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RESEARCH ARTICLE

Gastroprotective and ulcer healing potentials of Nigerian Bee Propolis flavonoid extract on acetic acid-induced gastric ulcers in albino rats (Wistar Strains)

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Abstract

Gastric ulcer is a serious global health challenge, and various natural products are being investigated to prevent and manage the condition. This study evaluated the gastroprotective and ulcer healing potentials of Nigerian bee propolis flavonoid-rich extract (NPE) on acetic acid-induced gastric ulcers in albino rats. Sixty adult male albino rats (222 ± 6.4 g) randomised into 5 groups ($n = 12$) were studied. Group A (SHAM) was left untreated, while gastric ulcer was induced in groups B (NPE), C (omeprazole) and D (saline). Group E (PRPE) was pre-treated with NPE prior to ulcer induction. The rate of ulcer contraction, volume and pH of gastric juice, and histopathological parameters were evaluated. The results showed a significantly higher rate of contraction ($P = 0.001$) between days 9 and 12 (NPE > OME > PRPE > SAL) and a significant decrease ($P = 0.003$) in the volume of gastric juice between days 9 and 12 (NPE < OME < PRPE). Gradual increase in pH was observed in all the groups from days 3 to 12, with a significantly higher rate ($P < 0.001$) between day 6 and 12 (SHAM > NPE > OME > PRPE > SAL). Histological evaluation showed significantly high neutrophils and macrophages on day 6 ($P = 0.006$) and lymphocytes ($P = 0.004$) between day 6 and 12 in the OME and NPE groups. NPE showed gastroprotective and ulcer healing properties by inhibiting ulcer formation and facilitating the curation of induced ulcers and is, therefore, a valuable alternative to conventional gastric ulcer therapy, especially in poor resource settings.

Keywords Flavonoids · Gastric ulcer · Gastroprotective · Nigerian propolis

Introduction

Gastric ulcers are defects in the stomach seen as sores or erosions in the gastric mucosa and sub-mucosa which may extend through the muscularis and serosa (Bukhari et al. 2011). Gastric mucosal injuries occur when a disequilibrium between gastric acid secretion and gastric mucosal defense systems leads to a disruption in the balance between the aggressive nature of the gastric acid and protective factors of the stomach (Lima et al. 2006; Tulassay & Herszényi 2010; Zatorski et al., 2017).

Gastric ulcers are induced by various factors, disease conditions and drugs with prolonged non-steroidal anti-inflammatory drug (NSAID) therapy as the most common inducing factor in dogs (Wallace, 1992; Laine et al. 2008). Other inducing factors include bacterial infection (*Helicobacter pylori*), nutritional deficiencies, ingestion of irritant 'chemicals' or drugs, stress, physical burns (Curling's ulcer) and age-related decline in prostaglandin levels (Belaiche et al. 2002). The condition may also be induced by pre-existing hepatic or renal disease (Kang 1994; Liang et al. 2014). The clinical signs in ulcer patients vary but mainly include vomiting, hematemesis (Fitzgerald et al. 2017; Pennick et al. 1997), abdominal pain, melena, erratic anorexia and weight

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loss (Pennick et al. 1997). Chronic gastritis, anaemia and shock may be present in severe cases (Pennick et al. 1997). Diagnosis of gastric ulcers basically involves patient's signalment, history and physical examination. Blood and serum analysis as well as diagnostic imaging techniques (radiography, endoscopy, gastro-camera photography and ultrasonography) may also provide vital information leading to definitive diagnosis of the condition (Parrah et al. 2013).

Medical and surgical methods have been employed to manage gastric ulcers (Fossum and Hedlund 2003). In the sequence of gastric ulcer management, it is usually expedient to exhaust conservative medical management before attempting surgical intervention, with proper consideration of the severity of the condition. Antacids, sucralfate, H_2-receptor antagonists and proton-pump inhibitors are current inorganic medical remedies used in the management of gastric ulcers (Manonmani et al. 1995; Bighetti et al. 2005). In more recent times, investigations have focused on exploring the gastric ulcer healing potentials of organic products, including propolis, following reports of efficacy in the management of several other health conditions (Kuropatnicki et al. 2013). Studies have since shown the efficacy of Brazilian green, Indian, Egyptian and southern-Poland propolis in the treatment of gastric ulcers in experimental rat models (de Barros et al 2007).

In Nigeria, however, little is known about propolis and only a few studies have been conducted using propolis (Babatunde et al. 2015). Although the cutaneous wound healing potential of the Nigeria bee propolis was recently investigated (Eyarefe et al. 2019a, b), its gastroprotective and ulcer healing potentials are yet to be reported. This requires further research that may lead to new discoveries of its compositions and possible applications. This study therefore investigated the gastroprotective and ulcer healing effects of Nigeria bee propolis flavonoid-rich extract on acetic acid- induced gastric ulcer in albino rats (Wistar strain).

Materials and methods

Ethical approval

Ethical approval (Ethic no. UI-ACUREC/19/0151) was obtained from the Animal Care and Use Ethical Committee, University of Ibadan before commencement of the study.

Experimental animal management

Sixty (60) male Albino rats (Wistar strain) weighing 222.2 ± 6.4 g were used for this study. They were acquired from a commercial breeding unit, housed in well ventilated 12 × 15 inches individual cages (27 ± 1 °C, and 12 h light/dark cycle) at the Laboratory Animal house, Faculty of Veterinary medicine, University of Ibadan (UI) and fed with commercial rat pellets (Breedwell feeds limited, Nigeria) and water ad-libitum. All animals received humane care and experiments were preceded by a 2-week acclimatization period, during which they were monitored once a day to check their health status as well as food intake and any behavioural changes.

Preparation of propolis extract

Crude Bee propolis was obtained from the Iwo, Osun state beehive site of the University of Ibadan Apiary unit and stored in a dark waterproof container. Extraction was carried out using a standard extraction technique (Couto 2001).

Study design

A simple randomized controlled design was adopted for this study. The rats were randomly assigned to one of five (5) treatment groups: Sham (SHAM), propolis extract (NPE), omeprazole (OME), saline (SAL) and propolis extract pretreated (PRPE).

Experimental induction of gastric ulcer

Anaesthesia: Each rat was fasted for 24 h and the body weight was determined using an electronic weighing scale (Camry Electrinic Limited, China) prior to anaesthetic induction. Anesthetic induction and maintenance were achieved by single doses each of 2% Xylazine HCl (Bioveta, Czech Republic) (5.0 mg/kg) and 5% Ketamine (Kwality Pharmaceuticals Limited, India) (35.0 mg/kg) via intramuscular injection at the quadriceps group of muscles (Eyarefe and Amid 2010).

Aseptic protocol: Following anaesthesia, the rats were placed on dorsal recumbency and the ventral abdomen of each rat was prepared for aseptic surgery by carefully shaving, followed by scrubbing and sterilization with alcohol and povidone iodine (Khoo et al. 2010).

Surgical Technique: The abdominal cavity was accessed via a 1.5–2 cm left paramedian incision. The stomach was exteriorized, stabilized with chalazion eye forceps and 0.03mls of normal saline was carefully injected into the gastric lumen of rats in Group A; while those in groups B to E were injected with 0.03 ml of 30% acetic acid solution at the area limited by the forceps. The stomach was returned into the abdominal cavity and the laparotomy incision closed with nylon sutures (Huaian Amgel Medical Instruments Co. Ltd, Jiangsu China) in cruciate suture pattern.

Post-operative care: Following surgical procedures, the rats were placed in plastic cages padded with cotton wool and warm water bags to provide a warm environment. Each

rat received 5 ml of Dextrose saline (Unique Pharmaceuticals Limited, Nigeria) by oral gavage four times at 15 min interval after recovery from anaesthesia to prevent hypoglycemia, and placed on blenderized diet (corn, edible common salt and milk with vanilla flavour) post-operatively till day 6. The rat pellets (Breedwell feeds limited, Nigeria) were gradual re-introduced from day 7 and water was provided ad-libitum.

Rats monitoring and establishment of gastric ulcer

The rats were carefully monitored for 3 days post-induction for clinical signs of gastric ulcers (dark tarry stool, reduced activities, arching of the back, vocalization) and scored (Table 1). Three rats each from groups A to D were sacrificed on day 3 to establish the presence of gastric ulcer, after which the treatment protocol was commenced.

Treatment protocol

Group A rats were left untreated, while rats in group B were treated with 30 mg/kg Propolis extract daily, those in group C were treated with 20 mg/kg Omeprazole daily and those in group D received 2mls normal saline daily, all by oral gavage.

Rats in group E however were pretreated with 30 mg/kg bw Propolis extract daily (till end of study) before the induction of gastric ulcers in 3 rats each on days 3, 6, 9 and 12. All drugs were administered orally.

Euthanasia

Three rats each in groups A to D were sacrificed with euthanizing doses of Xylazine (Bioveta, Czech Republic) (70 mg/kg) and Ketamine (Kwality Pharmaaceuticals Limited, India) (15 mg/kg) on days 6, 9 and 12 respectively followed by evaluation (gastric juice collection and measurement of ulcer size). The rats in group E were sacrificed 72 h post ulcer induction and evaluation was carried out as described for groups A to D.

Evaluation of gastric ulcer healing

Gastric juice collection and pH determination

Following euthanasia, a paramedian laparotomy incision was made to access the stomach, which was exteriorized, carefully tied around both openings (cardiac & pyloric sphincters) and harvested. The ligature at one end was loosened and the gastric content was carefully collected in sterile sample tubes and centrifuged at 500 rpm, for 5 min using a tube centrifuge (Celtech 800D Centrifuge, China). The supernatant was carefully aspirated using a Pasteur pipette and the volume of gastric juice was measured using graduated 1 ml syringe.

The pH of the gastric juice of each rat was evaluated using a pH strip with colour indicator (AtFipan pH Universal indicator paper).

Gross evaluation of gastric mucosa and ulcer size determination

The stomach of each rat was incised longitudinally along the greater curvature, gently rinsed with saline and examined grossly for ulcers. The size of the ulcer was measured (in cm^3) using a transparent graph sheet as previously described (Majeske 1992) to evaluate rate of ulcer contraction. The obtained ulcer size was used to calculate the percentage of wound contraction with the formula:

$$\%\text{Wound contraction} = \frac{\text{Day 0 wound size} - \text{Day } n \text{ wound size}}{\text{Day 0 wound size}} \times 100$$

Pictures of each wound were also taken on each day of observation.

Histopathological examination

After gross evaluation, the harvested stomach was fixed in 10% neutral buffered formalin and submitted for independent histological examination by pathologists blinded to the groupings. The lesions were scored and categorised

Table 1 Six-point scale for clinical signs (Parrah et al. 2013) and histological observations (Andrew et al., 2002) for gastric lesion/ulcer with modification following clinical signs and lesions observed in the study, respectively

Score	Clinical sign observed	Histopathological lesion observed
0	No clinical signs of gastric ulcers	Almost normal mucosa
1	Vocalisation, but no arching of the back or dark tarry faeces	Vascular congestion/ haemorrhage
2	Vocalisation and arching of the back, but no dark tarry faeces	Degenerative/ hyperplastic change (mucous, neck or pit cells)
3	Dark tarry faeces, but no vocalisation or arching of the back	Necrotic and a few inflammatory changes (mucous, neck or pit cells)
4	Dark tarry faeces and vocalisation, but no arching of the back	Erosions and inflammatory change
5	Dark tarry faeces, vocalisation and arching of the back	Intense mucosal defect (ulcer)

(Table 1) as described by Andrew et al. (2002) with slight modifications. Representative sections were stained with haematoxylin and eosin (H&E) for microscopic evaluation of inflammatory cells.

Statistical analysis

Data generated from rate of ulcer contraction, volume of gastric juice and pH were descriptively presented as 'mean ± standard deviation', using SPSS version 16.0. Significant differences between groups were assessed using one-way ANOVA followed by post-hoc multiple comparison test. Values of $P < 0.05$ were considered statistically significant. Fig 1.

Results

Clinical signs of gastric ulcers

Following induction of gastric ulcers, clinical signs such as dark tarry faeces, vocalisation and arching of the back attributed to the presence of gastric ulcers were prominent in the rats in NPE, OME, SAL and PRPE groups on day 3 compared with the SHAM group in which these signs were not as prominent (Table 2). The clinical signs progressively diminished following the commencement of treatments in the four groups. However, in SAL group, it was observed that the signs persisted for a longer period, not reducing at the same pace as the NPE, OME and PRPE groups, but no visible or palpable clinical signs were observed in the SHAM groups. Table 3.

Gastric ulcer contraction

Ulcers were not observed in the SHAM group. Fig 2 Ulcer contraction was gradual in all the other groups from day 3 to day 12, with a significantly higher rate of contraction ($P = 0.001$) between days 9 and 12 in the NPE, OME and PRPE groups compared with the SAL group with the trend being (NPE > OME > PRPE > SAL). Almost complete wound closure occurred on day 12 in the NPE group.

Volume of gastric juice

The volume of gastric juice obtained from the stomach of rats in the SHAM group was significantly lower when compared with the other groups. There was a significant decrease in the volume of gastric juice in the NPE, OME and PRPE groups ($P = 0.003$) between days 9 and 12, with the trend being (NPE < OM < PRPE), but there was non-significant decrease in the SAL group.

pH of gastric juice

There was gradual increase in pH in all the groups from day 3 to day 12, with significantly higher rate ($P < 0.001$) between days 6 and 12 in the NPE, OME and PRPE groups compared with the SAL group with the trend being (SHAM > NPE > OME > PRPE > SAL).

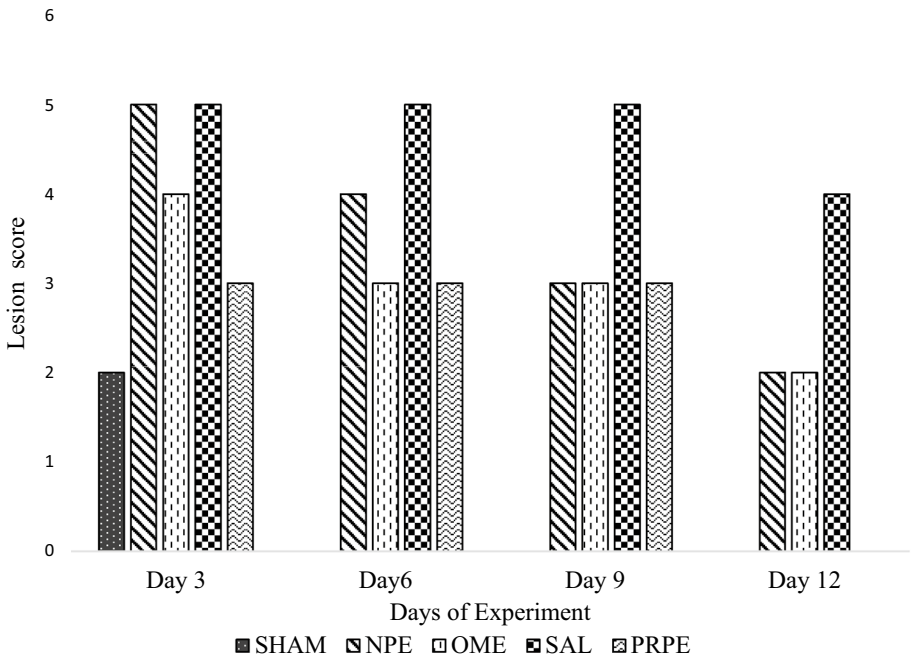


Fig. 1 Bar chart showing the severity of gastric ulcer lesions *using the histological lesion score* across the five groups on days 3, 6, 9 and 12 , *SHAM = Sham; NPE= Nigerian Propolis Extract; OME=Omeprazole; SAL= Saline; PRPE= Propolis Extract Pretreatment*

Table 2 Mean (± SD) values for gross parameters evaluated across the 5 groups on days 3, 6, 9 and 12

Gross Parameters	SHAM	NPE	OME	SAL	PRPE
CLINICAL SIGNS					
3	2.0 ± 1.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	4.0 ± 1.0
6	0.0 ± 1.0	1.0 ± 1.0	1.0 ± 0.0	4.0 ± 0.0	3.0 ± 1.0
9	0.0 ± 0.0*	1.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
12	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 1.0	2.0 ± 0.0	1.0 ± 1.0
RATE OF ULCER CONTRACTION					
3	0.00 ± 0.00	6.73 ± 0.31	6.30 ± 1.48	6.90 ± 0.26	3.23 ± 0.25
6	0.00 ± 0.00	4.83 ± 0.15	5.50 ± 0.30	6.27 ± 0.12	2.40 ± 0.20
9	0.00 ± 0.00	2.37 ± 0.15[a]	2.33 ± 0.15[b]	4.27 ± 0.12	2.13 ± 0.23[c]
12	0.00 ± 0.00	0.67 ± 1.15	1.57 ± 0.32	2.53 ± 0.15	1.93 ± 0.12
VOLUME OF GASTRIC JUICE					
3	0.15 ± 0.02	0.22 ± 0.02	0.25 ± 0.02	0.25 ± 0.03	0.25 ± 0.02
6	0.09 ± 0.02	0.22 ± 0.03	0.21 ± 0.02	0.24 ± 0.01	0.21 ± 0.01
9	0.06 ± 0.01	0.14 ± 0.02[a]	0.17 ± 0.02[b]	0.21 ± 0.02	0.15 ± 0.02[c]
12	0.05 ± 0.01	0.07 ± 0.01	0.10 ± 0.01	0.20 ± 0.01	0.11 ± 0.02
pH OF GASTRIC JUICE					
3	3.50 ± 0.00	2.17 ± 0.29	2.00 ± 0.00	2.00 ± 0.00	2.50 ± 0.00
6	3.83 ± 0.29	2.67 ± 0.29[a]	2.50 ± 0.50[b]	2.17 ± 0.29	2.67 ± 0.29[c]
9	5.17 ± 0.58	3.50 ± 0.50 [a]	3.67 ± 0.76 [b]	2.50 ± 0.50	3.50 ± 0.00 [c]
12	5.50 ± 0.00	4.83 ± 0.29	4.17 ± 0.29	3.17 ± 0.29	3.83 ± 0.29

Difference of values with superscript "a" is significantly higher than the differences between the values with superscripts "b and c". SHAM = Sham; NPE = Nigerian Propolis Extract; OME = Omeprazole; SAL = Saline; PRPE = Propolis Extract Pre-treatment

Table 3 The inflammatory cell response on days 3, 6, 9 and 12 across the five groups

Histologic Parameters	SHAM	NPE	OME	SAL	PRPE
DAY 3					
Neutrophil	25.48 ± 17.99	89.31 ± 42.09	113.68 ± 80.12	19.40 ± 25.53	30.24 ± 6.06
Macrophage	57.70 ± 43.53	87.01 ± 24.69	90.22 ± 21.67	42.99 ± 24.28	56.47 ± 2.58
Lymphocyte	50.92 ± 24.91	47.1 ± 34.36	65.46 ± 3.33	36.27 ± 14.32	56.85 ± 9.73
Plasma cell	14.88 ± 7.27	9.40 ± 0.35	10.77 ± 14.88	11.51 ± 4.91	12.43 ± 10.29
DAY 6					
Neutrophil	30.55 ± 10.51	57.33 ± 41.02[b]	52.25 ± 51.02 [a]	22.09 ± 26.94	20.63 ± 13.80
Macrophage	60.35 ± 10.02	63.56 ± 16.55 [b]	76.06 ± 30.25 [a]	36.62 ± 21.51	56.23 ± 26.29
Lymphocyte	61.16 ± 12.38	56.91 ± 29.22 [b]	54.04 ± 13.92 [a]	34.72 ± 24.91	46.61 ± 19.31
Plasma cell	9.17 ± 3.18	14.08 ± 6.43	9.65 ± 9.82	6.67 ± 5.69	18.53 ± 11.90
DAY 9					
Neutrophil	28.49 ± 16.34	21.61 ± 11.24	16.62 ± 16.40	7.56 ± 7.04	12.23 ± 6.94
Macrophage	65.67 ± 13.68	49.63 ± 10.16	52.44 ± 17.12	23.67 ± 16.01	45.53 ± 17.14
Lymphocyte	48.36 ± 7.10	37.26 ± 7.20 [b]	50.9 ± 14.22 [a]	20.33 ± 8.79	47.72 ± 16.81
Plasma cell	6.48 ± 5.66	10.45 ± 6.10	10.04 ± 11.00	5.77 ± 4.00	8.18 ± 6.12
DAY 12					
Neutrophil	28.51 ± 7.08	7.46 ± 9.21	33.44 ± 26.60	7.74 ± 6.34	4.79 ± 2.80
Macrophage	67.06 ± 11.73	27.23 ± 15.89	43.36 ± 31.16	26.60 ± 7.24	31.19 ± 7.78
Lymphocyte	61.71 ± 5.89	24.72 ± 14.14	42.78 ± 12.67	29.45 ± 13.04	28.47 ± 7.83
Plasma cell	8.39 ± 7.06	8.25 ± 7.25	10.42 ± 9.45	5.81 ± 2.93	8.31 ± 4.85

Difference of values with superscript "a" is significantly higher than the differences between the values with superscripts "b and c". SHAM = Sham; NPE = Nigerian Propolis Extract; OME = Omeprazole; SAL = Saline; PRPE = Propolis Extract Pre-treatment

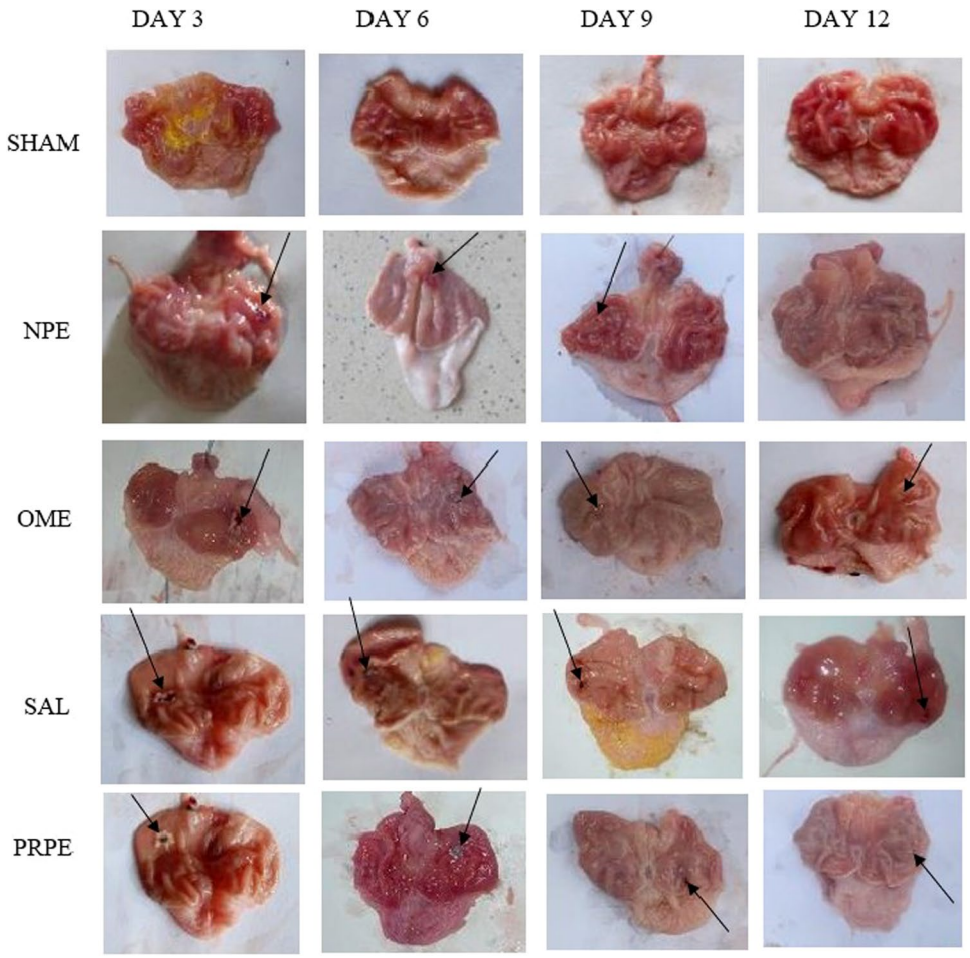


Fig. 2 Gross presentation of the gastric ulcers healing in rats within the five groups treatment from day 3 to 12 *SHAM = Sham; NPE = Nigerian Propolis Extract; OME = Omeprazole; SAL = Saline; PRPE = Propolis Extract Pre-treatment*

Inflammatory Cell response

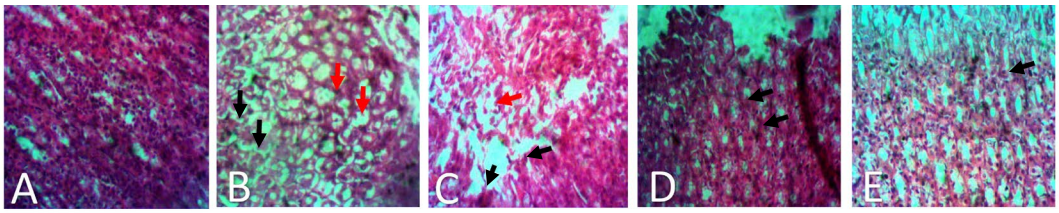
Across the five groups, inflammatory cellular response was significantly higher ($P < 0.05$) in the NPE, OME and SHAM groups when compared with the PRPE and SAL groups. The number of neutrophils was significantly high in the OME group ($P < 0.05$); macrophages was significantly high in the OME, SHAM and NPE groups ($P = 0.02$); Lymphocytes was significantly high in the SHAM and OM groups ($P = 0.006$).

The number of neutrophils and macrophages was significantly high in the OME and NPE group on day 6 ($P = 0.006$), with the trend being (OME > NPE > SHAM > PRPE > SAL). The number of lymphocytes was significantly high ($P = 0.004$) in the OME and NPE group between day 6 and day 12, with the trend being (SHAM > OME > NPE > PRPE > SAL). There was no significant difference in the Plasma cell count across the groups. Moreover, there was significant reduction in the neutrophil and macrophage counts observed in the NPE, OME and PRPE treatments ($P < 0.05$). Fig 3.

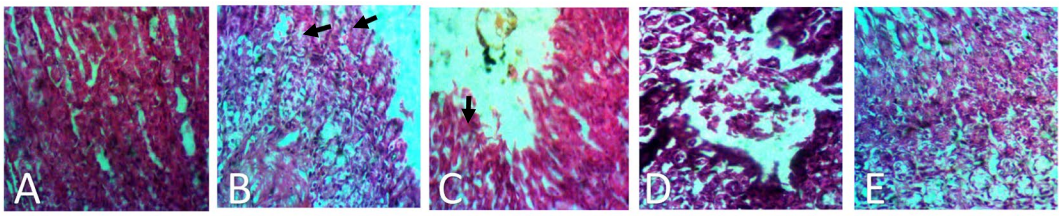
Discussion

Gastric ulcer disease is a global health challenge in both human and animal population with complications that include haemorrhage, perforation, gastrointestinal obstruction, and malignancy with high morbidity, mortality and economic loss to animal owners (Dimaline and Varro 2007). Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by different models which result in an imbalance between the gastric mucosa defence system and gastric acid production. The ability of the gastric mucosa to resist injury by endogenous secretions (acid, pepsin, and bile) and by ingested irritants can be attributed to several factors that have been generally referred to as mucosal defence system (Wallace 2001).

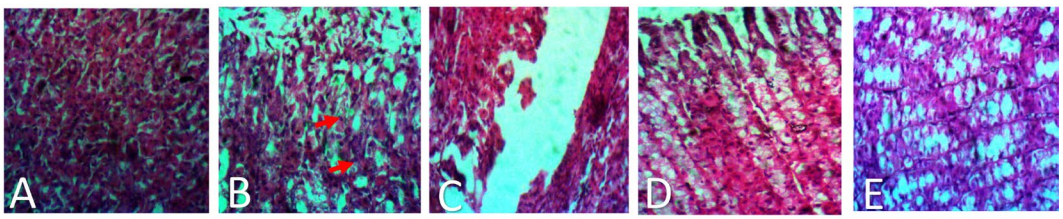
There has been a considerable interest in finding natural products for effective gastric ulcer management. In this study, Nigerian bee propolis elicited gastroprotective and ulcer-healing potential in acetic acid induced ulcer model. It is noteworthy that gastric ulcer has been most studied the rat model (Pillai et al. 2010). Injection of acetic acid into the stomach resulted in the induction of gastric ulcer as well as a



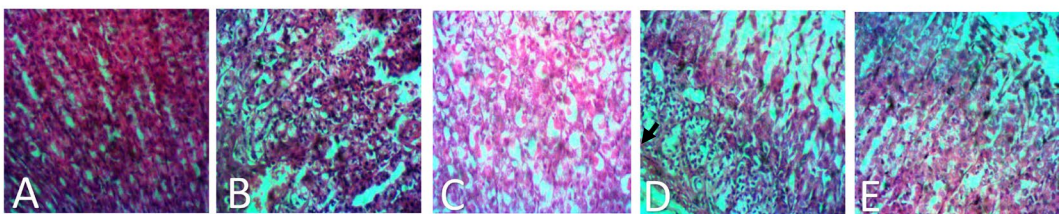
DAY 3: [A] (SHAM) showing normal gastric pit cells (chiefly oxynthic cells); [B] (NPE) showing hyperplasia of surface mucous cells and hypertrophy of neck (gastric pit) cells (red arrows). The extensive mucosal defect is bordered by a sharp indurated connective tissue; [C] (OME) showing necrosis of neck cells (black arrows) and gastric glands with mucosal defect (ulcer) and mucosal inflammation (red arrows); [D] (SAL) showing marked neck cell and glandular hyperplasia (arrow) with mucosal filling defect (ulcer); [E] (PRPE) Showing necrosis of surface and neck cells (arrows) and hyperplasia of gastric glands.
[Hematoxylin Eosin stain, x 400]



DAY 6: [A] (SHAM) showing no observable lesion; [B] (NPE) showing partial thickness epithelial loss (erosion) of the fundic mucosal, hyperplasia of surface mucous cells (black arrows) and hypertrophy of neck (gastric pit) cells; [C] (OME) showing necrosis of neck cells (arrows) and gastric glands with mucosal defect (ulcer); [D] (SAL) showing marked neck and glandular cell hyperplasia (arrow), apposition of the mucosal defect (ulcer) and connective tissue deposition; [E] (PRPE) showing atrophy of gastric glands.
[Hematoxylin Eosin stain, x 400]



DAY 9: [A] (SHAM) showing no observable lesion; [B] (NPE) showing infilling of mucosal defect characterized by shedding of surface mucous cells with focal coagulation necrosis of chief cells (arrows); [C] (OME) showing mucosal defect (ulcer), necrosis of neck cells with deposition of connective tissue; [D] (SAL) showing marked neck and gastric gland cell hyperplasia (arrow) with filling of mucosal defect (ulcer); [E] (PRPE) showing hyperplasia of surface mucous cells (black arrow), necrosis and atrophy of gastric glands with luminal ectasia (red arrow)
[Hematoxylin Eosin stain, x 400]



DAY 12: [A] (SHAM) showing no observable lesion; [B] (NPE) showing moderate focal erosion of the gastric mucosa with hyperplasia of mucous and neck cells (arrows); [C] (OME) showing keratinization of the gastric oesophageal junction and coagulation necrosis of gastric pit (chief) cells; [D] (SAL) showing marked atrophy of neck cells, hyperplasia of mucous cells and necrosis of gastric glands (black arrow) with infiltrates of inflammatory cells; [E] (PRPE) showing no observable lesion.
[Hematoxylin Eosin stain, x 400]

Fig. 3 Histological presentation of the gastric ulcers healing in rats within the five groups treatment from day 3 to 12

remarkably significant increase in severity, gastric juice production, and total gastric acidity. The clinical signs observed in this study following gastric ulcer induction such as: dark tarry faeces, vocalisation and arching of the back corroborates with previous findings by Parrah, et al., (2013). The ulcer induced by acetic acid has been attributed to generation of reactive oxygen species, initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis, and inhibition of prostaglandin synthesis (Bech-Hansen et al. 2000). Decreased prostaglandin levels impair almost all aspects of

gastro-protection and increases acid secretions which, in turn, aggravate the ulcer (Miller, et al 2012).

The Nigeria Bee Propolis produced both gastroprotective and ulcer healing potentials similar to that observed with the Egyptian Propolis (Pillai, et al. 2010; Abd El-Hady, et al. 2013). The gastroprotective and ulcer curative effects of propolis are ascribed to the anti-ulcer properties of flavonoids such as quercetin, naringenin and caffeic acid phenethyl ester (CAPE) present in bee propolis samples and known to reduce the production and release of histamine and other inflammatory mediators such as interleukins and tumour necrotic factor-∝ (Moura, 2011).

Nigerian Bee propolis accelerated the healing of gastric ulcers in rats (Wistar strain) at a rate similar to Omeprazole, a known ulcer healing agent that served as positive control in this study (Blandizzi et al. 1995; Biswas et al. 2003). Oral administration of Nigerian bee propolis significantly reduced the ulcer area, gastric acid output and acidity of the studied rats. Ulcer contraction that was gradual in all the groups from day 3 to day 12, was significantly higher between days 9 and 12 ($P = 0.001$) in the propolis extract, omeprazole and the pre-treated groups than in the saline treated group. This could be due to the ability of propolis to influence the production of transforming growth factor –alpha and beta 1 (TGF-∝ and TGF –ß1) by immune cells which stimulates cell growth (Martinotti and Ranzato 2015). Wound contraction was slower in the saline treated group which could be as a result of extended inflammatory and debridement phases (Rosique et al. 2015). There was a significant decrease in the volume of gastric juice in the NPE, OME and PRPE groups ($P < 0.001$) between days 9 and 12. The observed decrease in gastric acid volume in the NPE group could be attributed to propolis' ability to antagonise the binding of histamine to the H2 receptor on the parietal cells (Banji et al. 2010). The gradual increase in pH in all the groups from day 3 to 12, with significantly higher rate ($P = 0.001$) between days 6 and 12 in the NPE, OME and PRPE groups compared with the SAL group could be attributed to reduction in hydrogen ion concentration in the gastric juice (Lüllmann et al. 2000). This showed that propolis is a good gastroprotective agent since the gastric ulcer indices were lowest on the day of induction in the pre-treated group in comparison with the other groups.

The significant level of inflammatory cellular infiltrates (neutrophils and macrophages) in the Propolis and Omeprazole treated groups confirms an acute phase response to injury and healing of induced ulcer. The wound healing process is composed mainly of three overlapping phases: inflammation, proliferation and remodelling phases Baum and Arpey, 2005; Liu and Velazquez 2008). After injury ensues, platelets are activated at the site of blood vessel rupture, promoting clot formation to halt blood loss. Platelets also release factors that attract immune cells from the circulation into the wound. This marks the commencement of the inflammatory phase. Polymorphonuclear cells (neutrophils) arrive first, and then monocytes which rapidly differentiate into macrophages (Sindrilaru and Scharffetter-Kochanek 2013). Neutrophils produce high levels of reactive oxygen species (ROS), proteases and pro-inflammatory cytokines to sanitise the wound. When this process is complete, neutrophils apoptose and become phagocytosed by the newly arrived macrophages. There is considerable evidence that macrophages are key regulators of the wound healing process, during which they take on distinctive roles to ensure proper healing (Mosser and Edwards 2008).

Macrophages continue to phagocytose bacteria and debris to further clean the wound (Frykberg and Banks, 2015), during which the wound is sterilised and prepared for tissue regrowth in the proliferative phase (Baum and Arpey, 2005). Macrophages also play particularly important roles in vascularisation, by positioning themselves nearby newly emerging blood vessels and aiding in their stabilisation and fusion (Fantin et al. 2010; Ogle et al. 2016).

During the remodelling phase, Vascularised Extracellular Matrix (ECM) is laid down and gastric mucous cells migrate upon it to close the wound or ulcer (Falanga 2005). In the beginning of the final remodelling phase, macrophages release matrix metalloproteinases (MMPs) to breakdown the provisional extracellular matrix, and then apoptose so that the tissue or mucosal surface can mature to its original, non-wounded state (Vannella and Wynn 2017).

Also noteworthy is the lymphocytes number in the gastric mucosa of the NPE group that signifies some level of adaptive immunity. This immune cellular infiltration into the stomach tissues aids in autolytic debridement and growth of new tissues, thus the healing properties of propolis as observed in this study could also be attributed to propolis immune stimulating effect (Dimov et al. 1991). This information is of essence in clinical practice where Nigerian bee propolis could serve for prophylactic management patients at risk of gastric ulcer (Oyetayo et al. 2022).

In conclusion, results from this study showed that Nigerian bee propolis flavonoid extract is a potent gastroprotective and ulcer healing natural agent. It is therefore recommended as an alternative to current conventional antiulcer treatments and for prophylactic management of patients at risk of gastric ulcer disease.

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Declarations

Ethical approval Ethical approval (Ethic no. UI-ACUREC/19/0151) was obtained from the Animal Care and Use Ethical Committee, University of Ibadan for the use of experimental animals before the commencement of the study. All animals received humane care and all

methods were performed in accordance with the relevant guidelines and regulations stipulated in the ARRIVE guidelines.

Conflict of interest The authors declare that they have no competing interests.

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