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The Potential Antistress and Gastroprotective Effects of Berberine in Immobilization Stress Induced Gastric Ulcer in Rats

Heba Rady Salem[*1], Mona A. Kora[2], Suzan A. Khodir[1]

[1]Medical Physiology and [2]Pathology Departments, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.

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Keywords

- Berberine
- Gastric ulcer
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Abstract

Background: Stress has been implicated in pathophysiology of gastric ulcer. Berberine had shown promising gastroprotective and antistress effects. **Objective:** This study aimed to investigate the possible protective effect of berberine in immobilization stress induced gastric ulcer in male albino rats. **Methods:** Thirty-two adult male albino rats were randomly allocated into equal 4 groups (n=8): control group, stress, stress berberine-treated and stress omeprazole-treated groups. At the end of the study, rats were weighed, and blood samples were collected for measurement of serum corticosterone. Lastly, the rats were sacrificed. The adrenal glands were dissected out and weighed. The stomach was dissected, evaluated for gastric acidity and gastric lesions. Each stomach was divided into 2 parts. One part was prepared for histopathological and immunohistochemical analyses. The other one was homogenized for biochemical analysis. **Results:** Stressed rats showed significant increase of serum corticosterone, gastric acidity, gastric malondialdehyde and tumor necrosis factor-alpha compared with control group. Also, they showed significant decrease of gastric reduced glutathione, interleukin-10 and prostaglandin E2 compared with control group. Macroscopic and microscopic examination confirmed the presence of gastric ulcer. Histological examination of adrenal gland showed hyperplasia of zona fasciculata. Immunohistochemical study showed upregulation of gastric cyclooxygenase-2 in stressed rats and downregulation in both berberine and omeprazole treated rats. Both berberine and omeprazole supplementation to stressed rats improved serum corticosterone, oxidative stress, inflammatory markers, macroscopic and microscopic stress induced gastric ulcer and adrenal gland pathological changes. **Conclusion:** Berberine could protect against stress induced gastric lesions in rats via antistress, antioxidant, anti-inflammatory effects.

Corresponding author: Heba Rady Salem; ORCID: 0000-0003-2871-5190. Postal address: Medical Physiology Department, Faculty of Medicine, Menoufia University, Gamal Abd El-Naser Street, Shebin El-Kom, Menoufia governorate, 32511, Egypt. E-mail: heba.salem.12@med.menofia.edu.eg

INTRODUCTION

Stress is a state of endangered homeostasis evoked by external or internal stressful stimuli [1]. It activates hypothalamic pituitary adrenal axis (HPA-axis) and sympathetic nervous system resulting in a physiological and behavioural adaptive response [2]. Stress has been implicated in pathophysiology of various gastrointestinal disorders including gastric ulcer [3]. Different stress induced gastric lesions such as superficial mucosal damage, ulceration and bleeding often occurs in patients exposed to stress-related incidents such as shock, trauma, and burn [4].

The underlying pathophysiology for stress induced gastric ulcer is multifactorial. Inflammation and oxidative stress are involved, as well as decreased gastric prostaglandin production and suppression of mucosal proliferation [5]. Stress ulcers differs from ordinary peptic ulcer in involvement of mucosal ischemia and reperfusion injury in its pathogenesis [6].

Long-term use of classic antiulcer medications such as proton pump inhibitors have numerous side effects that limits their use for the prevention of stress-induced gastric ulcer [7]. Accordingly, search for safe anti-ulcer agents is required. A wide variety of medicinal plants with anti-peptic ulcer potential have been reported in the scientific literature [8]. Berberine is an alkaloid present in medicinal plants such as Berberis aristata Sims and Coptis chinensis Franch that had shown gastroprotective effects in rat models of gastritis and ethanol induced gastric ulcer [9, 10]. Moreover, it has antistress effect via inhibition of HPA-axis [11].

Thus, this study was designed to investigate for the first time the possible protective effect of berberine in immobilization stress induced gastric ulcer in male albino rats in comparison to proton pump inhibitor. We also aimed to explore the underlying mechanisms

Material and method

Animals

Thirty-two adult male albino rats, weighing 175-200 g each, were used in this study. They were housed in cages measuring 70x70x60 cm, 4 animals/cage, under natural light/dark cycle at room temperature. Rats had free access to water and standard rodent rat chow. All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press), and approved by the Ethics Committee for scientific research at the Faculty of Medicine, Menoufia University, Egypt.

Experimental design

Rats were left for 2 weeks for acclimatization, then they were randomly allocated into equal 4 groups (n=8): (1) Control group, rats were not exposed to stress and allowed to move freely. They received an equivalent volume of distilled water by oral gavage once daily for 14 days; (2) Stress group, rats were exposed to chronic immobilization stress by placing them in individual plastic restrainer for 2 h per day for 14 days, as previously described [12]; (3) Stress berberine-treated group (stress+BB), rats received berberine (Solaray Berberine 500 mg from Indian Barberry Root, Salt Lake City, Utah. USA) at a dose of 200 mg/kg/day dissolved in distilled water by oral gavage 1 hour before exposure to chronic immobilization stress for 14 days. The dose of berberine was based on previous study [13]; (4) Stress omeprazole-treated group (stress+Om), rats received omeprazole (Nexipro 20 mg sachet, Pharma Right Group, Cairo, Egypt) dissolved in

distilled water at a dose of 20 mg/kg/day by oral gavage 1 hour before exposure to chronic immobilization stress for 14 days [14].

All rats were weighed at the beginning and at the end of the study, and the change in body weight (BW) was calculated. At the end of the study, the rats were fasted overnight. Then, retroorbital blood samples were collected at 10 a.m. and serum was separated for measurement of serum corticosterone. Lastly, the rats were sacrificed. The adrenal glands were dissected out from the adhering tissues and weighed. The stomach was dissected along greater curvature, evaluated for gastric acidity and gastric lesions. Each stomach was divided into 2 parts. One part was prepared for histopathological and immunohistochemical analyses. The other one was homogenized for biochemical analysis.

Determination of gastric acidity

After scarification, each stomach was washed with 1 ml phosphate-buffered saline with a 1000 µl micropipette and the gastric juice was collected. The pH of the collected gastric juice was measured using a pH meter [15].

Macroscopic assessment of stress-induced gastric lesions

The mucosa was washed with normal saline and was inspected for the presence of ulceration and haemorrhage by the naked eye. The assessment of gastric mucosal damage was expressed as ulcer index as described by [16]. The ulcer index for each rat was calculated by multiplying total number of lesions by their respective severity factor. The severity factor was determined by using 0-3 scoring system based on the length of the lesion. If there were no lesions, severity factor = 0. For the lesions < 1 mm, severity factor = 1; for the lesions 1-4 mm, severity factor = 2; for the lesions > 4 mm, severity factor = 3.

Gastric homogenate preparation

Gastric specimens were weighed and homogenized separately using a tissue homogenizer (MPW120; MPW Medical Instruments, China). For estimation of gastric tumor necrosis factor-alpha (TNF-α), interleukin 10 (IL-10) and prostaglandin E2 (PGE2) levels, gastric tissues were homogenized in 50 mM phosphate-buffered saline (PBS), pH 7.4. For estimation of gatric MDA and GSH, gastric tissues were homogenized in 10 mM potassium phosphate buffer, pH 7.4. The crude tissue homogenate was centrifuged at 10,000 rpm for 15 min in an ice-cold centrifuge, and the resultant supernatant was collected and stored at −80°C for subsequent assays.

Biochemical analysis

Measurement of serum corticosterone level was performed using rat enzyme linked immunosorbent assay (ELISA) kit (catalog No. CSB-E07014r, CUSABIO Life Science Inc., Washington, DC, USA;) following manufacturer's instructions.

Measurement of TNF-α, IL-10, PGE2 levels in stomach homogenate was performed using their corresponding rat ELISA kits (TNF-α: ab100785, Abcam, Cambridge, UK), (IL-10: ab100765, Abcam, Cambridge, UK), (PGE2: MBS262150, MyBioSource, San Diego, CA, USA) following manufacturer's instructions.

Malondialdehyde (MDA) and reduced glutathione (GSH) in stomach homogenate were determined using colorimetric kits (Biodiagnostic Company, Giza, Egypt).

Histopathological analysis

The dissected gastric tissue and adrenal gland from all rats in different experimental groups were fixed in 10% phosphate-buffered formalin solution. They were stored in 10% neutral buffered formalin and then were embedded in paraffin for preparing 4-micron-thick sections. Both gastric tissue and adrenal gland were stained with ordinary Hematoxylin & Eosin (H&E) stain. Gastric tissue was also stained with Periodic Acid Schiff's (PAS) stain for histopathological examination.

Immunohistochemical (IHC) study

Gastric IHC staining for Cyclooxygenase-2 (COX-2)

Several sections were cut from the paraffin-embedded blocks with subsequent steps of deparaffinization and rehydration in xylene and a graded series of alcohol. Antigen retrieval was performed by boiling in 10 mL citrate buffer (pH 6.0) for 20 min, followed by cooling to room temperature. The slides were incubated overnight at room temperature with purified rabbit polyclonal anti-COX-2 antibody (Catalog No. A1253; Abclonal, Woburn, United States). The optimal dilution was 1:100 using PBS. Slides were de-paraffinized using xylene and then rehydrated in decreasing concentrations of ethanol. Antigen retrieval using microwave heating (20 min; 10 mmol/citrate buffer, pH 6.0) after inhibition of endogenous peroxidase activity (hydrogen peroxidase for 15 min) was used. The primary antibody was applied to the slides and incubated overnight at room temperature in a humidified chamber. Sections were washed with PBS and then incubated with secondary antibody for 15 min, followed by further washing with PBS. Finally, detection of bound antibody was accomplished using a modified avidin–biotin labeled reagent followed by 20 min washing with PBS. A 0.1% solution of diaminobenzidine was used for 5 min as a chromogen. Slides were counterstained with Mayer's hematoxylin for 5–10 min. Rat normal gastric tissue specimens were used as positive controls. Omission of the primary antibody served as a negative control.

Interpretation of COX-2 IHC results: Brown granular cytoplasmic staining involving any number of gastric mucosal cells was considered positive in the studied cases and control specimens. Gastric tissues in the four studied groups were assessed for:

1. **Expression percentage:** Positive cells were counted and given as a percentage of 200 cells of the whole section at 100× magnification in gastric tissues
2. **Intensity of staining:** Graded as mild (+), moderate (++), or strong (+++)
3. **Histo-score (H score):** H score was calculated in all positive specimens according to the following equation: H score = 1 × % of mildly stained cells + 2 × % moderately stained cells + 3 × % of strongly stained cells [17].

Statistical analysis

All data were expressed as mean ± standard deviation (SD). The data were analyzed using SPSS program version 16 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by post hoc Tukey test was used to detect significance between the groups. *P* value

≤ 0.05 was considered statistically significant.

Results

Change in body weight and adrenal gland weight

The mean value of change in BW in stress group was significantly lower than in control group (53.37 ± 8.25 vs 80.75 ± 4.68 g respectively, $P < 0.001$). In stress+BB group, it was significantly higher than stress group (73.37 ± 8.98 g, $P < 0.001$) and insignificantly different from control group ($P = 0.285$). Stress+Om group also revealed significantly higher values when compared with stress group (66.37 ± 9.59 g, $P = 0.017$), and significantly lower values when compared with control group ($P = 0.007$). There was insignificant difference between Stress+BB and stress+Om groups ($P = 0.329$), **(Fig.1A)**.

The mean value of adrenal gland weight in stress group was significantly higher than in control group (50.2 ± 0.58 vs 46.45 ± 0.35 mg respectively, $P < 0.001$). In stress+BB group, it was significantly lower than stress group (47.2 ± 0.37 mg, $P < 0.001$), but still significantly higher than control group ($P = 0.021$). Stress+Om also revealed significantly lower values when compared with stress group (48.26 ± 0.58 mg, $P < 0.001$), and significantly higher values when compared with control and stress+BB groups ($P = 0.001$), **(Fig.1B)**.

Serum corticosterone

The mean value of serum corticosterone in stress group was significantly higher than in control group (98.00 ± 6.07 vs 41.37 ± 3.5 ng/mL respectively, $P < 0.001$). In stress+BB group, it was significantly lower than stress group (61.5 ± 3.7 ng/mL, $P < 0.001$) but still significantly higher than control ($P < 0.001$) group. Stress+Om also revealed significantly lower values when compared with stress group (78.5 ±3.54 ng/mL, $P < 0.001$) and significantly higher values when compared with control and stress+BB groups ($P < 0.001$), **(Fig.1E).**

Gastric pH

The mean value of gastric pH in stress group was significantly lower than in control group (3.14 ± 0.18 vs 3.98 ± 0.37 respectively, $P < 0.001$). In stress+BB group, it was significantly higher than stress group (4.08 ± 0.27, $P < 0.01$) and insignificantly different from control group ($P > 0.05$). Stress+Om revealed significantly higher values (7.95 ± 0.25) when compared with control, stress and stress+BB groups ($P < 0.01$), **(Fig.1C)**.

Macroscopic ulcer index

The mean value of macroscopic ulcer index in stress group was significantly higher than in control group (28.13 ± 3.04 vs 0.25 ± 0.46 respectively, $P < 0.001$). In stress+BB group, it was significantly lower than stress group (15.88 ± 1.55, $P < 0.001$) but still significantly higher than control ($P < 0.001$) group. Stress+Om also revealed significantly lower values when compared with stress and stress+BB groups (3.88 ± 1.13, $P < 0.001$) and significantly higher values when compared with control group ($P = 0.002$), **(Fig.1D).**

Inflammatory markers

The mean value of gastric TNF-α in stress group was significantly higher than in control group (600.38 ± 11.51 vs 398.0 ± 11.6 pg/mg protein respectively, $P < 0.001$). In stress+BB group, it was significantly lower than stress group (498.38 ± 12.18 pg/mg protein, $P < 0.001$) but still significantly higher than control group ($P < 0.001$). Stress+Om also revealed significantly lower values when compared with stress group (543.75 ± 9.77 pg/mg protein, $P < 0.001$) and significantly higher values when compared with

control and stress+BB groups (P < 0.001), **(Fig.2A)**.

The mean value of gastric IL-10 in stress group was significantly lower than in control group (156.66 ± 15.03 vs 370.78 ± 22.71 pg/g tissue respectively, P < 0.001). In stress+BB group, it was significantly higher than stress group (233.49 ± 10.56 pg/g tissue, P < 0.01) but still significantly lower than control (P < 0.001) group. Stress+Om also revealed significantly higher values when compared with stress group (199.24 ± 6.60 pg/g tissue, P < 0.001) and significantly lower values when compared with control and stress+BB groups (P < 0.001). **(Fig.2B).**

The mean value of gastric PGE2 in stress group was significantly lower than in control group (16.63 ± 1.67 vs 33.3 ± 2.49 pg/mg tissue respectively, P < 0.001). In stress+BB group, it was significantly higher than stress group (21.46 ± 1.62 pg/mg tissue, P < 0.001) but still significantly lower than control (P < 0.001) group. Stress+Om group also revealed significantly higher values when compared with stress group (19.71 ± 0.56 pg/mg tissue, P = 0.007) and significantly lower values when compared with control group (P < 0.001). There was insignificant difference between stress+BB and stress+Om groups (P = 0.204), **(Fig.2E)**.

Oxidative stress markers

The mean value of gastric MDA in stress group was significantly higher than control group (8.89 ± 0.49 vs 6.09 ± 0.30 nmol/mg protein respectively, P < 0.001). In stress+BB group, it was significantly lower than stress group (7.13 ± 0.42 nmol/mg protein, P < 0.001) but still significantly higher than control (P < 0.001) group. Stress+Om also revealed significantly lower values when compared with stress group (8 ± 0.45 nmol/mg protein, P = 0.001) and significantly higher values when compared with control and stress+BB groups (P = 0.001), **(Fig.2C)**.

The mean value of gastric GSH in stress group was significantly lower than in control group (0.41 ± 0.03 vs 0.7 ± 0.03 mmol/mg protein respectively, P < 0.001). Level of gastric GSH in stress+BB group (0.6 ± 0.02 mmol/mg protein) was significantly higher than stress group (p < 0.01) but still significantly lower than control (P < 0.001) group. Stress+Om also revealed significantly higher values when compared with stress group (0.51 ± 0.03 mmol/mg protein, P < 0.001) and significantly lower values when compared with control and stress+BB groups (P < 0.001), **(Fig.2D)**.

Histopathological results

Gastric tissue H&E results

In the control group, H&E staining revealed normally oriented gastric mucosal glands free of dysplasia with intact surface mucous layer and resting on intact basement membrane with normal underlying submucosa and musclosa **(Figure 3A&B).** Stress group revealed severe mucosal erosions with disruption of gastric mucosal layer. Neutrophilic inflammatory infiltrate was noted. The submucosa exhibited oedema and congested vascular spaces **(Figure 3C&D).** Stress+BB group showed marked improvement in the form of regeneration of gastric mucosal glands, decreased submucosal oedema and congestion **(Figure 3E&F)** even better than Stress+Om group **(Figure 3G&H).**

Gastric tissue PAS results

Control group showed strong PAS staining of the mucosal cells of gastric glands **(Figure 3I).** Stress group showed areas of mucosal shedding, and

complete absence of PAS reactivity **(Figure 3J).** Stress+BB group showed regenerating mucosal covering, with strong PAS-reactive mucosal cells **(Figure 3K),** even a little stronger than Stress+Om group **(Figure 3L).**

Adrenal gland H&E results

Control group showed preserved architecture of adrenal gland with normal thickness of zona fasciculata, responsible mainly for corticosteroids secretion **(Figure 4A)**. Adrenocortical cells were bland looking with centrally located nucleus and granular eosinophilic cytoplasm **(Figure 4B).** Stress group exhibited increased thickness (hyperplasia) of zona fasciculata, compressed and attenuated zona glomerulosa, congested vascular spaces, haemorrhage and multiple foci of necrosis **(Figure 4C&D).** Stress+BB group **(Figure 4E&F)** as well as Stress+Om groups **(Figure 4G&H)** showed focal restoration of zona glomerulosa thickness compared to Stress group, regenerating adrenocortical cells of zona fasciculata together with absence of haemorrhage and congestion.

COX-2 IHC results

The mean value of COX-2 H scores was significantly higher in Stress group rats than in the control group (250.00 ± 31.94 vs. 96.25 ± 19.74, respectively, $P < 0.001$). Downregulation of the mean values of COX-2 H scores was noted in the Stress+BB group than in the Stress group (168.33 ± 15.71, $P < 0.05$). However, the mean values of COX-2 H scores in Stress+Om group was also downregulated than Stress group but didn't reach significant level (195.00 ± 5.77, $P > 0.05$) **(Figure 5)**.

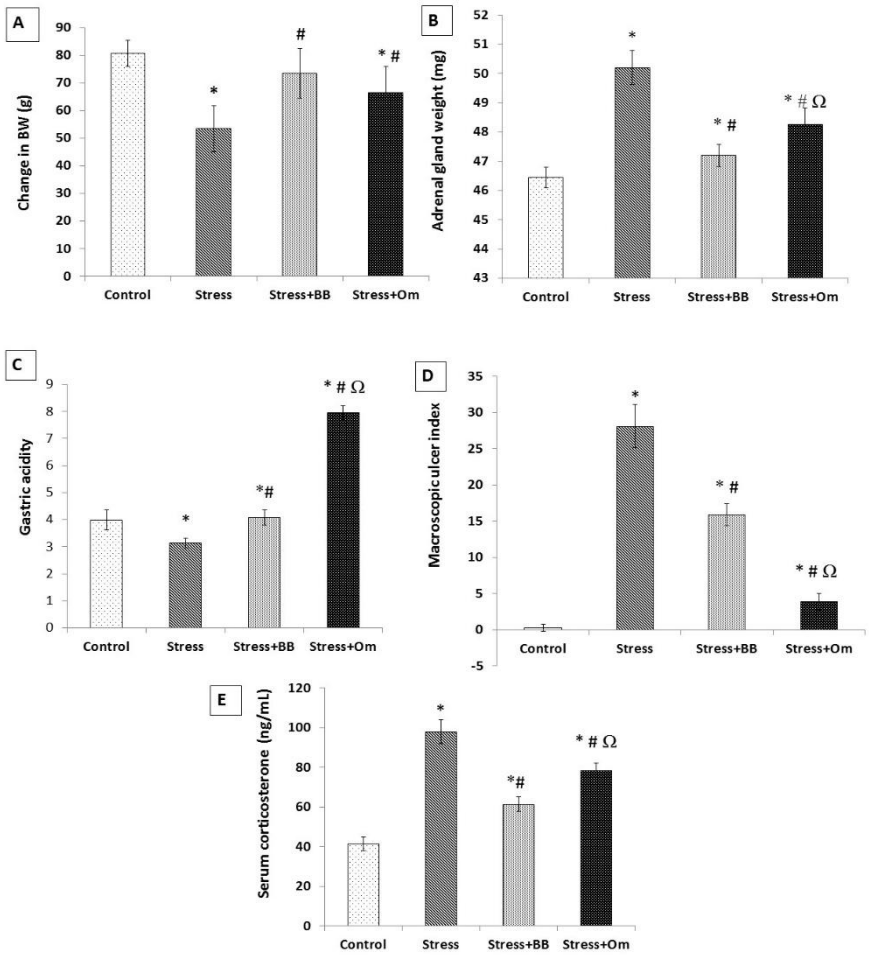

Figure 1

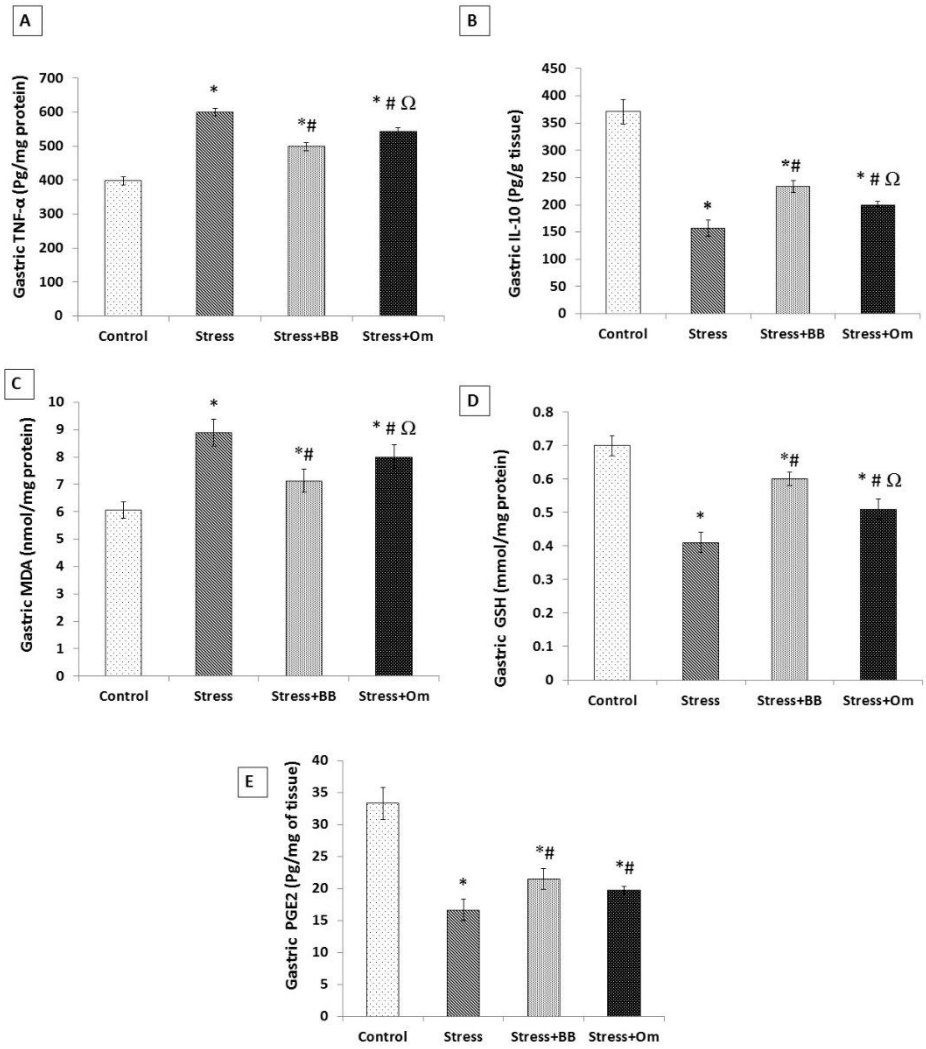

Figure 2

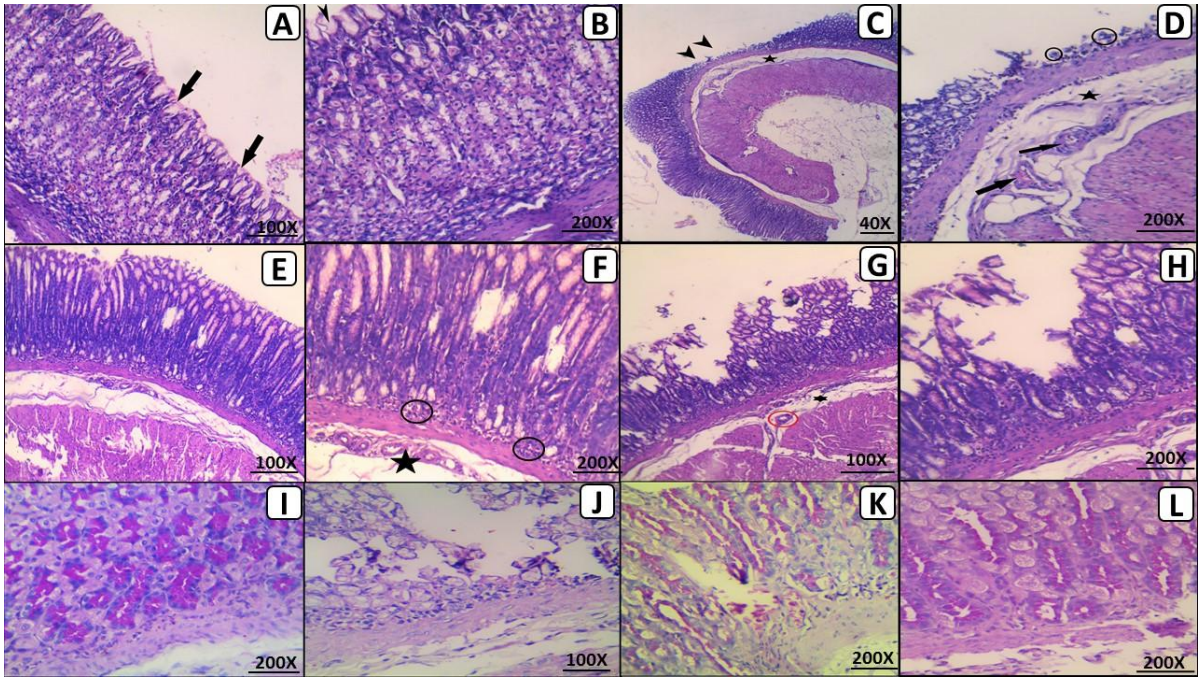

Figure 3

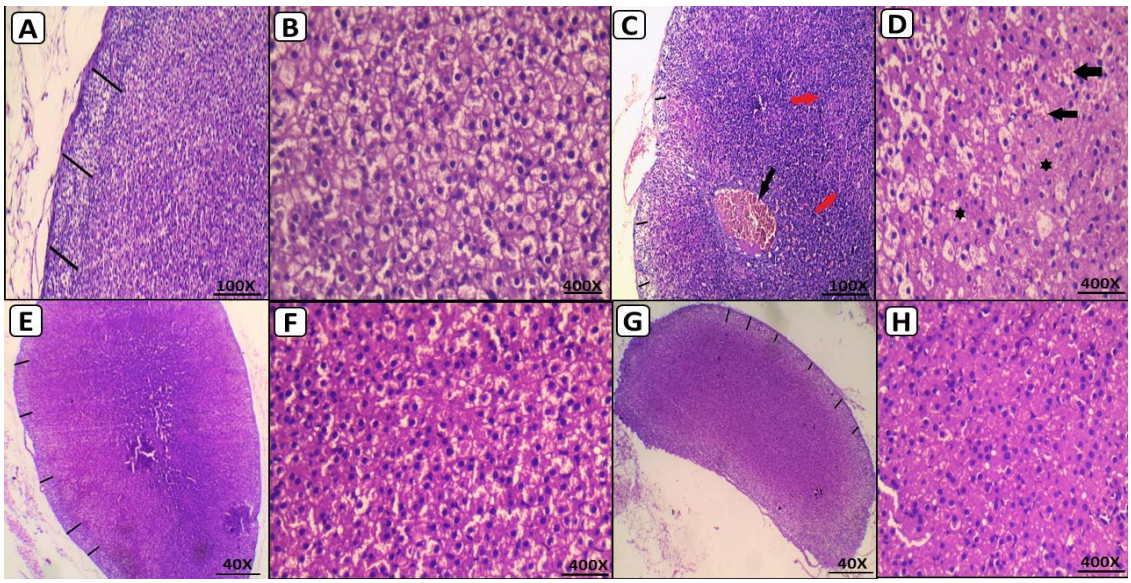

Figure 4

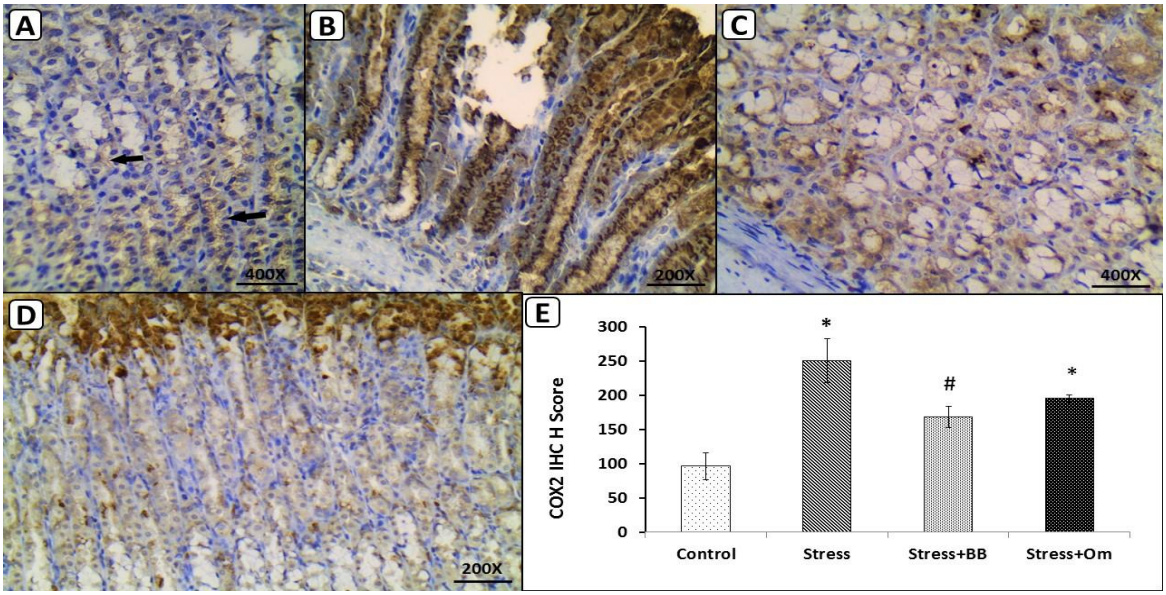

Figure 5

Figure legends

Fig. (1) Effect of berberine and omeprazole on A) Change in body weight (BW in g), B) Adrenal gland weight (mg), C) Serum corticosterone level (ng/mL), D) Gastric acidity and E) Macroscopic ulcer index among control, stress, stress berberine-treated (stress+BB) and stress omeprazole-treated (stress+Om) groups. Data are expressed as mean ± SD (n = 8). One-way ANOVA was used for comparison between groups. * $P < 0.05$ vs control, # $P < 0.05$ vs stress, Ω $P < 0.05$ vs stress+BB.

Fig. (2) Effect of berberine and omeprazole on A) Gastric tumour necrosis factor-alpha (TNF-α) in pg/mg protein, B) Gastric interleukin 10 (IL-10) in pg/g tissue, C) Gastric malondialdehyde (MDA) in nmol/mg protein and D) Gastric reduced glutathione (GSH) in mmol/mg protein and E) Gastric prostaglandine E2 (PGE2) in pg/mg tissue among control, stress, stress berberine-treated (stress+BB) and stress omeprazole-treated (stress+Om) groups. Data are expressed as mean ± SD (n = 8). One-way ANOVA was used for comparison between

groups. * $P < 0.05$ vs control, # $P < 0.05$ vs stress, Ω $P < 0.05$ vs stress+BB.

Fig. (3) Histopathological examination of gastric tissue after ordinary H&E and PAS staining in rats of the four groups, A) Normally oriented mucosal glands with preserved surface mucous layer (black arrows) in control group, B) Higher power view of control group highlighted healthy mucosal cells with intact mucous layer (arrow head), C) Stress group showed complete erosion of mucosal glands (arrow head) together with submucosal oedema and congestion (black star), D) Higher magnification of stress group highlighted neutrophiles in ulcer bed (circles), submucosal oedema (star) and congestion (black arrows), E) Stress+BB group showed marked improvement and regeneration of gastric mucosal glands except few neutrophiles in ulcer bed (circles), submucosal oedema and congestion (star) highlighted in higher magnification (F), G) Stress+Om group and higher magnification (H) exhibited improvement less marked than Stress+BB group (Magnification: 40x for C, 100× for A, E and G and 200× for B,D,F and H), I) Strong PAS staining of gastric mucosal glands of control group (PAS X200), J) Ulcerated mucosal covering in Stress group with complete absence of PAS reactivity (PAS X100), K) Strong PAS staining in regenerating mucosal glands of Stress+BB group (PAS X200), L) Regenerating mucosal covering in Stress+Om group with also strong PAS reactivity (PAS X200).

Fig. (4) Histopathological examination of adrenal gland (H&E staining) in rats of the four groups, A) Low power view of adrenal gland of control group with normal thickness of zona glomerulosa (black lines) and underlying zona fasciulata (H&E X100), B) Higher magnification of control group exhibiting normal morphology of adrenocortical cells of zona fasciulata (H&E X400), C) Low power view of Stress group showed attenuated zona glomerulosa (black lines), increased thickness of zona fasciulata, congested vascular spaces (black arrow) and foci of necrosis (red arrows) (H&E X100), D) Higher magnification of stress group highlighted haemorrahge (black arrows) and necrotic cells (stars) (H&E X400), E) Low power view of Stress+BB group showed increased thickness of zona glomerulosa (black lines) than Stress group in some areas and attenuated in others with absence of haemorrhage and congestion (H&E X40), F) Higher magnification of Stress+BB group showed regenerated adrenocortical cells of zona fasiculata with absence of haemorrahge and necrosis (H&E X400), G) Low power view of Stress+Om group with picture nearly similar to Stress+BB group (H&E X40), J**)** Higher magnification of Stress+Om group also showed regenerated adrenocortical cells of zona fasiculata (H&E X400).

Fig. (5) COX2 IHC staining of control, Stress, Stress+BB and Stress+Om groups, A) Mild granular cytoplasmic staining of mucosal cells in control group (black arrows) (IHC X400), B) Stress group showing strong granular cytoplasmic staining of majority of mucosal cells (IHC X200), C) Downregulation of COX2 IHC expression in stress+BB group (IHC X400), D) Mild granular cytoplasmic staining of majority of mucosal cells and strong staining in some mucosal cells (IHC X200), E) Effect of berberine and omeprazole on COX2 H Score among control, Stress, stress berberine-treated (stress+BB) and stress omeprazole-treated (stress+Om) groups. Data are expressed as mean ± SD (n = 8). One-way

ANOVA was used for comparison between groups. * $P < 0.05$ vs control, # $P < 0.05$ vs stress, Ω $P < 0.05$ vs stress+BB.

Discussion

Stress has been implicated in pathophysiology of various gastrointestinal disorders including gastric ulcer [3]. Berberine, a herbal extract, had shown promising gastroprotective and antistress effects [9-11]. Thus, this study aimed to investigate the the possible protective effect of berberine in immobilization stress induced gastric ulcer in male albino rats with reference to some underlying mechanisms.

In the current study, immobilization stress for 2 hours/day for 14 days induced significant increase of serum corticosterone and adrenal gland weight, and significant decrease of body weight in stress group compared with the control group confirming induction of chronic stress state, which is accordance with Tian et al. [12]. Also, it induced gastric stress lesions, which were proved by macroscopic and microscopic examination of the stomach. This is in agreement with previous study [18, 19].

The main outcome of this study was to explore whether berberine supplementation could protect against immobilization stress induced gastric lesions in rats. Thus, to explore the protective effect of berberine and underlying probable mechanisms, assessment of HPA-axis was done, serum corticosterone, inflammatory markers (gastric TNF-α and IL-10) and oxidative stress markers (gastric MDA and GSH), and PGE2 in gastric mucosa were measured. Also, COX-2 immunostaining in the stomach was performed. In comparison to omeprazole, the traditional antiulcer therapy, berberine showed better antistress, anti-inflammtory and antioxidant effects. But, its direct effect in decreasing gastric acidity was lower than omeprazole.

The results of the current study showed that both berberine and omeprazole supplementation to stressed rats had antistress effect. This was confirmed by the histological study of the adrenal gland and the significant decrease of serum corticosterone in the treated rats. Berberine could penetrate the blood brain barrier and yield pharmacological effects [20]. Previous study had shown that berberine has an inhibitory effect on HPA-axis leading to reduction of serum corticosterone in type 2 diabetic rats, which agrees with our results [11]. Another study had shown that omeprazole could protect against dexamethasone induced gastric ulcer by inhibiting both basal and adrenocorticotrophic hormone stimulated cortisol secretion, which supports our results [21].

Oxidative stress, inflammation and decreased gastric prostaglandin production contribute to pathophysiology for stress-induced gastric ulcer [5]. This is in line with the results of this study. Our results revealed a significant increase of MDA, significant decrease of GSH and PGE2 in the gastric mucosa of stressed rats compared with control group, which agrees with Kamer et al. [22]. In the current study, both berberine and omeprazole supplementation had shown antioxidant effects, as evidenced by significant decrease of MDA and significant increase of GSH in gastric mucosa. The antioxidant effect of berberine agrees with Jia et al.[23], who reported that berberine was able to increase GSH and decrease MDA in an experimental colitis rat model. Also, omeprazole was proved to have antioxidant activity in indomethacin-induced gastric ulcer in rats [24].

Different experimental studies has proved the gastroprotective effect of PGE2 in different models of stomach injury [25]. In accordance with previous study, the stressed rats showed significant reduction of PGE2 in gastric mucosa compared with stressed rats [26]. Omeprazole significantly increased PGE2 in stress+Om group compared with stress group, which agrees with previous study [27]. The current study revealed a new gastroprotective mechanism for berberine via enhancement of PGE2 secretion.

Stress induces changes in cytokines levels such as TNF-α and IL-10 that may be related to the pathogenesis of the associated organic diseases, which is in accordance with our results [28]. The current study revealed a significant increase of the inflammatory marker TNF-α and a significant decrease of the anti-inflammmtory marker IL-10 in gastric mucosa of stressed rats compared with control group. Both berberine and omeprazole supplementation had shown anti-inflammatory effects, as evidenced by significant decrease of TNF-α and significant increase of IL-10 in gastric mucosa. The anti-inflammatory activity of berberine in the digestive system had been shown in multiple investigations [29]. In accordance with our results, Jia et al.[23] reported the anti-inflammatory effect of berberine via reduction of TNF-α in colitis rat model. Also, Liu et al. [30] reported that omeprazole could improve gastric mucosal injury in rats via reduction of TNF-α and increase of IL-10, which agrees with our results.

In the present study, significant increase of gastric acidity was observed in stressed rats compared with control rats. This in agreement with Fatemeh et al. [31], who reported a significant increase of basal and stimulated gastric acid in stresses rats compared with control rats. Acid induces direct mucosal injury, increase of leucocyte infiltration and stimulation of TNF-α production [32]. Berberine significantly increased gastric pH in stress+BB group compared with control group. This may be contributed to its inhibitory effect on TNF-α production.

COX-2, the inducible isoform of cyclooxygenase, is a representative pro-inflammatory mediator in gastrointestinal damages [33, 34]. Current study declared upregulation of COX-2 expression in stress group compared with control group. Consistently, other studies previously detected increased COX-2 mRNA level in indomethacin induced gastric ulcer [34, 35], while other literature contradicted our results [36]. Moreover, Park, J. et al. [35] showed that the increased COX-2 expression in the damaged mucosa may have been brought on by the increased TNF-α level. Fortunately, berberine administration in current work resulted in COX-2 expression downregulation, probably through its anti-inflammatory mechanism of action.

The histopathological study was in line with the biochemical results. Stress group in current work revealed severe mucosal erosions with disruption of gastric mucosal layer. Neutrophilic inflammatory infiltrate was noted together with submucosal oedema and congested vascular spaces agreeing with previous studies [37-39]. Inflammatory markers e.g. TNF-α and COX-2 were postulated to mediate the cascade of stress induced mucosal injury and tissue damage by promoting lipid peroxidation [40, 41]. Berberine treated group showed marked improvement in the form of regeneration of gastric mucosal glands, decreased submucosal oedema and congestion, probably through antioxidant and anti-inflammatory properties.

Moreover, PAS staining results (detecting mucopolysaccharides in gastric mucosa and indicating its integrity [42]), confirmed the ordinary H&E stain with complete absence of PAS reactivity in stress group consistent with previous studies [37]. Stress+BB group exhibited strong PAS-reactive mucosal cells in line with other researchers after supplementation with gastroprotective agent treatments [43].

In conclusion, berberine could protect against immobilization stress induced gastric lesions in rats via antistress, antioxidant, anti-inflammatory effects. Thus, berberine may provide a therapeutic effect in the treatment of gastric stress ulcer.

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