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Gastro-Protective Effects of Epigallocatechin 3 –Gallate: Impact on Anti-oxidant, Anti-Inflammatory, and Anti-Apoptotic Actions, (*Invivo* and *Invitro* Study)

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ABSTRACT

Gastric ulcer disease is one of the major gastrointestinal diseases which occur because of unevenness between hostile and protective factors. Epigallocatechin 3 - gallate (EGCG) the most copious tea polyphenol is credited with anticancer, antidiabetic, and cardioprotective activities. The present examination was planned to assess the EGCG activity against pyloric ligation (PL) actuated gastric ulcer in rats.

Adult male albino rats, weighing 200g-250g were administered orally EGCG in two doses (5 mg/kg/day and 10 mg/kg/day) and ranitidine 80 mg/kg/day as a kind of perspective medication for seven consecutive days preceding subjection to PL.

The administration of EGCG in the two doses decreased the gastric injuries, ulcerative index, malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), Caspase 3 (Casp-3) levels and increment the levels of superoxide dismutase (SOD) and total antioxidant capacity (TAC) in a dose-dependent way. The immunohistochemical examination for an epidermal growth factor (EGF) demonstrated that EGCG increased EGF and diminished vascular endothelial growth factor (VEGF).

The ulcer defensive effect of EGCG was seen on the treated group and was compared to the ranitidine treated group; these effects might be because of its antioxidant, anti-inflammatory, and anti-apoptotic actions.

Keywords: Epidermal Growth Factor (EGF); Epigallocatechin 3 -gallate (EGCG); Gastric ulcer; Pyloric ligation; Ranitidine; Vascular Endothelial Growth Factor (VEGF).

Abbreviations: Casp-3: Caspase 3; EGF: Epidermal Growth Factor; EGCG: Epigallocatechin-3-gallate; MDA: Malondialdehyde; NO: Nitric Oxide; PGs: Prostaglandins; SOD: Superoxide Dismutase; TAC: Total antioxidant capacity; TNF-α: Tumor Necrosis Factor Alpha; UI: Ulcer Index; VEGF: Vascular Endothelial Growth Factor.

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INTRODUCTION

A Peptic ulcer disease is a group of disorders described by the presence of ulcers in any piece of gastrointestinal tract presented to acid inadequate concentration and period (1). A Peptic ulcer results from an obsessive condition in which the natural harmony between mucosal aggressive and guarded factors in the gastrointestinal tract is disturbed (2). The fundamental endogenous aggressive elements including gastric hydrochloric acid, reactive free radicals, oxidants, and leukotrienes. While the gastric barrier gives a mucosal safeguard and unsettling influences in blood perfusion of gastric mucosa results in the arrangement of disintegrations and ulcers. The principal factors, which manage gastric blood flow, are prostaglandins (PGs), vascular endothelial growth factor (VEGF), nitric oxide (NO), and epidermal growth factor (EGF) (3). Isolated tissues of gastrointestinal smooth muscle are known for their important place in pharmacological examinations, the most utilized model to be utilized in *invitro* tests of medication activity on the gastrointestinal tract are the rat stomach fundus and the guinea pig ileum (4). Histamine receptor antagonists, prostaglandins analogs, proton pump inhibitors, cytoprotective mediators, antacids, and anticholinergics, are ordinary medications utilized in the treatment of ulcers. As a large portion of these medications produce unwanted symptoms or medication interactions and may even modify biochemical mechanisms of the body upon perpetual utilization, herbal medicines are commonly utilized in extensive stretches.

Green tea was gotten from dried leaves of the plant *Camellia sinensis*, which is the most well-known drink around the world. Epigallocatechin-3-gallate (EGCG) is the richest and most biologically active catechin in green tea (5). It has different well-being advancing impacts, as an antioxidant and anti-inflammatory agent (6).

The present examination aims to explore the conceivable defensive impacts of EGCG in the pyloric ligation initiated gastric ulcer model. Ranitidine was picked as the reference standard in gastric ulcer protection and conceivable mechanism of activity of EGCG on isolated rat fundus strips and guinea pig ileum.

MATERIALS AND METHODS

Drugs and reagents

EGCG was acquired from Sigma-Aldrich (St. Louis, MO, USA), with clarity of ≥ 95%, and was dissolved directly into distilled water vehicle. EGCG was prepared fresh from powder just

before administration; because prolonged storage or freezing/thawing stock solutions seemed to cause debasement (e.g., darker staining of solution). Every other chemicals and solvent were of most noteworthy evaluation accessible.

Animals

Male Sprague Dawley rats and guinea pigs weighing 200-250 g were obtained from the animal house of El Nile Co. for pharmaceutical, El Amyria, Cairo, Egypt. The animals were kept at controlled ecological conditions in terms of steady temperature (24±1°C) and a 12/12 h light/dark They were acclimatized for one week before any trial strategies and were permitted standard rat chow (El-Nasr, Abu Zaabal, Cairo, Egypt) contained at the very least 20% protein, 5% fiber, 3.5% fat, 6.5% ash, and a vitamin blend, water was given not obligatory. The experimental protocol utilized in this examination was endorsed by the Animal Ethics Committee Faculty of Pharmacy, Al-Azhar University, Egypt.

Induction of Gastric ulcer

Animals have fasted overnight with free access to water. A midline ventral entry point 2 cm, beginning from xiphoid downwards, was made under chloral hydrate (300 mg/kg, ip) (7) anesthesia to uncover the stomach and duodenum, the intersection between the pylorus and the duodenum was picked up delicately. Pyloric ligation was completed by cautiously passing a silk string beneath the pylorus to avoid damage to the blood vessels or footing on the stomach. The abdominal wall was sutured and the wound was cleaned with refined water, dried, and secured with collodion and dry bandage dressing. The animal was put separately in its cage (8).

***Invivo* experiments (Evaluation of healing properties)**

Animals were arbitrarily separated into seven groups (8 rats for each group). Group I (Control): got normal saline orally. Group II (EGCG 5 (9), and III (EGCG 10 (10)) got EGCG (5 or 10 mg/kg, po), respectively for 7 days. Group IV (Pyloric ligated (P)), Group V (P/EGCG 5), and VI (P/EGCG 10) got EGCG (5 or 10 mg/kg, po) respectively for 7 days and pyloric ligation one hour after the last portion of EGCG. Group VII (P/ranitidine): got ranitidine (80 mg/kg, po) (11) as a reference standard drug for 7 days and pyloric ligation one hour after the last dose of ranitidine. The chosen regimen is based on the modified pyloric ligated rat method (12).

Four hours after pyloric ligation, all animals were sacrificed by decapitation. The abdomen was opened, the fundus of the stomach was dissected out and each stomach was analyzed for injuries in the forestomach divide and indexed by seriousness. Sections of the stomach were used for histopathological and immunohistochemical appraisals, generally stored at - 80° C for subsequent preparation of homogenates for biochemical testing.

Macroscopic evaluation of stomach

The stomachs were opened along the greater curvature, washed with saline to expel gastric substance and blood clumps, and inspected by a 10χ magnifier lens to evaluate the development of ulcers. The quantities of ulcers were tallied.

The scoring of ulcer will be made as pursue

Normal colored stomach (0), Red coloration (0.5), Spot ulcer (1), Hemorrhagic streak (1.5), Deep Ulcers (2), and Perforation (3).

The Mean ulcer score for every animal will be expressed as the ulcer index. The percentage of ulcer protection was resolved as pursues: Ulcer index (UI) was estimated by utilizing the following formula:

UI = UN + US + (UP X 10-1)

Where: UI= Ulcer Index; UN = Average number of ulcers per animal; US = Average number of seriousness score; UP= Percentage of animals with ulcers (13).

Percentage inhibition of ulceration was calculated as below

$$\% \text{ Inhibition of Ulceration} = \frac{(\text{Ulcer index Control} - \text{Ulcer index Test}) \times 100}{\text{Ulcer index Control}}$$

Biochemical Estimation

Assessment of inflammatory markers

Determination of Tumor Necrosis Factor Alpha (TNF-α) and Prostaglandin (PGE2) levels

The level of TNFα and PGE2 in serum were resolved by Rat TNF-alpha ELISA kit (RayBiotech, Inc., Norcross, Georgia, USA, Cat. No. ELR-TNF alpha-001C) and Rat PGE2 ELISA Kit (CUSABIO and CusAb, China Cat. No. CSB-E07967r) individually.

Assessment of antioxidant activity

Gastric superoxide dismutase (SOD) activity

Assurance of SOD activity in stomach homogenate supernatant was done according to Marklund (14). The changes in the absorbance at 420 nm were recorded at 1 min. interim for 3 min and results were expressed as U/mg protein.

Lipid peroxides concentration

Determination of lipid peroxide levels in stomach homogenate supernatant expressed as malondialdehyde (MDA), was done by the thiobarbituric acid test of Uchiyama and Mihara (15). The absorbance was recorded at 535 nm and the outcomes were communicated as nmol/g tissue.

Total antioxidant capacity

Determination of TAC levels in serum was carried out according to the modified Rice-Evans method (16). The absorbance was recorded at 405nm absorbance and the outcomes were expressed as µm/ml.

Caspase 3 level

The level of Caspase 3 in stomach homogenate supernatant was determined by Rat Caspase 3ELISA pack (CUSABIO and CusAb, China, Cat. No. CSB-E08857r)

Nitrite/nitrate ($NO2^-$/$NO3^-$) content

Determination of $NO2^-$/$NO3^-$ content in stomach homogenate supernatant was done according to Griess (17), Bredt and Snyder (18). The absorbance was recorded at 543 nm and the outcomes were communicated as µM/g tissue.

Immunohistochemical analysis

Detection of Epidermal Growth Factor (EGF)

Tissue specimens were fixed in formalin and installed in paraffin according to standard histological methods. Sections of 3 µm thick were cut, deparaffinized, rehydrated, and submitted to antigen recovery utilizing 0.1 M citrate buffer in a microwave at high temperature for 10 minutes. After antigen recovery, endogenous peroxidase activity was extinguished by immersing the sections in 3% H2O2 in

methanol for 20 minutes. At that point, sections were put in 10% ordinary goat serum for 30 minutes to block non-specific binding. A while later, the slides were incubated with primary antibodies coordinated against EGF (rabbit polyclonal, Cayman Chemical, Ann Arbor, MI; weakening 1:250) for 2 hours at room temperature. After washing in PBS, multiple times 5 minutes each, sections were incubated with secondary antibody (N-Histofine® Simple Stain MAX PO, Nichirei, Tokyo, Japan) for 30 minutes at room temperature. After a8 PBS wash, the color was created utilizing 3, 3'-diaminobenzidine tetrahydrochloride (Sigma) in 50 mmol Tris-HCl (pH 7.5) for 5 minutes. Counterstaining was finished utilizing hematoxylin and the sections were envisioned under the light magnifying microscope (Leica Microsystems, Germany) (19).

Immunohistochemical detection of Vascular Endothelial Growth Factor (VEGF)

Equivalent to in EGF aside from utilizing primary antibodies coordinated against VEGF (monoclonal, Santa Cruz Biotechnology, Inc., USA; dilution 1: 200 dilution) for 60 minutes (20).

Histopathological examinations

Stomach biopsies were fixed in 10% buffered formalin for 24 hours. The specimens were washed, dried out by alcohol, cleared in xylene, and implanted in paraffin. Sections 7 of 3 µm thickness were cut and stained with hematoxylin and eosin for histopathological examinations. All histopathological handling and appraisal of the specimen were performed by an experienced observer blinded to the identity of the examined samples to keep away from bias (21).

Masson's trichrome stain

Deparaffinized tissue sections were arranged and stained with H and E pursued by Masson's trichrome stain for the showing of collagen filaments. The thickness of collagen filaments was estimated in five non-overlapping fields of vision from each slide stained by Masson's trichrome of each group at X200 amplification utilizing Image J1.47v examination programming (22).

***Invitro* analyses**

Isolated rat stomach fundus strips

Preparation of the isolated rat stomach fundus strips was conveyed by Khan and Anwar (23), rats fasted overnight with free access to water. They were executed by decapitation and the stomach was removed. A bit of 2.5 cm long fundus strips was gotten and set in an oxygenated Tyrode's solution at 37°C. They were joined to Isometric force transducer (5 mg to 25 g Panlab, model number: TRI201, Spain) coupled to connect enhancer (ADInstruments, Model number: ML221, Australia) and Power Lab Data Acquisition framework (AD instruments, Model number: Power Lab 4/30, Australia).

Isolated guinea pig ileum

Preparation of the isolated guinea pig ileum was conveyed by Parry et al. (24), guinea pigs after 24h fasting were executed by a blow on the head and dying. The abdomen was opened and the ileum was evacuated and put in an oxygenated Tyrode's solution at 32°C. Bits of ileum 2.5 cm long were removed, joined as isolated rat stomach fundus. The isolated preparations (rat fundus strips and guinea pig ileum) were permitted to equilibrate for 30 to 45 min under a resting strain of 1g amid which the washing liquid was changed each 15 min. The normal contraction was monitored (at an inspecting rate of 40/sec). All *invitro* tests were performed in the Pharmacology Department, Faculty of Medicine, Cairo University.

Mechanism of activity of Epigallocatechin-3-gallate (EGCG) on isolated rat fundus strip

Two groups of isolated rat fundus strip, each comprises of 6 fundus strips were utilized as pursues:

Group I: Dose-response curve of Epigallocatechin-3-gallate (EGCG); EGCG was added in increasing concentrations by non-cumulative manner until maximum muscular responses occurred. The contact time of the EGCG with muscle strips before chronicling the impact of the tried drug was 10 minutes.

Group II: Site of action of Epigallocatechin-3-gallate (EGCG). The submaximal dose of EGCG was rehashed twice to get a regular response.

- 5-Hydroxytryptamine (5-HT) was included in a grouping of (10-9M) to test for the efficiency of 5-HT2-receptors (25), and ketanserin was included in a concentration of (10-6M) (26) to test for obstructing of 5-HT2 receptors, 5-HT was added in a concentration of (10-9M) to test for complete hindering of 5-HT2-receptors. Then a submaximal dose of EGCG was added without a wash.
- Histamine was added in a concentration of (10-4M) to test for the efficiency of H1-receptors (27) and mepyramine was added in a concentration of (10-6M) (28) to test for blocking of H1-receptors, histamine was added in a concentration of (10-4M) to test for complete hindering of H1 receptors. At that point, a submaximal dose of EGCG was included without a wash.
- Acetylcholine (Ach) was included in the e concentration of (10-4M) to test for the effectiveness of M2 and M3 receptors (29) atropine sulfate was added in a concentration of (10-6M) (28) to test for complete obstructing of M2 and M3 receptors, Ach was included in concentration of (10-4 M) to test for complete blocking of M2 and M3 receptors. At that point, a submaximal dose of EGCG was included without a wash.
- Verapamil, (Calcium Channel Blocker) was added in a concentration of (10-9M) for hindering of Ca+2 induced contractions (30). At that point, a submaximal dose of EGCG was added without a wash.

Mechanism of action of Epigallocatechin-3-gallate (EGCG) on guinea pig ileum

Two groups of disengaged guinea pig ileum, each comprises of 6 ileum pieces, were utilized as pursues:

Group I: Dose-response curve of Epigallocatechin-3-gallate (EGCG); EGCG was added in increasing concentrations by non-cumulative manner until maximum muscular responses occurred.

Group II: Site of action of Epigallocatechin-3-gallate (EGCG)

- Submaximal dose of EGCG was repeated twice to obtain a regular response.
- Histamine was added in a concentration of (10-8M) to test for the effectiveness of H1-receptors (31) and mepyramine was in a concentration of (2x10-7M) (28) to test for hindering of H1-receptors, histamine was included in a concentration of (10-8M) to test for complete blocking of H1-receptors. At that point, a submaximal portion of EGCG was included without a wash.

•5-Hydroxytryptamine (5-HT) was added in a concentration of (10-4 M) to test for the efficiency of 5-HT3 receptors (32) and ondansetron was included in a concentration of (10-7M)(25) to test for hindering of 5-HT3 receptors, 5-HT was included in a concentration of (10-4 M) to test for complete obstructing of 5-HT3 receptors. At that point, a submaximal dose of EGCG was added without a wash.

•Acetylcholine (Ach) was included in a concentration of (10-4 M) to test for the efficiency of M2 and M3-receptors (29) and atropine sulfate was added in a concentration of (10-6M) (28) to test for hindering of M2 and M3-receptors, Ach was added in a concentration of (10-4 M) to test for complete obstructing of M2 and M3-receptors. At that point, a submaximal dose of EGCG was included without a wash.

•Verapamil, (Calcium Channel Blocker) was added in a concentration of (10-9M) for the blocking of Ca+2 induced contractions (30). Then, a submaximal dose of EGCG was included without a wash.

Statistical analysis

Information analysis was accomplished by SPSS (version 21) statistical software. Information is communicated as mean ± SEM and the factual investigation was performed utilizing one-way ANOVA pursued by Tukey-Kramer as a post-hoc test. Statistical significance was set at $p < 0.05$.

RESULTS

First part: *Invivo* tests

Macroscopic examination of the stomach

Effect of Epigallocatechin 3-gallate (EGCG) on the ulcer index in pyloric ligated rats

The results are shown in table 1 and figure 1, pyloric ligation induced a significant increase in ulcer index by 277% compared to the sham group. Administration of ranitidine 80 mg/kg, EGCG 5mg/kg or EGCG 10mg/kg before pyloric ligation significantly decreased ulcer index by 30%, 31% and 52% respectively, compared to pyloric ligation. EGCG 10mg/kg significantly decreased ulcer index by 32% and 30%, respectively compared to ranitidine 80 mg/kg and EGCG 5 mg/kg treatments.

Stomach homogenate:

Effect of Epigallocatechin 3-gallate (EGCG) on gastric superoxide dismutase (SOD) activity in pyloric ligated rats

The results are shown in table 1, pyloric ligation induced a significant decrease in SOD activity by 25% compared to the sham group. Administration of EGCG 10mg/kg before pyloric ligation significantly increased SOD activity by 11.2%, compared to pyloric ligation. EGCG 10mg/kg persuaded a significant increase in SOD activity by 8% and 10%, respectively compared to ranitidine 80mg /kg and EGCG 5mg /kg treatments.

Effect of Epigallocatechin 3-gallate (EGCG) on gastric malondialdehyde (MDA) content in pyloric ligated rats

The results are shown in table 1, pyloric ligation significantly increased MDA by 95% compared to the sham group. Administration of ranitidine 80 mg/kg, EGCG 5mg/kg or EGCG 10mg/kg before pyloric ligation was significantly decreased MDA content by 33.59%, 40.92% and 108%, respectively compared to pyloric ligation. EGCG 10mg/kg significantly lessened MDA content by 36% and 32%, respectively compared to ranitidine and EGCG 5mg/kg treatments.

Effect of Epigallocatechin 3 -gallate (EGCG) on gastric nitrite/nitrate ($NO2^-/NO3^-$) content in pyloric ligated rats

The results are shown in table 1, pyloric ligation was significantly increased $NO2^-/NO3^-$content by 42% compared to the sham group. Administration of EGCG 5mg/kg or EGCG 10mg/kg before pyloric ligation induced a significant decrease in NO content by 8% and 48% respectively, compared to pyloric ligation. EGCG 10mg/kg significantly decreased $NO2^-$/ $NO3^-$content by 48% and 43%, respectively compared to ranitidine 80 mg/kg and EGCG 5mg/kg therapy.

Effect of Epigallocatechin 3 -gallate (EGCG) on gastric caspase 3 (Casp-3) content in pyloric ligated rats

The results are shown in table 1; pyloric ligation was significantly increased Casp-3 content by 396% compared to the sham group. Administration of ranitidine 80mg/kg, EGCG 5mg/kg or EGCG 10mg/kg before pyloric ligation induced a significant decrease in Casp-3 content by 33%, 31%and 52% respectively, compared to pyloric ligation. EGCG 10mg/kg was significantly decreased casp-3 contents by 29% and 30%, respectively compared to treatment by ranitidine 80 mg/kg and EGCG 5mg/kg.

Serum parameters

Effect of Epigallocatechin 3-gallate (EGCG) on serum Prostaglandin E2 (PGE2) level in pyloric ligated rats

The results are graphically presented in figure 2A, Pyloric ligation was significantly increased PGE2 level by 496% compared to the sham group. Administration of ranitidine 80mg/kg, EGCG 5mg/kg or EGCG 10mg/kg respectively, before pyloric ligation significantly decreased PGE2 by 41%, 28% and58% compared to pyloric ligation. EGCG 10mg/kg before pyloric ligation induced a significant decrease in PGE2 level by 29% and 42%, respectively compared to ranitidine 80mg/kg and EGCG 5mg/kg treatment.

Effect of Epigallocatechin 3-gallate (EGCG) on serum tumor necrosis factor-alpha (TNF-α) level in pyloric ligated rats

The results are graphically represented in figure 2B, pyloric ligation was significantly increased TNF-α level by 485% compared to the sham group. Administration of ranitidine 80mg/kg, EGCG 5mg/kg or EGCG 10mg/kg before pyloric ligation significantly decreased TNF-α by 41%, 26% and 56%, respectively compared to pyloric ligation. EGCG 10mg/kg induced significant decline in TNF-α level by 26% and 41% compared to ranitidine 80mg/kg and EGCG 5mg/kg treatment.

Effect of Epigallocatechin 3-gallate (EGCG) on serum total antioxidant capacity (TAC) level in pyloric ligated rats

The results are graphically represented in figure 2C, pyloric ligation was significantly increased TAC level by 87% compared to the sham group. Administration of ranitidine 80 mg/kg, EGCG 5mg/kg or EGCG 10mg/kg before pyloric ligation significantly increased TAC level by 212%, 108% and 370%, respectively compared to pyloric ligation. EGCG 10mg/kg was significantly decreased TAC level by 51% and 126%, respectively compared to ranitidine 80mg/kg and EGCG 5mg/kg therapy.

Histopathological alterations of stomach

The results are presented as a photomicrograph in figure 3**.**

Effect of Epigallocatechin 3-gallate (EGCG) on gastric collagen deposition in pyloric ligated rats

The results are presented as a photomicrograph of

a transverse section of rat stomach and graphically presented in figures 4, pyloric ligation was significantly increased collagen deposition by 234% compared to the sham group. Administration of ranitidine 80 mg/kg, EGCG 5mg/kg or EGCG 10mg/kg before pyloric ligation collagen deposition was significantly decreased by 67%, 65%, and 63%, respectively compared to pyloric ligation.

Immunohistochemical parameters

Effect of Epigallocatechin 3-gallate (EGCG) on gastric epidermal growth factor (EGF) in pyloric ligated rats

The results are presented as a photomicrograph of a transverse section of rat stomach and graphically presented in figures 5, pyloric ligation was significantly increased EGF by 1045% compared to the sham group. Administration of ranitidine 80 mg/kg, EGCG 5mg/kg or EGCG 10mg/kg before pyloric ligation significantly decreased EGF by 38%, 11% and 17%, respectively compared to pyloric ligation. EGCG 10mg/kg significantly increased EGF by 91% and 31%, respectively compared to ranitidine 80 mg/kg and EGCG 5mg/kg treatment.

Effect of Epigallocatechin 3-gallate (EGCG) on gastric vascular endothelial growth factor (VEGF) in pyloric ligated rats

The results are presented as a photomicrograph of a transverse section of rat stomach and graphically presented in figures 6, pyloric ligation was significantly increased VEGF by 1861% compared to the sham group. Administration of ranitidine (80mg/kg), EGCG 5mg/kg or EGCG 10mg/kg before pyloric ligation significantly decreased by 47%, 76% and 97%, respectively compared to pyloric ligation. EGCG 10mg/kg significantly decreased VEGF by 94% and 86%, respectively compared to ranitidine (80mg/kg) and EGCG (5 mg/kg) therapy.

Second part: *invitro* experiments

Effect of Epigallocatechin 3-gallate on isolated rat fundus strips and guinea pig ileum

Table 2 shows the effect of epigallocatechin 3-gallate on isolated rat fundus strips and guinea pig ileum. Epigallocatechin 3-gallate produced concentration-dependent contractions of isolated rat fundus (70-279µM) and guinea pig ileum (210-350 µM). The maximum contractile response in isolated rat fundus strips obtained at 279 µM was 3.4 gm while the maximum contractile response in isolated guinea pig ileum obtained at 315µM was 3.7gm. The contractions were not affected by blocking of muscarinic receptors by atropine sulfate or by the histaminergic receptor with mepyramine or by serotonergic receptors by ketanserin (fundus strips) and ondansetron (ileum pieces), respectively. Complete blocking of ECGC induced contraction produced by verapamil (CCB); figures 7A and 7B.

DISCUSSION

Pyloric ligation causes accumulation of acid and pepsin prompting autodigestion of gastric mucosa and ulceration (33). The pathogenesis of pyloric ligation-prompted ulcer includes the generation of reactive oxygen species (ROS) (34). The lipid peroxidation and presence of malondialdehyde (MDA) in the blood and gastric juice could result from ROS-started chain responses that stifle the antioxidant ability to scavenge ROS. Moreover, ROS could straightforwardly upset the mitochondrial membrane that consequently prompts the activation of caspase and apoptosis course, lastly prompts cell death through apoptosis (35). The present examination was intended to research the impact of EGCG at a dose of 5 and 10 mg/kg on pyloric ligation-incited peptic ulcer. Information of the present work demonstrated that EGCG administration at previous doses before pyloric ligation significantly diminished the ulcer index, compared to pyloric ligation rats; these results are following those of Sharkawi et al. (36).

Macroscopic examination of the stomach was utilized to evaluate the creation of ulcers. In the present investigation stomach of the pyloric ligation, the group demonstrated the proximity of hemorrhagic streak and profound ulcer. Conversely, EGCG administration before pyloric ligation indicated fewer ulcer numbers; this result is as per Abboud et al. (37). Superoxide dismutase (SOD) is viewed as the primary line of protection against the pernicious impact of ROS in cells. It catalyzes the dismutation of superoxide radical (O2.) to either normal atomic oxygen (O2) or hydrogen peroxide H2O2 (38). In the present investigation, pyloric ligation significantly diminished SOD, which prompting oxidative stress; a comparable example was recorded by Zaghlool et al. (39). In this investigation, administration of EGCG 5 mg/kg before pyloric ligation demonstrated no significant increase in SOD activity compared to the sham group while administration of EGCG 10mg/kg before pyloric ligation showed a significant increase in SOD activity, this outcome as per Charoenchon et al. (40) who announced that EGCG upregulated serum SOD activity in ulcer model.

Malondialdehyde (MDA) is the last result of lipid peroxidation. It is generally utilized as a marker to decide the level of lipid peroxidation (41). Lipid peroxidation happens when the initiated ROS assaults the unsaturated fatty acids of cell membrane phospholipids, making harm to the membrane phospholipid, prompting cell damage (42). In the present work, gastric MDA content in pyloric ligation was expanded essentially significantly compared to the sham group. A comparable example was recorded by Zaghlool et al. (39) who found that lipid peroxidation and MDA substance were raised in pyloric ligated rats, in consequence, promoting oxidative pressure. In this investigation, EGCG administration before pyloric ligation prompted a significant diminishing in MDA content compared to pyloric ligation, this outcome is in concurrence with Adhikary et al. (9).

Neutrophils cause endothelial harm by creating different free radicals including NO that significantly increase oxidative burst (43). It is currently well-perceived that the enhanced generation of NO by the iNOS may add to the pathogenesis of different gastroduodenal disorders including peptic ulcers (44). High concentrations of NO may be endorses inflammation via mucosal swelling and epithelial harm (45). In this examination pyloric ligation was significantly increased $NO2^-/NO3^-$ content compared to the sham group, a comparable example was recorded by Sherif et al. (46).

In the present investigation, administration of EGCG before pyloric ligation actuated a significant reduction in $NO2^-/NO3^-$content contrasted with pyloric ligation. This outcome is in concurrence with Paquay et al. (47) who announced that NO can be directly scavenged by green tea extract with EGCG being the best. Besides, Adhikary et al. (9) announced that EGCG created a decrease in the total NOS activity and nitrite level. Receptive oxygen species (ROS) generation has been accounted for to assume a basic role in pathogeneses of pyloric ligation-incited peptic ulcer (34).

The consequences of the present investigation demonstrated that pyloric ligation caused a significant decrease in the total antioxidant capacity (TAC) as compared with the sham group; this is as per Wang et al. (48). Such increased activity of ROS frequently prompts mucosal harm with the consequent obliteration of the epithelial basement membrane. The watched increment in TAC level in a group which got EGCG before pyloric ligation is as per Lin et al. (49). In the present investigation, EGCG has clear cell antioxidant activities related to the diminished quantity of MDA, a decrease of NOS activity, increment in SOD activity, and increased TAC. Caspase-3 is one of the effectors' caspases found in the apoptotic cell, which is initiated by the activity of upstream signaling promoting cell termination (50). In this examination, pyloric ligation prompted a significant increment in casp-3 content compared to the sham group, a comparative example was recorded by Brzozowski et al. (51). In this investigation administration of EGCG before pyloric ligation initiated a significant abatement in casp-3 content contrasted with the pyloric ligation group. This outcome is in concurrence with Park et al. (52) who revealed that EGCG repressed numerous purposes of the apoptotic sequence, incorporating caspase 3 in humans. Brzozowski et al. (51) announced that inhibitors of apoptosis quicken recuperating of stress injuries and might be effective operators in the mending of the harmed gastric mucosa.

The biosynthesis of PGE2 at inflammatory locales includes the release of arachidonic acid from membrane phospholipids by cytosolic phospholipase A2, oxygenation of arachidonic acid to PGH2, especially by COX-2, and further transformation to PGE2 by mPGES-1-B (53), PGE2 additionally can prompt the generation of other proinflammatory mediators including cytokines, nitric oxide, and connective tissue degrading enzymes (Oliva, et al. 2020). Consequences of the present investigation revealed that pyloric ligation caused a significant increment in PGE2, as compared with the sham group. The observed decline in PGE2 level in the group which got EGCG before pyloric ligation is as per Koeberle et al. (54) who concluded that restraint of mPGES-1 may represent the principle mechanism of EGCG for suppressing inducible PGE2 formation in biological systems, and this may add to the anti-inflammatory and anti-carcinogenic capability of EGCG.

Tumor necrosis factor-alpha (TNF-α) is a significant mediator of endotoxin-actuated tissue damage and is believed to be a proximal go mediator inflammatory reaction that triggers the release of several mediators that start an enormous number of occasions prompting shock and tissue damage (55). Additionally, it was accounted for that TNF-α, is engaged with intense gastric damage (56). In the present investigation, pyloric ligation incited a significant increment in TNF-α level compared to the sham group. This finding is per Sood and Muthuraman (57) who detailed that pyloric ligation showed increased TNF-α level compared with control animals. Administration of EGCG before pyloric ligation instigates significant decline in TNF-α level, this outcome is as per Adhikary et al. (9) who revealed that EGCG could diminish the mucosal TNF-α expression as well as the circulating TNF-α level. Concealment of TNF-α would encourage ulcer mending by upgrading epithelial cell proliferation and gastric blood flow and diminishing epithelial apoptosis.

Hematoxylin-Eosin staining was utilized to assess the degree of stomach histopathological alterations in rats. In the current investigation, we found that pyloric ligation caused necrosis and atrophy of gastric mucosal layer just as submucosal edema with few inflammatory cells infiltration. In addition to congestion of mucosal and submucosal blood vessels as well as submucosal fibroblasts proliferation. These outcomes are as per a few changes revealed by Chandra et al. (58) who established that pathological changes in the stomach brought about by pyloric ligation are congestion, edema, and necrosis in gastric mucosa. The present work showed that all pathophysiological changes delivered by pyloric ligation were improved by administration of EGCG before pyloric ligation, however to various degrees.

(61) Masson trichrome staining was utilized to assess the degree of stomach collagen deposition in rats. Miao et al. (59) revealed that fibroblast proliferation and collagen deposition were found to happen when epithelial cells were seriously harmed and there was a postponement in the epithelial repair process. In the current investigation, we found that pyloric ligation caused a significant increment in collagen deposition as compared to the sham group. This is as per Xing et al. (60) who disclosed that the increased rate of ulcerations may result from diminished mucosal blood flow delivered by increased deposition of connective tissue (collagen fibers) in lamina propria mainly around blood vessels. The present work showed that collagen amount was significantly diminished in the group gotten EGCG before pyloric ligation contrasted with pyloric ligation group. This is as per You et al. (61) who announced that EGCG treatment enormously diminished collagen depositions in irradiation-provoked pulmonary fibrosis in adult rats.

Epidermal growth factor EGF is an endogenous substance that restrains the secretion of gastric acid advances epithelial proliferation and improves the nourishment into the tissue to forestall gastric mucosa damage. Besides, EGF not just shields the gastric mucosa from harm factors and keeps up the intactness of the stomach mucosa, yet additionally animates the migration and proliferation of cells to increase the rate of the mending procedure of gastric ulcers (62). In the present study, pyloric ligation initiated a significant increase in EGF level compared with the sham group. This finding is as per Suo et al. (63) who detailed that mucosal harm increased the expression of EGF protein and EGFR mRNA in the gastric mucosa.

The present work demonstrated that EGCG administration before pyloric ligation was significantly increased EGF level contrasted with the pyloric ligation group. Engevik (64) announced that the overexpression of growth factor (EGF and TGFα) harmonizes with the restraint of gastric secretion and increased blood flow at the ulcer margin, demonstrating that these elements influence gastric secretion and blood flow throughout ulcer healing. From the past outcome, it was concluded that increased quality of ulcer mending by EGCG (10 mg/kg) is ascribed to overexpression of EGF, this outcome is by Kangwan et al. (65).

Vascular endothelial growth factor (VEGF) is the most significant and best perceived angiogenic factor. It is created by endothelial cells, fibroblasts, macrophages, smooth muscle cells, and neoplastic cells, and is associated with both physiological and neurotic guideline of angiogenesis (66). It introduces all the angiogenic factor properties, for example, it has explicit receptors on endothelial cells and its presence improves angiogenesis while its absence smothers the procedure (67).

In the present investigation, pyloric ligation actuated a significant increment in VEGF level compared with the sham group. This finding is as per Malara et al. (68) who revealed

that as early as one day after the advancement of ulcers there is a significant increment in the expression of the VEGF protein in the stress-prompted ulcers model in rats. Additionally, Lee et al. (69) announced that VEGF creation was expanded in the mucosa- bordering necrosis following 24 hours from the induction of ulcer. Administration of EGCG 10mg/kg before pyloric ligation indicated a significant decline in VEGF level contrasted with pyloric ligation gathering. This outcome is as per Mojzis et al. (70) who revealed that EGCG may exert at least part of its impact by hindering angiogenesis through obstructing the production of VEGF. The present work demonstrated that all pathophysiological changes delivered by pyloric ligation-prompted ulcer were improved by EGCG 5 and 10 mg/kg, yet to various degrees. Besides, The study of isolated rat stomach fundus and guinea pig ileum demonstrated that EGCG created focus subordinate withdrawals of segregated rodent fundus (70 - 279 µM) and detached guinea pig ileum (210 - 315µM). The constrictions incited by the submaximal dose of EGCG in the two arrangements were not influenced by hindering of muscarinic receptors by atropine sulfate or the histaminergic receptor by mepyramine or serotonergic receptors by ketanserin (rodent fundus strip) and ondansetron (guinea pig ileum). Complete obstructing of EGCG withdrawal was conveyed by verapamil. This outcome is as per Wang et al. (71) who announced that EGCG caused a dose-dependent activation in intracellular Ca2+ in the study of the impacts of EGCG on Ca2+ signals in hippocampal neurons. From past outcomes, It was concluded that EGCG induced contractions were mediated through calcium channel activation.

CONCLUSIONS

In conclusion, because of the trial finding of the present investigation, it was established that pyloric ligation of rat initiated mucosal damage. Administration of EGCG has a defensive impact against gastric ulcers, potentially through their anti-oxidant and anti-inflammatory impact as confirm by decreased oxidative stress, as well as inflammatory biomarkers.

The present study concluded that impacts of 7 days treatment with EGCG 5 and 10 mg/kg before pyloric ligation created significant defensive impact against pyloric ligation-initiated peptic ulcer. Where EGCG in high dose (10 mg/kg); conveyed better defensive impact on mucosal ulcer damage than EGCG (5mg/kg). EGCG at dosages 10 mg/kg is concluded to possess more anti-inflammatory, antioxidant, and anti-apoptotic effects than ranitidine. This examination proposes that EGCG has antiulcer activity and gastro defensive impact conceivably through anti-oxidant, anti-apoptotic, and anti-inflammatory effects.

CONFLICT OF INTEREST

None.

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AUTHOR CONTRIBUTIONS

Ekram N. Abd Al-Haleem developed the research idea, designed the experiments, supervised the execution of the experiments, and wrote the manuscript; **Gellan A. Mohamed** performed the experiments, collected the data, analyzed the data, and performed the graphical and statistical analysis. **Azza S. M. Awad** shared developing the research idea and designing the experiments, supervised the experiment execution. **Ragia A.M. Taha** suggested the research idea, supervised the execution of the experiments, supervised the data analysis, and revised the manuscript. All authors have approved the article for submission and they certify that this article has been subjected to professional language editing.

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TABLES

Table1. Effect of Epigallocatechin 3 -gallate (EGCG) on ulcer index, gastric superoxide dismutase (SOD) activity, malondialdehyde (MDA), nitrite/nitrate ($NO2^-/NO3^-$) and Caspase 3 (Casp-3) content in pyloric ligated rats.

Groups\ Parameters	Ulcer Index	SOD (U/g tissue)	MDA (nmol/g tissue)	$NO2^-/ NO3^-$ (µM/g tissue)	Casp-3 (ng /g tissue)
Sham group	5.142±0.3	2.842±0.02	2.038 ± 0.03	145.1±2.50	7.417±0.28
EGCG (5mg/kg)	4.623±0.3	2.435±0.02 [a]	2.758±0.04 [a]	199.7±2.91[a]	11.0±0.4[a]
EGCG (10mg/kg)	5.143±0.5	2.499±0.01[a]	2.892± 0.02 [a]	217.7±0.7 [a]	12.64±0.16[a]
Pyloric ligation (P)	19.38±0.2 [a,b,c]	2.134±0.01[a,b,c]	3.967±0.03[a,b,c]	205.7±0.7[a]	36.75±0.78[a,b,c]
Ranitidine+ P	13.62±0.3 [a,b,c,d]	2.193±0.01[a,b,c]	2.970±0.05[a,d]	205.3±1.377[a]	24.73±0.51[a,b,c,d]
EGCG (5mg /kg) + P	13.31±0.2 [a,b,c,d]	2.163±0.02[a,b,c]	2.815±0.05[a,d]	189.5±3.03[a,b,c,d,e]	25.32±0.56[a,b,c,d]
EGCG (10mg/kg) +P	9.277±0.2[a,b,c,d,e,f]	2.374±0.02[a,c,d,e,f]	1.907±0.1[b,c,d,e,f]	107.3±0.73[a,b,c,d,e,f]	17.64±0.28[a,b,c,d,e,f]

Values are mean ± SEM (n=8).
a, b, c, d, e or f: significantly different from sham, EGCG (5mg/kg), EGCG (10mg/kg), pyloric ligation (P), Ranitidine +P or EGCG (5mg/kg)+P treated groups respectively at $p<0.05$ using one-way analysis of variance (ANOVA) followed by Turkey-Kramer test for multiple comparisons.

Table 2. Effect of Epigallocatechin 3-gallate on isolated rat fundus strips, and guinea pig ileum.

Effect of epigallocatechin-3-gallate on isolated rat fundus strips			Effect of epigallocatechin-3-gallate on isolated guinea pig ileum		
EGCG(µM)	**Tension (gm)**	**% Maximal contraction**	**EGCG (µM)**	**Tension (gm)**	**% Maximal contraction**
70	1.412±0.01	41.22±0.3	210	2.2±0.01	60.39±0.53
105	2.779±0.01	81.09±0.1	245	3.028±0.04	82.52±0.9
140	3.427±0.02	100±0.1	280	3.688±0.05	100±0.6
279	3.427±0.02	100±0.1	315	3.688±0.05	100±0.6

The muscle strips were allowed to equilibrate for 30 to 45 min in an oxygenated Tyrode's solution under a resting tension of 1g during which the bathing fluid was changed every 15 min. The normal contraction was monitored (at a sampling rate of 40/sec). The force of contractions was measured using an isometric force transducer.

Values are expressed as mean ± SEM of n=6 preparations.

FIGURES

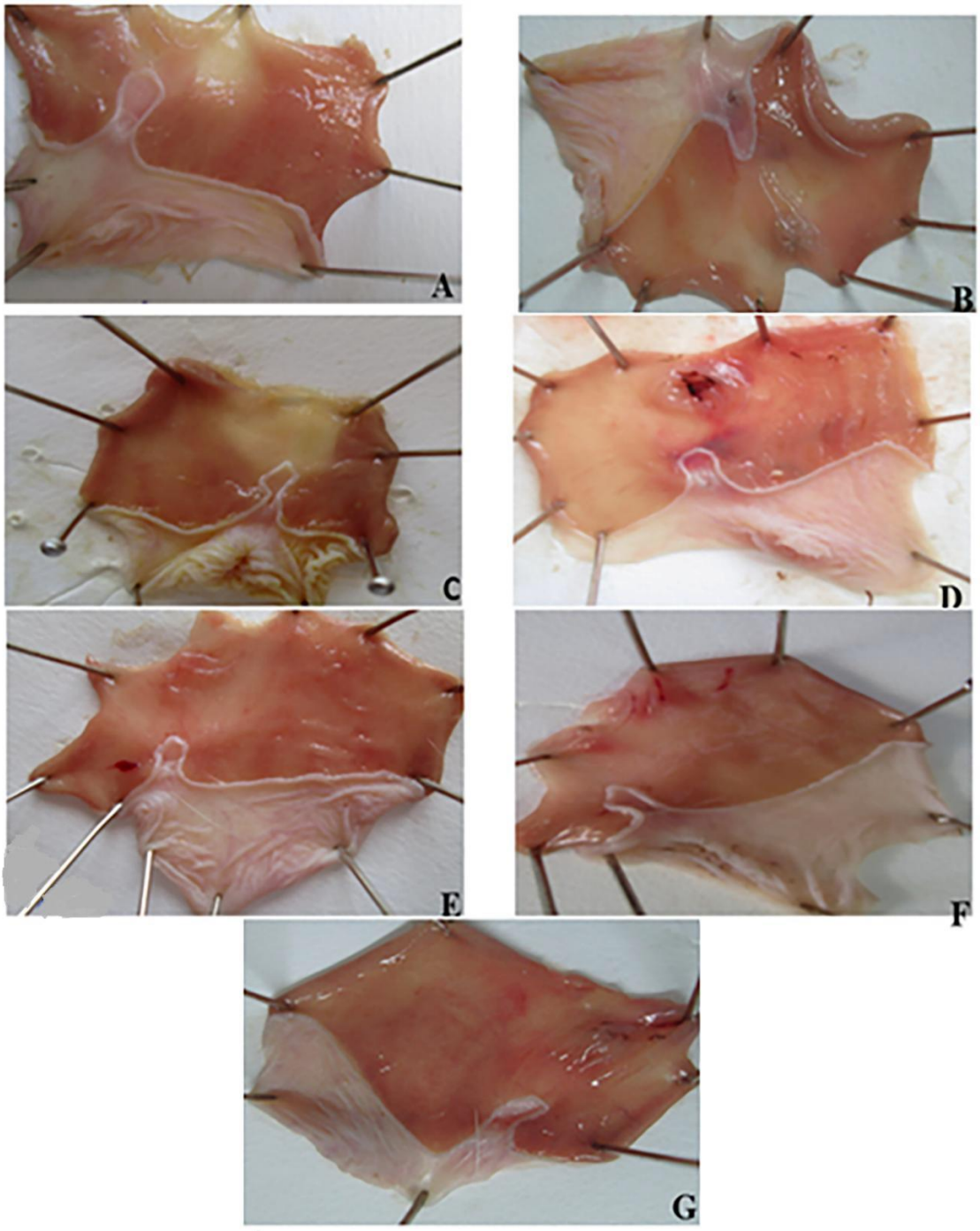

Figure 1. Effect of Epigallocatechin 3-gallate (EGCG) on macroscopic examination in pyloric ligated rats.
(A): Stomach rat from sham group. (B): Stomach of EGCG (5 mg/kg) group. (C): Stomach of EGCG (10 mg/kg) group. (D): Stomach of rat from pyloric ligation group (P). (E): Stomach of rat from ranitidine +P group. (F): Stomach of rat from EGCG (5 mg/kg) +P group. (G): Stomach of rat from EGCG (10 mg/kg) + P group.

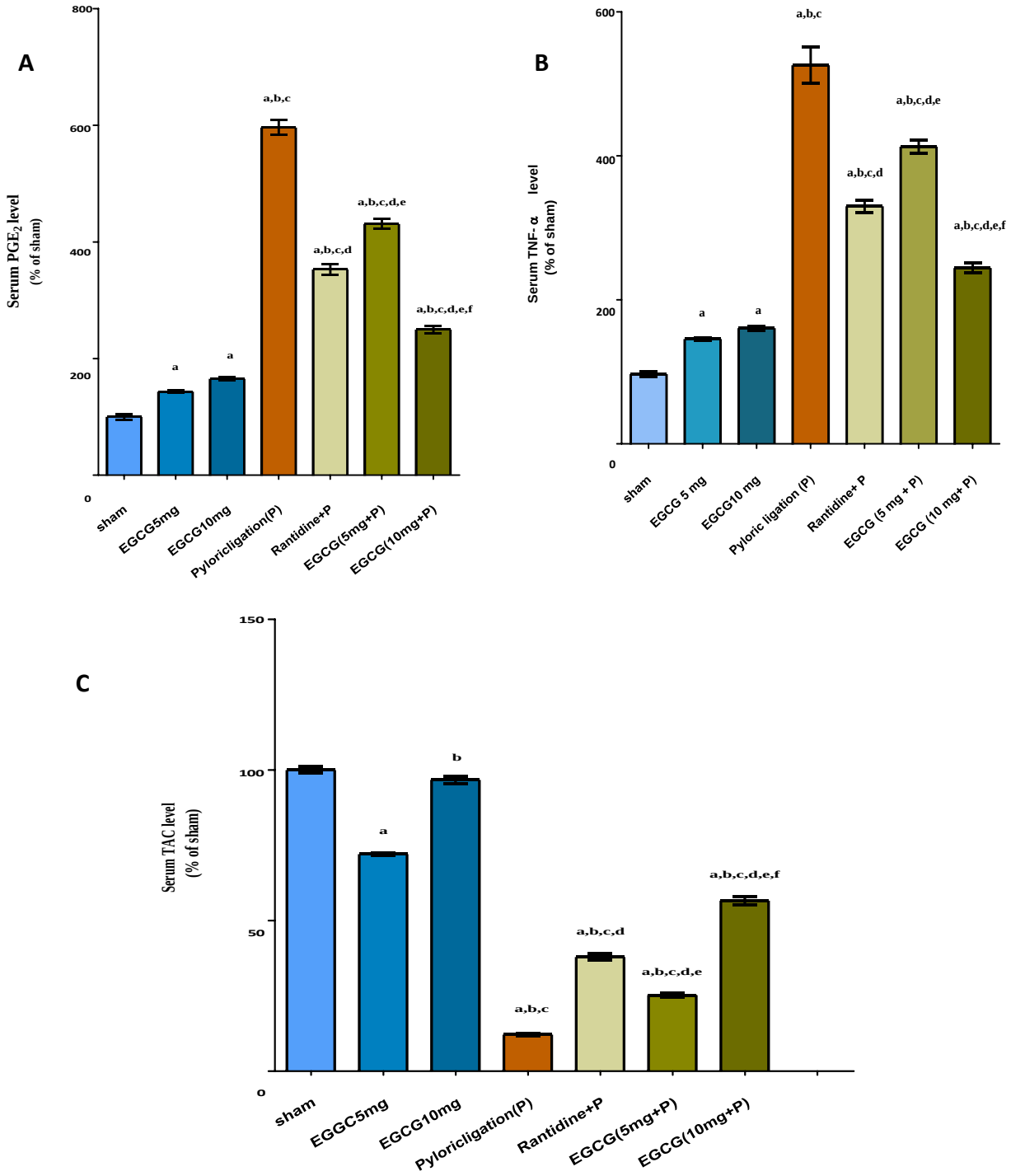

Figure 2. Effect of Epigallocatechin 3-gallate (EGCG) on (A) serum Prostaglandin E2 (PGE2), (B) serum tumor necrosis factor-alpha (TNF-α), and (C) serum total antioxidant capacity (TAC) levels in pyloric ligated rats.

Values are mean ± SEM (n=8).

a, b, c, d, e or f: significantly different from sham, EGCG (5mg/kg), EGCG (10mg/kg), pyloric ligation (P), ranitidine (80 mg/kg) +P or EGCG (5mg/kg)+P treated groups respectively at $p<0.05$ using one-way analysis of variance (ANOVA) followed by Turkey-Kramer test for multiple comparisons.

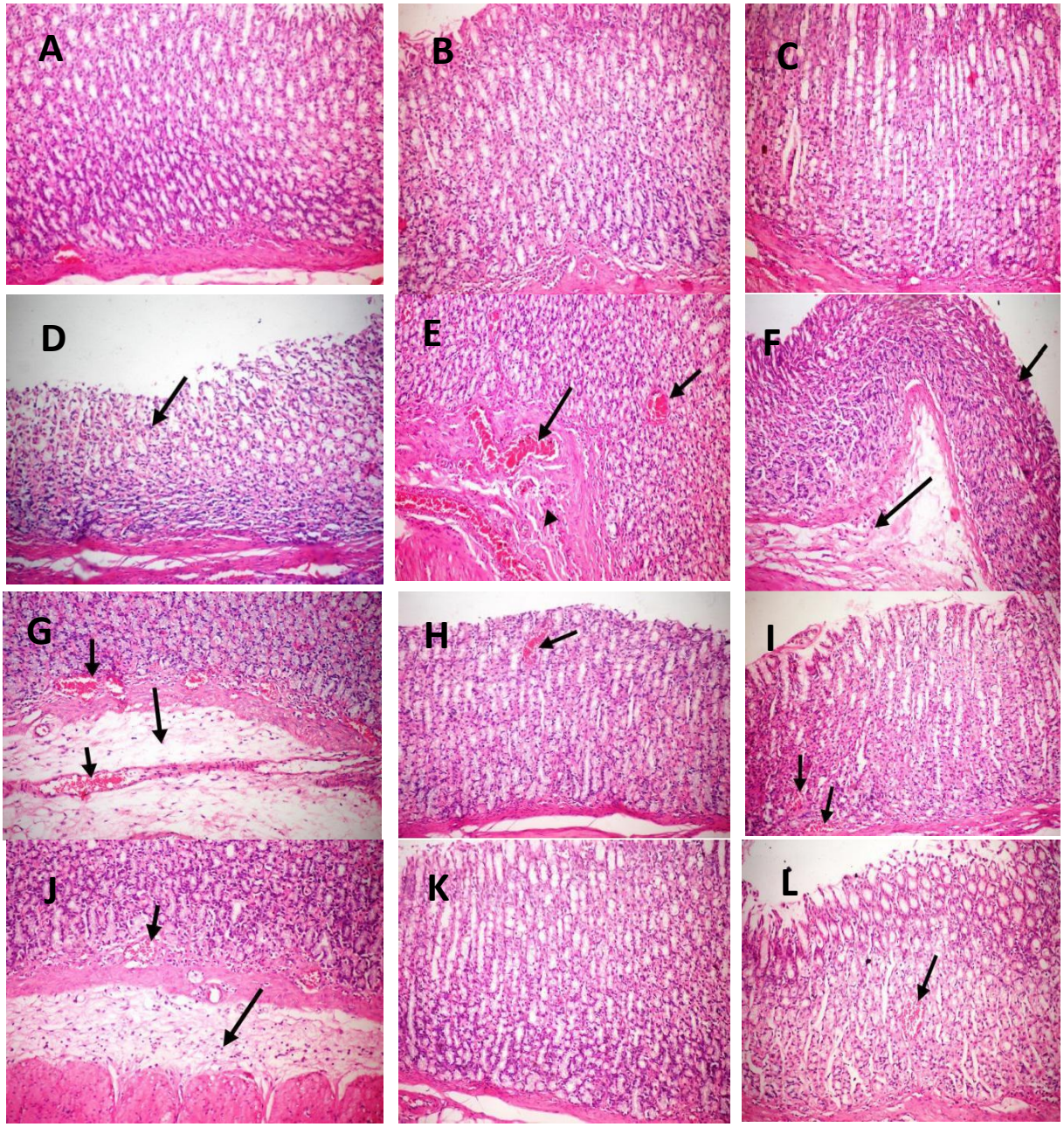

Figure 3. Representative photomicrographs of stomach sections stained by H & E (x200).
Transverse Sections were taken from rat stomach in the sham group showing the normal histological structure of gastric layers (A), TS of rat stomach treated with ECGG 5 mg/kg or ECGG 10 mg/kg for 7 days showing no histopathological changes (B, C). TS of rat stomach in pyloric ligation group showing necrosis and atrophy of gastric mucosal layer. As well as submucosal edema with few inflammatory cells infiltration. There congestion of mucosal and submucosal blood vessels as well as submucosal fibroblasts proliferation (D, E, and F). While, TS of the stomach of pyloric ligated rats treated with ranitidine 80 mg/kg for 7 days showing no histopathological changes and congestion of mucosal and submucosal blood vessels associated with submucosal edema (G, H).TS of the stomach of pyloric ligated rats treated with ECGG 5mg/kg for 7 days showing no histopathological changes and congestion of mucosal blood vessels. There is submucosal edema associated with few inflammatory cells infiltration (I and J). TS of the stomach of pyloric ligated rats treated with ECGG 10mg/kg for 7 days showing no histopathological changes and congestion of mucosal blood vessel (K and L).

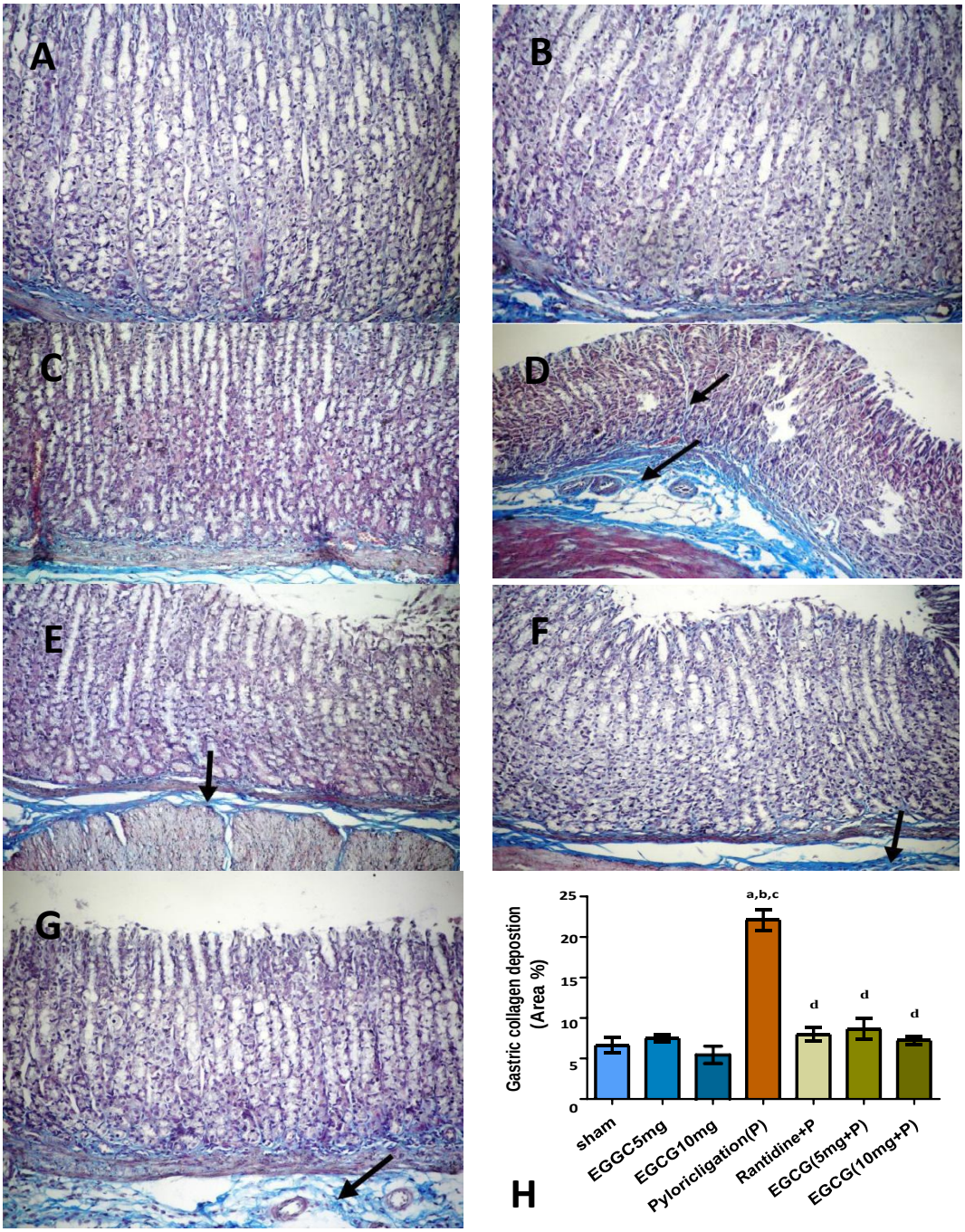

Figure 4. Effect of Epigallocatechin 3 -gallate (EGCG) on gastric collagen deposition in pyloric ligated rats. (Masson's Trichrome stain X 200).

Photomicrographs from transverse sections of rat stomach in the sham group, rat stomach treated with ECGG 5 mg/kg or ECGG 10 mg/kg for 7 days showing weak positive immunohistochemical reaction for collagen fibers (A, B, and C), respectively. TS of rat stomach in pyloric ligation group showing strong positive immunohistochemical reaction for collagen fibers (D).TS of rat stomach in groups received ranitidine 80 mg/kg, EGCG 5mg/kg or EGCG 10mg/kg before pyloric ligation for 7 days showing weak positive immunohistochemical reaction for collagen fibers (E, F, and g). (H) Showing percentage of the area of gastric collagen deposition. Values are given as mean ± SEM of eight rats. a, b, c, d, e or f: significantly different from sham, EGCG (5mg/kg), EGCG (10mg/kg), pyloric ligation (P), Ranitidine +P or EGCG (5mg/kg) + P treated groups respectively at $p<0.05$ using one-way analysis of variance (ANOVA) followed by Turkey-Kramer test for multiple comparisons.

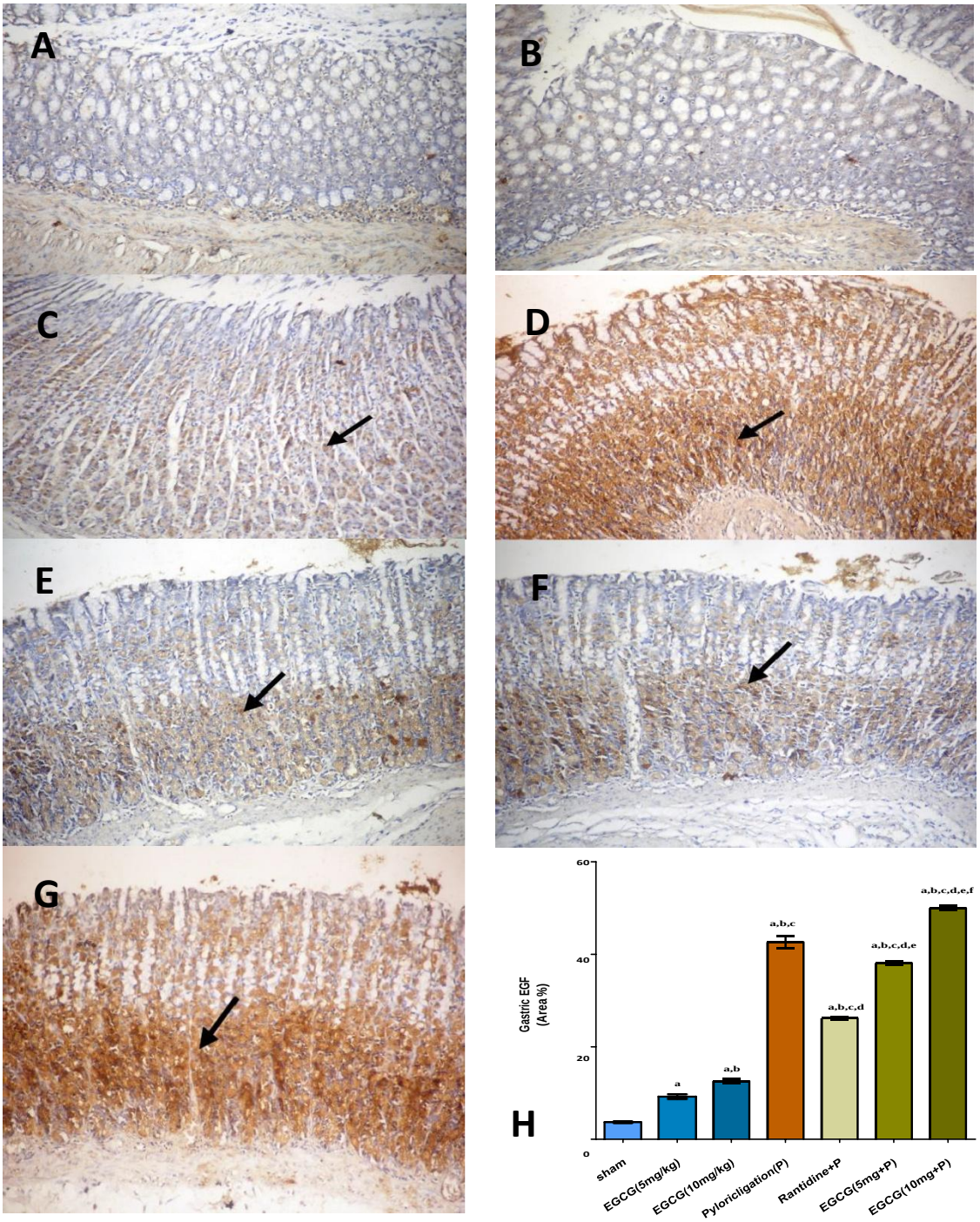

Figure 5. Effect of Epigallocatechin 3 -gallate (EGCG) on gastric epidermal growth factor (EGF) in pyloric ligated rats. Expression of EGF by immunohistochemical staining (magnification 200).

Photomicrograph of a transverse section of rat stomach in the sham group or rat stomach treated with ECGG 5 mg/kg for 7 days showing no expression (negative immunoreaction) of EGF (A and B).TS of rat stomach treated with ECGG 10 mg/kg for 7 days showing weak positive immunoreaction of EGF (C). TS of rat stomach in pyloric ligation group showing strong positive immunoreaction of EGF (D).TS of rat stomach in pyloric ligated rat treated with ranitidine 80 mg/kg or ECGG 5 mg/kg for 7 days showing weak positive immunoreaction of EGF (E and F). TS of rat stomach in pyloric ligated rat treated with ECGG 10 mg/kg for 7days showing strong positive immunoreaction of EGF (G). (H) Showing Percentage of the area of the immuno-positive reaction of EGF. Values are given as mean ± SEM of eight rats. a, b, c, d, e or f: significantly different from sham, EGCG (5mg/kg), EGCG (10mg/kg), pyloric ligation (P), Ranitidine +P or EGCG (5mg/kg) + P treated groups respectively at $p<0.05$ using one-way analysis of variance (ANOVA) followed by Turkey-Kramer test for multiple comparisons.

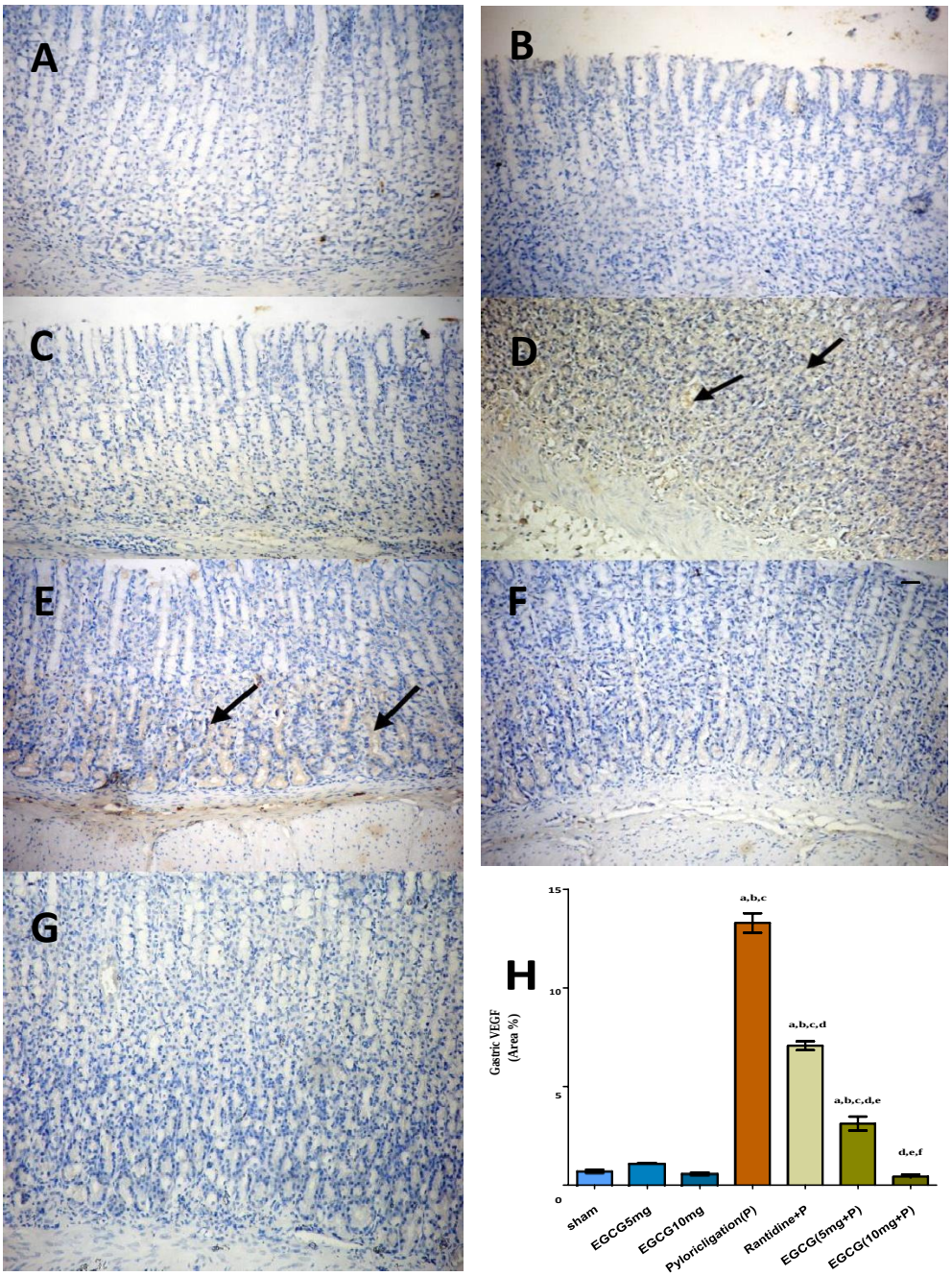

Figure 6. Effect of Epigallocatechin 3 -gallate (EGCG) on gastric vascular endothelial growth factor (VEGF) in pyloric ligated rats. Expression of VEGF by immunohistochemical staining (magnification 200).

Photomicrograph of a transverse section of rat stomach in the sham group, rat stomach treated with ECGG 5 mg/kg or ECGG 10 mg/kg for 7 days showing no expression (negative immunoreaction) of VEGF(A, B, and C).TS of rat stomach in pyloric ligation group showing moderate positive immunoreaction of VEGF (D). TS of rat stomach in pyloric ligated rat treated with ranitidine 80 mg/kg for 7 days showing weak positive immunoreaction of VEGF (E).TS of rat stomach in pyloric ligated rat treated with ECGG 5 mg/kg or ECGG 10 mg/kg for 7days showing no expression (negative immunoreaction) of VEGF (F and G). (H) showing the percentage of the area of the immuno-positive reaction of VEGF. Values are given as mean ± SEM of eight rats. a, b, c, d, e or f: significantly different from sham, EGCG (5mg/kg), EGCG (10mg/kg), pyloric ligation (P), Ranitidine +P or EGCG (5mg/kg) + P treated groups respectively at $p<0.05$ using one-way analysis of variance (ANOVA) followed by Turkey-Kramer test for multiple comparisons.

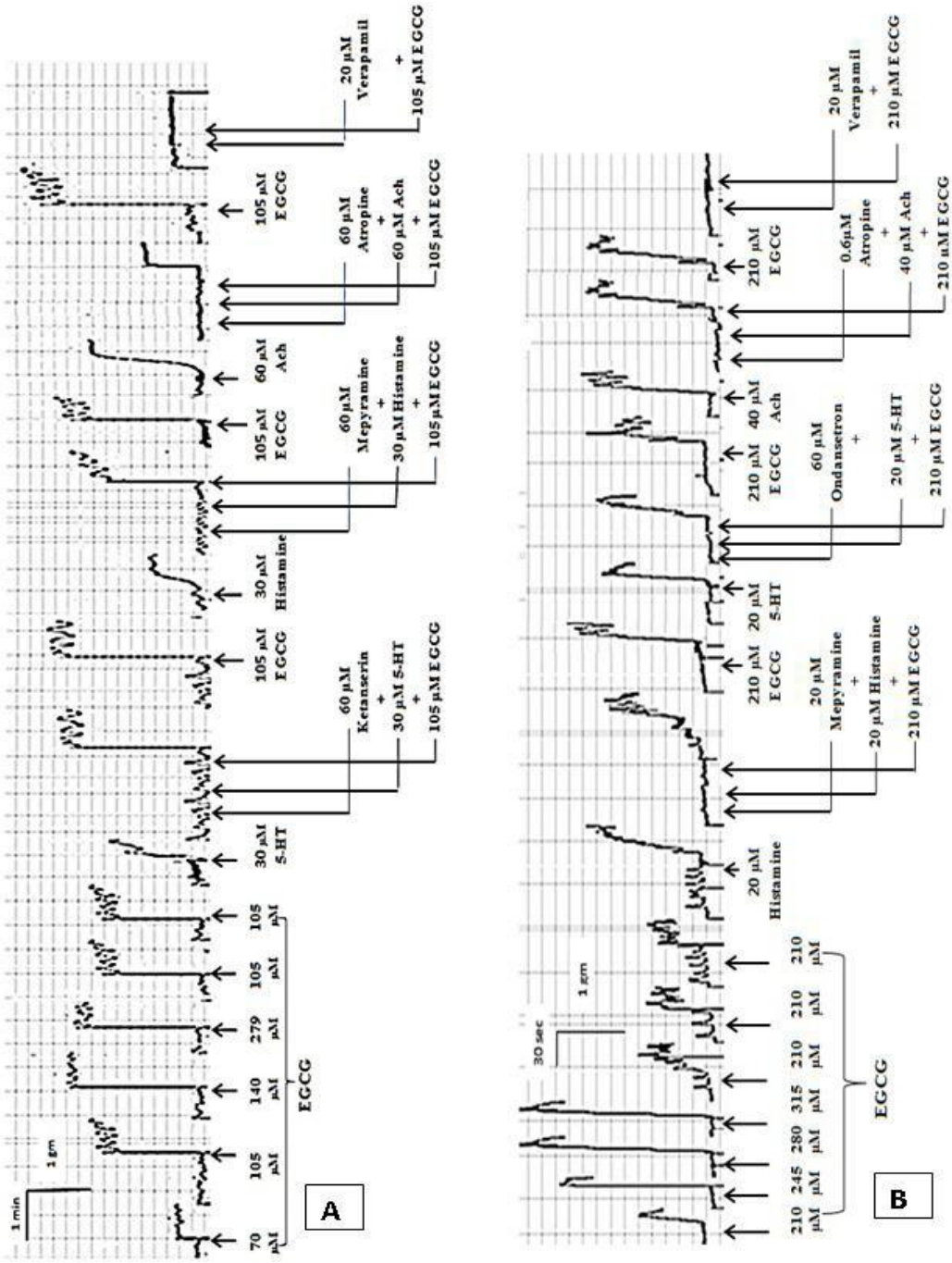

Figure 7. (A) Site of action of EGCG on rat fundus strip.

Fundus strip was suspended in oxygenated Tyrode's solution at 37^{O}C.

The 1 min. scale represents the speed of recording and 1g – scale represents the calibration–tension scale.

(B) Site of action of EGCG on isolated guinea pig ileum.

Ileum was suspended in oxygenated Tyrode's solution at 32^{O}C.

The 30sec. scale represents the speed of recording and 1g – scale represents the calibration–tension scale.