EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 7
620-631
Year: 2020

GASTROPROTECTIVE ACTIVITY OF BROWN PROPOLIS EXTRACT AGAINST ASPIRIN AND ETHANOL- INDUCED GASTRIC ULCERS IN ADULT WISTAR RATS BY TARGETING OXIDATIVE STRESS AND PRO-INFLAMMATORY SIGNALING

Mrunal Patil and Kedar Prabhavalkar*

Department of Pharmacology, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Vile Parle, Mumbai, Maharashtra, India.

***Corresponding Author: Kedar Prabhavalkar**
Department of Pharmacology, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Vile Parle, Mumbai, Maharashtra, India.

Article Received on 20/05/2020 **Article Revised on 10/06/2020** **Article Accepted on 01/07/2020**

ABSTRACT
Aspirin is a commonly prescribed non steroidal anti-inflammatory drug and alcohol is consumed by large number of population throughout the world but its prolonged use injures the gastric mucosa. This study was carried out to investigate the therapeutic effect of Brown Propolis Extract (BPE) against aspirin and ethanol- induced gastric ulcers in Wistar rats. Gastric ulcer was induced by oral administration of aspirin (200 mg/kg/day) for 3 days. Pretreatment with BPE (250 and 500 mg/kg bw) was given orally for 10 days. Ranitidine at 50 mg/kg orally was used as standard. The various parameters like gastric volume, gastric pH, free acidity, total acidity, ulcer index, percent inhibition were estimated for assessment of anti-secretory and gastroprotective effects of BPE. At the same time antioxidant parameters like superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) and reduced glutathione (GSH) in addition to that pro-inflammatory parameters such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) were also estimated according to their respective method of estimation using analyzing kit. BPE helped in improving the gross morphology, histology and mucous layer of gastric tissue, antioxidants and by reducing tissue levels of the lipid peroxidation marker (malondialdehyde) and inflammatory mediators. In conclusion, BPE has a therapeutic potential in aspirin and ethanol- induced gastric injury by alleviating oxidative stress and inflammation.

KEYWORDS: Brown Propolis Extract, gastric ulcers, pro-inflammatory, antioxidant, malondialdehyde.

1. INTRODUCTION
Gastric ulcer is a common gastrointestinal disorder, affecting many people throughout the world. It can be defined as damage to the mucosa that ruptures the muscle layer and forms an injury followed by inflammation.[1] Ulcers can occur in the stomach, where they are called as gastric ulcers or they can occur in the first portion of the intestine called as duodenal ulcers.[2] Generally normal gastric mucosa is exposed to various offensive factors like increased secretion of hydrochloric acid and pepsin, reactive oxygen species, improper dietary habits, administration of non-steroidal anti-inflammatory drugs, consumption of alcohol, stressful conditions and infection with *Helicobacter pylori* infection, whereas it decreases the activity of defensive factors like mucin, adequate blood flow, nitric oxide, prostaglandin secretion, bicarbonate and growth factors.[3] This may results from an imbalance between defensive factors and offensive factors present in the gastric mucosal layer.[4] Gastric ulcers are known to be induced by excessive use of several Non-Steroidal Anti-Inflammatory Drugs (NSAID's) like aspirin, indomethacin, and ibuprofen.[5] NSAID's are responsible for the production of gastric ulcers by inhibiting prostaglandin synthesis via cyclooxygenase (COX) pathway.[6] Prostaglandin plays an important and protective role in the production of mucus and secretion of bicarbonate, which maintains mucosal blood flow and regulates mucosal cell integrity.[7] Thus the suppression of production of prostaglandins by NSAIDs leads to the gastric mucosal damage and gastric ulcers. The mechanism behind NSAIDs -induced gastric ulceration includes blocking activity of the cyclooxygenase enzymes (COX-1 and COX-2), by NSAIDs, which further leads to the decreased mucus and bicarbonate secretion, impaired platelet aggregation, decreased mucosal blood flow, changes in microvascular structures leading to epithelia damage.[8] Increased reactive oxygen species (ROS) and lipid peroxidation (LPO) are also responsible for gastric mucosal damage.[9] NSAID's specially those are having chemically acidic nature, can exert direct cytotoxic effects on epithelial cells, which disrupt surface active phospholipids on the mucosal surface thus making the mucosa more susceptible to

damage by luminal acid.[10] Ethanol has been commonly used as a damaging agent to gastric mucosa for the induction of gastric ulcers. Administration of absolute ethanol (>99%) has been used as a reproducible method to induce gastric mucosal damage in experimental animals.[11] The pathology behind ethanol-induced gastric ulcer generally involves three main parameters: inflammatory response, oxidative stress and apoptosis.[12]

In the gastrointestinal tract, the motility of the esophagus, stomach, and gut as well as the capacity of gut absorption can be severely affected by alcohol exposure. It can cause severe mucosal damage and even carcinogenesis especially gastric cancer.[12] Ethanol causes severe gastric damage by producing several instabilities in the gastric mucosal layer such as a decreased bicarbonate secretion, gastric blood flow and mucus production. Ethanol can stimulate gastric acid secretion, resulting in microvascular injuries which facilitate vascular permeability, through release of gastrin and histamine from sensitive nerve terminals present in the gastric mucosa.[13] Ethanol ruptures gastric mucosal integrity through exfoliation of cells, which leads to increase in mucosal permeability and in somehow causes bleeding.[14] Intra-gastric administration of absolute ethanol results in severe gastric mucosal injury characterized by disturbances in microcirculation, mast cell secretary products, inhibition of prostaglandin synthesis, reduction in mucus production and reactive oxygen species. Neutrophil infiltration in the gastric mucosa is also responsible for the production of lesions induced by absolute ethanol.[15]

Oxidative stress plays a significant role in alcohol-induced gastric mucosal damage.[16] Ethanol is also known to increase cellular oxidative stress and produce changes in gastric cell calcium levels.[17] which may lead to the pathogenesis of gastric mucosal injury. Ethanol directly increases the levels of free radicals that can alter the cell structure and function and can also gives its direct toxic effects on the gastric mucosa resulting in reduced bicarbonate secretion and gastric mucus production.[18]

A number of phytochemicals have shown potent anti-ulcer as well as gastroprotective activity against several ulcer causing agents when they were investigated. The epidemiological evidence regarding the consumption or use of natural phytochemicals as gastroprotective agents showed positive results. Natural products and their compounds are highly effective as anti- ulcer agents as well as gastroprotectives. Several natural products acts by significant suppression of inflammatory cytokines like IL-2, TNF-α, COX-2 and nitrosative stress.[19]

Propolis, this word has been derived from Greek literature, where ‘Pro’ means ‘‘at the entrance to” and ‘polis’ means ‘‘community’’ or “city’’ which means defense for hive. Propolis is collected by worker honey bees by mixing these secretions with their own saliva and enzymatic secretions.[20] Honey bees use these resinous secretions as cementing material for sealing the cracks and crevices occurring in their hives.[21,22] Apart from blocking the cracks, sealing the spaces and smoothening the internal walls of their hives, honey bees use this propolis as an antiseptic[23,24,25] to protect bees larvae, honey and hive from bacterial infections.[26] Currently propolis is used as an anti-bacterial, anti-inflammatory, anti-viral[27], anti-oxidant, anti-protozoal, anesthetic, anti tumoural, anti-cancer, anti-fungal[28], antiseptic, anti-mutagenic, anti-hepatotoxic in addition to being used for cytotoxic activity, etc.[29]

The aim of this study was to investigate the gastroprotective effects of BPE against aspirin and ethanol-induced gastric ulcers in Wistar rats using ranitidine as a standard drug. In addition, the probable mechanism by which BPE showed its efficacy in terms of oxidative stress and pro-inflammatory signaling using indicators like Malondialdehyde (MDA), Reduced glutathione (GSH), Superoxide dismutase (SOD) and Catalase activity (CAT), TNF-α and IL-1β.

2. MATERIAL AND METHODS

2.1. Experimental animals

Male wistar rats weighing 150-200 g were procured from National institute of Biosciences, Pune. Animals were housed in Perspex cages at SVKM’s Animal House Facility, Mumbai and maintained under general conditions (12h: 12h light/ dark cycle, room temperature 20-220C, 50-70% humidity). Animals were fed on standard food pellets and water ad libitum. Before starting an experiment animals were acclimatized for the period of one week. The entire experimental protocol was reviewed and approved by an Institutional Animal Ethics Committee registered under “Committee for the purpose of Control and Supervision of Experiments on Laboratory Animals” (CPCSEA), Ministry of Environment and Forests, Government of India.

2.2. Plant material and chemicals

Brown Propolis Extract was obtained as a gift sample from Pharma Base S.A. Switzerland. Ranitidine was obtained as a gift sample from Neon laboratories Ltd. Mumbai. Aspirin was obtained as a gift sample from The Andhra Sugars Limited, Tanuku, Andhra Pradesh, India and Absolute Ethanol was also obtained as a gift sample from Research-Lab Fine Chem Industries, Mumbai.

2.3. Preparation of drug solution

Brown Propolis Extract and aspirin were homogeneously suspended in 1% carboxymethylcellulose (1% CMC) solution and standard drug Ranitidine was dissolved in normal saline. The drug solution was freshly prepared just before each dosing session. Vehicle (1% CMC) and drugs were administered orally (*p.o.*).

2.4. Phytochemical analysis of the extract

The preliminary phytochemical screening of the obtained Brown Propolis Extract (BPE) was carried out in order to determine the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins, tannins, terpenoids, coumarins, saponins according to standard procedures.[30]

2.5. Acute toxic study

Brown Propolis Extract was studied for its acute toxicity in Wistar rats (170–200 g) by a fixed dose method as per OECD guideline No. 420, 2001. Brown Propolis Extract was administered at the dose 5, 50, 300, 2000 and 5000 mg/kg body weight, to determine mortality rate and animals were observed for 14 consecutive days.[30]

2.6. Experimental design

Animals were divided into following groups, each group contains six rats. Pre-treatment with the different doses of BPE was given. The extract was administered orally once daily using oral feeding tube of 18 gauge with the ad libitum provision of food and water throughout the experimental period.

2.7. Antiulcer activity of BPE in aspirin-induced gastric ulceration in wistar rats.

Group I	Control Group
Group II	Vehicle group -- 1% carboxymethylcellulose (CMC) solution
Group III	Negative Control -- Aspirin (200 mg/kg/day)
Group IV	Standard -- Ranitidine (50mg/kg) + Aspirin (200 mg/kg/day)
Group V	Brown Propolis Extract (250 mg/kg) + Aspirin(200 mg/kg/day)
Group VI	Brown Propolis Extract (500 mg/kg) + Aspirin (200 mg/kg/day)

2.8. Antiulcer activity of BPE in ethanol-induced gastric ulceration in Wistar rats.

Group I	Control Group
Group II	Vehicle group -- 1% carboxymethylcellulose (CMC) solution
Group III	Negative Control -- Ab. Ethanol (1.0 ml/animal)
Group IV	Standard -- Ranitidine (50mg/kg) + Ab. Ethanol (1.0 ml/animal)
Group V	Brown Propolis Extract (250 mg/kg) + Ab. Ethanol (1.0 ml/animal)
Group VI	Brown Propolis Extract (500 mg/kg) + Ab. Ethanol (1.0 ml/animal)

3. INDUCTION OF ULCER

3.1. Aspirin-induced gastric ulcer

Aspirin induced gastric ulcer model was chosen for the investigation of anti-ulcerative activity. Treatment drug, BPE was given once daily for 10 consecutive days. BPE was administered in two doses i.e. 250 mg/kg and 500 mg/kg in order to investigate its efficacious dose. Aspirin was given once daily at a dose of 200 mg/kg for last three days i.e. from day 8 to day 10.[31]

Experimental protocol

After acclimatization, rats were randomly divided into six groups with six animals (n=6) in each group. Group I served as normal control group while group II received only vehicle i.e. 1% CMC solution for 10 days. Rats from group V and VI were orally pretreated with Brown Propolis Extract while group IV was pretreated with Ranitidine (50 mg/kg) for 10 consecutive days. Then starting from day 8 to day 10, Aspirin (200 mg/kg) was administered to group III, IV, V, VI. On day 10, twenty four hour fasted animals were sacrificed five hour after the last dose of aspirin and their stomachs were removed and gastric juice was collected and it was then opened along with a greater curvature for the determination of gastric lesions. The severity of gastric damage was then scored.

3.2. Ethanol-induced gastric ulcer

Ethanol induced gastric ulcer model was chosen for the investigation of gastroprotective activity. Treatment drug, Brown Propolis Extract was administered in two doses i.e. 250 mg/kg and 500 mg/kg in order to investigate its efficacious dose. Brown Propolis Extract was administered one hour prior to ethanol.[32]

Experimental protocol

After acclimatization, rats were randomly divided into six groups with six animals (n=6) in each group. Group I served as normal control group while group II received only vehicle i.e. 1% CMC solution for 10 days. Rats were fasted for 48 hrs, water was withheld 2 hrs before the commencement of experiment. Rats from group V and VI were orally pretreated with Brown Propolis Extract while group IV was pretreated with Ranitidine (50 mg/kg) one hour prior to Absolute Ethanol (1 ml/animal). One hour after the dose of absolute ethanol, animals were sacrificed and their stomachs were removed and gastric juice was collected and it was then opened along with a greater curvature for the determination of gastric lesions. The severity of gastric damage was then scored.

3.3. Determination of gastric volume, gastric acidity and gastric lesions

The gastric mucosal layer of the stomach was viewed under a magnifying lens (10×) to evaluate the gastric ulcers. The ulcerated areas were counted and scored using the method described by Kulkarni. The score was graded as: 0- Normal colouration, 0.5- Red colouration, 1.0- Spot ulcers, 1.5- Haemorrhagic streaks, 2.0- Deep ulcers- 3.0- Perforations.[30]

Mean Ulcer Index (MUI) was calculated as total sum of ulcer scores assigned to gastric lesions. Percentage of ulcer inhibition was calculated by using the formula:
Inhibition=(MUIcontrol-MUItest)÷MUIcontrol × 100%.

After sacrifice, rat stomach was removed and the gastric content was collected. The gastric volume was measured by measuring cylinder (10 ml). The stomach was opened along the greater curvature and gastric content was drained into a centrifuge tube. The resultant solution was centrifuged at 3000 rpm for 10 min and the supernatant was collected. The pH of gastric juice was determined using a pH meter. Collected supernatant was used for the determination of free acidity and total acidity. Free acidity and total acidity of collected gastric juice was estimated by titrating gastric juice with 0.01 N NaOH, using Topfer's reagent and phenolphthalein as indicator. 0.01 N NaOH was prepared and poured into the burette. Collected gastric content was poured in a beaker containing 50 ml of distilled water. From this solution, 25 ml of gastric juice was pipette out in a conical flask and two to three drops of Topfer's reagent was added for the estimation of free acidity. Prepared NaOH was titrated against the acidic gastric content and observed until yellow coloration was obtained. The total volume of NaOH used was noted and free acidity was calculated.[33]

The above mentioned procedure was repeated for estimation of total acidity using phenolphthalein as an indicator. In this experiment, the color change was from colorless to pink and the total volume of alkali added was recorded for total acidity.

3.4. Evaluation of antioxidant activity

The stomach samples from the rats were homogenized in a 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% potassium chloride, followed by centrifugation of the homogenate at 10,000 rpm for 15 min at −4 °C. The supernatant was collected to allow estimation of Lipid peroxidation or Malondialdehyde (MDA), Reduced glutathione (GSH), Superoxide dismutase (SOD) and Catalase activity (CAT).

Measurement of malondialdehyde (MDA)

Lipid peroxidation was measured as malondialdehyde (MDA) according to the method described by Ohkawa et al. To each 0.5 mL supernatant, 0.5 mL phosphate buffer (0.1 M, pH 8.0) and 0.5 mL 24% TCA was added. The resulting mixture was incubated at room temperature for 10 min, followed by centrifugation at 2000 rpm for 20 min. An aliquot of 1 mL of the resulting supernatant was mixed with 0.25 mL 0.33% TBA in 20% acetic acid and the resulting mixture boiled at 95 °C for 1 h. The resulting pink colored product was cooled and the absorbance was read at 532 nm.[33]

Estimation of reduced glutathione (GSH)

Reduced glutathione (GSH) was determined using the method described by Beutler. 1 mL of collected supernatant was added to 0.5 mL Ellman's reagent (10 mM), followed by addition of 2 mL phosphate buffer. And then produced yellow colored solution was read at 412 nm against a blank containing 3.5 mL phosphate buffer. In a similar way, a series of standards were prepared. The amount of GSH was expressed as µg/mg protein.[33]

Estimation of superoxide dismutase (SOD)

The supernatant was collected for superoxide dismutase (SOD) activity by using the method described by Misra and Fridovich.[32] Aliquots of 2.5 mL buffer, 30 mM EDTA, and 300 µL 2 mM pyrogallol were added to 200 µL of the lysate. Any increase in absorbance was recorded at 420 nm for 3 min using a spectrophotometer. The activity of SOD was expressed as µg/mg protein.[33]

Estimation of catalase activity (CAT)

The method to determine the catalase (CAT) activity was based on the measurement of hydrogen peroxide consumption in the studied sample, as described by Chance and Oshino (1971), with adaptations. The supernatant was collected as described above and 2 µl was added to 191 µl of phosphate buffer. This mixture was read in the spectrophotometer, and 7 µl of hydrogen peroxide was added. The reaction was read at 240 nm for 2 min, and the data was expressed as the CAT activity/mg of proteins. The protein content was determined using bovine serum albumin as a standard, according to the method described by Bradford (1976).[33]

3.5. Evaluation of pro-inflammatory parameters

Determination of IL-1β levels

Stomach samples were rinsed in Phosphate buffer Saline (PBS) (pH 7.4) to remove excess blood, Weighed and stored at -800c before homogenization. Tissues were minced homogenize them in PBS (pH 7.4) with a glass homogenizer on ice. Thaw at 2-80c or freeze at -200c. Centrifuge at 2000-3000 rpm for approximately 20 minutes. Further procedure was carried as per the instructions given in commercial available ELISA kit by Krishgen Biosystems. Optical density of each well was determined immediately using a microplate reader set to 450 nm within 30 min after adding the stop solution.

Determination of TNF-α levels

Stomach samples were rinsed in Phosphate buffer Saline (PBS) (pH 7.4) to remove excess blood, Weighed and stored at -800c before homogenization. Tissues were minced homogenize them in PBS (pH 7.4) with a glass homogenizer on ice. Thaw at 2-80c or freeze at -200c. Centrifuge at 2000-3000 rpm for approximately 20 minutes. Further procedure was carried as per the instructions given in commercial available ELISA kit by Krishgen Biosystems. Optical density of each well was determined immediately using a microplate reader set to 450 nm within 30 min after adding the stop solution.

3.6 Histopathology of stomach samples

Stomach was excised, washed and cleaned with 0.9% saline and kept in 10% neutral Formalin and given for histopathological examination by staining with Haematoxylin and Eosin (H & E).

3.7. Statistical analysis

The statistical evaluation will be performed with the help of GraphPad Prism version 8.0.1. for 64 bit Windows. All the evaluation data will be compared to access the statistical significance using One-way ANOVA (Analysis of Variance). The data will be represented as mean ±SEM values.

4. RESULTS

4.1 Phytochemical analysis of the extract

The BPE was analyzed for different phytochemical constituents as per standard procedure at institute laboratory and the results are given in Table 1.

Table 1: Phytochemical analysis of BPE.

Sr No.	Phytochemicals	Tests	BPE
1.	Flavonoids	Shinoda test Sulphuric acid test Lead acetate test	+ + +
2.	Tannins	Lead acetate test FeCl3 test Dil. Iodine solution test	- + +
3.	Phenols	FeCl3 test Potassium dichromate	+ +
4.	Glycosides	Libermanns test Foam test	- +
5.	Steroids	Libermanns test	-
6.	Alkaloids	Dragendorff's test Mayer's test Hager's test Wagner's test	+ + + +

4.2 Acute oral toxicity study

All animals were alive and showed no any visible signs of toxicity in higher doses. No any behavioral changes were found in animals. No change was observed in animal body weight. Thus the median lethal dose was considered to be greater than 2000 mg/kg body weight.

4.3. Ulcer index, ulcer inhibition and gastric lesions

Aspirin and ethanol induced ulcers observed clearly in the negative control groups, whereas standard drug ranitidine and BPE treated groups showed the relatively normal structure (Figs. 1 and 2).

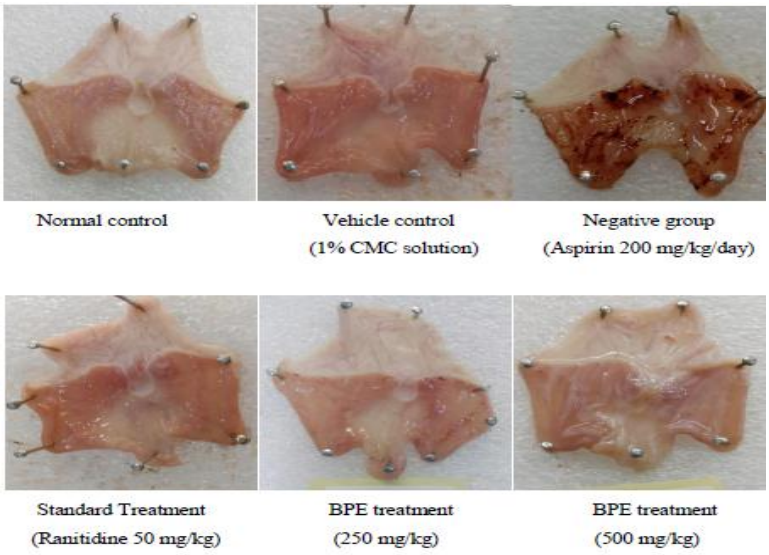

Figure 1: Macroscopic examination of aspirin induced gastric ulcers.

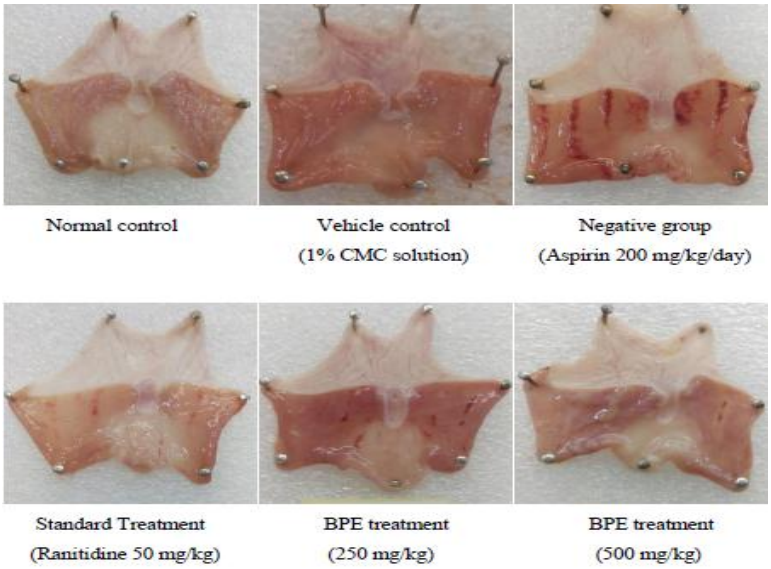

Figure 2: Macroscopic examination of ethanol induced gastric ulcers.

The effects of BPE on ulcer index and inhibition are reported in Table 2 and 3 for both aspirin and ethanol induced gastric ulcer groups respectively. Ulcer index in the ulcer control group was significantly ($p< 0.001$) increased as compared to the control group. The severity of gastric ulcers in models was significantly ($p< 0.001$) reduced by pretreatment with BPE. However, in ranitidine pretreated group, maximum inhibition was observed, which was comparable to the 500 mg/kg BPE.

4.4. Gastric volume and gastric acidity

The effects of BPE on gastric volume, gastric pH, free acidity and total acidity are reported in Table 2 and 3 for both aspirin and ethanol induced gastric ulcer groups respectively.

The gastric juice volume was significantly increased ($P < 0.001$) in the negative group when compared against normal control group. The group pretreated with 250 mg/kg BPE produced no significant decrease in gastric juice volume but standard treated group (ranitidine 50 mg/kg) and BPE (500 mg/kg) treated group showed significant decrease ($p< 0.01$ and $p< 0.05$ respectively) in total gastric volume as compared to negative group while BPE (500 mg/kg) treated group showed not much significant ($p< 0.01$) difference when compared against normal control group.

The gastric pH was significantly decreased ($P < 0.001$) in the negative group when compared against normal control group. The group pretreated with 250 mg/kg BPE produced no significant increase in gastric pH but standard treated group (ranitidine 50 mg/kg) and BPE (500 mg/kg) treated group showed significant increase ($p< 0.001$ and $p< 0.01$ respectively) in gastric pH as compared to negative group while BPE (500 mg/kg) treated group showed not much significant ($p< 0.01$) difference when compared against normal control group.

Table 2: Effect of BPE in aspirin induced gastric ulcer in wistar rats.

Group	Gastric volume (ml)	Gastric pH	Free Acidity (Meq/L)	Total Acidity (Meq/L)	Ulcer Index	% inhibition
I	2.733 ± 0.1282	3.487 ± 0.297	29.53 ± 2.890	45.66 ± 2.298	-	-
II	3.553 ± 0.092	4.017 ± 0.060	27.53 ± 1.331	47.06 ± 1.469	-	-
III	4.667 ± 0.246***	1.270 ± .131***	63.70 ± .704***	85.10 ± .743***	73.36 ± 1.928***	-
IV	3.533 ± 0.095##	3.335 ± 0.186###	34.88 ± .136###	48.59 ± .8218###	26.51 ± 0.6878###	61.36
V	4.195 ± 0.357***	1.998 ± 0.157***	51.01 ± .830***#	73.69 ± 2.721***##	56.67 ± 2.046	22.75
VI	3.783 ± 0.107**#	2.445 ± 0.200**##	45.78 ± .458**###	55.33 ± 2.831*###	35.42 ± 1.953	51.72

Table 3: Effect of BPE in ethanol induced gastric ulcer in wistar rats.

Group	Gastric volume (ml)	Gastric pH	Free Acidity (Meq/L)	Total Acidity (Meq/L)	Ulcer Index	% inhibition
I	2.717 ± 0.1851	3.835 ± 0.1645	17.81 ± 1.479	46.60 ± 4.885	-	-
II	3.000 ± 0.1693	4.102 ± 0.1371	16.57 ± 0.2438	40.68 ± 0.7376	-	-
III	4.733 ± 0.1892***	1.517 ± 0.1672***	34.88 ± 3.136***	86.10 ± 4.028***	72.22 ± 1.444***	-
IV	2.767 ± 0.2140###	3.660 ± 0.2371###	19.77 ± 1.574 ###	59.86 ± 1.053*###	32.96 ± 2.381###	52.93
V	3.807 ± 0.1774**##	2.365 ± 0.1549***#	27.43 ± 1.608**	71.02 ± 1.026 ***##	51.36 ± 3.915	45.96
VI	3.633 ± 0.1382*#	2.817 ± 0.1558**###	24.75 ± 1.154 ##	63.54 ± 1.730**###	34.44 ± 1.717	51.35

Values are represented as mean ± SEM (n=6). Data was analysed by using one way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test; significance is denoted by ; * p< 0.05, **p< 0.01, ***p< 0.001 when compared against Normal control group and # p< 0.05, ## p< 0.01, ### p< 0.001 when compared with negative group.

Free acidity was found to be significantly increased (P < 0.001) in the negative group when compared against normal control group. The group pretreated with 250 mg/kg BPE produced not much significant decrease (p<0.05) in free acidity but standard treated group, ranitidine (50 mg/kg) and BPE (500 mg/kg) treated group showed significant decrease (p< 0.001 and p< 0.001 respectively) in free acidity as compared to negative group while BPE (500 mg/kg) treated group showed not much significant (p< 0.01) difference when compared against normal control group.

Total acidity was found to be significantly increased (P < 0.001) in the negative group when compared against normal control group. The group pretreated with 250 mg/kg BPE produced significant decrease (p<0.01) in total acidity but standard treated group (ranitidine 50 mg/kg) and BPE (500 mg/kg) treated group showed significant decrease (p< 0.001 and p< 0.001 respectively) in total acidity as compared to negative group while BPE (500 mg/kg) treated group showed not much significant (p< 0.05) difference when compared against normal control group.

4.5. Evaluation of antioxidant activity

The effect of BPE on MDA, GSH, SOD and CAT were shown in Fig. 3 and 4 for aspirin and ethanol-induced gastric ulcer groups.

Tissue malondialdehyde (MDA) levels were found to be significantly increased (P < 0.001) in the negative group when compared against normal control group. The group pretreated with 250 mg/kg BPE produced not much significant decrease (p<0.01) in tissue malondialdehyde (MDA) levels but standard treated group (ranitidine 50 mg/kg) and BPE (500 mg/kg) treated group showed significant decrease (p< 0.001 and p< 0.001 respectively) in tissue malondialdehyde (MDA) levels as compared to negative group while BPE (500 mg/kg) treated group showed not much significant (p< 0.05) difference when compared against normal control group.

Reduced Glutathione (GSH) levels were found to be significantly decreased (P < 0.001) in the negative group when compared against normal control group. The group pretreated with 250 mg/kg BPE produced significant decrease (p<0.001) in reduced glutathione (GSH) levels and also standard treated group (ranitidine 50 mg/kg) and BPE (500 mg/kg) treated group showed significant decrease (p< 0.001 and p< 0.001 respectively) in reduced glutathione (GSH) levels as compared to negative group while BPE (500 mg/kg) treated group showed not much significant (p< 0.05) difference when compared against normal control group.

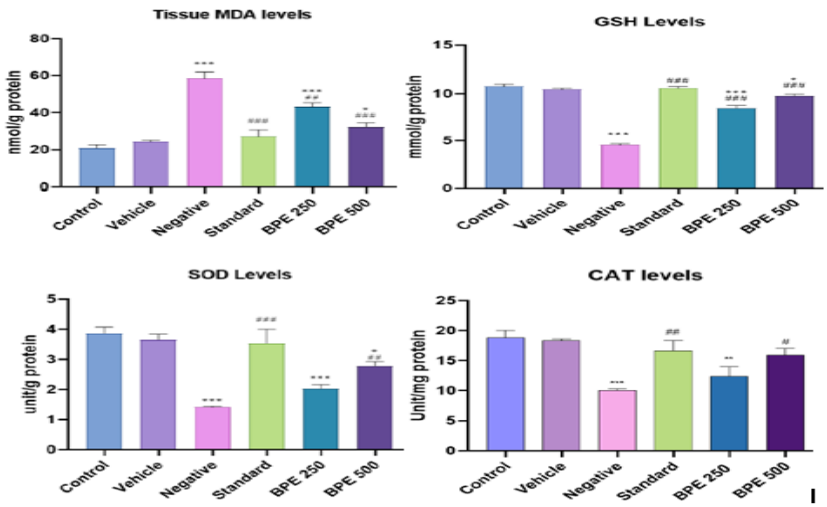

Figure 3: Antioxidant activity of BPE in aspirin induced gastric ulceration in wistar rats. ***Values are represented as mean ± SEM (n=6). Data was analysed by using one way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test; significance is denoted by ; * p< 0.05, **p< 0.01, ***p< 0.001 when compared against Normal control group and # p< 0.05, ## p< 0.01, ### p< 0.001 when compared with negative group.***

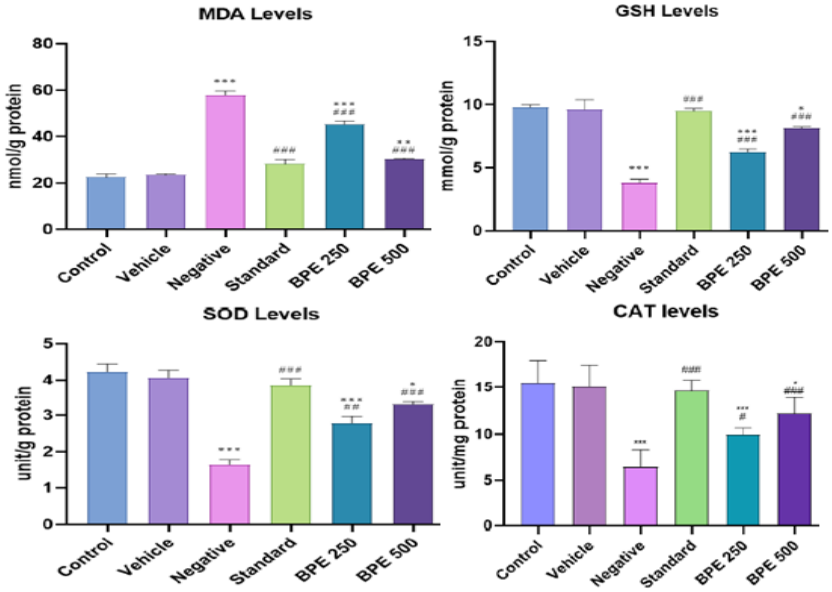

Figure 4: Antioxidant activity of BPE in ethanol induced gastric ulceration in wistar rats. ***Values are represented as mean ± SEM (n=6). Data was analysed by using one way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test; significance is denoted by ; * p< 0.05, **p< 0.01, ***p< 0.001 when compared against Normal control group and # p< 0.05, ## p< 0.01, ### p< 0.001 when compared with negative group.***

Superoxide dismutase (SOD) levels were found to be significantly decreased (P < 0.001) in the negative group when compared against normal control group. The group pretreated with 250 mg/kg BPE produced no significant decrease in superoxide dismutase (SOD) levels but standard treated group, ranitidine (50 mg/kg) and BPE (500 mg/kg) treated group showed significant decrease (p< 0.001 and p< 0.01 respectively) in superoxide dismutase (SOD) levels as compared to negative group while BPE (500 mg/kg) treated group showed not much significant (p< 0.05) difference when compared against normal control group.

Catalase (CAT) levels were found to be significantly decreased (P < 0.001) in the negative group when compared against normal control group. The group pretreated with 250 mg/kg BPE produced no significant decrease in catalase (CAT) levels but standard treated group, ranitidine (50 mg/kg) and BPE (500 mg/kg) treated group showed significant decrease (p< 0.01 and p< 0.05 respectively) in catalase (CAT) levels as compared to negative group while BPE (500 mg/kg) treated group showed not much significant (p< 0.05) difference when compared against normal control group.

4.6. Evaluation of pro-inflammatory parameters

The effect of IL-1β and TNF-α were shown in Fig. 5 and 6 for aspirin and ethanol-induced gastric ulcer groups.

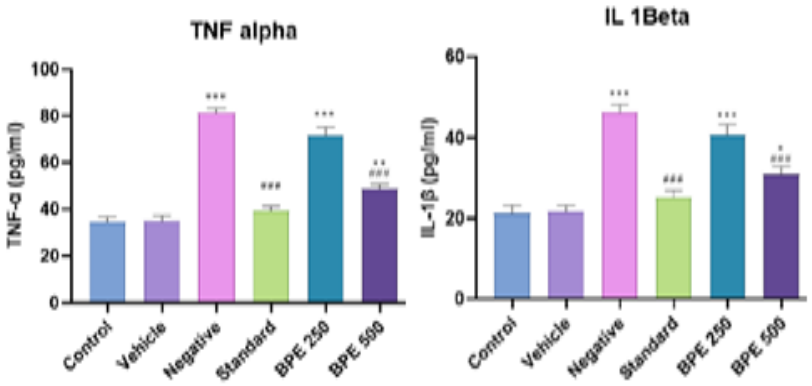

Figure 5: Effect of BPE on pro-inflammatory biomarkers in aspirin induced gastric ulceration in Wistar rats. ***Values are represented as mean ± SEM (n=6). Data was analysed by using one way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test; significance is denoted by ; * p< 0.05, **p< 0.01, ***p< 0.001 when compared against Normal control group and # p< 0.05, ## p< 0.01, ### p< 0.001 when compared with negative group.***

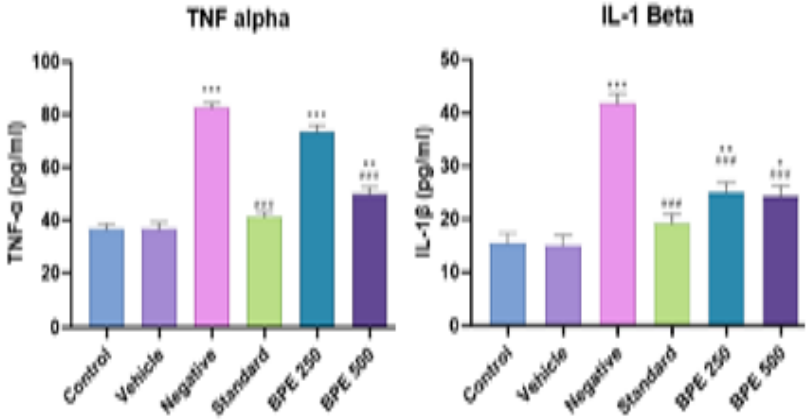

Figure 6: Effect of BPE on pro-inflammatory biomarkers in ethanol induced gastric ulceration in Wistar rats. ***Values are represented as mean ± SEM (n=6). Data was analysed by using one way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test; significance is denoted by ; * p< 0.05, **p< 0.01, ***p< 0.001 when compared against Normal control group and # p< 0.05, ## p< 0.01, ### p< 0.001 when compared with negative group.***

TNF-α levels were elevated in negative control group. BPE 250 mg decreased TNF-α levels as compared to negative control group. Significant decrease in TNF-α level was seen at dose of BPE 500 mg/kg. Standard drug, ranitidine, also showed significant decrease in TNF-α level.

IL-1β levels were elevated in negative control group. BPE 250 mg did not decrease IL-1β levels as compared to negative control group. Significant decrease in IL-1β level was seen at dose of BPE 500 mg/kg. Standard drug, ranitidine, also showed significant decrease in IL-1β level.

4.7. Histological findings

Microscopic examination of gastric mucosa of rats in aspirin and ethanol induced ulcer group revealed occurrence of epithelial erosion, separation and exfoliation of cells lining gastric pit, inflammatory infiltrates and suffusion of blood into the mucosa. Pre-treatment with single administration of BPE alleviated the injury caused by aspirin and ethanol and its restorative effect matched that of ranitidine except mild

suffusion of blood where ranitidine was more effective than BPE. The results are shown in Figs. 7 and 8.

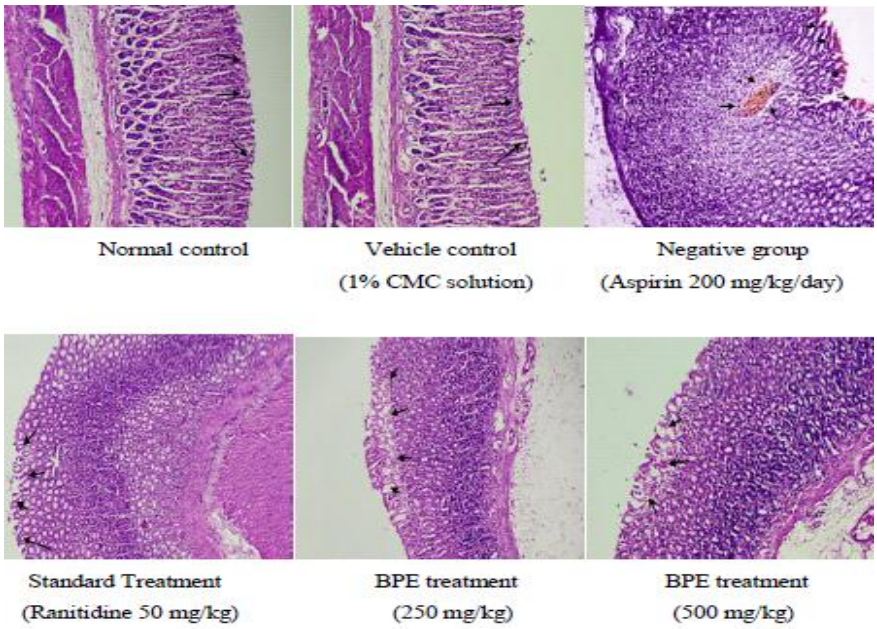

Figure 7: The histological evaluation of effect of BPE on gastric mucosal staining in aspirin induced gastric ulcer in Wistar rats.

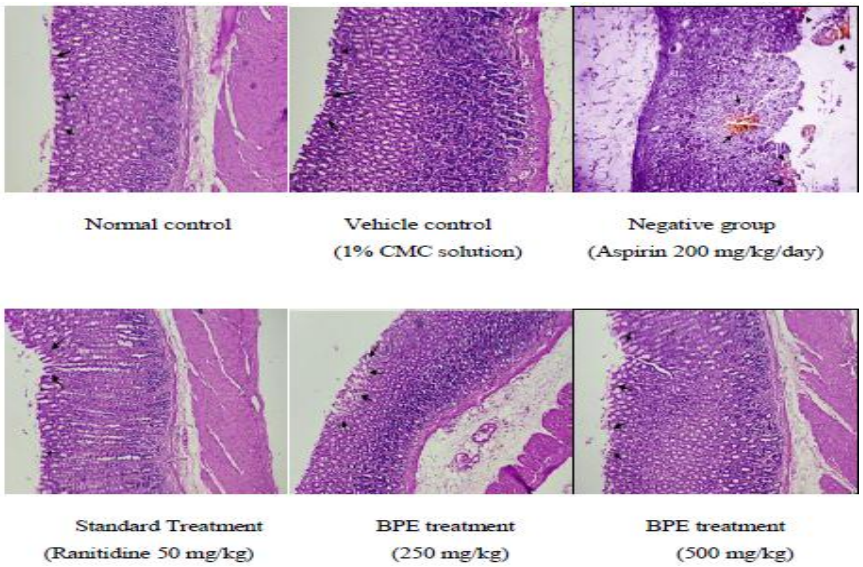

Figure 8: The histological evaluation of effect of BPE on gastric mucosal staining in ethanol induced gastric ulcer in Wistar rats.

5. DISCUSSION

The pathogenic effects of both the models of gastrointestinal ulcers have been established to include increased secretion of gastric acid, which resulted in increased gastric volume, reduced pH, and increased total acidity, acid output and ulcer index. Findings from this study showed that in aspirin as well as ethanol induced ulcer rats, the BPE pretreated groups (250 and 500 mg/kg) produced a significant decrease in acid output, free acidity, total acidity, and volume of gastric content with a corresponding increase in pH compared to the ulcer control group. This decrease in gastric juice volume in the BPE pretreated groups may be due to a decrease in acid production as evidenced from the gastric juice total acidity. This is an indication that BPE contains some biological compounds that reduce the acidity of gastric secretions which had been increased by aspirin and ethanol.

From this study, the results showed that BPE can significantly prevent the gastric damaging effects of aspirin by protecting the mucosa with a significant decrease in ulcer index, and an increase in mucus production in comparison to the ulcer control group. This

observed cytoprotection offered by the extract may be mediated by strengthening of the mucosal barrier (gastric mucus restoration) through increase in prostaglandin synthesis, enabling it to resist the toxic effects of aspirin. Also, a consensus has reached that ethanol inflicting gastric ulcer primarily attributes to oxidative stress. Reactive oxygen species (ROS) is responsible for oxidative stress.[34] When ethanol stimulates gastric mucosa, excessive ROS would generate including superoxide anions, hydroxyl radical and hydrogen peroxide and strike the *in vivo* balance between oxidation and anti-oxidation.[17] The level of lipid peroxidation in gastric tissue was determined by MDA, a metabolite for oxidative stress. On the other hand, GSH, SOD and CAT constitute an endogenous antioxidant system and can be applied to scavenge excessive oxygen-derived free radicals and maintain them at physiological levels.[35] In this study, pre-treatment with BPE showed a significant decrease in MDA and an increase in GSH, SOD and CAT, as compared with ulcerated group, indicating that BPE exerted potent gastric protection by alleviating oxidative stress in aspirin as well as ethanol induced gastric ulcer model.

Histological evaluation revealed that the gastric mucosa is protected and infiltration of leucocytes into the submucosa is inhibited or reduced in rats pretreated with BPE. The gastric mucosa is otherwise extensively damaged by aspirin and ethanol, leading to increased infiltration of neutrophils into the submucosa. Inflammatory mediators are mainly formed by neutrophils that can release free radicals, which are harmful to cells and tissues. A decrease in infiltration of neutrophils into ulcerated gastric tissue enhances gastric ulcer healing in rats. Gastric ulcer healing in rats is inhibited by oxygen-free radicals released by neutrophils that infiltrated into ulcerated gastric tissues This inhibition of leucocyte infiltration by BPE into the submucosa suggests that the extract may possess anti-inflammatory properties, which could also play a role in gastric ulcer prevention.

6. CONCLUSION

In conclusion, the study suggests that BPE possesses significant gastroprotective effects against aspirin as well as ethanol induced gastric ulcers in rats. The observed gastroprotective effect might be possibly due to its antisecretory, cytoprotective and antioxidative properties. Our findings may have beneficial application in the management of gastric mucosal ulcers associated with aspirin and ethanol -induced gastric ulceration.

7. ACKNOWLEDGEMENT

Authors are thankful to Dr. Munira Momin, Principal and Dr. Lokesh kumar Bhatt, HOD Pharmacology Department, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Vile Parle, Mumbai for their kind support. Authors are also greatly thankful to the Mr. Kishor Parekh, PharmaBase S.A. Switzerland, Neon laboratories Ltd., The Andhra Sugars Limited and Research-Lab Fine Chem Industries, Mumbai for providing drug samples and chemicals to carry out this research work.

8. REFERENCES

1. Aro P, Storskrubb T, Ronkainen J, et al. Peptic ulcer disease in a general adult population: The kalixanda study: A random population-based study. *Am J Epidemiol*, 2006; 163(11): 1025-1034. doi: 10.1093/aje/kwj129.
2. Prabhu V, Shivani A. An overview of history, pathogenesis and treatment of perforated peptic ulcer disease with evaluation of prognostic scoring in adults. *Ann Med Health Sci Res*, 2014; 4(1): 22. doi: 10.4103/2141-9248.126604.
3. Sowndhararajan K, Chul S. Protective effect of ethyl acetate fraction of Acacia ferruginea DC. against ethanol-induced gastric ulcer in rats. *J Ethnopharmacol*, 2013; 148(1): 175-181. doi: 10.1016/j.jep.2013.04.007.
4. Aslan A, Halici M, Odabasoglu F, et al. The inhibition of gastric mucosal lesion, oxidative stress and neutrophil-infiltration in rats by the lichen constituent diffractaic acid. *Phytomedicine*, 2005; 13(8): 584-590. doi: 10.1016/j.phymed.2005.07.002.
5. Rainsford KD. The effects of 5-1ipoxygenase inhibitors and leukotriene antagonists on the development of gastric lesions induced by nonsteroidal antiinflammatory drugs in mice. *Agents Actions*, 1987; 21: 316-319.
6. Matsui H, Shimokawa O, Kaneko T, Nagano Y, Rai K, Hyodo I. The pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and intestine. *J Clin Biochem Nutr*, 2011; 48(2): 107-111. doi: 10.3164/jcbn.10.
7. Bjarnason [Hayllar and I. NSAIDs, Cox-2 inhibitors, and the gut. *Lancet*, 1995; 346(8982): 1105-1106. doi: 10.1016/S0140-6736(95)91782-9.
8. Wallace JL, McKnight W, Reuter BK, Vergnolle N. NSAID-Induced gastric damage in rats: Requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology*, 2000; 119(3): 706-714. doi: 10.1053/gast.2000.16510.
9. Whittle BJR. Gastrointestinal effects of nonsteroidal anti-inflammatory drugs. *Fundam Clin Pharmacol*, 2003; 17(3): 301-313. doi: 10.1046/j.1472-8206.2003.00135.x.
10. Wallace JL. Prostaglandins, NSAIDs, and Gastric Mucosal Protection: Why Doesn't the Stomach Digest Itself? *Physiol Rev*, 2008; 88(4): 1547-1565. doi: 10.1152/physrev.00004.2008.
11. Yu C, Mei XT, Zheng YP, Xu DH. Gastroprotective effect of taurine zinc solid dispersions against absolute ethanol-induced gastric lesions is mediated by enhancement of antioxidant activity and endogenous PGE2production and attenuation of NO production. *Eur J Pharmacol*, 2014; 740: 329-336. doi: 10.1016/j.ejphar.2014.07.014.
12. Bode C, Bode JC. Alcohol's role in gastrointestinal tract disorders. *Alcohol Health Res World*, 1997; 21(1): 76-83. doi: 10.1007/s13398-014-0173-7.2.

13. Albino de Almeida AB, Melo PS, Hiruma-Lima CA, et al. Antiulcerogenic effect and cytotoxic activity of semi-synthetic crotonin obtained from Croton cajucara Benth. *Eur J Pharmacol*, 2003; 472(3): 205-212. doi: 10.1016/S0014-2999(03)01909-5.
14. Guslandi M. Effects of ethanol on the gastric mucosa. *Dig Dis*, 1987; 5(1): 21-32.
15. La Casa C, Villegas I, Alarcón De La Lastra C, Motilva V, Martín Calero MJ. Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions. *J Ethnopharmacol*, 2000; 71(1-2): 45-53. doi: 10.1016/S0378-8741(99)00174-9.
16. Arda-Pirincci P, Bolkent S, Yanardag R. The role of zinc sulfate and metallothionein in protection against ethanol-induced gastric damage in rats. *Dig Dis Sci*, 2006; 51(12): 2353-2360. doi: 10.1007/s10620-006-9301-3.
17. Wong Sh, cho ch, ogle cw. calcium and ethanol-induced gastric mucosal damage in rats Department of Pharmacology, Faculty of Medicine, University of Hong Kong, Hong Kong. *Pharmacol Res*, 1991; 23(1): 71-79.
18. Marhuenda E, Martin MJ, Alarcon Lastra CD La. Antiulcerogenic activity of aescine in different experimental models. *Phyther Res*, 1993; 7(1): 13-16. doi: 10.1002/ptr.2650070105.
19. Kangwan N, Park J, Kim E, et al. Quality of healing of gastric ulcers : Natural products beyond acid suppression. *World J Gastrointest Pathophysiol*, 2014; 5(1): 40-47. doi: 10.4291/wjgp.v5.i1.40.
20. Sforcin JM. Propolis and the immune system: a review. *J Ethnopharmacol*, 2007; 113(1): 1-14. doi: 10.1016/j.jep, 2007.05.012.
21. Wagh. Propolis: A wonder bees product and its pharmacological potentials. *Adv Pharmacol Sci*, 2013; 2013: 1-11.
22. Elnakady YA, Rushdi AI, Franke R, et al. Characteristics, chemical compositions and biological activities of propolis from Al-Bahah, Saudi Arabia. *Sci Rep*, 2017; 7(2016): 1-13. doi: 10.1038/srep41453.
23. Akbay E, Özenirler Ç, Çelemli ÖG, Durukan AB, Onur MA, Sorkun K. Effects of propolis on warfarin efficacy. *Exp Cardiovasc Lung Reasearch*, 2017; 14(1): 43-46. doi: 10.5114/kitp.2017.66929.
24. Alonso-Salces RM, Cugnata NM, Guaspari E, et al. Natural strategies for the control of Paenibacillus larvae, the causative agent of American foulbrood in honey bees: a review. *Apidologie*, 2017; 48(3): 387-400. doi: 10.1007/s13592-016-0483-1.
25. Kasiotis KM, Anastasiadou P, Papadopoulos A, Machera K. Revisiting Greek propolis: Chromatographic analysis and antioxidant activity study. *PLoS One*, 2017; 12(1): 1-27. doi: 10.1371/journal.pone.0170077.
26. Omar R, Igoli JO, Zhang T, et al. The Chemical Characterization of Nigerian Propolis samples and Their Activity Against Trypanosoma brucei. *Sci Rep*, 2017; 7(1): 1-10. doi: 10.1038/s41598-017-01038-2.
27. Sforcin JM, Bankova V, Kuropatnicki AK. Medical Benefits of Honeybee Products. *Evidence-based Complement Altern Med*, 2017: 1-2. doi: 10.1155/2017/2702106.
28. Sforcin JM. Biological Properties and Therapeutic Applications of Propolis. *Phyther Res*, 2016; 30(6): 894-905. doi: 10.1002/ptr.5605.
29. Toreti VC, Sato HH, Pastore GM, Park YK. Recent progress of propolis for its biological and chemical compositions and its botanical origin. *Evidence-based Complement Altern Med*, 2013: 1-13. doi: 10.1155/2013/697390.
30. Hatware KV, Sharma S, Patil K, Shete M, Karri S, Gupta G. Evidence for gastroprotective, anti-inflammatory and antioxidant potential of methanolic extract of Cordia dichotoma leaves on indomethacin and stress induced gastric lesions in Wistar rats. *Biomed Pharmacother*, 2018; 103(2017): 317-325. doi: 10.1016/j.biopha.2018.04.007.
31. Balogun ME, Besong EE, Obimma JN, Mbamalu OS, Djobissie FSA. Protective roles of Vigna subterranea (Bambara nut) in rats with aspirin-induced gastric mucosal injury. *J Integr Med*, 2018; 16(5): 342-349. doi: 10.1016/j.joim.2018.07.004.
32. Boutemine IM, Amri M, Amir ZC, 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: 273-282. doi:10.1016/j.jep.2018.05.040.
33. Adefisayo MA, Akomolafe RO, Akinsomisoye SO, et al. Gastro-protective effect of methanol extract of Vernonia amygdalina (del.) leaf on aspirin-induced gastric ulcer in Wistar rats. *Toxicol Reports*, 2017; 4: 625-633. doi: 10.1016/j.toxrep.2017.11.004.
34. Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Brazilian J Med Biol Res*, 2002; 35(5): 523-534. doi: 10.1590/S0100-879X2002000500003.
35. Marhuenda E, Martin MJ, Alarcon Lastra CD La. Antiulcerogenic activity of aescine in different experimental models. *Phyther Res*, 1993; 7(1): 13-16. doi:10.1002/ptr.2650070105.