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RESEARCH ARTICLE

Status of inflammatory markers and growth factor in gastric ulcer protective effects of *Punica granatum* L. peel extract in rat

Indal Chauhan[1], Sudhanshu Agrawal[2], Raj Kumar Goel[3]

[1]Department of Pharmacology, Institute of Medical Sciences, Varanasi, Uttar Pradesh, India, [2]Department of Physiology, Heritage Institute of Medical Sciences, Varanasi, Uttar Pradesh, India, [3]Department of Pharmacology, Heritage Institute of Medical Sciences, Varanasi, Uttar Pradesh, India

Corresponding to: Raj Kumar Goel, E-mail: rkgoelbhu50@gmail.com

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ABSTRACT

Background: Inflammatory markers, namely, myeloperoxidase (MPO), cytokines (tumor necrosis factor-alpha [TNF-α], and interleukin-1 beta [IL-1β]) and vascular endothelial growth factor (VEGF) have been reported to play an important role in ulcerogenesis and healing. Recently, we reported that the gastric ulcer (GU) protective and healing effects of 50% ethanol (EtOH) extract of *Punica granatum* peel (PGE) in rats were predominantly due to strengthening of mucosal defense and antioxidants status. **Aims and Objectives:** The present study incorporates the effects of PGE on rat gastric mucosal inflammatory markers (MPO, TNF-α, and IL-1β) and angiogenic factor (VEGF) in GU induced by cold-restraint stress (CRS) and EtOH in rats. **Materials and Methods:** PGE (100 mg/kg) was administered orally once daily to rats for 7 days before induction of CRS and EtOH GU. Ulcer index (UI), gastric mucosal MPO, TNF-α, and IL-1β, and VEGF were estimated. **Results:** CRS rats showed increase in UI and MPO (419.5, $P < 0.001$) compared with unstressed rats while PGE caused decrease in UI (46.1%, $P < 0.05$) and MPO (51.1%, $P < 0.001$) compared with CRS rats. EtOH rats showed increase in UI, TNF-α (936.5%, $P < 0.001$), IL-1β (37.2%, $P < 0.05$), and VEGF (13.0%, $P < 0.05$) compared with control normal saline-treated rats. PGE-treated EtOH rats showed decrease in UI (68.9%, $P < 0.05$), TNF-α (77.9%, $P < 0.01$), and IL-1β (24.8%, $P < 0.05$) but tended to decrease VEGF (7.3%, $P < 0.2$) compared with EtOH rats. **Conclusion:** Both the inflammatory markers and VEGF were found to increase in GU rats while PGE EtOH extract decreased the status of inflammatory markers with little change in VEGF level with concomitant decrease in ulceration indicating their role in ulcer healing and repair.

KEY WORDS: *Punica granatum* Peel; Gastric Ulcer Healing; Myeloperoxidase; Tumor Necrosis Factor-alpha; Interleukin-1 Beta; Vascular Endothelial Growth Factor

INTRODUCTION

Gastric lesions are resultant of mucosal damage produced by a number of factors and are associated with cellular influx, free radical generation, cytokines, and growth factors. Acute inflammatory marker, myeloperoxidase (MPO), and pro-inflammatory cytokines (tumor necrosis factor-alpha [TNF-α], interleukin-6 [IL-6], and IL-1 beta [IL-1β]) play a major role in gastric ulceration.[1,2] Stress has been reported to increase the gastric mucosal level of MPO[3] while ethanol (EtOH) ingestion was reported to cause gastric mucosal damage through the inflammatory and oxidative processes due to increase levels of malondialdehyde, nitric oxide, and TNF-α and IL-1β.[4] Growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and transforming growth factor-α (TGF-α) are having

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a major role in tissue healing processes including that of gastric ulcers (GU).[5] We have earlier reported the healing effects of EtOH extract of dried fruit pulp of plantain banana (*Musa sapientum* var. *paradisiaca*) on acetic acid-induced GU in rat and found the role of cytokines, TNF-α, IL-1β, and growth factor, TGF-α in healing GU.[6]

Punica granatum L. (family - Punicaceae, PG) is native to Iran, Afghanistan, Baluchistan, Mediterranean regions, and Himalayas in Northern India. PG fruits have been reported to have pharmacological properties such as antioxidant, anti-diarrheal, anti-inflammatory, anticancer, antibacterial, antifertility, angiogenesis, apoptotic, antiulcer, and wound-healing activity.[7] There are reports of GU protective effects of PG peel (PGE), rind and seeds against EtOH- and non-steroidal anti-inflammatory drugs-induced GU in rats which could be due to decreased acid secretion and promotion of mucin secretion and adhered mucin and status of mucosal antioxidants.[8,9] Recently, we reported, in our "in press" work, the GU protective and healing properties of 50% EtOH extract of PGE against acute GU induced by various experimental physical and chemical ulcerogens in rats and demonstrated the role of defensive mucin secretion and mucosal glycoproteins and antioxidants.[10] The present work is, thus, in continuation of our earlier work on PGE and is further carried out to study the role of MPO, cytokines (IL-1β and TNF-α), and VEGF in the ulcer-protective effects of PGE against cold-restraint stress (CRS)-and EtOH-induced GU in rats.

MATERIALS AND METHODS

Animals

Inbred Charles-Foster (CF) albino rats (150-200 g) of either sex were obtained from the central animal house of Institute of Medical Sciences, Banaras Hindu University, Varanasi. They were kept in the departmental animal house at ambient temperature of 26°C ± 2°C and relative humidity of 44-56%, with light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Pashu Aahar, Ramnagar, Varanasi) and the food was withdrawn 18-24 h before the experiment though water was allowed *ad libitum*. "Principles of laboratory animal care" (NIH Publication No. 82-23, revised 1985) guidelines were followed. Approval from the Institutional Animal Ethical Committee was taken before the experimental work (Notification No.: Dean/13-14/CAEC/333 dated 20.11.2013).

Collection of Fruits and Extraction

Fruit of PG was collected in the months of September-November from Ayurvedic Gardens, Banaras Hindu University. Peel of PG was removed from the fruits, dried in shade, and blended to form of fine powder. PGE was prepared by adding 200 g of dried fine powder of PG in 1000 ml of EtOH (500 ml) and distilled water (500 ml) mixture. The mixture was shaken at intervals and the extract so obtained was filtered after an interval of two days. The procedure was repeated twice at an interval of two days. PGE so obtained each time was mixed and later dried at 40° C in an incubator. The yield was about 20.1% (w/w). PGE was stored at −20°C until further use.

Drugs and Chemicals

Omeprazole (OMZ, Merck India Ltd., Mumbai, India), sucralfate (SCF, Reckitt and Colman India, Kolkata), pentoxifylline (PTX, Merck India Ltd., Mumbai, India), and all other chemicals and reagents were used of analytical grade.

GU and Inflammatory Markers Studies

The dose of PGE and the standard anti-ulcer drugs, OMZ (antisecretory, 2 mg/kg), SCF (ulcer healer, 500 mg/kg), TNF-α antagonist, and PTX (10 mg/kg), was selected as reported earlier.[1,2,10] PGE, OMZ, SCF, and PTX were suspended in 0.5% carboxymethyl cellulose (CMC). The animals received the drugs orally with the help of an orogastric tube in the volume of 10 ml/kg body weight and were given orally once daily for 7 days. The experiments were conducted on day 7, one hour after the last dose of PGE/standard drugs to 18 h fasted rats while control rats received CMC only. The effects of PGE were studied on gastric ulceration and gastric mucosal MPO in CRS-induced GU rats while gastric ulceration and cytokines, TNF-α and IL-1β, and VEGF were studied in EtOH-induced GU.

Anti-ulcer Study

Acute GUs like CRS and EtOH were produced following the methods as reported earlier.[11] Briefly, CRS was given by strapping the rats on a wooden plank and keeping them for 2 h at 4-6°C while EtOH (1 ml/200 g, 1h) was given orally to the overnight fasted rats for 1h. The animals were euthanized with over dose of anesthetic ether after 2 and 1 h of CRS and EtOH. The stomachs were dissected out for ulcer scoring. In CRS rats, ulcer index (UI) in CRS rats was calculated by adding the number of ulcer per stomach plus the severity of ulcer converted as one plus per stomach by a person unaware of the experimental protocol,[12] while in EtOH treated rats, UI was scored, based on the product of length and width of the ulcers present in the glandular portion of the stomach (mm^2/rat).[11] The stomachs were dissected out, and the mucosal scrapings were taken from the glandular portion of the stomach and homogenized in respective buffered solution for the estimation of various biochemical paradigms.

Gastric mucosal MPO estimation in CRS rats: Gastric mucosal protein (mg/g wet tissue) and MPO (mU/mg protein) were estimated in the gastric mucosal homogenate of unrestraint stress (URS, negative control) or CRS (Control) rats treated

rats with oral CMC; PGE (test extract) and SCF and OMZ (positive controls). Briefly, the gastric mucosa was scrapped and homogenized in normal saline (NS) (20 mg/ml)/0.5% HTAB with 50 mM potassium phosphate buffer (pH 6) (100 mg/ml) for the estimation of protein[13] and MPO,[14] respectively. The 0.05 ml of solution of alcoholic precipitate of gastric mucosa in 0.1 N NaOH was added to 0.95 ml of distilled water. Out of this 1 ml solution, 0.4 ml was taken into another test tube. To this, 4 ml of alkaline reagent was then added and kept for 10 minutes. Then, 0.4 ml of the phenol reagent was added and again 10 minutes were allowed for color development. Readings were taken against the blank prepared with water at 610 nm. The protein content was calculated from the standard curve prepared with bovine albumin and expressed in terms of mg/g wet mucosa. For MPO estimation, mucosal scrap was homogenized as above and was freeze-thawed three times and sonicated for 10 seconds and then centrifuged at 14000 × g for 45 minutes at 4°C. The resulting supernatant was used for estimation of MPO. A unit of MPO activity is defined as that converting 1 μmol of H_2O_2 to water in 1min at 25°C. The results were expressed as nM/mg protein.

Gastric Mucosal Cytokines and Growth Factor Studies in EtOH-treated Rats

Gastric mucosal protein (mg/g wet tissue),[13] TNF-α (pg/mg protein),[15] IL-1β (pg/mg protein),[16] and VEGF (pg/mg protein)[17] were estimated in the gastric mucosal homogenate of NS (negative control)- or EtOH (Control)-treated rats with oral CMC; PGE (test extract) and SCF and OMZ, and PTX (positive controls) using standard kits [Cat#ELR- TNF-α-CL; Cat#ELR-IL-1β-CL; Cat#ELR-VEGF-CL; Ray Biotech, Inc., GA].

Statistical Analysis

Results are expressed as mean ± SEM (n=4). Data were analyzed by one-way ANOVA followed by Dunnett's test for multiple comparison and significance was declared at $P < 0.05$.

RESULTS

Effect of PGE on GU and Gastric Mucosal Proteins and MPO in CRS Rats

UI

CRS rats showed a significant increase in UI compared with URS rats while CRS-induced UI was reduced with pre-treatments of PGE, OMZ, and SCF compared with CRS alone (Table 1).

Proteins and MPO

Rats of URS, CRS, or PGE-/OMZ-/SCF-treated CRS rats showed little or no change in the gastric mucosal protein content. MPO was increased significantly in rats subjected to CRS (419.5% increase, $P < 0.001$) compared with URS rats while rats subjected to CRS after treatments with PGE, OMZ, and SCF showed significant decrease in the level of MPO (38.8-51.1% decrease, $P < 0.01$ to $P < 0.001$) compared with CRS rats (Table 1).

Effect of PGE on GU and Gastric Mucosal Protein, Cytokines, and VEGF in EtOH Rats

UI

EtOH (UI-22.3 ± 3.89) caused significant gastric ulceration compared with NS treated alone (UI 0.0 ± 0.0). Pre-treatment of rats with PGE, OMZ, SCF, and PTX in EtOH-induced GU indicated significant ulcer reduction which ranged from 68.9 to 82.9% ($P < 0.05$ to $P < 0.01$), respectively (Table 2).

Gastric mucosal proteins, TNF-α, IL-1β, and VEGF

Gastric mucosal homogenate of CMC-NS-treated rats showed protein as 54.5 ± 1.36 mg/g wet tissue while VEGF, TNF-α, and IL-1β were 146.4 ± 3.30, 630.7 ± 61.7, and 391.9 ± 38.7pg/mg protein, respectively. CMC- EtOH-treated rats did not show any change in protein content (58.0 ± 1.55 mg/g wet tissue) while VEGF, TNF-α, and IL-1β were increased to 165.4 ± 5.56, 6537 ± 911, and 537.5 ± 31.6 pg/mg protein, respectively (13.0 to 936.5% increase, $P < 0.05$ to $P < 0.001$), compared with CMC-NS-treated rats (Table 2). PGE, OMZ, SCF, and PTX showed little or no change in their protein content compared with control EtOH group. PGE, OMZ, SCF, and PTX showed significant decrease in both TNF-α and IL-1β levels (21.5-87.6% decrease, $P < 0.05$ to $P < 0.001$) compared with control EtOH group. VEGF tended to decrease in PGE, OMZ, SCF, and PTX (6.6-9.4% decrease, $P < 0.1$ to $P < 0.2$) compared with control EtOH group (Table 2).

DISCUSSION

PGE was earlier found to protect the incidence and severity of GU induced by both physical (CRS and pylorus ligation) and chemical agents (Aspirin & EtOH) and healed the chronic ulcer induced by acetic acid, predominantly by strengthening of mucosal defense and antioxidant status.[10] As cytokines, MPO and growth factors were reported to play an important role in ulceration and healing, so their roles were studied in the ulcer protective and healing effects of PGE using CRS and EtOH rat GU models.

MPO activity is closely associated with neutrophil-dependent inflammatory response in experimental ulcer and invasion of gastric tissues by neutrophils, marked with increased MPO activity, contributes to gastric mucosal damage and was found to increase concomitantly with the gastric lesion.[18] The result of the present study indicated an increase in gastric mucosal MPO in CRS rats indicating mucosal inflammation

Table 1: Effects of PGE, OMZ, and SCF on UI, mucosal protein, and MPO in CRS-GU rat

Parameters (mg/kg, once daily×7 days)	UI	Protein (mg/g wet tissue)	MPO (mU/mg protein)
CMC 0.5%+URS	0.0±0.0	58.9±2.61	11.3±0.52
CMC 0.5%+CRS	25.8±2.97^{c}	60.3±2.56	58.7±4.49^{c}
PGE 100+CRS	13.9±2.01^{x}	61.3±2.17	28.7±2.07^{z}
OMZ 2+CRS	8.93±1.67^{y}	64.7±2.73	35.9±3.03^{y}
SCF 500+CRS	10.1±2.03^{y}	59.6±2.81	33.2±3.89^{y}

Values are mean±SEM of 6 animals in each group. $^{c}P<0.001$ compared with CMC+URS group and $^{x}P<0.05$, $^{y}P<0.01$ and $^{z}P<0.001$ compared with CMC+CRS group. SEM: Standard deviation, PGE: *Punica granatum* peel extract, OMZ: Omeprazole, SCF: Sucralfate, CMC: Carboxymethyl cellulose, URS: Unrestraint stress, UI: Ulcer index, CRS: Cold-restraint stress, MPO: Myeloperoxidase, GU: Gastric ulcer

Table 2: Effects of PGE, OMZ, SCF, and PTX on UI and gastric mucosal protein, VEGF, TNF-α, and IL-1β in rat EtOH-induced GU

Parameters (mg/kg, od×7 days)	UI	Protein (mg/g tissue)	TNF-α (pg/mg protein)	IL-1β (pg/mg protein)	VEGF (pg/mg protein)
CMC 0.5%+NS	0.0±0.0	54.5±1.36	630.7±61.7	391.9±38.7	146.4±3.30
CMC 0.5%+EtOH	22.3±3.89^{c}	58.0±1.55	6537±911^{c}	537.5±31.6^{a}	165.4±5.56^{a}
PGE 100+EtOH	6.93±1.79^{y}	61.8±2.70	1446±394^{y}	404.2±25.7^{x}	153.3±5.02
OMZ 2+EtOH	6.23±1.81^{y}	56.2±4.36	1456±397^{y}	422.1±242^{x}	154.7±4.76
SCF 500+EtOH	4.16±1.57^{y}	54.6±3.57	1129±376^{y}	414.2±26.3^{x}	153.7±3.81
PTX 10+EtOH	3.82±0.93^{z}	53.7±2.19	808±216^{z}	373.9±17.3^{y}	149.9±3.93

Values are mean±SEM of 6 animals in each group. $^{a}P<0.05$ and $^{c}P<0.001$ compared with respective carboxymethyl cellulose (CMC)+NS group and $^{x}P<0.05$, $^{y}P<0.01$ and $^{z}P<0.001$ compared with respective CMC+EtOH group. SEM: Standard deviation, PGE: *Punica granatum* peel extract, OMZ: Omeprazole, SCF: Sucralfate, CMC: Carboxymethyl cellulose, URS: Unrestraint stress, UI: Ulcer index, CRS: Cold-restraint stress, MPO: Myeloperoxidase, GU: Gastric ulcer, NS: Normal saline, PTX: Pentoxifylline, EtOH: Ethanol, TNF-α: Tumor necrosis factor-alpha, IL-1β: Interleukin-1 beta, VEGF: Vascular endothelial growth factor

and subsequent damage compared with URS rats. PGE-, OMZ-, and SCF-treated CRS rats showed a decrease in MPO which could be due to decrease in mucosal damage and reduction in inflammation as evidenced by decrease in UI. Our study does indicate the protective effects of PGE which could be mediated through decrease in MPO-induced oxidative stress and gastric mucosal inflammation and damage.

Oxidative stress has been implicated in the development of EtOH-induced gastric injury where reactive oxygen species generated by activated leukocytes triggers mucosal damage through lipid peroxidation and depletion of antioxidant defenses such as reduced glutathione, catalase, and total antioxidant capacity[19] In addition, depletion of mucosal cytoprotective moieties, including PGE_2 and glycoproteins, has been linked to EtOH consumption.[19,20] Although the mechanisms underlying EtOH-induced GU have not been fully elucidated yet, mounting evidence have indicated that pro-inflammatory cytokines, oxidative stress, and apoptosis play important roles in its pathogenesis.[21] Activation of neutrophils is associated with an up-regulated inflammatory response with increased gastric expression of nuclear factor kappa B (NF-kB), which controls the generation of pro-inflammatory cytokines including TNF-α and IL-1β. These events amplify the inflammatory cascade through triggering the release of other pro-inflammatory mediators and enhancing further recruitment of macrophages and neutrophils, thereby exacerbating the gastric damage.[22] Pro-inflammatory cytokines, TNF-α, and IL-1β are thus involved in the induction of inflammation and injury the gastric mucosa with sustained inflammatory reaction delaying healing process at the ulcer area. Rather TNF-α stimulates caspase-3 in epithelial and endothelial cells of gastric mucosa and thus contributes to apoptosis and subsequent damage. PTX decreased the levels of TNF-α and IL-1β significantly in EtOH-induced ulcerated gastric mucosa, and the above observation is in confirmation with the reported work of other workers.[2,23,24] TNF-α is reported to stimulate the upregulation of IL-1β levels, so by controlling the TNF-α expression, the IL-1β expression can be controlled. This may be the mechanism by which PTX decreases both the cytokines levels. In the present study, we have observed increased levels of pro-inflammatory cytokines TNF-α and IL-1β in the gastric mucosa of EtOH-induced rats and treatments with PGE, OMZ, SCF, and PTX in EtOH-treated rats showed significant decrease in both TNF-α and IL-1β levels compared with control EtOH group indicating the role played by cytokines in ulceration and healing.

Angiogenesis is a prerequisite for the healing of EtOH-induced deep gastric mucosal damage. EtOH-induced injury

to the gastric mucosa activates VEGF gene expression as reflected by increases in VEGF at both the transcriptional and translational levels. Increased VEGF expression in the gastric mucosa in response to EtOH injury strongly suggests the importance of VEGF as a mediator of the angiogenesis crucial for the repair of gastric mucosal erosion[25] Treatment with PGE, OMZ, SCF, and PTX in EtOH-treated rats tended to decrease VEGF level in the gastric mucosa compared with EtOH group which may be consequence to decrease damage to the gastric mucosa leading to non-stimulation of VEGF synthesis. However, VEGF level in all the test drugs showed its level more than NS-treated group indicating that their effects on VEGF are not inhibitory.

Many chemical constituents were isolated from PGE by different workers and found a higher amount of phenolic compounds than in the fruit pulp.[26] These compounds include flavonoids (anthocyanins, catechins, cyanidin, kaempferol, and other complex flavonoids) and hydrolyzable tannins such as punicalin, pedunculagin, punicalagin, gallic, and ellagic acid[27] Punicalagin and ellagic acid, the main bioactive constituents in the pomegranate husk, have shown antioxidant, antiproliferative, and apoptotic activities.[28,29] Successful *in vitro* and *in vivo* assays indicated that pomegranate peel extract and hydrolysable tannins, in the form of standardized active components, are very effective treatment measure against various inflammatory disorders.[27] The antioxidant and anti-inflammatory properties of PGE may be due to polyphenols (tannins and flavonoids), and this could be helpful in protecting gastric mucosa to ulceration.

CONCLUSION

We earlier reported PGE having significant GU protective and healing activities through an increase in gastric juice mucoproteins and mucosal glycoproteins and decrease in oxidative stress-induced mucosal damage. At present, PGE was found to decrease the pro-inflammatory cytokines, TNF-α and IL-1β, and MPO and mucosal damage in rats. VEGF was found to increase in EtOH-induced injury, a consequent induction of VEGF synthesis to acute damage and decrease in VEGF level with PGE, OMZ, and SCF and PTX indicated prevention of tissue damage and consequent decrease in VEGF induction. Further, in depth studies are required to confirm the above hypothesis by doing more study at the cellular and biochemical levels.

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REFERENCES

1. Mohan Kumar M, Joshi MC, Prabha T, Dorababu M, Goel RK. Effect of plantain banana on gastric ulceration in NIDDM rats: Role of gastric mucosal glycoproteins, cell proliferation, antioxidants and free radicals. Indian J Exp Biol. 2006;44(4):292-9.
2. Kumar M, Gautam MK, Singh A, Goel RK. Healing effects of *Musa sapientum* var. *Paradisiaca* in diabetic rats with co-occurring gastric ulcer: Cytokines and growth factor by PCR amplification. BMC Complement Altern Med. 2013;13:305.
3. Qui BS, Mei QB, Liu L, Tchou-Wong KM. Effects of nitric oxide on gastric ulceration induced by nicotine and cold-restraint stress. World J Gastroenterol. 2004;10(4):594-7.
4. Amirshahrokhi K, Khalili AR. The effect of thalidomide on ethanol-induced gastric mucosal damage in mice: Involvement of inflammatory cytokines and nitric oxide. Chem Biol Interact. 2015;225:63-9.
5. Tarnawski AS. Cellular and molecular mechanisms of gastrointestinal ulcer healing. Dig Dis Sci. 2005;50 Suppl 1:S24-33.
6. Kumar MM, Singh A, Gautam MK, Goel RK. Role of cytokines and growth factor in gastric ulcer healing effects of unripe plantain banana (*Musa sapientum* var. *Paradisiaca*). In:, editors. Translational Research in New Drug Development. 1st ed., Vol. 1. New Delhi, India: Vidyanilayam Prakashan; 2012. p. 269-84.
7. Bhandari PR. Pomegranate (*Punica granatum* L). Ancient seeds for modern cure? Review of potential therapeutic applications. Int J Nutr Pharmacol Neurol Dis. 2012;2:171-84.
8. Alam MS, Alam MA, Ahmad S, Najmi AK, Asif M, Jahangir T. Protective effects of *Punica granatum* in experimentally-induced gastric ulcers. Toxicol Mech Methods. 2010;20(9):572-8.
9. Gautam R, Sharma SC. Anti-ulcer activity of *Punica granatum* Linn in diabetic rats. Int J Pharm Pharm Sci. 2012;4(3):S459-61.
10. Chauhan I, Sharma A, Gangwar M, Gautam MK, Singh A, Goel RK. Gastric antiulcer and ulcer healing effects of *Punica granatum* L. Peel extract in rats: Role of offensive and defensive mucosal factors and oxidative stress. Int J Pharm Pharm Sci. 2016. In Press.
11. Dorababu M, Prabha T, Priyambada S, Agrawal VK, Aryya NC, Goel RK. Effect of *Bacopa monniera* and *Azadirachta indica* on gastric ulceration and healing in experimental NIDDM rats. Indian J Exp Biol. 2004;42(4):389-97.
12. Sanyal AK, Pandey BL, Goel RK. The effect of a traditional preparation of copper, tamrabhasma, on experimental ulcers and gastric secretion. J Ethnopharmacol. 1982;5(1):79-89.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75.
14. Bradley PP, Christensen RD, Rothstein G. Cellular and extracellular myeloperoxidase in pyogenic inflammation. Blood. 1982;60(3):618-22.
15. Brouckaert P, Libert C, Everaerdt B, Takahashi N, Cauwels A, Fiers W. Tumor necrosis factor, its receptors and the connection with interleukin 1 and interleukin 6. Immunobiology. 1993;187(3-5):317-29.
16. Dinarello CA. Interleukin-1 and interleukin-1 antagonism. Blood. 1991;77(8):1627-52.

17. Kim KJ, Li B, Houck K, Winer J, Ferrara N. The vascular endothelial growth factor proteins: Identification of biologically relevant regions by neutralizing monoclonal antibodies. Growth Factors. 1992;7(1):53-64.
18. Selvamathy SM, Geetha A, Saranya P. Gastroprotective effect of *Swertia chirayita*-a study with ulcer induced rats. Pharmacol Online. 2010;1:332-55.
19. Liu Y, Tian X, Gou L, Fu X, Li S, Lan N, et al. Protective effect of l-citrulline against ethanol-induced gastric ulcer in rats. Environ Toxicol Pharmacol. 2012;34(2):280-7.
20. Golbabapour S, Gwaram NS, Hassandarvish P, Hajrezaie M, Kamalidehghan B, Abdulla MA, et al. Gastroprotection studies of Schiff base zinc (II) derivative complex against acute superficial hemorrhagic mucosal lesions in rats. PLoS One. 2013;8(9):e75036.
21. Arab HH, Salama SA, Omar HA, Arafa ES, Maghrabi IA. Diosmin protects against ethanol-induced gastric injury in rats: Novel anti ulcer actions. PLoS One. 2015;10(3):e0122417.
22. Sangiovanni E, Vrhovsek U, Rossoni G, Colombo E, Brunelli C, Brembati L, et al. Ellagitannins from *Rubus berries* for the control of gastric inflammation: *In vitro* and *in vivo* studies. PLoS One. 2013;8(8):e71762.
23. Ohba R, Otaka M, Odashima M, Jin M, Komatsu K, Konishi N, et al. Effect of cilostazol, a selective Type-III phosphodiesterase inhibitor, on water-immersion stress-induced gastric mucosal injury in rats. J Gastroenterol. 2006;41(1):34-40.
24. Brzozowska I, Targosz A, Sliwowski Z, Kwiecien S, Drozdowicz D, Pajdo R, et al. Healing of chronic gastric ulcers in diabetic rats treated with native aspirin, nitric oxide (NO)-derivative of aspirin and cyclooxygenase (COX)-2 inhibitor. J Pharm Pharmacol. 2004;55:773-90.
25. Jones MK, Itani RM, Wang H, Tomikawa M, Sarfeh IJ, Szabo S, et al. Activation of VEGF and ras genes in gastric mucosa during angiogenic response to ethanol injury. Am J Physiol. 1999;276:G1345-55.
26. Li Z, Wang C, Jiao X, Lu Y, Fu M, Quong AA, et al. Cyclin D1 regulates cellular migration through the inhibition of thrombospondin 1 and ROCK signaling. Mol Cell Biol. 2006;26(11):4240-56.
27. Ismail T, Sestili P, Akhtar S. Pomegranate peel and fruit extracts: A review of potential anti-inflammatory and anti-infective effects. J Ethnopharmacol. 2012;143(2): 397-405.
28. Lu J, Wei Y, Yuan Q. Preparative separation of punicalagin from pomegranate husk by high-speed countercurrent chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2007;857(1):175-9.
29. Madrigal-Carballo S, Rodriguez G, Krueger CG, Dreher M, Reed JD. Pomegranate (*Punica granatum*) supplements: Authenticity, antioxidant and polyphenol composition. J Funct Foods. 2009;1(3):324-9.

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