopharmacology. 2021;29(1):317-32. doi: 10.1007/s10787-020-00749-9, PMID 32914383.
6. Fahmi AA, Abdur-Rahman M, Aboul Naser AF, Hamed MA, Abd-Alla HI, Shalaby NMM, *et al.* Chemical composition and protective role of *Pulicaria undulata* (L.) C.A. Mey. subsp. undulata against gastric ulcer induced by ethanol in rats. Heliyon. 2019;5(3):e01359. doi: 10.1016/j.heliyon.2019.e01359, PMID 30957042.
7. Arab HH, Salama SA, Eid AH, Kabel AM, Shahin NN. Targeting MAPKs, NF-κB, and PI3K/AKT pathways by methyl palmitate ameliorates ethanol-induced gastric mucosal injury in rats. J Cell Physiol. 2019;234(12):22424-38. doi: 10.1002/jcp.28807, PMID 31115047.
8. Armah FA, Henneh IT, Alake J, Ahlidja W, Amoani B, Ofori EG, *et al. Allanblackia floribunda* Seed extract attenuates the ethanol-induced gastric ulcer in rats via the inhibition of TNF-α and INF-γ levels and modulation in the expression of Ki67 protein. BioMed Res Int. 2021; 2021:6694572. doi: 10.1155/2021/6694572, PMID 33521129.
9. Asaad GF, Mostafa RE. Lactoferrin mitigates ethanol-induced gastric ulcer via modulation of ROS/ICAM-1/Nrf2 signaling pathway in Wistar rats. Iran J Basic Med Sci. 2022;25(12):1522-7. doi: 10.22038/IJBMS.2022.66823.14656, PMID 36544526.
10. Hama Amin RR, Aziz TA. Gastroprotective effect of azilsartan through ameliorating oxidative stress, inflammation, and restoring hydroxyproline, and gastrin levels in ethanol-induced gastric ulcer. J Inflam Res. 2022;15:2911-23. doi: 10.2147/JIR.S365090, PMID 35592072.
11. Hu J, Luo J, Zhang M, Wu J, Zhang Y, Kong H, *et al.* Protective effects of radix *Sophorae flavescentis* Carbonisata-based carbon dots against ethanol-induced acute gastric ulcer in rats: anti-inflammatory and antioxidant activities. Int J Nanomedicine. 2021;16:2461-75. doi: 10.2147/IJN.S289515, PMID 33814910.
12. Izhar H, Shabbir A, Shahzad M, Mobashar A, Ahmed SS. *Phyllanthus reticulatus* prevents ethanol-induced gastric ulcer via downregulation of IL-8 and TNF-α levels. Evid Based Complement Alternat Med. 2021; 2021:1734752. doi: 10.1155/2021/1734752, PMID 34608395.
13. Jin Y, Zhang M, Wang Y, Lu Y, Liu T, Yang G, *et al.* Protective effect and potential mechanism of the traditional Chinese medicine Shaoyao-Gancao decoction on ethanol-induced gastric ulcers in rats. Evid Based Complement Alternat Med. 2022; 2022:3069089. doi: 10.1155/2022/3069089, PMID 35449820.
14. Mousa AM, El-Sammad NM, Hassan SK, Madboli AENA, Hashim AN, Moustafa ES, *et al.* Antiulcerogenic effect of *Cuphea ignea* extract against ethanol-induced gastric ulcer in rats. BMC Complement Altern Med. 2019;19(1):345. doi: 10.1186/s12906-019-2760-9, PMID 31791313.
15. Ofusori AE, Moodley R, Jonnalagadda SB. Antiulcerogenic effects of *Celosia trigyna* plant extracts on ethanol-induced gastric ulcer in adult Wistar rats. J Tradit Complement Med. 2020;10(6):586-93. doi: 10.1016/j.jtcme.2019.11.004, PMID 33134135.
16. Ostovaneh A, Eslami F, Rahimi N, Dejban P, Shafaroodi H, Abbasi A, *et al.* Gastroprotective effect of sumatriptan against indomethacin-, stress- and ethanol-induced gastric damage in male rats: possible modulatory role of 5-hydroxytryptamine 1-B/1D receptors and pro-inflammatory cytokines. Basic Clin Pharmacol Toxicol. 2023;133(2):156-67. doi: 10.1111/bcpt.13905, PMID 37248787.
17. Liu X, Quan S, Han Q, Li J, Gao X, Zhang J, *et al.* Effectiveness of the fruit of *Rosa odorata* sweet var. Gigantea (Coll. et Hemsl.) Rehd. et Wils in the protection and the healing of ethanol-induced rat gastric mucosa ulcer based on Nrf2/NF-κB pathway regulation. J Ethnopharmacol. 2022;282:114626. doi: 10.1016/j.jep.2021.114626, PMID 34517064 (Coll. et Hemsl.).
18. Manal Ahmad A, Yasser Ibrahim K, Manal Mohammad A. Efficacy of extract from *Ononis spinosa* L. on ethanol-induced gastric ulcer in rats. J Tradit Chin Med. 2021;41(2):270-5. PMID 33825407.
19. Moawad H, El Awdan SA, Sallam NA, El-Eraky WI, Alkhawlani MA. Gastroprotective effect of cilostazol against ethanol- and pylorus ligation-induced gastric lesions in rats. Naunyn Schmiedebergs Arch Pharmacol. 2019;392(12):1605-16. doi: 10.1007/s00210-019-01699-y, PMID 31372695.
20. Mohan S, Hobani YH, Shaheen E, Abou-Elhamd AS, Abdelhaleem A, Alhazmi HA, *et al.* Ameliorative effect of Boesenbergin A, a chalcone isolated from *Boesenbergia rotunda* (Fingerroot) on oxidative stress and inflammation in ethanol-induced gastric ulcer *in vivo*. J Ethnopharmacol. 2020;261:113104. doi: 10.1016/j.jep.2020.113104, PMID 32565307.
21. Gilani SJ, Bin-Jumah MN, Al-Abbasi FA, Albohairy FM, Nadeem MS, Ahmed MM, *et al.* The ameliorative role of hibiscetin against high-fat diets and streptozotocin-induced diabetes in rodents via inhibiting tumor necrosis factor-α, interleukin-1β, and malondialdehyde level. Processes. 2022;10(7):1396. doi: 10.3390/pr10071396.
22. Alzarea SI, Afzal M, Alharbi KS, Alzarea AI, Alenezi SK, Alshammari MS, *et al.* Hibiscetin attenuates oxidative, nitrative stress and neuroinflammation via suppression of TNF-α signaling in rotenone induced parkinsonism in rats. Saudi Pharm J. 2022;30(12):1710-7. doi: 10.1016/j.jsps.2022.09.016, PMID 36601498.
23. Mahdi WA, AlGhamdi SA, Alghamdi AM, Imam SS, Alshehri S, Almaniea MA, *et al.* Neuroprotectant effects of hibiscetin in 3-nitropropionic acid-induced Huntington's disease via subsiding oxidative stress and modulating monoamine neurotransmitters in rats brain. Molecules. 2023;28(3):1402. doi: 10.3390/molecules28031402, PMID 36771072.

24. Ran XL, Zhang M, Liu W, Qiu L, Wang Y, Bhandari B, *et al.* Effects of hibiscetin pretreatment on the color and anthocyanin level of microwave vacuum dried edible roses. Drying Technol. 2021;39(9):1231-9. doi: 10.1080/07373937.2021.1880433.
25. Ragi C, Muraleedharan K. Antioxidant activity of Hibiscetin and Hibiscitrin: insight from DFT, NCI, and QTAIM. Theor Chem Acc. 2023;142(3):30. doi: 10.1007/s00214-023-02970-5.
26. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, *et al.* The ARRIVE guidelines 2.0. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. J Cereb Blood Flow Metab. 2020;40(9):1769-77. doi: 10.1177/0271678X20943823, PMID 32663096.
27. Sistani Karampour N, Arzi A, Rezaie A, Pashmforoosh M, Kordi F. Gastroprotective effect of zingerone on ethanol-induced gastric ulcers in rats. Medicina (Kaunas). 2019;55(3):64. doi: 10.3390/medicina55030064, PMID 30862060.
28. Sanpinit S, Chonsut P, Punsawad C, Wetchakul P. Gastroprotective and antioxidative effects of the traditional Thai polyherbal formula Phy-Blica-D against ethanol-induced gastric ulcers in rats. Nutrients. 2021;14(1):172. doi: 10.3390/nu14010172, PMID 35011049.
29. Raish M, Shahid M, Bin Jardan YA, Ansari MA, Alkharfy KM, Ahad A, *et al.* Gastroprotective effect of sinapic acid on ethanol-induced gastric ulcers in rats: involvement of Nrf2/HO-1 and NF-κB signaling and antiapoptotic role. Front Pharmacol. 2021;12:622815. doi: 10.3389/fphar.2021.622815, PMID 33716749.
30. Rubab F, Ijaz H, Hussain S, Munir A, Stuppner S, Jakschitz T, *et al.* Gastroprotective effects of *Caragana ambigua* stocks on ethanol-induced gastric ulcer in rats supported by LC-MS/MS characterization of formononetin and biochanin A. J Sci Food Agric. 2022;102(15):7030-8. doi: 10.1002/jsfa.12064, PMID 35689485.
31. Salaheldin AT, Shehata MR, Sakr HI, Atia T, Mohamed AS. Therapeutic potency of ovothiol A on ethanol-induced gastric ulcers in Wistar rats. Mar Drugs. 2022;21(1):25. doi: 10.3390/md21010025, PMID 36662198.
32. Guzmán-Gómez O, García-Rodríguez RV, Quevedo-Corona L, Pérez-Pastén-Borja R, Rivero-Ramírez NL, Ríos-Castro E, *et al.* Amelioration of ethanol-induced gastric ulcers in rats pretreated with phycobiliproteins of *Arthrospira* (Spirulina) Maxima. Nutrients. 2018;10(6):763. doi: 10.3390/nu10060763, PMID 29899291.
33. Zhang Y, Yuan Z, Chai J, Zhu D, Miao X, Zhou J, *et al.* ALDH2 ameliorates ethanol-induced gastric ulcer through suppressing NLPR3 inflammasome activation and ferroptosis. Arch Biochem Biophys. 2023;743:109621. doi: 10.1016/j.abb.2023.109621, PMID 37209766.
34. Zhou D, Yang Q, Tian T, Chang Y, Li Y, Duan LR, *et al.* Gastroprotective effect of gallic acid against ethanol-induced gastric ulcer in rats: involvement of the Nrf2/HO-1 signaling and anti-apoptosis role. Biomed Pharmacother. 2020;126:110075. doi: 10.1016/j.biopha.2020.110075, PMID 32179202.
35. Zou Y, Cui X, Xiang Q, Guo M, Liang Y, Qu Y, *et al.* Protective effect of against ethanol-induced gastric ulcer and its mechanism. Zhejiang Da Xue Xue Bao Yi Xue Ban. 2021;50(5):561-7. doi: 10.3724/zdxbyxb-2021-0055, PMID 34986535.
36. Lian YZ, Lin IH, Yang YC, Chao JC. Gastroprotective effect of *Lycium barbarum* polysaccharides and C-phycocyanin in rats with ethanol-induced gastric ulcer. Int J Biol Macromol. 2020;165(A):1519-28. doi: 10.1016/j.ijbiomac.2020.10.037, PMID 33058973.
37. Alimi H, Mabrouk FH, Zouari N, Sakly M, Rhouma KB. LC-ESI-MS phenolic contents assessment, antioxidant, and protective ability of *Punica granatum* root bark extract against ethanol-induced gastric ulcer in rats: *in silico* H+, K+-ATPase inhibitory pathway study. Toxicol Res (Camb). 2023;12(2):189-200. doi: 10.1093/toxres/tfad006, PMID 37125332.
38. Al-Sayed E, Michel HE, Khattab MA, El-Shazly M, Singab AN. Protective Role of Casuarinin from *Melaleuca leucadendra* against Ethanol-Induced Gastric Ulcer in Rats. Planta Med. 2020;86(1):32-44. doi: 10.1055/a-1031-7328, PMID 31689719.
39. Alzokaky AA, Abdelkader EM, El-Dessouki AM, Khaleel SA, Raslan NA. C-phycocyanin protects against ethanol-induced gastric ulcers in rats: role of HMGB1/NLRP3/NF-κB pathway. Basic Clin Pharmacol Toxicol. 2020;127(4):265-77. doi: 10.1111/bcpt.13415, PMID 32306544.
40. Arab HH, Eid AH, El-Sheikh AAK, Arafa EA, Ashour AM. Irbesartan reprofiling for the amelioration of ethanol-induced gastric mucosal injury in rats: role of inflammation, apoptosis, and autophagy. Life Sci. 2022;308:120939. doi: 10.1016/j.lfs.2022.120939, PMID 36115582.
41. Badr AM, El-Orabi NF, Ali RA. The implication of the crosstalk of Nrf2 with NOXs, and HMGB1 in ethanol-induced gastric ulcer: potential protective effect is afforded by Raspberry ketone. PLOS ONE. 2019;14(8):e0220548. doi: 10.1371/journal.pone.0220548, PMID 31404064.
42. Bakry SM, Naser AFA, Negoumy SIE, Kassem MES, Abdel-Sattar E, Meselhy MR. Phenolic acids-rich fraction from *Ficus drupacea* leaves for the prevention and treatment of ethanol-induced gastric mucosal injury in rats. Inflammopharmacology. 2023;31(3):1423-36. doi: 10.1007/s10787-023-01158-4, PMID 36840885.
43. Beiranvand M, Bahramikia S. Ameliorating and protective effects mesalazine on ethanol-induced gastric ulcers in experimental rats. Eur J Pharmacol. 2020;888:173573. doi: 10.1016/j.ejphar.2020.173573, PMID 32956646.
44. Beiranvand M, Bahramikia S, Dezfoulian O. Evaluation of antioxidant and anti-ulcerogenic effects of *Eremurus persicus* (Jaub and Spach) Boiss leaf hydroalcoholic extract on ethanol-induced gastric ulcer in rats. Inflammopharmacology. 2021;29(5):1503-18. doi: 10.1007/s10787-021-00868-x, PMID 34435283.
45. Boutemine IM, Amri M, Amir ZC, Fitting C, Mecherara-Idjeri S, Layaida K, *et al.* Gastro-protective, therapeutic and anti-inflammatory activities of *Pistacia lentiscus* L. Fatty oil against ethanol-induced gastric ulcers in rats. J Ethnopharmacol. 2018;224:273-82. doi: 10.1016/j.jep.2018.05.040, PMID 29859303.
46. Fahmy NM, Al-Sayed E, Michel HE, El-Shazly M, Singab ANB. Gastroprotective effects of *Erythrina speciosa* (Fabaceae) leaves cultivated in Egypt against ethanol-induced gastric ulcer in rats. J Ethnopharmacol. 2020;248:112297. doi: 10.1016/j.jep.2019.112297, PMID 31606535.
47. Fatima SF, Ishtiaq S, Lashkar MO, Youssef FS, Ashour ML, Elhady SS. Metabolic profiling of *Heliotropium crispum* aerial parts using HPLC and FTIR and *in vivo* evaluation of its anti-ulcer activity using an ethanol induced acute gastric ulcer model. Metabolites. 2022;12(8):750. doi: 10.3390/metabo12080750, PMID 36005621.
48. Fu YH, Hou YD, Duan YZ, Sun XY, Chen SQ. Gastroprotective effect of an active ingredients group of *Lindera reflexa* Hemsl. On Ethanol-Induced gastric ulcers in Rats: involvement of VEGFR2/ERK and TLR-2/Myd88 signaling pathway. Int Immunopharmacol. 2022;107:108673. doi: 10.1016/j.intimp.2022.108673, PMID 35259712.
49. Lebda MA, Mostafa RE, Taha NM, Abd El-Maksoud EM, Tohamy HG, Al Jaouni SK, *et al.* *Commiphora myrrh* Supplementation Protects and Cures Ethanol-Induced Oxidative Alterations of Gastric Ulceration in Rats. Antioxidants (Basel). 2021;10(11):1836. doi: 10.3390/antiox10111836, PMID 34829707.
50. Li C, Wen R, Liu D, Yan L, Gong Q, Yu H. Assessment of the potential of *Sarcandra glabra* (Thunb.) Nakai. in treating ethanol-induced gastric ulcer in rats based on metabolomics and network analysis. Front Pharmacol. 2022;13:810344. doi: 10.3389/fphar.2022.810344, PMID 35903344.
51. Zhang J, Yin Y, Xu Q, Che X, Yu C, Ren Y, *et al.* Integrated serum pharmacochemistry and investigation of the anti-gastric ulcer effect of Zuojin pill in rats induced by ethanol. Pharm Biol. 2022;60(1):1417-35. doi: 10.1080/13880209.2022.2098345, PMID 35938492.
52. Zhang X, Wang Y, Li X, Dai Y, Wang Q, Wang G, *et al.* Treatment mechanism of Gardeniae Fructus and Its carbonized product against ethanol-induced gastric lesions in rats. Front Pharmacol. 2019;10:750. doi: 10.3389/fphar.2019.00750, PMID 31333466.
53. Park HS, Seo CS, Baek EB, Rho JH, Won YS, Kwun HJ. Gastroprotective effect of myricetin on ethanol-induced acute gastric injury in rats. Evid Based Complement Alternat Med. 2021; 2021:9968112. doi: 10.1155/2021/9968112, PMID 34630623.
54. Pineda-Peña EA, Capistran-Amezcua D, Reyes-Ramírez A, Xolalpa-Molina S, Chávez-Piña AE, Figueroa M, *et al.* Gastroprotective effect methanol extract of *Caesalpinia coriaria* pods against indomethacin- and ethanol-induced gastric lesions in Wistar rats. J Ethnopharmacol. 2023;305:116057. doi: 10.1016/j.jep.2022.116057, PMID 36574790.
55. Rahman Z, Dwivedi DK, Jena GB. Ethanol-induced gastric ulcer in rats and intervention of tert-butylhydroquinone: involvement of Nrf2/HO-1 signalling pathway. Hum Exp Toxicol. 2020;39(4):547-62. doi: 10.1177/0960327119895559, PMID 31876185.
56. Rahman Z, Dwivedi DK, Jena GB. The intervention of tert-butylhydroquinone protects ethanol-induced gastric ulcer in type II diabetic rats: the role of Nrf2 pathway. Can J Physiol Pharmacol. 2021;99(5):522-35. doi: 10.1139/cjpp-2020-0173, PMID 33095998.
57. Salama RM, Ahmed RH, Farid AA, AbdElSattar BA, AbdelBaset RM, Youssef ME, *et al.* Gastroprotective effect of dapagliflozin in ethanol-induced gastric lesions in rats: crosstalk between HMGB1/RAGE/PTX3 and TLR4/MyD88/VEGF/PDGF signaling pathways. Int Immunopharmacol. 2023;115:109686. doi: 10.1016/j.intimp.2023.109686, PMID 36623411.
58. Sánchez-Mendoza ME, López-Lorenzo Y, Cruz-Antonio L, Cruz-Oseguera A, García-Machorro J, Arrieta J. Gastroprotective effect of Juanislamin on ethanol-induced gastric lesions in rats: role of prostaglandins, nitric oxide and sulfhydryl groups in the mechanism of Action. Molecules. 2020;25(9):2246. doi: 10.3390/molecules25092246, PMID 32397642.
59. Shams SGE, Eissa RG. Amelioration of ethanol-induced gastric ulcer in rats by quercetin: implication of Nrf2/HO1 and HMGB1/TLR4/NF-κB pathways. Heliyon. 2022;8(10):e11159. doi: 10.1016/j.heliyon.2022.e11159, PMID 36311358.
60. Shin MS, Lee J, Lee JW, Park SH, Lee IK, Choi JA, *et al.* Anti-inflammatory Effect of *Artemisia argyi* on Ethanol-Induced Gastric Ulcer: analytical, *in vitro* and *in vivo* Studies for the Identification of Action Mechanism and Active compounds. Plants (Basel, Switzerland). 2021;10(2):332. doi: 10.3390/plants10020332, PMID 33572173.
61. Sidahmed HMA, Vadivelu J, Loke MF, Arbab IA, Abdul B, Sukari MA, *et al.* Anti-ulcerogenic activity of dentatin from *Clausena excavata* Burm.f. against ethanol-induced gastric ulcer in rats: possible role of mucus and antioxidant effect. Phytomedicine. 2019;55:31-9. doi: 10.1016/j.phymed.2018.06.036, PMID 30668441.
62. Wu X, Huang Q, Xu N, Cai J, Luo D, Zhang Q, *et al.* Antioxidative and anti-inflammatory effects of water extract of *Crostichum aureum* Linn. against ethanol-induced gastric ulcer in rats. Evid Based Complement Alternat Med. 2018; 2018:3585394. doi: 10.1155/2018/3585394, PMID 30643529.
63. Xie L, Guo YL, Chen YR, Zhang LY, Wang ZC, Zhang T, *et al.* A potential drug combination of omeprazole and patchouli alcohol significantly normalizes oxidative stress and inflammatory responses against gastric ulcer in ethanol-induced rat model. Int Immunopharmacol. 2020;85:106660. doi: 10.1016/j.intimp.2020.106660, PMID 32559721.

64. Yu L, Li R, Liu W, Zhou Y, Li Y, Qin Y, *et al.* Protective effects of wheat peptides against ethanol-induced gastric mucosal lesions in rats: vasodilation and anti-inflammation. Nutrients. 2020;12(8):2355. doi: 10.3390/nu12082355, PMID 32784583.
65. Gao F, Gan S, He Y, Chen Z, Liu X, *et al.* Chemical characterization and gastroprotective effect of an isolated polysaccharide fraction from *Bletilla striata* against ethanol-induced acute gastric ulcer. Food Chem Toxicol. 2019;131:110539. doi: 10.1016/j.fct.2019.05.047, PMID 31158404.
66. Kim YS, Lee JH, Song J, Kim H. Gastroprotective effects of Inuleae Flos on HCl/Eethanol-induced gastric ulcers in rats. Molecules. 2020;25(23). doi: 10.3390/molecules25235623, PMID 33260419.

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