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Honokiol mitigates gastric ulcer induced by indomethacin in rats via suppression of inflammatory biomarkers and reactive oxygen species

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Abstract

Gastrointestinal toxicity of nonsteroidal anti-inflammatory drugs (NSAIDs) as indomethacin has been frequently associated with gastric injury. Honokiol (HK) has demonstrated marked hepatoprotective features; however, its effects on indomethacin induced gastric injury have not been studied. The aim of the study was to evaluate the potential gastroprotective activity of honokiol against indomethacin evoked gastric mucosal damage. Thirty rats were randomly divided into five groups, 6 animals each, and treated for 21 days: the first group (control group), the second group received honokiol only, the third group received indomethacin, the fourth group received honokiol and indomethacin, the fifth group received Omeprazole and indomethacin. The rat's stomachs were examined in terms of the inflammatory and oxidative perturbations. Results demonstrated that HK attenuated the gross gastric damage, scores of ulcer index, area of mucosal lesions and histopathology outcomes; actions which were similar to the reference antiulcer drug omeprazole, also pretreatment with HK hampered tumor necrosis factor-α (TNF-α) and significantly decreased Malondialdehyde (MDA) level associated with a significant increase in GSH content, total antioxidant capacity (TAC) level and rise in antioxidants enzyme activities namely; catalase (CAT) and glutathione peroxidase (GPx). In conclusion, the available data in this research propose that the extracts of Honokiol evidenced to be capable of ameliorating indomethacin-induced gastric ulceration and the possible mechanisms are via anti-oxidative and anti-inflammatory.

Keywords: Ulcer, Indomethacin, Honokiol, Antioxidant, Anti-inflammatory

1. Introduction

Peptic ulcer is one of the supreme vital and combined syndromes of the alimentary system occurring owing to damages in mucus and submucosal layers of the alimentary tract[1]. This disorder results from inconsistency between protective factors comprehending bicarbonate ,mucus, antioxidants, prostaglandins, mucosal blood flow and invasive ones as acid secretion, pepsin, and *Helicobacter pylori*. Annual incidence of the syndrome is entirely about 8% and its epidemic rate is about 10% through the life[2]. For many years, non-steroidal anti-inflammatory drugs (NSAIDs) have been applied as the another risk aspect in gastric ulcers aetiology which can ultimately result in bleeding and/or further gastrointestinal complications[3]. By fading synthesis of prostaglandins, decreasing blood flow in mucus and submucosal layers of the alimentary system, and improving acid secretion and pepsin activity, NSAIDs cause such conditions in stomach which in conclusion lead to formation of peptic ulcer[4]. Indomethacin-induced mucosal damage may cause severe mucosal erosions and ulcerative injuries[5]. Gastrointestinal mucosal damage via NSAIDs arises fundamentally due to a systematic effect[6]. Oral intake of NSAIDs primes to relief of intercellular adhesionmolecule-1 in vascular endothelial cells of gastric mucosa. In concern of the mechanism, massive amounts of neutrophils stick to vascular endothelial cells owing to inflammatory cytokines like tumour necrosis factor α (TNF-α) [7]. The generation and elimination of reactive oxygen species (ROS) processes are

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thoroughly associated. Living organisms have finely controlled systems to keep very little ROS levels; nevertheless, under certain conditions this balance can be disturbed. There are some causes for that: (i) increased level of endogenous and exogenous complexes arriving autoxidation united with ROS release; (ii) exhaustion of reserves of low molecular mass antioxidants; (iii) inactivation of antioxidant enzymes; (iv) decline in production of antioxidant enzymes and low molecular mass antioxidants; and, finally, (v) certain combinations of two or more of the listed above factors. Definitely, increase in steady-state ROS level, which results from imbalance between generation and elimination processes, can disturb many, if not all, living processes. Also reactive oxygen species as superoxide anion radical (O2·−), hydrogen peroxide (H2O2), hydroxyl anion (OH−), released by neutrophils, show a significant role in oxidation of biomolecules. As a result of the phenomena, vascular endothelial damage declines blood flow in gastrointestinal mucosa and in the termination breaks down hemodynamic microcirculation [8]. Moreover, oxidative stress shows an important role in gastrointestinal mucosal injury caused by NSAIDs, and strong antioxidants may suppress oxidative damage correlated to NSAIDs[9] .The root and stem bark of the oriental herb *Magnolia officinalis* (moreover well-known as Houpo) have been used in complementary Chinese and Japanese medicine for the management of numerous diseases due to its muscle relaxant[10], anti–gastric ulcer[11],and antithrombotic properties[12]. Honokiol, one of the bioactive ingredients of *M. officinalis*, has concerned an excessive deal of research awareness due to its miscellaneous biological special effects[13].The well-recognized pharmacologic properties of honokiol contain inhibition of platelet aggregation[14], anti-inflammatory, antioxidative effects [16]. Though the mechanism underlying the pharmacological activities has not been obviously interpreted, it must be noted that an unusual biphenyl compound, neolignan, shown numerous in vivo and in vitro biophysiological actions, proposing that there is a possible to progress powerful derivatives from its simple and reactive chemical structure[13]. Although many lines of verifications supporting the valuable effect of Honokiol, its gastroprotective effect against indomethacin induced gastric ulcer has not been yet thoroughly studied. Therefore, the present study was accompanied by evaluation the therapeutic potential of Honokiol in peptic ulcer induced by indomethacin using Omeprazole as a reference standard drug.

2. Material and methods

2.1. Materials

- Honokiol of 99% purity was purchased from Xi'an Biof Bio-Technology Co., Ltd. (China).
- Indomethacin (Hikma Pharmaceutical Co, Egypt) and Omperazole(Amriya Co. Egypt).
- All other chemicals materials and diagnostics kits were commercially available and of high quality.

2.2. Animals.

Adult male Sprague Dawley rats (180-200 g) were obtained from the breeding colony maintained at the animal house of the National Organization for Drug Control and Research (NODCAR, Cairo, Egypt). Animals were maintained at controlled conditions of 25 ± 2 °C temperature and 40-60 % relative humidity with 12 hours alternative dark and light cycles. They had free access to water ad libitum and standard diet. Rats were habituated two weeks before the experiment. The standard guidelines of NODCAR were followed in handling the experimental animals and this by conforming to the Guide for Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication no. 85-23, revised 1996).The study protocol was approved by The Institutional Research Ethics Committee (NODCR-REC) at the National Organization of Drug Control and Research (NODCAR/I/55/19)

2.3. Experimental design.

Animals were randomly divided into five groups, 6 animals each, and treated for 21 days as follows:

The first group (Control group) was given mixture of DMSO and corn oil as vehicle for honokiol in ratio of 1:9 per oral gavage for 21 days.

The second group was given honokiol only (10mg/Kg, per oral gavage;[17].

The third group (the diseased group)(INDO group) was given single injection of indomethacin (20mg/kg, Ip)[18] once on the 21th day.

The fourth group (Treated group) was given honokiol (10mg/kg, per oral gavage) for 21 days, and a single injection of indomethacin (20mg/kg, I.p; [18]once on the 21th day one hour after the last dose of honokiol.

The fifth group was given omeprazole as a standard agent (20mg/kg, per oral gavage)[18] for 21 days, and a single injectionin of domethacin (20mg/kg, I.p) on the 21th day one hour after the last dose of omeprazole.

2.4. Ulcer Induction:

Animals were kept alone and fasted for 36h in wide mesh bottom cages, permitted free access to water excepting for the latest hour before the last dose of the medication. Rats were injected intraperitoneally by indomethacin (20 mg/kg) 1 h after the last dose of the treatments [19].

2.5. Collection of gastric juice and determination of acidity

Indomethacin was injected and rats were euthanized 4 h later by cervical disclocation under deep ether anesthesia, Immediately thereafter, the pyloric ligation was carried out according to the method of [20] for the collection of gastric juice, following ligature of the oesophagocardiac junction, stomachs were excised and gastric juice was collected after an incision at the greater curvature. Following gastric ulcer assessment gastric mucosal homogenates were prepared using in phosphate buffered saline (PBS) buffer (pH=7.4) to obtain 10% homogenate, for the assessment of oxidative and inflammatory parameters. A portion of each stomach tissue was fixed in 10% formalin for histopathological examination.

2.6. Assessment of gastric lesion scoring

The rat was anesthetized and a midline notch was made and the stomach is removed, then the stomach was opened along the greater curvature, stretched reasonably by pinning on a cork board, and then the gastric mucosa was observed by naked eye and enlarging lens to: count the number of lesions and define the ulcer acuity for each group. Gastric lesions were recorded according to Fayad et. al. 2014 [21]**.**

2.7. Determination of pH of gastric juice

Gastric juice was gathered, centrifuged and the supernatant was titrated with 0.01 N NaOH by methyl orange as an indicator till yellowish orange color come out and the result indicated free acidity. At that point phenolphthalein was added as an indicator and continues titrating till red color returns however the total volume of alkali added indicated total acidity as defined before[22]. Furthermore, pH of gastric juice was determined by digital pH meter

2.8. Assessment of oxidative stress biomarkers in stomach homogenate

Reduced glutathione (GSH), Malondialdehyde (MDA), glutathione peroxidase and total antioxidant capacity (TAC) contents were assessed using Biodiagnostic kits (Cairo, Egypt;) Cat. NO. (GR 25 11, MD 25 29, GP 2425, TA 25 13 respectively) in stomach homogenate GSH was assessed according to manufacturer's procedure where GSH reduces Ellman's reagent [5,5′-dithiobis (2-nitrobenzoic acid)] (DTNB) to form a stable yellow product (5-mercapto-2-nitrobenzoic acid), which can be measured colorimetrically at 412 nm . Additionally, lipid peroxidation was determined by estimating the level of thiobarbituric acid reactive substances (TBARS) measured as MDA, according to the method described by manufacture. GPx content was assessed using assay kit.Total antioxidant capacity was assessed followed by manufacture's protocol.

2.9. Estimation of catalase

Catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase (HRP), remaining H_2O_2 reacts with 3,5-Dichloro -2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample. according to biodiagnostics kit (CA 2517)

2.10. Assessment of TNF-α in stomach homogenate

Rat TNF-α was measured by the ELISA kits obtained from RayBiotech Inc. (Parkway, LaneSuiteNorcross, GA).

2.11. Histopathological method:

Specimens from stomach were fixed in 10% formalin then dehydrated and embedded in paraffin for light microscopic examination. Sections of 5micron thickness were cut and stained by Hematoxylin and Eosin (H&E) for general histological structure and counterstain which was done using eosin stain[26].

2.12. Statistical analysis:

Data are presented as mean ± standard error. The data were analyzed using one-way ANOVA, followed byTukey post-hoc test, using GraphPad Prism data analysis program (GraphPad software, Inc., San

Diego, CA, USA). A value of $p \leq 0.05$ was considered statistically significant.

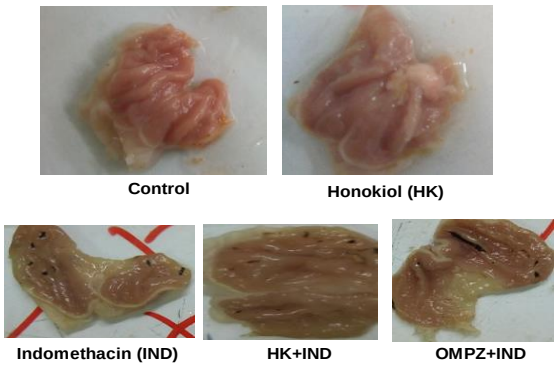
3. Results

3.1. Physiological Results

3.1.1. Ulcerative index

For the scoring of ulcerative index, administration of indomethacin (IND) strongly increased ulcerative index as compared with that of control group, extensive and detectable hemorrhagic lesions were observed in gastric mucosa figure (1). To the contrary, pretreatment with honokiol (HK) or omeprazole (OMPZ) showed significant reduction compared with INDO group figure (2).

3.1.2. Effect on gastric acidity



Administration of indomethacin strongly induced gastric acidity as compared with that of control group. To the contrary, pre-treatment with honokiol or omeprazole showed significant increase in gastric juice PH compared with INDO group (figure 3).

3.2. Biochemical Results:

3.2.1. Changes of Reactive Oxygen species (ROS):

Table (1) revealed the effects of honokiol extract on the lipid peroxidation, GSH and GPX activity of stomach tissue of indomethacin ulcerated rats. MDA level was significantly increased ($p < 0.05$) in the ulcerated animals. A significant reduction ($p< 0.05$) was also observed in the activity of GSH and GPx in the indomethacin-induced animals. Commendably, honokiol extract resulted in a significant improvement ($p < 0.05$) in these parameters and the observable effects compared favourably well with both normal control and standard drug (omeprazole) employed in the study.

3.2.2. Changes of TNF alpha:

Figure (4) showed significant increase in INDO group when compared to control group ($p<0.05$), on the other hand ulcerated group that treated with honokiol and group that treated with omeprazole demonstrated significant decrease when compared to INDO group.

3.2.3. Changes of Total Antioxidant Capacity (TAC) activity of stomach tissue:

Indomethacin administration brought about a significant ($p < 0.05$) decrease in TAC of stomach tissue when compared to control group. The observed changes in that parameter were significantly attenuated ($p < 0.0005$) in the honokiol extrac and omeprazole ulcerated group.

Treatment with omeprazole revealed more potent efficacy in the modulation of TAC of ulcerated rats as shown in figure (5).

3.2.4. Changes of Catalase activity of stomach tissue

Ulcerative group treated with Honokiol extract and other one with omeprazole showed significant amelioration effect revealed by increase of catalase level when compared to INDO group which showed significant decrease in that enzyme when compared to control group (p value<0.05) figure 7

3.3. Histological studies

Histopathological examination of stomach sections for normal and INDO induced ulcer rats with or without administration of tested therapeutic agents stained by H&E (x100)

Stomach of indomethacin -treated rats showed marked histological changes where focal necrosis and ulceration of gastric mucosa (small arrow) replaced by inflammatory cells infiltration in the mucosa and submucosa (large arrow), however, by supplementation of both honokiol or omperazole as a preventive regimen the histlogical changed were reduced and nearly restored near to normal and stomach tissue show submucosal oedema (small arrow) and inflammatory cells infiltration (large arrow) (X 100) figure (7).

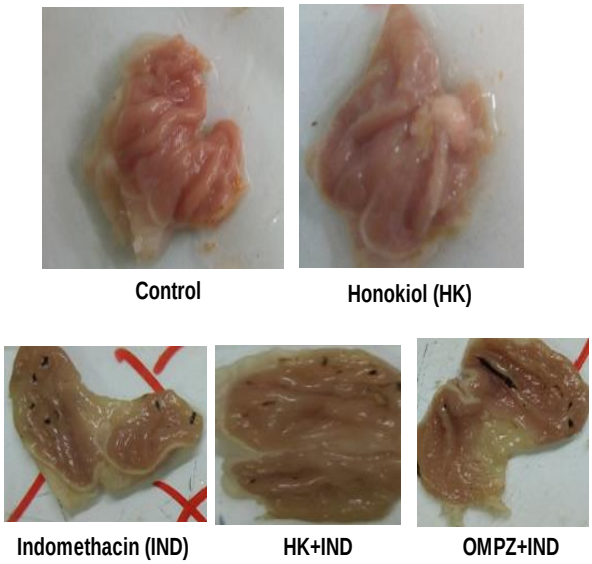


Figure (1) stomach tissue showed ulcer and erosion by naked eye.

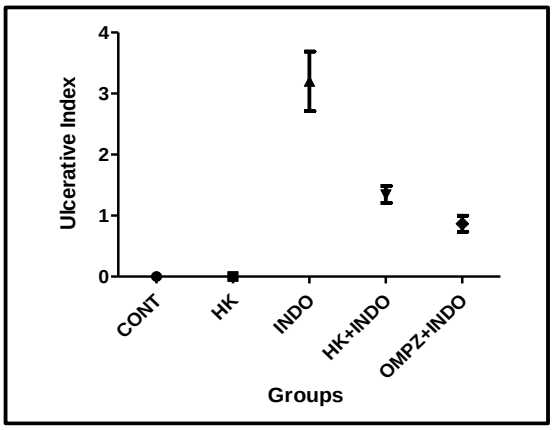


Figure (2) ulcerative index of different group.

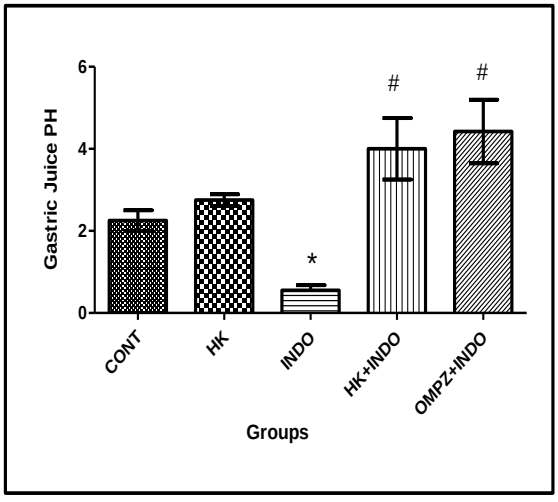


Figure (3) Gastric Juice PH of different group ($p < 0.05$).

* Significance difference from control group.

Significance difference from INDO group.

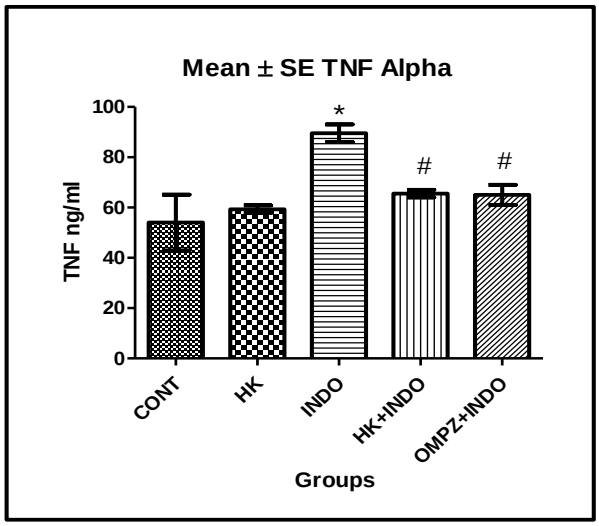


Figure (4): Mean ±SE of TNE alpha ($p < 0.05$).

* significance difference from control group.

signifinance difference from INDO group.

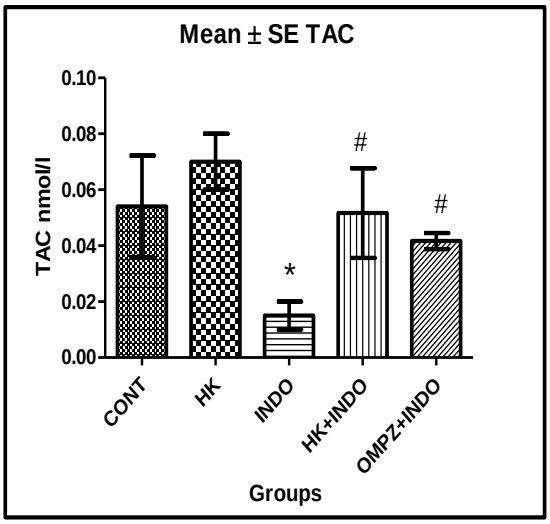


Figure (5): Mean ±SE of TAC ($p < 0.05$).

* significance difference from control group.

signifinance difference from INDO group.

4. Discussion

Indomethacin is an Aryl acetic acid derivative recognized as one of the utmost powerful NSAIDs with numerous alimentary argumentative effects[5] due to its inhibitory effect on prostaglandins synthesis[2]. It can harm stomach tissue by increasing gastric acid and pepsin activity[24]. A thoughtful of these events might be of ultimate relevance in designing new antiulcer drugs. With the characteristic adverse side effects and significantly high cost of synthetic drugs, developing natural products of plant source which are thought to be non-toxic, effective and

reasonable will be most suitable in the treatment of gastric ulcer. Phytotherapy is promptly gaining grounds in satisfying human health and in the prevention[25]. Analysis of gastric secretions (for ulcerative index and, pH) is usually engaged to establish its status subsequent exposure to biochemical analysis[26].

Table (1) Mean±SE of GSH, MDA and GSPX content of stomach tissue. ($p < 0.05$)

	GSH nmol/g tissue	MDA nmol/g tissue	GPX mU/ml
CONT	31.9± 1.1	21.1±2 .7	145.6± 10.5
HK	39.5± 2.4	28.2±1 .8	151.9± 16
INDO	21±1. 4*	44.14± 5.6*	78±11 *
HK+IND O	79.7± 3.6#	19.6±1 .6#	141±2 #
OMPZ+I NDO	41.8± 1.6#	33.7±2 .7#	132±3. 7#

* significance difference from control group.
# signifinance difference from INDO group.

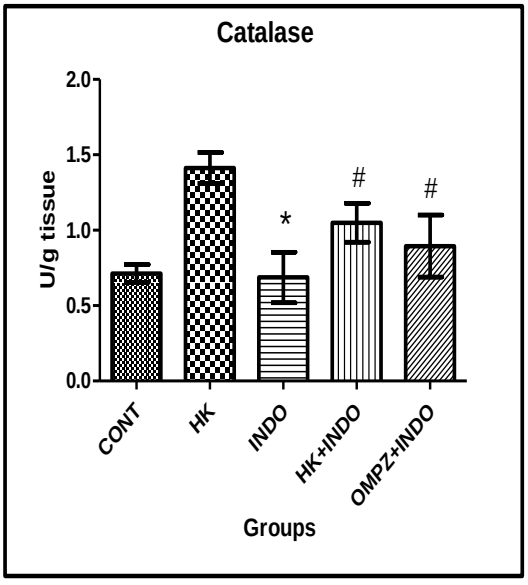
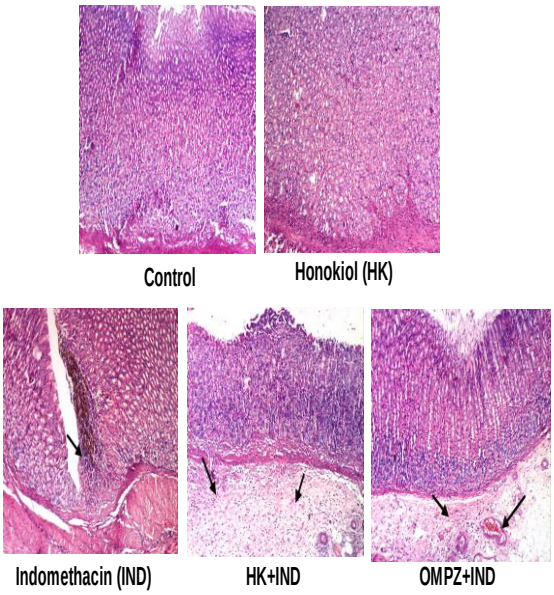


Figure (6) Mean±SE of Catalase ($p < 0.05$).
* significance difference from control group.
# signifinance difference from INDO group.



Figure(7) histological examination of stomach tissue by H&E (x100), necrosis and ulceration of gastric mucosa (small arrow),inflammatory cells infiltration in the mucosa and submucosa (large arrow).

The pH gives an idea of the level of acidity and volume of gastric secretions. Low pH value is an index of declined hydrogen ion concentration in gastric juice[20]. As presented in this study, Indomethacin administeration cause increased in ulcerative index and gasric acidity this is attributed to disruption of mucus phospholipids and lead to the seperation of mitochondrial oxidative phosphorylation, thus initiating mucosal injury. When exposed to acidic gastric juice (pH 2), Indomethacin became to protonated form and pass lipid membranes to go into epithelial cells (pH 7.4), wherein they ionize and release H^+ so can't pass the lipid membrane, and are trapped in epithelial cells, leading to the uncoupling of oxidative phosphorylation, reduced mitochondrial energy production, improved cell permeability, and decreased cell integrity. level of MDA in stomach tissue was significantly increase (p-value<0.05) in indomethacin group when compared to control group, this is due to increasing lipid peroxidation and oxidative stress by producing free radicals in mucus[2].This is concurrent with previous researches which reported that oxidative damage come to be more marked with lipid peroxidation[1][25], which is essential in physiopathology of gastric destruction[26], regarding injury to the cell and the cell membrane[30]. In previous studies the quantity of

lipid peroxidation was revealed to be related to gastric mucosal injury induced by indomethacin as well as enzyme activity such as catalase[7] and glutathione peroxidase[6] which were exhausted by indomethacin as demonestrated in this study.Thus, consumption of substances that can surge the activity of these enzymes for protection of the gastric mucosa from the effects of indomethacin is a prospective solution in the first step[7]. pro-inflammatory cytokines as,Tumor necrosis factor α(TNF-α) is a participant of the cytokine family[27] that motivates the acute phase reaction[8]. It is produced essentially by activated macrophages, though it can be produced by CD^{4+} lymphocytes, NK cells, neutrophils, mast cells, eosinophils, and neurons[6]. This is in agreement with our study as indomethacin group showed significant increase in TNF-α when compared to control group[25]. Gastric ulcer appears to be produced from over-secretion of gastric juice and imbalance of protective and forceful factors convoluted in sustaining gastric mucosal integrity[28]. Therefore, acid production managers such as proton pump inhibitors as omeprazole widely used in clinic seem to hasten the curative of gastric ulcers and gastritis by powerful and long lasting antisecretory actions[29]. Omeprazole was displayed gastroprotective effect against indomethacin induced ulcer by diminishing lipid peroxidation[5]. Honokiol is one of two major groups of bioactive composites isolated from the cortex of *Magnolia officinalis*[11].Thus these results suggest that the mechanism of honokiol isolated from MB by which it inhibited gastric ulcer and gastritis may be due to suppression of aggressive factors [13](i.e., inhibition of gastric acid secretion) and in turn, acceleration of protective factors, like as proton pump inhibitors [30]. In this study honokiol revealed gastroprotective activity through reducing TNF-α and declining MDA[10]. These results are harmony with Huang et al 2017[31] who reported that Honokiol expressively inhibited indomethacin induced gastric lesions through motivation of mucus secretion.The extract of honokiol has revealed the acid-neutralizing capacity and antioxidant activity[32]. Honokiol displayed a robust antioxidant effect that may elucidate clinical suggestion for defense of stomach from ulcer and bleeding[16].Earlier studies indicated that antioxidant effect of honokiol was 1000 times higher than those of α-tocopherol[33]. Furthermore, the polyphenols of Honokiol may stop formation of hydroxyl radical by chelating the transition metals such as copper and iron or healing molecules after free radical attack[34]. In this study Honokiol improved glutathione peroxidase (GPx) activity and catalase activity. Moreover, Honokiol exhibits anti-inflammation effects via decreasing tumor necrosis factor-α [14]. Honokiol has revealed strong antioxidative[33] and anti-inflammatory properties[35] mediated by numerous approaches of action. Antioxidant protection system through direct detoxification ROS[14] or via a GPx[36] catalyzed mechanism. The physiological role of GSH[34] is as a vital intracellular reducing mediator for the maintenance of reducing state of thiol groups and other antioxidant molecules[34]. The results of the current study revealed that the honokiol extract have a tendency to rise GSH concentration in the stomach of rats. previous researches reported that Honokiol has been found to have a protective effect against indomethacin-induced gastric ulcer[18], which is used as an alternative approach to oxidative stress, acting by inhibiting intracellular GSH depletion. Total antioxidant capacity (TAC) reflects the capacity of the non-enzymatic antioxidant defense system[16]. Honokiol extract has increased TAC for the stomach. This is in concurrent with our results.the previous studies showed that the honokiol extract increased the activities of both the non-enzymatic and enzymatic anti-oxidant defense systems and has potential for use as a natural antioxidant.

5. Conclusion: The present work affirms the beneficial gastroprotective effects of HK in gastric mucosal injury triggered by indomethacin in rats. These benfecial effects were chiefly mediated via curbing oxidative stress induced by indomethacin and augmentation of antioxant acitivty associated with attenuation of gastric inflammatory aberrations.

Conflicts of interest

"There are no conflicts to declare".

Formatting of funding sources

NA

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