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Research Article
Europinidin Enhances Healing through Modulating Antioxidant Processes in Experimentally Induced-Stomach Ulcer Condition

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Abstract

Background and Objective: Anthocyanidin, a flavonoid generated from plants, has a wide spectrum of therapeutic benefits, according to research. Europinidin is a natural water-soluble pigment derived from delphinidin, an anthocyanidin with an O-methyl ring. In this study, the anti-ulcerative properties of flavonoids were assessed using an experimental animal model of ethanol-induced stomach ulcers. **Materials and Methods:** In an experimental rat model, the therapeutic effectiveness of flavonoid 10 mg kg^{-1} was investigated using a 10 days procedure for pharmacological evaluation ulcer score, pH, total acidic content and biochemical markers such as superoxide dismutase (SOD), catalase activity (CAT), glutathione (GSH), myeloperoxidase (MPO), malondialdehyde (MDA), Tumour Necrosis Factor-α (TNF-α), nitric oxide (NO) and prostaglandin E2 were all investigated. **Results:** When compared to the control cluster of ethanol, stomach parameters such as ulcer score, pH and acidic content were maintained in the flavonoid clusters. In comparison to the normal cluster, gastric ulcers have a considerable rise in serum levels, GSH, SOD and CAT as well as a decrease in MDA, TNF-α and prostaglandin E2 characteristics. In the test clusters, flavonoids significantly improved all serum parameters. **Conclusion:** The results of this study showed that it is possible to produce cost-effective phytochemical alternatives for the treatment of chemically generated stomach ulcers.

Key words: Europinidin, anthocyanidin, ethanol, flavonoid, gastric ulcer, myeloperoxidase, nitric oxide

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The most prevalent gastrointestinal tract ailment in the world is a gastric ulcer. Men are three to four times as likely as women to get ulcers, which usually emerge as they become older[1,2]. Local lesions of various etiologies have been reported to spread laterally into the stomach ulcer if left untreated, owing to a clinical imbalance between physical, chemical and psychological elements linked to gastric protective factors[3]. Chronic alcohol usage, cigarette chewing, excessive use of NSAIDs as well as *Helicobacter pylori* infections and severe stress are the most prevalent contributory aggressive type factors involved[4]. The most common cause of stomach mucosa injury is excessive and uncontrolled alcohol consumption[5]. Furthermore, those who consume ethanol have an increased chance of developing ulcers[6]. As a result, the ethanol-induced experimental animal model will be the most extensively used procedure for anti-ulcer activity screening[7-9]. Despite the abundance of synthetic medicines commercially accessible for the treatment of many human diseases, there is a global movement to recognize the importance of alternative and traditional medical systems in the treatment of human disorders[10]. Medication interactions, rising medication costs, microbial resistance and adverse and hazardous events linked with their usage are only a few of the challenges that have plagued accessible drug therapy[11,12]. The stomach mucosa is made up of several layers and any injury to this layer causes aberrant gastric secretion, which includes gastric acid, the generation of free radicals via nitric oxide synthase and lipid peroxidation[13]. Stress, alcohol and cigarette usage, sedentary lifestyles, irrational medicine use, chronic illness conditions and bile salt reflux are all pathogenic variables that contribute to the formation of stomach ulcers[14]. The pathogenesis of ethanol-induced gastric ulcers is linked to stomach cell necrosis, vascular damage and the establishment of gastric ulcers. As a result of the ethanol metabolism process, numerous free radicals are produced inside the body[15,16]. Similarly, persistent ethanol use activated specific biomarkers linked to inflammation, including cytokines as well as nitric oxide production[17]. Trefoil Factor Family 2 (TFF2), a peptide found mostly in the gastrointestinal system, especially in mucosal neck cells and epithelial cytoplasm, is known to have therapeutic properties against stomach ulcers by stabilizing the mucin gel layers[18,19]. Proinflammatory cytokines such as Tumour Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) have been proven to have a variety of inflammatory and immunological effects[20]. One of the transcription factors (NF-κB) is involved in the overproduction of proinflammatory biomarkers such as cytokines, growth factors and chemokines from ulcer tissues[21,22]. The implications of phytoconstituent as an alternative in clinical interventions for prevention and therapy have been widely acknowledged in the contemporary environment[23-25]. According to recent research, gallic acid has a gastroprotective action against stomach ulcers caused by ethanol in rats shows that it is effective in an ethanol-induced stomach acid model[26]. Previous research on the plant ingredient indicated its promise as an anti-asthmatic[27], anti-inflammatory[28], anti-oxidant neuroprotective[29] and capacity to block tyrosinase[30]. Mother earth will be the first source for examining the wide collection of agents originating from plant sources for hundreds of years[31]. All gastrointestinal illnesses, including gastric ulcers, are known to be effectively treated using phytoconstituents having therapeutic qualities of secondary metabolites[32]. Recent research on plant-derived elements suggested that secondary metabolites found in medicinal plants may have gastroprotective properties[33]. Flavonoids are a class of bioactive chemicals present in a variety of diet foods based on plants. Regular intake has already been related to a decreased risk of cardiovascular disease, cancer, neurological disorders, gastric peptic ulcer and diabetes, among other chronic conditions[34,35]. Among other things, flavonoids protect cells from free radicals, inflammation, bacterial infection and also from harmful acids secreted in the stomach[35]. Flavonoids increase the antioxidant defense system GPx, GSH, SOD, CAT and the nuclear Nrf2 protein level to scavenge ROS, reducing lipid peroxidation and maintaining cell membrane and gastrointestinal tissue integrity[35]. Immunomodulator, protection from neuronal damage and free radical scanvangering actions have been revealed, in addition to therapeutic benefits against a variety of disorders such as diabetes, cancer and inflammation in anthocyanins, clan of flavonoid and they have been found to be useful in preventing various neurological conditions like Alzheimer's disease and Parkinson's disease[36].

Europinidin, a new anthocyanidin discovered in the petals of *P. europea*. Both are monomethyl and 5, 3'dimethyldelphinidin, respectively[37]. Europinidin is a bluish-red coloured water-soluble plant dye derived from delphinidin. It is an anthocyanidin with an O-methyl ring that is deemed crucial[38]. However, this bio-considerable constituent's antioxidant and antiulcer potential in ethanol-induced stomach ulcer experimental animals has yet to be reported. As a result, the proposed scheme looks at the role of europinidin in ulcer protection in a peptic ulcer experimental animal model.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Pharmacology, Trans-genica, India from November, 2021 to January, 2022.

Animals: A total of 24 male Wistar rats (150-200 g), were randomly bred and then acclimatized to regular laboratory settings in this study. The health of the procured animals was assessed and rats of around 2 months of age were chosen for the current study. Cages made from polypropylene materials were utilized to keep the experimental animal with steel stop grills and feed grills with nozzle orifices, as well as autoclaved husk as bedding materials. Throughout the inquiry, a 12:12 light:dark cycle was maintained, with ambient temperatures not exceeding 30°C and relative humidity not exceeding 50%. Experimental animals were fed conventional pellet food and given free access to drinking water. The Institutional Ethics Committee approved the current study (IAEC/TRS/PT/021/009).

Chemicals: Merk Pvt., Ltd., in India supplied the ethanol. The commercially available biochemical estimation kits were purchased from Modern Lab, M.S. India. The experiment was performed using standard reagents and chemicals.

Acute toxicity study: Europinidin's acute oral toxicity was assessed using the OECD ANNEX-423 criteria (LD_{50}). Europinidin was given orally to rats (200 mg kg^{-1} b.wt.), according to previous toxicity studies[39].

Treatment procedure and experimental design: The experimental design of the current investigation is based on a recently published study by Rahman *et al.*[7], with certain modifications in a well-connected manner. The gastric ulcers in rats were generated by administering 5 mL kg^{-1}, per oral a solitary dosage of ethanol by gastric gavage on day 10 after 24 hrs of fasting. The animals were separated into four clusters, cluster-1 (vehicle solution control), cluster-2 (ethanol control), cluster-3 (europinidin control 10 mg kg^{-1}), cluster-4 (ethanol+europinidin 10 mg kg^{-1}). Before generating a stomach ulcer in all experimental animals in clusters 2 and 4 by promptly dosing 5 mL kg^{-1} ethanol on day 10, before ethanol administration, all animals got the per se and scheduled dose, cluster-wise, for up to 10 days. The vehicle solution control cluster received simply the solution vehicle, the europinidin control cluster received 10 mg kg^{-1} per oral europinidin and the treatment cluster received flavonoid europinidin 10 mg kg^{-1} per oral. The animals were given 5 mL kg^{-1} of 100% ethanol orally after fasting for 24 hrs.

Preparation and collection of biological samples: Following a 60 min ethanol infusion, decapitated the animals. To eradicate clotting, the guts of animals were extracted, dissected along the greater curvature and cleansed with saline-cooled phosphate buffer solutions. The materials of the stomach have been taken to determine the pH. To determine the ulcer area, the tissues of the stomach were wiped, glued on cardstock made of paraffin and then images have been taken. Each piece of stomach tissue was split into two similar halves, one on the right and one on the left. For histological and immune histochemical analysis, all of the animals' right halves were immersed in a formal saline solution. The biochemical properties of the left half were examined after homogenization.

Biochemical analysis

Ulcer index: According to Zhang *et al.*[40], the extent of stomach mucosal ulcer index was assessed and scored. The ulcer index was scaled up using ulcer scorings that appeared as follows.

The 0 indicates free from ulcer condition, 1 indicates superficial mucosal erosion, 2 indicates deep ulcer or transmural necrosis and 3 indicates a perforated or penetrated ulcer[41].

Measurement of pH: The gastrointestinal contents of all animal clusters were extracted and centrifuged and the gastric juices from the supernatant liquid were measured using a digital pH meter as suggested by Bigoniya and Singh[42].

Total acidity: To quantify total acidic content, we used the approach published by Santin *et al.*[43], which included mixing 1 mL water with 1 mL centrifuged stomach content. After that, add of phenolphthalein and 0.01N NaOH was used to titrate the solution until it became pink. It was determined how much titar (NaOH 0.01N) was required and total acidity is calculated.

Pepsin activity: Based on previously reported, a stop-point bioassay of denatured haemoglobin hydrolysis was utilised to test pepsin activity[44]. A total of 10 mg of p-nitro phenyl sulfite was dissolved in 2 mL acetonitrile to make a substrate stock solution. For at least 3 hrs, this combination should be stored on ice. The procedure for performing the test is as follows: 100 L of the substrate solution is aliquot into 10 mL of a 0.01 M glycine hydrochloride buffer. When the pH was

between 1.8 and 2.0, the enzymatic hydrolysis frequency was optimum. As a consequence, for this experiment, pH 1.9 was employed. The existence of a little amount of turbidity in the substrate indicates that it hasn't been hydrolyzed yet, so it might be utilized in the experiment. Because this functioning substrate solution hydrolyzes entirely in less than 8 min, the next stages are carried out as promptly as feasible. The functioning substrate liquid is put into a standard cuvette and the same solution is promptly added to the specimen cuvette. The specimen cuvette is immediately placed in a spectrophotometer with a cell chamber kept at 25°C and 1-10 IL of pepsin. The amount of p-nitro phenol produced following exclusively enzymatic hydrolysis of the precursor is estimated using the rate of change of absorbance at 320 nm.

Malondialdehyde (MDA): The approach described in 1979 was used to determine how much malondialdehyde (MDA) was present in the stomach[45,46]. The tissue was homogenised in a buffer solution of phosphate and centrifuged at 10,000 r/min and MDA was calculated from the supernatants. The supernatant was incubated for 1 hr at 95°C with 0.8% thiobarbituric acid, 8.1% SDS and 20% acetic acid and absorbance was measured spectrophotometrically at 532 nm. The standard curve was created using known quantities of MDA standard (1,1,3,3-tetra methoxy propane) and was expressed in micrometres of MDA per gram of tissue.

Myeloperoxidase (MPO) activity: The MPO levels were measured using a modified technique based on myeloperoxidase, a neutrophil infiltration marker[46]. The procedure involves resuspending pellets made from gastric tissue homogenate in PBS (50 mM) at a pH of 6.0 and adding hexadecyl trimethyl ammonium bromide to the mix (0.5%). The resulting mixture was frozen and sonicated using a sonicator. The final suspension solution was centrifuged again (4°C/15 min/15000 rpm) and the resultant supernatant was analysed at 532 nm for MPO activity measurement using o-dianisidine dihydrochloride and 0.005% hydrogen peroxide.

Superoxide dismutase (SOD) activity: According to McCord and Fridovich's technique described in a previously published paper[46], gastric mucosa samples were homogenised (1:150) in a solution of 50 mM PBS and 100 mM EDTA (pH 7.8). A 0.1% Triton solution was added to the homogenate. At pH 7.8 and 25°C, the test technique employed 10 M ferricytochrome c, 50 M xanthine as an O_2 source and enough milk xanthine oxidase (5 nM) to achieve a rate of rising in absorbance of 0.025/min[47]. At 550 nM and a rate of 0-70 sec, the reaction kinetics was monitored in a spectrophotometer. The results are given in units of SOD per milligramme of tissue. The quantity of enzyme that inhibits cytochrome c reduction by 50% is defined as one unit of SOD.

Nitric oxide: The nitric oxide (NO) assessment is based on a previously published study of the diazotization process, in which the concentration was determined by measuring the nitrite generated as a result of nitric oxide oxidation[48].

Reduced glutathione (GSH): The method described by Rehaman *et al.*[7], was used for the estimation of glutathione. The 0.25 mL of test sample supernatant was combined with sulphosalicylic acid (5%) and held on ice for half-hour to allow the protein to precipitate before being centrifuged at 12,000 r/min for 12 min at 5°C. The supernatant was combined with 5, 50-dithiobis (2-nitro benzoic acid) and incubated for 15 min at 37°C. For the creation of the standard curve, product absorbance was measured at 412 nm and estimated using known amounts of reduced glutathione (GSH)[7]. The obtained results were expressed in mg per gram of tissue.

Catalase activity: According to a previously established approach by Lemos *et al.*[49], CAT activity was assessed by a reduction in hydrogen peroxide. The stomach was homogenised at 1:10 (w/v) with cold 50 mM phosphate buffer pH 7.0 and centrifuged at 12,000 rpm for 10 min at 5°C. The quartz cuvette was filled with a 10 mM H_2O_2 solution (2 mL) in 50 mM phosphate buffer pH 7.0 and 0.02 mL of sample. Readings at 240 nm were started right away and continued every minute for 4 min. From the standard curve of H_2O_2, the absorbance values were extrapolated to calculate the concentration of H_2O_2. The obtained results were expressed in units per mg of tissue.

Prostaglandin E2: The estimation of prostaglandin E2 was done by the previously described method by Trabadela *et al.*[46], with some modifications. The stomach mucosa tissue was extracted and washed with the cold saline solution immediately. The tissue was weighed and homogenised in a 6 mL TEAP buffer (pH 3.24) with a cyclooxygenase inhibitor, lysine acetylsalicylate. Then centrifuged for 10 min at 5°C at 5000 rpm, the supernatant was removed and the eluate was collected after passing it through a reverse-phase octadecyl silica C18 Sep Pak cartridge that had been cleaned with 10 mL distilled water, 10 mL 15% ethanol, 10 mL hexane and

10 mL ethyl acetate. After each ethyl acetate fraction was vaporised, the residue was redissolved in ethanol. The PGE2 was measured using an Elisa kit. The findings were calculated using PGE2/mg tissue.

Tumour Necrosis Factor-α: Biomarkers for proinflammatory mediators such as TNF-α (Tumour Necrosis Factor-α) were investigated in the ethanol and other treatment clusters. The levels of the proinflammatory biomarker TNF-α in a homogenate made from rat stomach tissues were measured using commercially available ELISA kits.

Statistical analysis: This study's data was analyzed with GraphPad prism 5.02 edition software, which has a 5.02 licence version. The data were shown as the Standard Deviation of Mean (SEM). The degree of significance between distinct variables within each experimental cluster was depicted using the statistical technique of ANOVA, accompanied by a Tukey's test, which is a one-way analysis of variance. The p-value represented the degree of significance in statistical analysis, a p-value of not more than 0.05 being considered significant.

RESULTS

Acute toxicity study: Europinidin has been proven harmless up to a maximum intake of 200 mg kg^{-1} b.wt., in acute toxicity tests in rats. During the 15 days acute toxicity period, no morbidity or clinical signs were seen. Based on the results of the acute oral toxicity research, we decided to conduct the main study with 10 mg kg^{-1} of europinidin.

Calculation of ulcer index: The impact of europinidin therapy on ulcer index in rats with ethanol-induced stomach ulcers was illustrated in Fig. 1. The animals in the ethanol-control cluster had a substantial increase in ulcer index when compared with treatment clusters ($p<0.01$). In addition, the animal clusters that were given europinidin treatment medication before ethanol induction had a significantly lower ulceration index ($p<0.001$). According to ANOVA with one-way effects and a *post hoc* test, the europinidin treatment cluster, 10 mg kg^{-1} ($p<0.001$) a considerable reduction in ulceration among the respective animal clusters.

pH measurement: The impact of flavonoid therapy on pH estimation in ethanol-treated animals was shown in Fig. 2. On the 10th day, the control ethanol cluster had a significantly acidic pH when compared to the usual treatment clusters ($p<0.01$). Similarly, in comparison to ethanol-control clusters, the animal clusters that were given europinidin as medication therapy before induction of ethanol showed substantial alkaline mode pH restoration ($p<0.001$). Following a *post hoc* test ($p<0.001$), the europinidin treatment cluster with 10 mg kg^{-1} ($p<0.01$) showed a considerable increase in restoring alkaline pH similar to the normal treatment cluster.

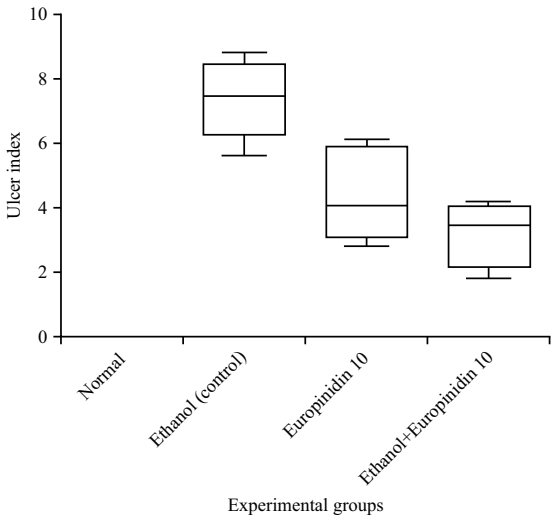

Fig. 1: Effect of europinidin on ulcer index in ethanol-induced gastric ulcers in rats
Values are expressed as Mean±SEM (n = 6) and values are statistically significant at $p<0.05$ vs. ethanol control rats group, respectively

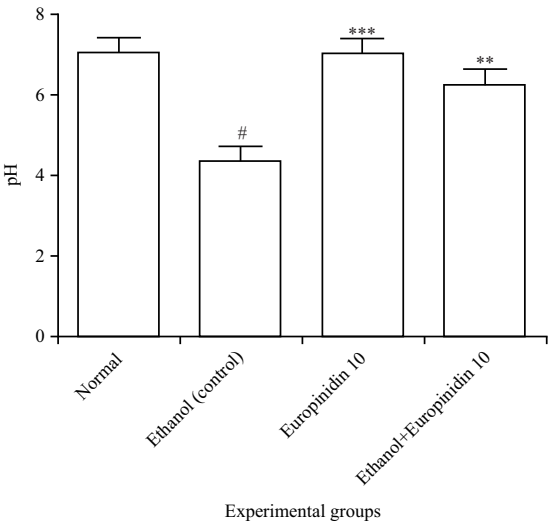

Fig. 2: Effect of europinidin on pH measurement in ethanol-induced gastric ulcers in rats
Values expressed as Mean±SEM (n = 6), #p<0.001 vs. normal rats, **p<0.01 vs. ethanol control rats, ***p<0.001 vs. ethanol control rats and one-way ANOVA was followed by Tukey's test

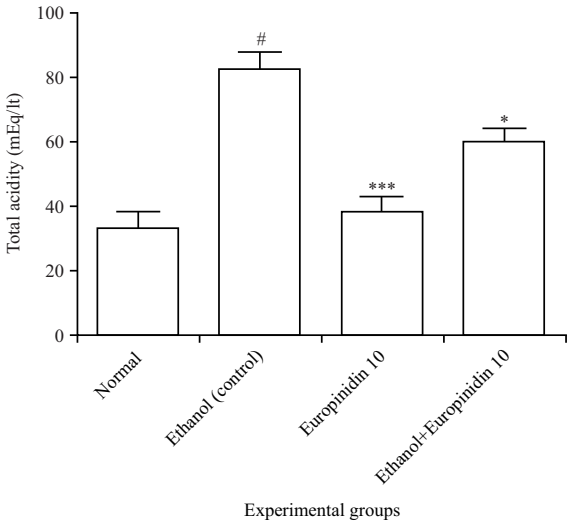

Fig. 3: Effect of europinidin on total acidity in ethanol-induced gastric ulcers in rats

Values expressed as Mean±SEM (n = 6), #p<0.001 vs. normal rats, *p<0.05 vs. ethanol control rats, *** p<0.001 vs. ethanol control rats and one-way ANOVA was followed by Tukey's test

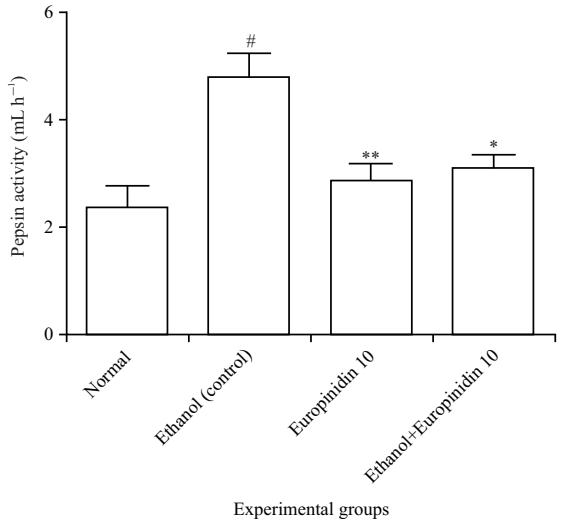

Fig. 4: Effect of europinidin on pepsin activity in ethanol-induced gastric ulcers in rats

Values expressed as Mean±SEM (n = 6), #p<0.001 vs. normal rats, *p<0.005 vs. ethanol control rats, **p<0.01 vs. ethanol control rats and one-way ANOVA followed by Tukey's test

Calculation of total acidity: The influence of europinidin treatment on total acidity parameters in rats with ethanol-induced gastric ulcers was shown in Fig. 3. On the tenth day, the ethanol-treated cluster had considerably greater total acidic content than the control cluster ($p<0.001$). Furthermore, when compared to the ethanol-control clusters, the animal clusters that were given europinidin medication therapy before ethanol induction showed considerable restoration of total acidity ($p<0.001$). Europinidin (10 mg kg^{-1}) considerably reduced total acidity inside the treatment cluster when compared to the ethanol-control cluster ($p<0.01$) as determined by a *post hoc* test.

Pepsin activity: The effect of flavonoid treatment on pepsin activity parameters in rats with gastric ulcers produced by ethanol treatment was depicted in Fig. 4. When compared to the europinidin treatment clusters, the ethanol-treated cluster had considerably greater levels of pepsin activity ($p<0.001$). Following a *post hoc* test, europinidin (10 mg kg^{-1}) was shown to be significantly effective in restoring pepsin levels in comparison to the ethanol-control clusters ($p<0.001$).

Biochemical indicators assessment

Effect of europinidin on biochemical indicators of ulceration: The impact of europinidin administration on biochemical measures of ulceration in ethanol-induced stomach ulcers in rats was proven in a clinical demonstration at the end of the experimental phase in Fig. 5a-d. During the test, the ethanol-treated cluster had considerably higher MDA levels ($p<0.001$). As a result, the ethanol-control cluster had lower levels of SOD, GSH and CAT ($p<0.001$) than the europinidin treatment clusters. Furthermore, as compared to the ethanol-control clusters, the animal clusters that were given regular europinidin medication therapy before induction of ethanol showed a substantial rise in levels of SOD, GSH and CAT ($p<0.001$) and decreased levels of MDA ($p<0.001$).

Effect of europinidin on myeloperoxidase (MPO) activity: The impact of flavonoid administration on myeloperoxidase (MPO) activity in ethanol-induced stomach ulcers in rats was demonstrated in clinical demonstrations after the experimental protocol (Fig. 6). The MPO activity was substantially higher in the ethanol-control cluster than in the europinidin treatment clusters in this investigation ($p<0.001$). Furthermore by *post hoc* test, when compared to the ethanol-control clusters, the animal clusters that received europinidin as a medicine therapy (10 mg kg^{-1}) before induction of ethanol showed substantial restoration of MPO activity by reducing levels ($p<0.001$).

Effect of europinidin on nitric oxide assay: The impact of flavonoid administration on total acidity measures in gastric

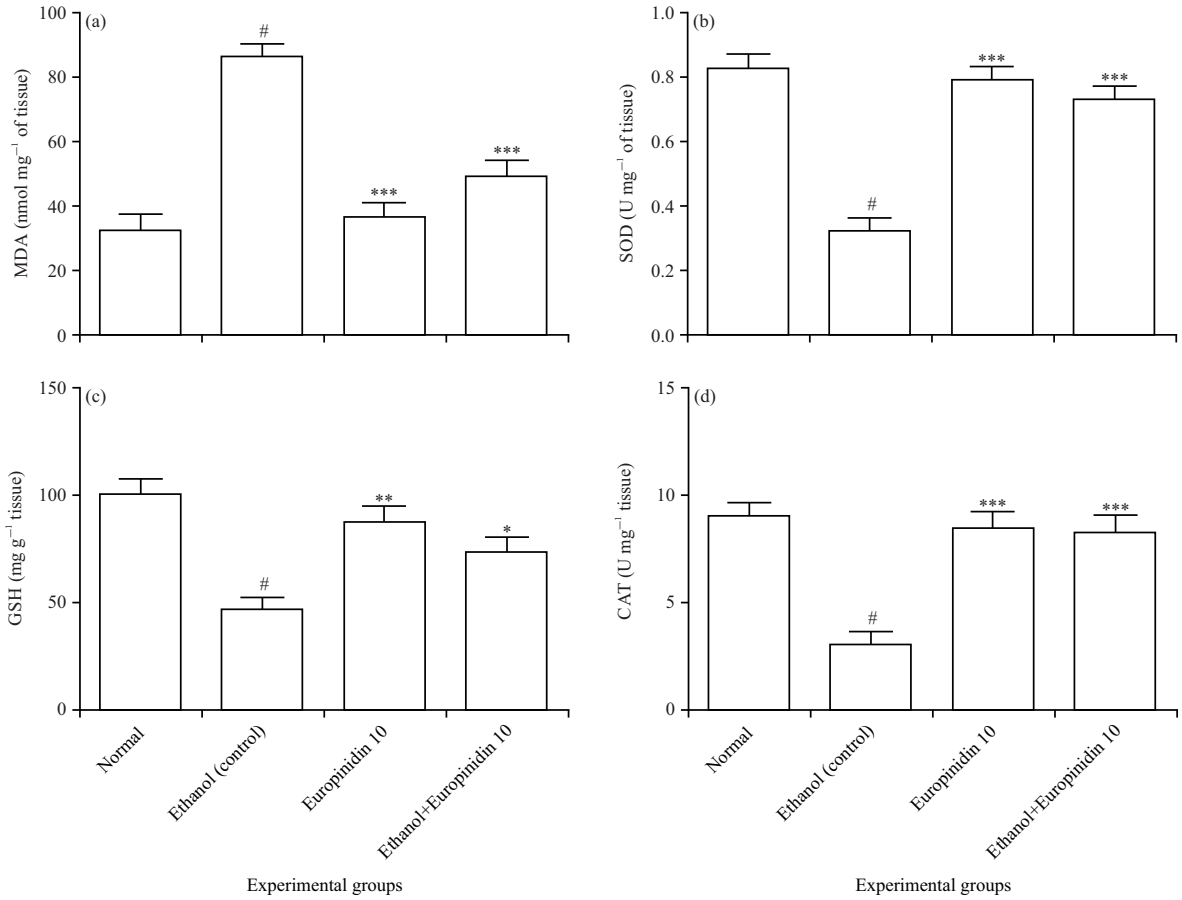

Fig. 5(a-d): Effect of europinidin on biochemical indicators in ethanol-induced gastric ulcers in rats, (a) Malondialdehyde (MDA), (b) Superoxide dismutase (SOD), (c) Glutathione (GSH) and (d) Catalase activity (CAT)

Values expressed as Mean±SEM (n = 6), #p<0.001 vs. normal rats, ***p<0.001, **p<0.01, *p<0.05 vs. ethanol control rats and one-way ANOVA was followed by Tukey's test

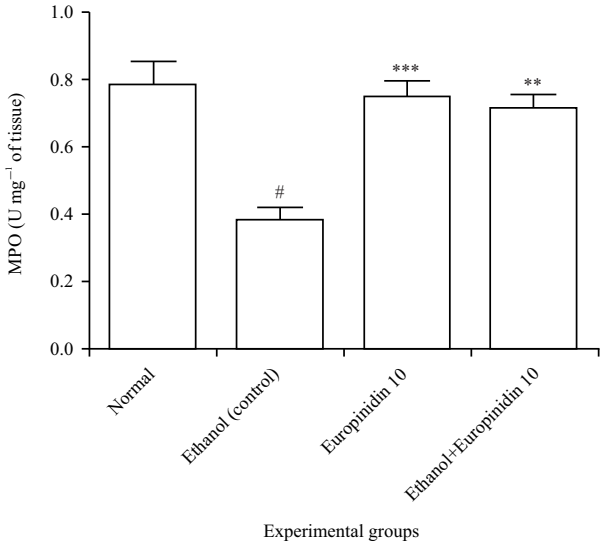

Fig. 6: Effect of europinidin on MPO levels in ethanol-induced gastric ulcers in rats

Values expressed as Mean±SEM (n = 6), #p<0.001 vs. normal rats, **p<0.01 vs. ethanol control rats, ***p<0.001 vs. ethanol control rats and one-way ANOVA was followed by Tukey's test

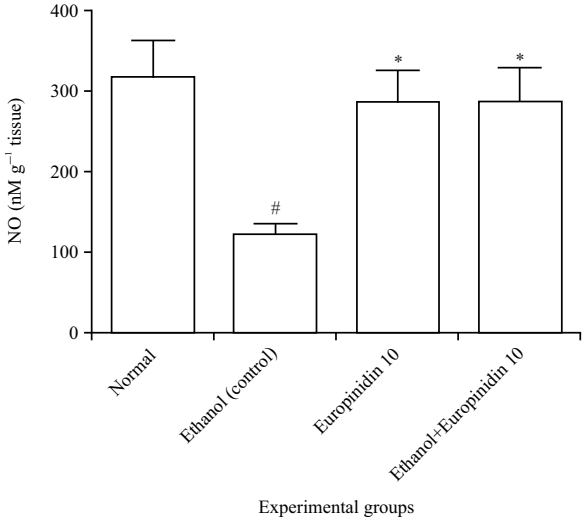

Fig. 7: Effect of europinidin on nitric oxide levels in ethanol-induced gastric ulcers in rats

Values expressed as Mean±SEM (n = 6), #p<0.001 vs. normal rats, *p<0.005 vs. ethanol control rats and one-way ANOVA was followed by Tukey's test

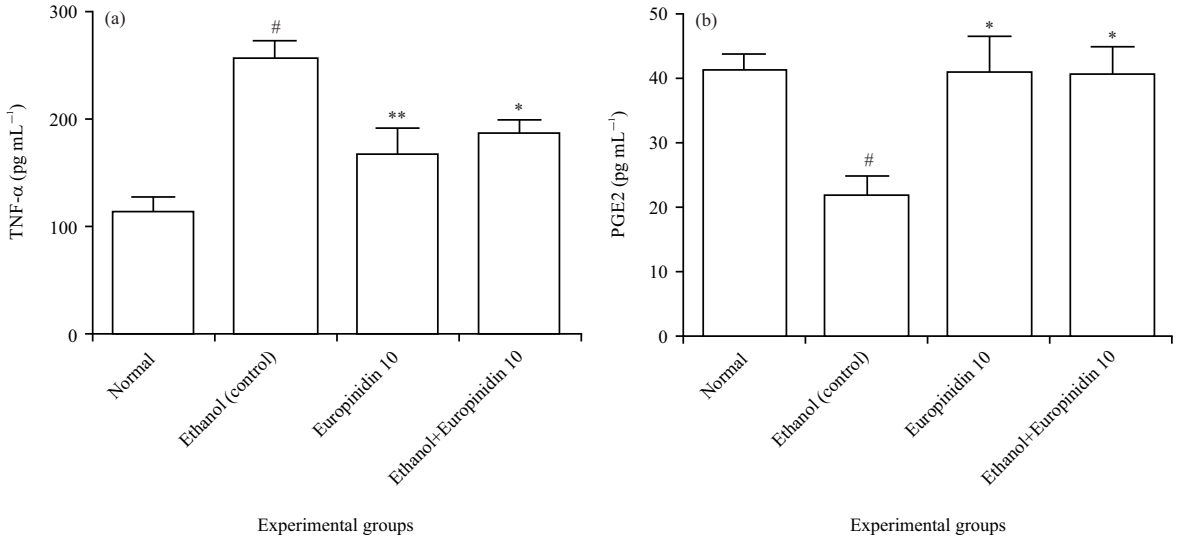

Fig. 8(a-b): Effect of europinidin on biochemical parameters in ethanol-induced gastric ulcers in rats, (a) Tumour necrosis factor-α (TNF-α) level and (b) Prostaglandin E2 (PGE2) level

Values expressed as Mean±SEM (n = 6), #p<0.001 vs. normal rats, ***p<0.001, **p<0.01, *p<0.05 vs. ethanol control rats and one-way ANOVA followed by Tukey's test

shown in Fig. 7. In this study, the ethanol-treated cluster had significantly lower levels of NO compared to the europinidin treatment clusters (p<0.001). In a separate series of experiments, animal clusters given europinidin (10 mg kg^{-1}) before ethanol induction showed considerable restoration of normal NO levels when compared to ethanol-control animals (p<0.001).

Effect of europinidin on tumour necrosis factor-α and prostaglandin E2: The impact of flavonoid administration on proinflammatory indices of ulceration in ethanol-induced stomach ulcers in rats was proven in a clinical demonstration at the end of the experimental phase (Fig. 8a-b). The TNF-α level in the ethanol treatment cluster was substantially higher (p<0.001) than in the control cluster. As a result, the ethanol-

control cluster had lower levels of PGE2 ($p<0.001$) than the europinidin treatment clusters. Furthermore, as compared to the ethanol-control clusters, the animal clusters that were given europinidin (10 mg kg^{-1}) before ethanol induction showed substantial increases in levels of PGE2 ($p<0.001$) and lower levels of TNF-α ($p<0.001$).

DISCUSSION

The flavonoid europinidin was investigated as a possible gastro-protective in animals with ethanol-induced gastric ulceration. Acute toxicity tests for europinidin were conducted as part of the evaluation to see whether there were any toxicities connected to europinidin. In rats, europinidin was determined to be safe up to limit the dosage of 200 mg kg^{-1}, with no clinical effects. Histomorphology by avoiding additional necrosis caused by ethanol administration, as compared to a higher europinidin flavonoid dosage.

Chronic, uncontrolled alcohol consumption has been recognized as the primary cause of stomach ulcers across the world. Many preclinical investigations in animal models to explore stomach ulcers induced by ethanol induction have recently been conducted to look at the relevance in humans. Concurrently, a cluster of researchers acknowledged the need of exploring the effectiveness of plant-derived compounds in antiulcer activities as novel therapy options[35]. The oral introduction of ethanol in laboratory animals resulted in significant gastric wall damage. The gastric wall is involved in the damaging molecular process[7,50]. The prevalence of haemorrhage, epithelial erosion, capsular oedema and disruption to gastric villi have all been used to characterize gastric lesions in patients[51,52].

The present investigation measures up normal control and ethanol-control clusters in rats, leading to the conclusion that ethanol-treated cluster animals display signs of gastric ulceration, such as increased ulcer index, total acidic content and MPO activity, as well as unusual levels of biosensors such as SOD, GSH, MDA, CAT, TNF-α and PGE2. Additionally, rapid administration of absolute ethanol resulted in structural abnormalities in the stomach, with a considerable increase in gastric villi defects. Ethanol overdose produces hemorrhagic cellular changes in mouse models and alters the levels of various pro-inflammatory markers. According to an earlier study by Rahman *et al.*[7], ethanol also produces necrotic coagulations of the stomach mucosa, according to laboratory research[52]. A further set of studies predicted whether generating ethanol induced a substantial increase in lesion index when scarification was done on day 10 after acute ethanol administration, showing a comparable animal model for stomach ulceration. This medical explanation is useful in choosing the optimum method for evaluating flavonoids against an ethanol-induced peptic ulcer in the current research. Plant-derived flavonoids are important in modifying the pH of the stomach in previous studies, which was due to their gastro-protective action[53,54]. Likewise, a recent study showed the role of flavonoids in comparative gastric pH regulation and peptic protection. The results of this study showed that providing europinidin flavonoid before ethanol induction induces a substantial boost in pH and total acid content in the flavonoid-treated cluster, comparable to the normal saline control cluster. Moreover, the ethanol-control cohort had a significant increase in pepsin activity when compared to the normal control cluster, which is consistent with previous research that revealed abnormal peptic activity in the context of chronic ethanol use[7]. The pathophysiology of peptic ulcers was mediated by particular inflammatory processes marked by the buildup of some proinflammatory mediators in gastric linings as well as damage to the gastric epithelium[55]. The TNF-α and PEG2 are some of the most important proinflammatory produced by macrophages during inflammation[20]. Based on that result, the existing research entails calculating this proinflammatory cytokine in ethanol-induced rat models and analyzing the effect of europinidin flavonoid on this cytokine, with a dose of 10 mg kg^{-1} demonstrating significance in all of the above parameters.

Another research has discovered that ROS generation is important in the development of mitochondrial gastric mucosal damage, as well as a biochemical mechanism linked to the pathophysiology of ethanol-induced oxidative depilation[15,16]. A few scientists have also pointed to the role of superoxide anion (ROS) in oxidative damage as a source of abnormal biochemical markers[16,56].

The existence of oxidative damage biological markers in the ethanol-control cluster, such as elevated levels of MDA, was noticed in the ethanol-treated cluster, whereas ethanol-induced peptic ulceration led to a substantial decrease in SOD, GSH and CAT levels, implying that ethanol plays a role in lipid peroxidation. Furthermore, europinidin flavonoid treatment (10 mg kg^{-1}) exhibited reduced MDA activity and restored levels of GSH, SOD and CAT in experimental clusters.

In the latest research, the function of the enzyme MPO, the important marker for neutrophil infiltration was also emphasized. According to the findings, it was postulated that ethanol administration generates an aberrant rise in MPO activity, which then in mouse models is efficiently counterbalanced by flavonoids.

Nitric oxide, an intrinsic regulator produced by nitric oxide synthase, maintains the stomach mucosa intact[57]. This feature of NO has been studied before of its relevance in decreasing neutrophil invasion[26]. Current results in the animal studies showed that ethanol administration generates an abnormal decrease in NO production, which is successfully countered by flavonoids.

CONCLUSION

The present study looks at a variety of clinical data that suggested that europinidin flavonoids have substantial gastro-protective properties, such as the ability to reduce acidic content, proinflammatory indicators and elevated biochemical parameters connected to peptic ulcers. This might open the opportunities for sustainable development of low-cost phytochemical treatments for chemical-induced peptic ulcers.

SIGNIFICANCE STATEMENT

This study was performed to examine the antiulcer effects of europinidin against experimentally induced-stomach ulcer conditions in rodents. This study will help the researcher and other healthcare practitioners to uncover the protective influences of this phytoconstituent and to correct the proinflammatory indicators that many researchers were not able to explore in gastric ulcers.

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