RESEARCH ARTICLE

REVISED The potential effects of cranberry extract on indomethacin-induced gastric ulcer in rats

[version 2; peer review: 1 approved, 2 approved with reservations]

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Abstract

Background

Indomethacin belongs to nonsteroidal anti-inflammatory drugs (NSAIDs) prescribed for treatment of rheumatoid diseases and linked to the development of gastric ulcers in many people. Cranberry is a rich source of polyphenols and flavonoids, which have powerful antioxidant and anti-inflammatory properties.

Methods

This study aimed to evaluate the activity of cranberry aqueous extract on indomethacin-induced gastric ulcers in albino rats. 20 adult male rats were sequentially assigned to four groups of 5 each. The control group consumes distilled water (DW) orally for 15 days. The induction group received a single oral dosage (60 mg/kg) of IND. The omeprazole group got 60 mg/kg of indomethacin as a single oral dose and then 20 mg/kg/day of omeprazole for 15 days. The cranberry group was given a single dose of indomethacin 60 mg/kg orally and subsequently 200 mg/kg/day of cranberry aqueous extract for 15 days. Rats were euthanized on day 15, and gastric tissues were removed for biochemical and histopathological evaluations.

Results

Cranberry extract considerably ameliorated the severity of indomethacin-induced gastric ulcerations and fixed histological deteriorations. Furthermore, indomethacin-exposed rats treated with cranberry extract exhibited dramatically lower serum levels of inflammatory biomarkers like TNF-α and IL-6, but higher levels of anti-

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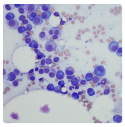
oxidative biomarkers like SOD and GPx. The bioactive flavonoids and polyphenols content of cranberry extract could possibly account for its profound gastroprotective effects. The anti-oxidative and anti-inflammatory properties of cranberry extract could be a promising strategy for ameliorating the indomethacin-aggravated gastrotoxicity.

Keywords
Cranberry extract; Omeprazole; Gastric ulcer; Indomethacin



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REVISED **Amendments from Version 1**

This article has been revised depending on the comments of reviewers; there are few changes in the introduction, methods, and discussion sections; however, there are no changes in the results section. Of note, the study limitations were further clarified.

Any further responses from the reviewers can be found at the end of the article

Introduction

Despite recent advancements in pharmaceutical technology, the gastrotoxicity of non-steroidal anti-inflammatory drugs (NSAIDs), which frequently result in stomach ulcers and delayed healing, continues to be a significant issue.[1,2] Gastric ulcers caused by the use of NSAIDs are caused by several factors, including the inhibition of prostaglandin-E2 (PGE2) or angiogenesis, the enhancement of the generation of free radicals, the induction of cyclooxygenase-2 (COX-2) expression, and the production of cytokines that are responsible for pro-inflammatory effects.[3–5] In addition, it has been demonstrated that NSAIDs decrease the rate at which ulcers heal by inhibiting the development of pro-angiogenic factors.[6] These pro-angiogenic factors include vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and essential fibroblast growth factor (bFGF).[7–12] Indomethacin (IND) was found to have a more significant propensity to induce damage to the stomach than traditional NSAIDs.[13]

Omeprazole, which is regarded as an important drug by the World Health Organization (WHO), is utilized extensively throughout the world to treat a variety of gastrointestinal conditions. Omeprazole inhibits the production of stomach acid by inhibiting a proton pump inside the stomach.[14] Furthermore, it lowers the levels of endogenous oxidative stress as well as cytokines that promote inflammation.[15] In recent years, several natural products have been introduced that, due to their anti-oxidative qualities, make it possible to ease gastrointestinal illnesses.[16] In this regards, several illnesses were effectively managed with botanical medicine, which entails the administration of plant-derived products.[17–20] The natural therapies are safer and display minimal undesirable effects than manufactured drugs.[21–25]

Natural substances and their medically active elements are extensively researched and evaluated as possibly successful therapies for a variety of diseases.[26–31] These substances are being used to offer gastro-protection in a variety of contexts, notably the avoidance of chronic progressive gastric disease and acute stomach ulcers.[32]

Cranberries, also known as Vaccinium macrocarpon, are an important source of phytochemicals, particularly polyphenols, citric and malic acids, vitamin C, and triterpenoids, which are required for the antioxidant activity of the fruit.[4,33] In addition to phenolic acids and benzoates, the red cranberry contains a high concentration of flavonoids, specifically proanthocyanins, anthocyanidins, and flavanols.[34,35] There are also phenolic acids and benzoates present. Cranberry can treat a variety of other problems, including gingivitis, diarrhoea, and cardiovascular health.[36] To the best of our knowledge, no previous studies were carried out to investigate the impact of cranberry extract on NSAID-induced gastric ulceration. This study aimed to evaluate the efficacy of cranberry extract in treating indomethacin-induced gastric ulcers in albino rats by exploring its effects on antioxidant enzymes, inflammatory markers, and histological findings. Furthermore, the possible mechanisms underlying its therapeutic effects are being investigated.

Methods

Materials

Experimental animals

Twenty male albino rats aged between 6 and 12 months with an average weight of 200 ± 15 g, were used in this study. The animals were acquired from the animal house of the Iraqi Centre for Cancer Research and Medical Genetics– Baghdad – Iraq and housed in it. They were placed in polyethylene cages with stainless steel covers and raised to prevent coprophagy. Rats were kept for acclimatization for one week before the experiment. They were maintained in standard laboratory conditions (25°C, 12-hour light-dark cycle) and had free access to food from a chow pallet and tap water. They fasted for 24 hours before indomethacin admiration and were allowed free access to water. The study was started at the beginning of January 2024 and finished in February 2024. This study was approved by the ethical committee for experimental studies at the College of Medicine/University of Baghdad.

Drugs and reagents

Indomethacin 60 mg/kg was purchased from Sigma Aldrich; Omeprazole 20/kg mg was obtained from its capsules marked by Acino, Zurich, Switzerland. Cranberry Extract was obtained from its tablet, Adrien Gagnon, Canada, and sodium carboxymethylcellulose was obtained from Loba Chemie, India.

Methods

Preparations of pharmaceutical solutions

According to previous studies, an oral administration of 60 mg/kg of indomethacin is needed to induce gastric ulcers.[3,4] The suspension had a concentration of 24 mg/ml. Experimentation requires a volume of 0.5 mL to provide a dose of 60 mg/kg to rats.

Regarding omeprazole, previous studies relied on an oral dose of 20 mg/kg.[37] An oral suspension was prepared for administering omeprazole orally. The suspension was made with 0.5% Na-CMC as the suspending agent, and omeprazole capsules (20 mg) were used as the source of the active component. The suspension had a concentration of 8 mg/ml. To provide a dose of 20 mg/kg for experimental rats with an average weight of 200 ± 15 g, a volume of 0.5 ml is required.

To administer cranberry aqueous extract orally, an oral suspension was made by utilizing 0.5% Sodium salt of carboxymethylcellulose (Na-CMC) as a suspending agent and cranberry extract tablets (270 mg) as a source of the active ingredient. The amount of cranberry extract was calculated based on the results of an oral acute toxicity study,[38] in which rats were given a dose of 2000 mg/kg. It showed no evidence of toxicity in their bodies. Therefore, the study utilized one-tenth of this dose, which is equivalent to 200 mg/kg, to validate the safety of the substance, as stated by another study.[39] An oral suspension of the powder was made by combining powder cranberry extract with 0.5% Na-CMC to provide a concentration of 80 mg/mL. For experimental rats with an average weight of 200 ± 15 g, a volume of 0.5 ml is necessary to provide a dose of 200 mg/kg according to standard protocols.

Experimental design

This research project was carried out in the Department of Pharmacology, College of Medicine, University of Baghdad, as well as the Iraqi Laboratory for Cancer and Biomedical Genomic Research. The current examination began on January 10, 2024 and lasted around June 30, 2024. Experimental rats were randomly assigned into four groups with each group consisting of five animals as the following:

- Group 1(n=5) is the standard control group, which was kept under normal laboratory conditions and received 0.5 mL of oral 0.5% Na-CMC suspension for 15 days by oral gavage.
- Group 2(n=5) is the ulcer induction group, which received 60 mg/kg of indomethacin at day 0 and oral 0.5% Na-CMC suspension for 15 days by oral gavage.
- Group 3(n=5) is the standard oral Omeprazole-treated group, which received 60 mg/kg of indomethacin at day 0 and omeprazole oral suspension (20 mg/kg) in 0.5% Na-CMC for 15 days by oral gavage.
- Group 4(n=5) is the cranberry extract-treated group, which received 60 mg/kg of indomethacin at day 0 and cranberry extract oral suspension (200 mg/kg) in 0.5% Na-CMC for 15 days by an oral gavage.

Tissue and blood sample collection

The animals were put under anesthesia with 87 mg of ketamine/kg of body weight and 13 mg of xylazine per kg at the end of the experiment, which was day 15.[40–42] Every attempt was established to reduce the overall number of animals employed for the experiments and minimize their misery by keeping them in private, clean boxes with a broadened metal mesh floor beneath appropriate spots, making sure they had a 12-hour span of daylight and darkness, and giving them anesthetic medications to ease any kind of discomfort or pain they may have experienced. The samples of blood were taken by performing a direct heart puncture with plastic syringes containing 5 milliliters, and then they were placed into gel tubes. The tubes were then subjected to centrifugation for ten minutes at a speed of three thousand revolutions per minute throughout the entire process.[43–47] After the complete separation of the blood, the serum was removed, then deposited into plastic tubes with a capacity of 2 mL that had not been treated, and then stored at a temperature of -20 °C for further analysis.[48–51]

Assessment of inflammatory and anti-oxidative parameters

The serum concentrations of superoxide dismutase (SOD) and glutathione peroxidase (GPx), as well as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), were measured for all experimental groups[1–4] after 15 days of the experiment. The ELISA kits employed in this investigation were generally obtainable and were prepared according to the supplier's directions (Elabscience® Laboratory, China). The first part of the procedure is to add anti-marker antibodies to a plate with 96 holes. The tubes were filled with specimens and standards, and the packaging antibodies drew any TNF-α, IL-β, SOD, or GPx from the circulation. Following detaching the unpaired biotin-associated antibody,

streptavidin and horseradish peroxidase (HRP) were properly applied to the prepared plates.[52–57] The total amount of indicators in each collection was estimated by comparing optical density to traditional charts. The plates were cleaned once again, and TMB-substrate blends were used to show the matched marker quantities utilizing the resulting color. The absorbance of the specimens was determined employing a microplate reader spectrophotometer. The color intensity is calculated at 450 nm when the color changes from blue to yellow with a stop solution.[58–63]

Haematoxylin and eosin staining

Gastric tissue slices from different rat groups have been treated with hematoxylin and eosin. The technique involves warming aluminum potassium sulfate and dispersing it in purified water. Hematoxylin was submerged in alcohol concurrently. Following heating, the two mixtures were incorporated and withdrawn from heat. A tiny quantity of mercuric oxide was progressively poured while spinning before being immersed in frigid water.[64–68]

2 grams of eosin powdered form were dissolved in 25 millilitres of distilled water, and then 475 millilitres of 100% alcohol were added to produce 0.5 litters of eosin. It produces reddish or pink colors in the cytoplasm and components of the extracellular matrix.[69–72]

Assessment of histopathological changes

The stomachs of all experimental groups[1–4] were taken on the 15th day of the trial. Following the administration of ketamine and xylazine anaesthesia, the stomachs of the animals were extracted and preserved in 10% formalin for histological inspection.[73–76] Afterward, the samples were immersed in paraffin, cut into slices that were 5 μm in thickness, and then treated with haematoxylin and eosin (H&E) stain.[77–80]

The histological slides were analyzed using standard light microscopy techniques. An experienced pathologist examined the treatment group without any prior knowledge, and only one sample slide was selected for each group.

The semi-quantitative score based on inflammation severity (0-3), haemorrhagic spot (0-3), sub-mucosal edema (0-3), and superficial mucosal ulcers (0-3) was used to assess changes in the stomach tissue between the experimental groups after 15 days for comparison purposes.[81–84]

Statistical analysis

Statistics were done in Graph Pad Prism 9. Data were introduced in SPSS version 22 (Statistical Program for the Social Sciences). Mean SD is employed to determine descriptive statistics. A statistical assessment was conducted, and various charts and tables were prepared to account for unanticipated variables. To determine group associations, ANOVA and post hoc Tukey's multiple-comparisons tests were used. Statistical significance required a P-value below 0.05. The histopathological scoring system employed median and interquartile ranges, but all other data were presented as mean and standard deviation. To analyze histological group scores, Dunn's multiple comparisons test followed the non-parametric Kruskal-Wallis test.[85]

Results

The results revealed an apparent induction of peptic ulcer with considerable macroscopic alterations in gastric tissue sections of the indomethacin group (G2) compared to the control group (G1). However, both the omeprazole (G3) and cranberry extract (G4) groups exhibited fewer macroscopic stomach modifications than the indomethacin group, culminating in a much lower severity and extent of the experimentally generated ulcer, as depicted in Figure 1.

The indomethacin group (G2) disclosed significantly reduced serum levels of antioxidant enzymes SOD and GPX in comparison with the normal control group (G1) ($p < 0.05$). Nonetheless, omeprazole (G3) and cranberry (G4) treatment groups demonstrated significantly higher serum levels of antioxidant enzymes SOD and GPX in comparison with the indomethacin induction (G2) group ($p < 0.05$). However, there were no substantial differences in SOD or GPX levels between the omeprazole (G3) and cranberry extract (G4) groups ($P > 0.05$) as seen in Figure 2.

Furthermore, serum levels of the inflammatory cytokine indicators TNF-α and IL-1β were substantially elevated in the indomethacin group (G2) when compared with the normal control group (G1) ($p < 0.05$). The omeprazole (G3) and cranberry (G4) treatment groups, however, presented a substantial reduction in serum levels of inflammatory markers TNF-α and IL-1β when compared with the indomethacin induction (G2) group ($p < 0.05$). On the other hand, it was demonstrated that the group treated with cranberry extract (G4) had substantially diminished serum levels of inflammatory mediators, including TNF-α and IL-1β, than the group treated with omeprazole (G3) ($p < 0.05$) as illustrated in Figure 3.

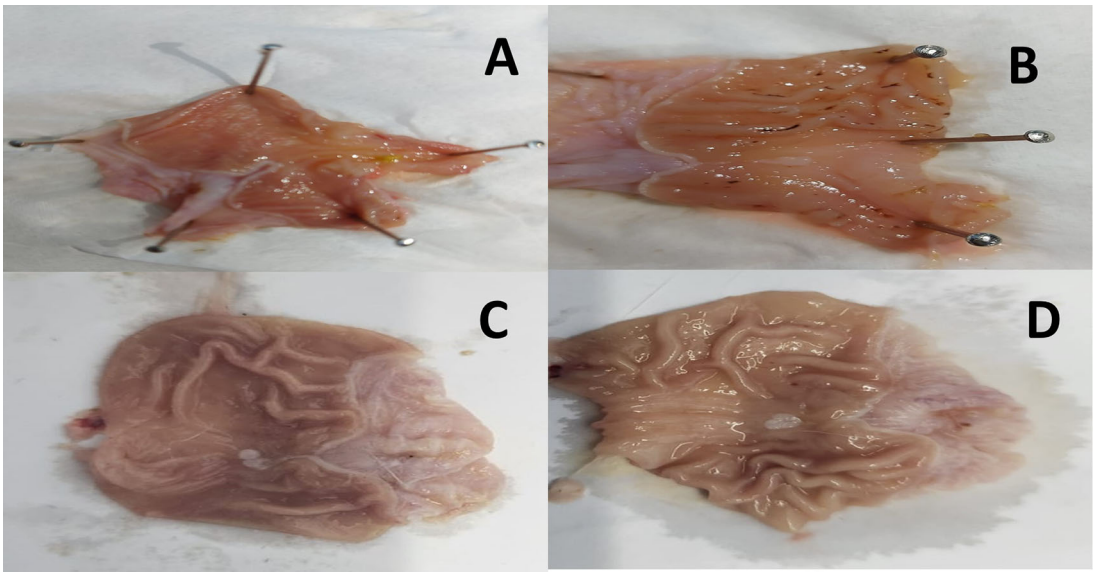

Figure 1. Representative photographs of the macroscopic structure of the stomach in rats. A = Normal control group (G1), B = Indomethacin group (G2), C = Omeprazole group (G3), D = Cranberry treatment group (G4).

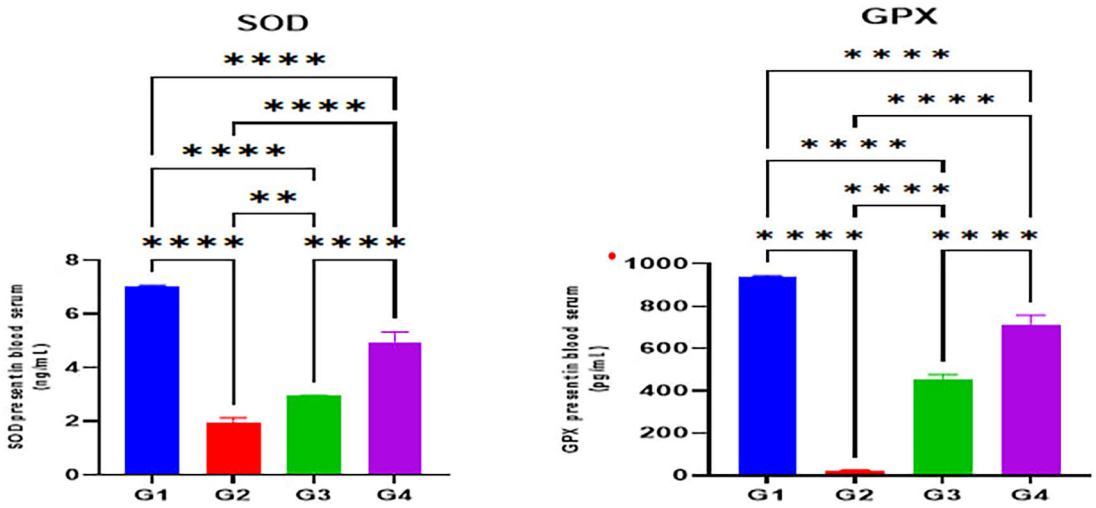

Figure 2. Effects of studied drugs on serum levels of antioxidant enzymes (SOD and GPX) estimated at day 15 of the experiment for the control (G1), indomethacin induction (G2), omeprazole treatment (G3), and cranberry treatment (G4) groups. Data were presented as Mean±SD; ****= significant differences ($p < 0.05$), n = 5 animals/group.

In addition, the histological outcomes of the present investigation demonstrated that gastric specimens from normal rats had normal appearance of the stomach mucosa, submucosa, and muscularis, as clarified in Figure 4A and Table 1.

In contrast, the rat stomach tittue section of the indomethacin group (G2) enjoyed substantial histopathological abnormalities characterised by extensive mucosal surface necrosis, profound congestion with mononuclear leukocyte infiltration, and marked degeneration of digestive glands as compared to the control group (G1), as shown in Figure 4B and Table 1.

The omeprazole treatment group (G3), on the other hand, displayed a major reductionS in indomethacin-induced histopathological irregularities as evidenced by mild congestion, mild edema, minor gastric bleeding, moderate inflammatory cell infiltration, and slight necrotic changes, as seen in Figure 4C and Table 1.

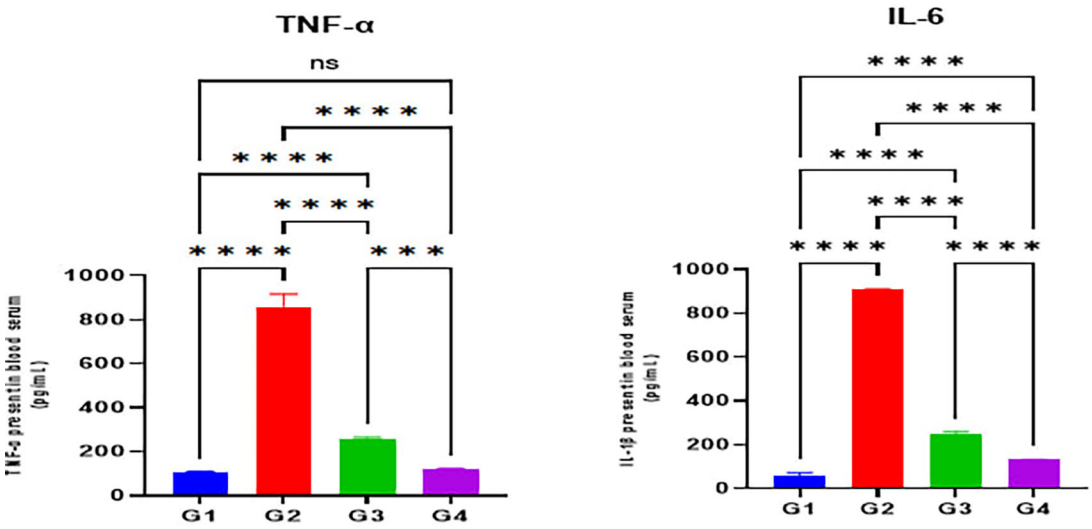

Figure 3. Effects of studied drugs on serum levels of inflammatory mediators (TNF-α and IL-1β) estimated at day 15 of the experiment for the control (G1), indomethacin induction (G2), omeprazole treatment (G3), and cranberry treatment (G4) groups. Data were presented as Mean±SD; **** = significant differences ($p < 0.05$), n = 5 animals/group.

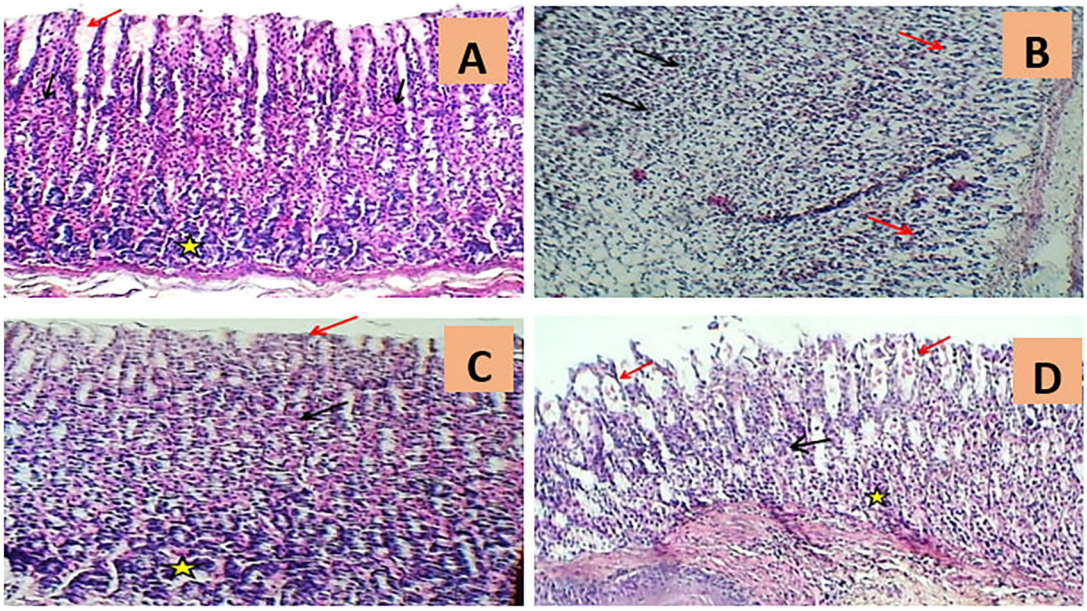

Figure 4. Exemplary photomicrographs of histopathological scores from different experimental groups of rats. A. The rat stomach histology of normal controls (G1) demonstrated the typical appearance of gastric pits (red arrow), parietal cells (black arrows), and chief cells (asterisk) (H&E 100X). B. The rat histological stomach segment of the indomethacin induction group (G2) exhibited severe massive necrotic gastritis characterized by marked mucosal surface necrosis and leukocyte infiltration (black arrows) and extensive degeneration of gastric glands (red arrows) (H&E 100x). C. The rat histological stomach segment treated with omeprazole (G3) displayed regular gastric pits (red arrow), parietal cells (black arrow), and chief cells of gastric glands (asterisk) (H&E 100X). D. The rat histological skin slice from the cranberry treatment group (G4) indicated intact gastric pits (black arrow), mild luminal sloughing of parietal cells (red arrows), and minimal cellular swelling of chief cells (asterisk) (H&E 100X).

The rat gastric section of the cranberry extract group (G4) indicated dramatically diminished histopathological modifications, including mild sloughing of parietal cells, minor cellular swelling of chief cells, little gastric haemorrhage with minimal leukocyte infiltration, congestion, and oedema. Yet, the cranberry extract group's stomach mucosa seemed to be comparable to that of the ontrol group, exhibiting normal cytoarchitecture as illustrated in Figure 4D and Table 1.

Table 1. Effects of studied drugs on the total histopathological scores of the stomach tissue in the control group (G1), indomethacin induction group (G2), Omeprazole treatment group (G3), and Cranberry treatment group (G4) on day 15 of the study.

Groups	Score in median (interquartile ranges)
G1	0(0-0.5)
G2	12(11.5-12) *
G3	0(0-1)**
G4	3(2.5-3.5)
p-value	0.000676

*Significant difference ($p<0.05$) versus control group (G1).
**Significant difference ($p<0.05$) versus indomethacin induction group (G2).

Discussion

It has been demonstrated beyond a reasonable doubt that oxidative stress and inflammation play a significant part in the etiology of indomethacin-induced injury to the stomach.[86,87] In the current study, the level of antioxidant enzymes (SOD and GPX) was found to be considerably lower in the group that was subjected to ulcer induction (G2) compared to the group that served as the control (G1) and the other treatment groups (G3, G4). Under typical circumstances, the oxidant and antioxidant defense mechanisms of the organism are in equilibrium with one another for the group that serves as the control.[88] Diminished activities of SOD and GPx indicate impaired antioxidant pathways, which are frequently seen in many inflammatory diseases.[89–91] These enzymes are necessary for neutralizing deleterious reactive oxygen species (ROS), and their absence can lead to further oxidative stress and inflammation.[92–95]

The injection of an induction dosage of indomethacin results in the production of ROS and produces oxidative stress.[96] According to research, indomethacin can bind to a region in the mitochondrial electron transport chain that is close to the complex and ubiquinone. This results in the uncoupling of the oxidative phosphorylation process and the formation of reactive oxygen species.[97] Consequently, reactive oxygen species (ROS) are responsible for the inactivation of mitochondrial aconitase, which leads to the generation of free iron, which in turn generates more mitochondrial •OH.[98] There is a correlation between oxidative stress and mitochondrial malfunction, the creation of mitochondrial permeability transition pores, and the generation of mitochondrial oxidative stress (MOS).[99–102]

Regarding the omeprazole treatment group (G3), there was a significant increase in the level of antioxidant enzymes (SOD and GPX) as compared to the ulcer induction group (G2). These findings are consistent with the findings of another research investigation, which demonstrated that omeprazole possesses antioxidant activity in addition to its antisecretory characteristics. Omeprazole was found to be a powerful scavenger of hypochlorous acid (HOCl) even at a drug concentration, and it also showed significant inhibition of iron- and copper-driven oxidant damage at pH 5.3 and 3.5, respectively, according to research that was carried out to investigate the in-vitro antioxidant effects of omeprazole at specific pH levels.[103] Another study discovered that OMP, because of its antioxidant activity, is characterized by the overexpression of superoxide dismutase in gastric mucosal cells.[104]

The cranberry treatment group (G4) showed enhanced levels of antioxidant enzymes, SOD and GPX; as compared to the Indomethacin ulcer induction group (G2). Cranberry extract contains several bioactive compounds, such as anthocyanin and flavonoids, which are well known for their antioxidant activity.[105] Anthocyanins were found to induce the expression of several antioxidant enzymes, often mediated by Nrf2-dependent pathways responsible for inducing cytoprotective responses. In an earlier investigation to test the anti-oxidant function of Syzygium cumin (L.) Skeels on indomethacin-induced acute stomach ulceration, results indicated that the anthocyanin content of this plant dramatically upregulates SOD and GPx levels as compared to untreated rats.[106] Another study conducted to explore this finding found that the mRNA and protein levels of Nrf2 in anthocyanins treatment groups were near to the control group and higher than the NSAIDs- ulcer induction group. This is related to the ability of these phytochemicals to induce Nrf2 expression or inhibit its proteasomal degradation by modifying the Nrf2–Keap1 complex.[107]

Several other flavonoids with antioxidant ability were reported in cranberries, such as rutin, apigenin, and quercetin.[108] Rutin is among the flavonoids that exert antioxidant and free radical scavenging activities.[109,110] In a study investigating the antioxidant activity in rats with indomethacin-induced ulcers, rutin could significantly lessen the oxidative stress biomarkers deteriorated by indomethacin treatment.[111] Rutin has been proven in several laboratory investigations to boost glutathione levels and superoxide dismutase activity by capturing superoxide anions and scavenging free radicals, while also modulating TNF-α and IL-6 concentrations.[112–114]

Regarding quercetin, the most potent antioxidant flavonoid, studies demonstrated that it has a beneficial effect in attenuating indomethacin-induced gastric ulcers in rats by increasing the antioxidants enzymes activity (Catalase, SOD, and GPX).[91,94] The mechanisms behind the antioxidant activity are scavenging oxygen radicals, protecting against lipid peroxidation, and chelating metal ions.[115–119]

In addition to oxidative stress, inflammation is also a crucial factor in the pathophysiology of gastropathy that is generated by nonsteroidal anti-inflammatory drugs.[120–122] Based on the findings of the present study, it was observed that the level of inflammatory markers, specifically TNF-α and IL-1β, was considerably greater in the group that was subjected to ulcer induction (G2) compared to the group that served as the control (G1) and the other treatment groups (G3, G4). There is a direct connection between the formation of reactive oxygen species (ROS) and the oxidative stress that leads to a rise in the expression of TNF-α and IL-6 genes, which in turn leads to an increase in their levels through the nuclear factor kappa (NF-κB) dependent pathway.[39,123,124] More specifically, TNF-α acts to facilitate immune system responses and cellular proliferation. It encourages the translocation-related process of NF-κB, which aids communicating signals during inflammation.[20,125–128]

Regarding the Omeprazole treatment group, there was a significant decrease in TNF-α and IL-6 levels compared to the ulcer induction group (G2). This indicates that omeprazole has an anti-inflammatory effect independent of suppressing gastric secretion. The suggested mechanism behind these effects is postulated to be related to the down-regulation of nuclear factor kappa (NF-κB) with subsequent suppression of pro-inflammatory cytokines, as reported by other researchers.[129,130]

Presently available therapeutic options for ulceration of the stomach feature a significant relapse probability. Natural remedies for ulceration management and therapy are not rare, since communities have historically used plant components.[131] A large fraction of the vegetable variety stays untapped for medicinal purposes. Antiulcer capabilities in botanical products exhibit potential, and many animal models are employed to assess their efficacy.[132] Competent experiments are required to evaluate the ulcer-preventing properties of botanicals and medicines. Such models are useful for understanding the pathological causes of wounds, as well as the antioxidative capabilities of critical medications or compounds with antiulcer effects.[133] There are several models for assessing anti-ulcer medications, rendering it difficult to pick an acceptable model.

The group that received cranberry treatment (G4) demonstrated a noteworthy reduction in the levels of TNF-α and IL-6 when compared to the levels that were observed in the group that was subjected to Indomethacin ulcer induction (G2). There is a connection between this discovery and the anti-inflammatory properties of cranberry extracts, which are primarily comprised of anthocyanins and proanthocyanins.[39,134] The principal mechanisms by which anthocyanin compounds diminish inflammation involve hampering NF-κB, a transcriptional element that is vulnerable in terms of inflammatory and oxidative processes.[135] NF-κB is a substance produced by cells and located in the innermost part of the cell. It is inert due to its strong-affinity suppressor, IκB, which retains it in the cytoplasm and prevents its liberation.[136] Once triggering events, like as oxidative damage, occurs, a substantial signaling chain is initiated. The chain of reactions promotes IKK-a and IKK-b, two kinases that metabolize IκB.[129] Phosphorylation of IκB causes its disassociation, allowing NF-κB to relocate to the nucleus and attach to κB activation regions. This drives gene expression of chemotactic cytokines involving TNF-α and IL-1β.[137] Additionally, the phosphorylation of Mitogen-activated protein kinase (MAPKs) enzymes was suppressed by anthocyanin extract, and as a result, activation is necessary to mitigate the inflammatory response.[138] MAPKs, which include ERKs, c-JNKs, and p38, are a family of enzymes that react to various stimuli, one of which is inflammation. These enzymes, in turn, govern a wide variety of cellular responses, such as cell differentiation, mitosis, and apoptosis.[139] It is necessary to phosphorylate MAPKs for them to become active, as their base form is inactive from a catalytic perspective.[140]

Furthermore, it has been proposed that the anti-inflammatory action that is brought about by cranberry extracts is due to the flavonoid component of the cranberry, which prevents neutrophils from initiating the infiltration process.[141] Several factors can lead to neutrophil infiltration, including the reduction in mucosal blood flow after IND administration.[142] According to the findings of a study, the flavonoid product known as rutin helped to boost the activity of cNOS, which in turn led to an increase in the levels of nitric oxide in the mucosal tissues of the stomach. Nitric oxide that is produced from cNOS can increase mucosal blood flow and tissue perfusion, which ultimately results in a significant reduction in neutrophil infiltration.[143]

According to the results obtained, the highest ulcer index score was reported for the Indomethacin ulcer induction group (G2), which was significantly higher than the control, Omeprazole, and cranberry treatment groups. This is due to gastrotoxicity of a high dose of indomethacin inducing an inflammatory response and oxidative stress damaging the

gastric mucosa coupled with decreasing the mucus and bicarbonate layer as a result of NSAIDs use which makes gastric tissue more liable to acidic damage of the gastric secretions.[13] On the other hand, the Omeprazole treatment group (G3) showed less ulcer score than the indomethacin ulcer induction group (G2) since omeprazole is a PPI that inhibits Na +/K+ ATPase enzyme leading to suppression of acid secretion.[14] Furthermore, from the previous results, omeprazole displayed antioxidant and anti-inflammatory actions that collectively contributed to their anti-ulcer effects.[15] The cranberry extracts treatment group showed an ulcer score that is significantly lower than the Indomethacin ulcer induction group (G2) and Omeprazole treatment group (G3) because of their potent antioxidant and anti-inflammatory effects owing to their flavonoid content.[36] The limitations of the current study could be summarized by the small number of animals used and the lack of estimation of the exact molecular mechanism through which cranberry extract exerts its gastro-protective effect. Estimation of Malondialdehyde (MDA) tissue levels, being the product of lipid peroxidation and the expression level of nuclear factor erythroid 2-related factor 2 (Nrf2) mRNA is usually used to predict the pathways of enhancing antioxidant activity while the nuclear factor kappa (NF-κB) mRNA to estimate the anti-inflammatory activity of the extract. Moreover, it is advised that future studies on female rats be conducted to identify gender-related differences. Furthermore, there is an urgent need for future research to explore the composition of phytomolecules present in cranberry extract using various separation techniques such as HPTLC-MS, NMR, LC-MS, etc.

Conclusions

The current study's findings revealed that cranberry extract proved its efficacy as a potential treatment for gastric ulcers caused by nonsteroidal anti-inflammatory drugs (NSAIDs) due to its anti-inflammatory and antioxidant properties, as confirmed by biochemical and histological analysis.

Ethical approval

The research project has been approved by the Institutional Review Board (IRB) of the University of Baghdad's College of Medicine. The Declaration of Helsinki's requirements and guidelines were scrupulously adhered to in the course of conducting the current investigation. The ethical authority at University of Baghdad's College of Medicine confirmed the required documentation and client data with approval number (UoB.Med.03-29) on December 31, 2023.

Author contributions

Zaid Mahmood Abdul Majeed conducted the investigation, wrote and refined the first draft of the document, participated in its design and provided financing and other forms of assistance, donated supplies, equipment, and lab animals, and finished the final copy of the research article.

Mohammed Qasim Yahya Malallah A. Al-atrakji created the theoretical framework for the researched project, specified the parameters of the exploratory analysis, and outlined the main goals through an in-depth assessment of the findings, supplemented with insightful criticism and supervision.

Data availability statement

Underlying data

Figshare: The Potential Effects of Cranberry Extract on Indomethacin-induced Gastric Ulcers in Rats https://doi.org/10.6084/m9.figshare.28236107.v4[144]

This project contains following underlying dataset:

1. dr.zaid results.xlsx

2. histopathology of stomach.png

Reporting guidelines

Figshare repository: ARRIVE checklist for 'Potential Effects of Cranberry Extract on Indomethacin-induced Gastric Ulcers in Rats'. https://doi.org/10.6084/m9.figshare.28236107.v4[144]

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Open Peer Review

Current Peer Review Status: ✔ ? ?

Version 2

Reviewer Report 25 June 2025

https://doi.org/10.5256/f1000research.183327.r390699

?

Vishal Dubey
Sundyota Numandis Probioceuticals Pvt. Ltd, Ahmedabad, Gujarat, India

The manuscript entitled "**The potential effects of cranberry extract on indomethacin-induced gastric ulcer in rats**" aims to evaluate the effect of cranberry extract in preventing the severity of gastric mucosal ulcers resulting from indomethacin-induced gastric toxicity. In the revised manuscript, the authors have significantly improved overall readability and addressed most major queries. However, the following observations remain:

1. **Inconsistency in study timeline:** The manuscript mentions that the study was conducted between January and February 2024 in one section, and between January and June 2024 in another.
2. **Method of gastric tissue collection:** In the Methods section, describe how the gastric tissue was collected. If gastric tissue was stored for certain duration, then provide details of storage condition as well.
3. **Cytokine inconsistency (IL-6 vs IL-1ß):** Both IL-6 and IL-1ß are mentioned throughout the manuscript. Confirm which cytokine was actually studied and ensure consistency across the entire text.
4. **Histological scoring:** If only one histological slide was examined per group, clarify how the median and IQR of histological scores were calculated.
5. **Unexplained symbol in Figure 2:** The asterisk symbol ("**") used in Figure 2 is not defined in the figure legend.
6. **"ns" in Figure 3:** The term "ns" appears in Figure 3 but is not defined in the figure legend.
7. **Inconsistent results reporting:** The Results section states that the levels of SOD and GPx in the cranberry and omeprazole groups are comparable ($p > 0.05$). However, Figure 2 suggests that the cranberry group shows significantly higher SOD and GPx levels than the omeprazole group.
8. **Labeling error in Figure 3:** The title of Figure 3 is "IL-6," while the axis label refers to "IL-1ß." Confirm whether IL-6 or IL-1ß.
9. **Contradiction in histological scores:** The Results section states that the histological score of the cranberry group is equal to that of the control group. However, Table 1 shows that the omeprazole group has a score equal to the control group.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Herbal and Dietary Supplements; Pharmacology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 1

Reviewer Report 03 June 2025

https://doi.org/10.5256/f1000research.174603.r385778

?

Vijayakumar A. R
Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India

DEAR AUTHOR,
The following point is to be clarified

1. What is the rationale behind the use only in male animals?

2. What is the normal lab temperature condition in Iraq (because 232°F was mentioned in the text)?

3. Is there no significant effect on the control and cranberry-treated group in the TNF-alpha study (figure 3)?

4. In the discussion, it was mentioned that the extract significantly reduced inflammatory markers, such as TNF-alpha; however, the result in Figure 3a showed no significant change in TNF-alpha.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pharmacology and Toxicology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 29 May 2025

https://doi.org/10.5256/f1000research.174603.r385782

?

Vishal Dubey
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The manuscript entitled *"The potential effects of cranberry extract on indomethacin-induced gastric ulcer in rats"* aimed to evaluate the effect of cranberry extract in preventing the severity of gastric mucosal ulcers due to indomethacin-induced gastric toxicity. The authors have utilized histopathological tests and antioxidant and anti-inflammatory biomarkers to identify the effect of cranberry extract compared to appropriate standard, disease, and normal control groups. The results of the study are promising that supports the antioxidant and anti-inflammatory property of cranberry, further supporting its putative application in preventing NSAIDs induced gastric toxicity. While, the study is described in sufficient quantity and the observations of the study support the study conclusions, extensive language and grammatical errors observed in the entire manuscript. Great amount of work is required for updating the language of the manuscript. But certain major observations were identified as described below:

ABSTRACT

1. In methods section, it is mentioned that 24 rats were included and randomized in four groups with six rats per group, while in methods it is described that 20 rats were included with five rats per group.
2. In methods section, it is mentioned that rats were euthanized on day 16, while in manuscript it is mentioned that euthanasia was performed on day 15.

INTRODUCTION

1. Many words were identified for abbreviations which are not again used in the entire manuscript. For example: PGE2, VEGF, PDGF, bFGF. In academic writing, abbreviations must be identified for only those words that are being used for at least three or more times. Kindly check the entire manuscript for such abbreviations.
2. The abbreviations for indomethacin and omeprazole has been identified in the introduction section, but still many instances are observed that full word “indomethacin” and “omeprazole” instead of IND and OMP. In academic writing, the identification of abbreviations must ensure that the abbreviations are used consistently throughout the manuscript instead of the word. Kindly check the entire manuscript for such inconsistency.
3. The statement “... particularly polyphenols, which are required for the antioxidant activity of the fruit” can be rephrased as now it is providing the impression that only polyphenols are the components present in the fruit that is responsible for the antioxidant activity, while there are other ingredients as well (like citric and malic acids, vitamin c, and triterpenoids) that plays important role in the antioxidant effect of the fruit.
4. The statement “The traditional usage of cranberry for treating .... Treatment of NSAID-induced gastric ulcers” is confusing. Is the statement trying to explain the historical use of cranberry in disease conditions like UTI or describing the aim of the study. Separate statements are required for both the instances to prevent confusion.
5. The statement “As far as our knowledge, no previous studies were carried.” Is incomplete. What are the literature or scientific gap that is getting highlighted by this statement?

METHODS

1. The title of this section “methods” is identified twice. So suggestion to change the heading to “Materials” instead of “methods”
2. In experimental animals sub-section, any reason for including only male rats? If the study is based on any previous study methodology, then the limitations of that study methodology is required to be mentioned in the limitation section of this study as well, including this point as well.
3. The statement “They fasted for 24 hours ....free access to water.” does not belongs to this section and needs to be placed in the experimental design section.
4. Kindly provide the ethics committee approval number or protocol number for future study identification.
5. In drugs and reagents sub-section, the individual dose of IND and OMP (60 and 20 mg/kg) is not required to be mentioned in this section, as it will definitely let confusion on the strength of the drugs used.
6. Kindly mention the %strength of IND and OMP used in this study. Strength of drug (as %Assay) is crucial information that helps better replication of the current study in future.
7. Typo-error, use “marketed by Acino” instead of “marked by Acino”.
8. Identify the abbreviation (Na-CMC) for sodium carboxymethylcellulose in this section, as it is appreared for the first time in this section rather than in the latter sections. In academic writing, abbreviations must be identified on the first identification of the word and then abbreviation used consistently.

METHODS

1. The title of this section is already identified previously. Kindly make appropriate changes.
2. In preparation of pharmaceutical solutions sub-section, describe the method by which the

IND suspension was prepared. It is just mentioned that IND suspension of strength 24 mg/ml was prepared but method is not described.

3. In preparation of pharmaceutical solutions sub-section, for OMP and cranberry solutions, commercially available products were utilized. But as pharmaceutical preparations comprises of excipients as well, was the method of solution preparation adjusted to meet the dose of 20 mg/kg for OMP and 200 mg/kg for cranberry. Or just the pharmaceutical preparation was suspended in Na-CMC and the prepared suspension was supplemented. In such case, the dose might not reach to the limit identified for OMP and cranberry. It is advised that in case the commercially available products are used as investigational therapy, detailed method of preparation is mandatory, along with mentioning the type of excipients are present in the formulation (if the data can be obtained from the manufacturer). The process of investigational therapy preparation is critical for study future replicability.
4. In preparation of pharmaceutical solutions sub-section, the statement "To provide a dose of .....a volume of 0.5 ml is required" can be rephrased as "To provide a dose of 20 mg/kg/rat, a dose of 0.5 ml of prepared suspension was administered". Authors must always try to minimize the use of repetitive information in the entire manuscript, especially in the methods and results section, in this case is the average weight of rats which is already described in previous section. Similar observation is evident in following paragraphs as well, so changes in entire manuscript is expected.
5. In experimental design sub-section, the site "Iraqi Laboratory for Cancer and Biomedical Genome Research" is identified as one of the study sites, while in the previous experimental animals sub-section, the site "Iraqi Centre for Cancer Research and Medical Genetics" was identified as site for animal housing. Are both the sites same or different? If different, then the role of the site "Iraqi Laboratory for Cancer and Biomedical Genome Research" is required to be mentioned.
6. In experimental design sub-section, the study is identified to last till June 30, 2024, while in previous experimental animals sub-section, it is mentioned that study finished in February 2024. Authors necessarily will have to conduct rigorous evaluation of entire manuscript to identify and rectify such major inconsistencies.
7. In experimental design sub-section, identify the group abbreviations as G1, G2, G3, and G4 in this section rather than in the results section. For example, it can be written as Group 1 (G1; n=5)
8. In tissue and blood sample collection sub-section, the day of euthanasia is missing. As per abstract, euthanasia is performed on day 16, while anaesthesia is done on day 15. When was euthanasia done? If euthanasia is done on day 15, then were the animals supplemented with their designated therapy and then euthanasia performed, or if done in day 16, then were the animals fasted overnight?
9. In tissue and blood sample collection sub-section, method of euthanasia is missing.
10. In tissue and blood sample collection sub-section, method of stomach and gastric tissue collection is missing.
11. In academic writing, numbers from 0-9 are written as full name (zero, one, ...), while any other value is written as numerical. In tissue and blood sample collection sub-section, the value "three thousand revolutions" can be written as "3000 revolutions".
12. In tissue and blood sample collection sub-section, the statement "Every attempt was established .....pain they may have experienced" can be removed, as this is the responsibility of the animal EC. Animal EC ensures this principle and only then provides the study approval. The description of animal EC role and responsibility in this section is not

recommended.

13. In assessment of inflammatory and anti-oxidative parameters sub-section, the inflammatory biomarker evaluated were TNF-a and IL-1b or IL-6. In many instances (like in the results section), the use of IL-1b is identified, while in certain instances (like title of Figure 3), IL-6 is identified. Kindly confirm, IL-1b or IL-6, and make changes in entire manuscript accordingly.
14. In assessment of inflammatory and anti-oxidative parameters sub-section, what were the analytical sensitivities of the ELISA kits for the evaluated biomarkers. This is essential for study replicability.
15. In assessment of inflammatory and anti-oxidative parameters sub-section, were the collected serum directly utilized for biomarker analysis or further serum processing was performed. Kindly mention the same in this section, and if processing was performed than describe the method in sufficient detail to ensure future study replication.
16. In haematoxylin and eosin staining sub-section, identify the abbreviation H&E here rather than in the following sections.
17. In academic writing, the SI units are always written in abbreviated form and not mentioned in full form. So in the haematoxylin and eosin staining sub-section, the grams, and millilitres, litters can be written as gm, ml, L, respectively.
18. In assessment of histopathological changes sub-section, the statement “An experienced pathologist examined the treatment group without any prior knowledge” can be phrased as “An experienced pathologist who was blinded to the therapy in9tervention, examined the H&E stained tissues”.
19. In statistical analysis sub-section, it is defined that median and IQR was calculated for histological scores. But in previous sub-section, it is mentioned that only one sample slide was selected and evaluated from each group. How is it possible that only one sample is evaluated and median and IQR is calculated?

RESULTS

1. In Figure 2, the symbol ** in SOD graph is not defined in the figure legend. Also, it is advised that five * can be replaced with single * in the graph to make the figure less cluttered.
2. As per Figure 2, cranberry therapy showed significantly higher SOD and GPx levels compared to OMP group, while in results section it is mentioned that no difference between is observed between OMP and cranberry groups.
3. Consider changes in figure 3 similar as figure 2 regarding use of * instead of ****. Also, identify “ns” in the figure legend.
4. In figure 3, it is mentioned IL-6 as title, while figure Y-axis is termed as IL-1b.
5. In the statement “In contrast, the rat stomach tittue section ….”, try use alternative term to the word “enjoyed” as the described statement belongs to rats in the IND-group. Also, rectify the spell error “tittue” which is “tissue”.
6. As per table 1, the OMP group showed histological scores similar to normal control group, while in the results section, the statement “Yet, the cranberry extract group’s stomach ….. as illustrated in Figure 4D and Table 1” denotes that the cranberry group showed histological scores similar to normal control group.

DISCUSSION

1. More emphasis must be given on the strength and limitations of the study.

2. The study did not evaluated the effect of OMP + Cranberry therapy (to identify synergism or additive effect), the optimal dose of cranberry for preventing IND-induced gastric toxicity was not identified (only one dose was evaluated), the effect of therapies on symptomatic parameters (food and water intake level) was not presented. All these points are potential limitations of the current study. Identifying potential limitations of study does not down-grade the observations of the current study, but provides potential pathway for future experimental studies. It helps researchers identify the potential limitations, that might remain undermined if not elaborated clearly in this section.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Herbal and Dietary Supplements; Pharmacology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 17 April 2025

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Suraj N Mali
Dr. D.Y. Patil Deemed to Be University, Navi, Mumbai, Maharashtra, India

Authors herein elaborated more on 'The potential effects of cranberry extract on indomethacin-induced gastric ulcer in rats'. However, I would like to see the phytomolecules present in them. In any way, authors can provide the composition by HPTLC-MS, NMR, LC-MS,etc.
Why only male rats have been chosen? In order to see more gender specific outcomes, they can include Female as well. However, authors can give a proper reference with justification.
A discussion part lacks detailing on phytocompositions. Please go through articles and update the literature in the introduction section: *Biol Trace Elem Res* (2024). [Ref 2]; [Ref 1]

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Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Anti-Ulcer agents

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.