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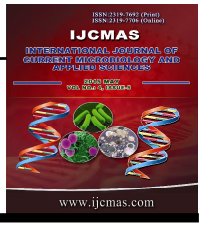

Original Research Article

Gastroprotective and Antioxidant Potential of Montelukast against Acetyl Salicylic Acid Induced Gastric Ulcer Model in Male Rabbits

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Keywords

Gastric Ulcer, Oxidative Stress, Montelukast, Antioxidant Enzymes

A B S T R A C T

This study was aimed at investigation the gastroprotective effects of montelukast against acetylsalicylic acid -induced gastric mucosal damage. Thirty local domestic male rabbits had been used in this study, divided into 5 groups as follows: control group, acetylsalicylic acid (ASA) group, Omeprazole pretreated group, montelukast pretreated group and montelukast alone treated group. At the end of the experiment, the stomach of rabbits were removed and the tissue homogenate was prepared.The results revealed that after the administration of ASA significantly increase the MDA concentration and XO activity along with decrease in SOD, GPX and GST activities. But in presence of montelukast there is a significant decreased in oxidant parameter (MDA,XO) along with increase in antioxidant enzyme activity (SOD, GPX, GST). It was concluded that the free radicals and decreased activity of antioxidant enzymes play an important roles in gastric damage induced by ASA. Increased xanthine oxidase activity plays a major role in free radicals formation. Montelukast protects against ASA-induced gastric damage and this can be attributed to its ameliorating effect on oxidative damage

Introduction

Gastric ulcer is an erosion of the gastric mucosal layer or excavation of the surface of gastric tissue as a result of the sloughing of inflammatory necrotic tissue (Ejam *et al.*, 2015). Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs worldwide. NSAID's are known as one of the most common pathogenic factors associated with gastric mucosal damage (Konturek, 2003).

Acetylsalicylic acid (Aspirin, ASA), is one of the NASID which is widely used for the treatment of rheumatoid arthritis and related diseases as well as the prevention of cardiovascular thrombotic diseases (Heibashy *et al.*, 2014).

It damages gastrointestinal mucosa by their suppression of prostaglandin synthesis through cyclooxygenase (COX) inhibition,

and direct topical damage by a non-prostaglandin mediated mechanism; however, the exact pathogenic mechanism remains to be elucidated (Wallace, 2001).

Oxidative stress is defined as tissue damage resulting from imbalance between the production of reactive oxygen species (ROS) and antioxidant defense mechanisms (Betteridge, 2000). Oxidative stress has been reported to play a part in the pathogenesis of various diseases including gastric ulcer (Al Rashdi *et al.*, 2012). Lipid peroxidation as expressed by Malondialdehyde (MDA) mediated by oxygen free radicals is believed to be an important cause of destruction to cell membranes (Arulkumaran *et al.*, 2007).

Antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase are present in normal healthy cells which are the first line of cellular defense against oxidative injury decomposing $O_2^{-\bullet}$ and H_2O_2 before they interact to form more reactive radicals (Amin, 2008). Montelukast is an anti-inflammatory agent most commonly used in the treatment of allergic rhinitis and asthma (Benninger and Waters, 2009). It is a 5-LOX inhibitor as well as a reversible cysteinyl leukotriene D4–receptor antagonist. Montelukast was reported to have beneficial effects in management of experimental gastric mucosal damage (Dengiz *et al.*, 2007). This study is aimed at investigating the effect of montelukast on some oxidant and antioxidant parameters in stomach tissue of rabbits with gastric ulcer and its role in gastroprotection.

Materials and Methods

Preparation of acetylsalicylic acid dosage form

Acetylsalicylic acid powder (schuchardt Company, Germany) was suspended in 5 ml distilled water and given orally through stomach tube in a dose of 500 mg /kg.

Preparation of omeprazole dosage form

Omeprazole 40 mg vial (Cipla, india) mixed with 5 ml distilled water and given (i.p) in a dose of 20 mg / kg.

Preparation of montelukast dosage form

Montelukast (MSD, united kingdom) was suspended in 5 ml distilled water; ten tablets of this drug were crushed and suspended in distilled water to prepare a fresh solution immediately before administration, and used in a dose of 20 mg /kg administered orally through a stomach tube.

Experimental animals

Thirty local domestic male rabbits had been used in this study. Their body weights ranged from 1.5 to 2.5 kg. They were housed in the animal house in College of Medicine / University of Babylon under controlled condition of temperature 25+4°C. They were fed with standard chow diet and they had free access to drink water.

Experimental protocol

After two weeks of adaptation period, the animals were randomly separated into 5 groups (6 rabbits in each group) and treated as follows:

Group 1 (**Normal control group):** all rabbits in this group were received distilled water (DW) 5 ml orally through stomach tube during an experimental period.

Group 2 (**Active control group**): all rabbits in this group were received acetylsalicylic acid (500 mg/kg b.w.) orally through stomach tube as single dose**.**

Group 3 (Omeprazole pretreated group): all rabbits in this group were received omeprazole (20 mg/kg b.w.) intraperitoneally (i.p) one hour before acetylsalicylic acid administration.

Group 4 (**Montelukast pretreated group**): all rabbits in this group were received montelukast (20 mg/kg b.w.) orally through stomach tube one hour before acetylsalicylic acid administration.

Group 5 (Montelukast alone treated group): all rabbits in this group were received montelukast (20 mg/kg b.w) orally through stomach tube one hour before administration of 5 ml DW.

The omeprazole and montelukast were continually given once daily for three days. After one hour of the last dose (3rd day) of thirty six hours fasted animals, ASA was administered orally to the animals (except normal control group and montelukast alone treated group) in a dose of (500 mg / kg b.w.), then all the animals were sacrificed five hour later. All experiments were performed during the same time of the day to avoid diurnal variations of putative regulators of gastric functions.

Collection and preparation of sample

At the end of the experiment, the animals were sacrificed by an overdose of chloroform vapors and the stomach was separated from the surrounding viscera then washed with physiological saline solution pH 7.4.

Gastric tissues were homogenized in cold phosphate buffer saline pH 7.4 to obtain 10% of tissue homogenate (using a glass-teflon homogenizing tube) at 6000 rpm for 10 minutes.

Measurement of different parameters

Gastric tissue malondialdehyde concentration: The measurement has been done according to method illustrated by Carl and Edward, (1999).

Gastric tissue xanthine oxidase activity: The measurement has been done according to method illustrated by Ackermann and Brill, (1974).

Gastric tissue superoxide dismutase activity: The measurement has been done according to method illustrated by Marklund and Marklund (1974).

Gastric tissue glutathione peroxidase activity: The measurement has been done according to method illustrated by Rotruck *et al.* (1973).

Gastric tissue glutathione-S-transferase activity: The activity of GST was determined spectrophotometrically by the method of Habig *et al.* (1974).

Statistical analysis of data

The results were expressed as mean ± standard deviation (mean ± SD). Statistical analysis was carried out using one way ANOVA followed by least significance difference (L.S.D.) test for multiple comparisons between groups by using the 19 edition of SPSS program. A value of $p < 0.05$ was considered to indicate a significant difference between groups.

Results and Discussion

The administration of ASA significantly increase the gastric tissue MDA concentration and XO activity ($P< 0.05$) (4.3 $\pm$ 0.71 µmol/l) (127.03 $\pm$ 12.19 U/L) respectively when compared with the

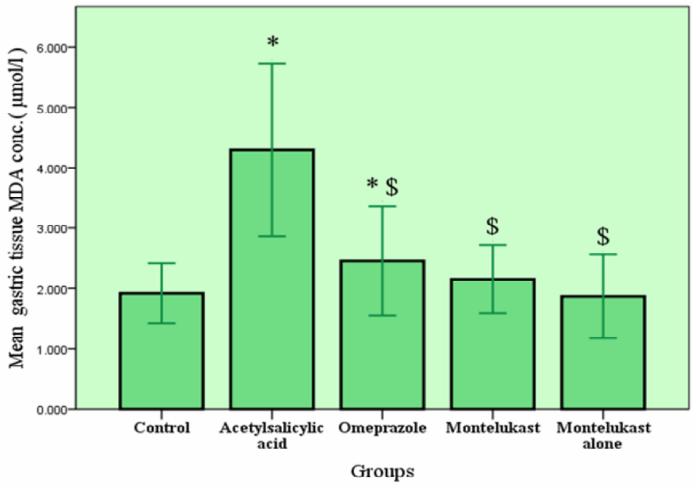
normal control group (1.92 ± 0.25 µmol/l) (110.25 ± 8.83 U/L) respectively, (Figure 1, 2). From the results of present study, increased gastric level of MDA in ASA treated group (Figure 1), indicating that lipid peroxidation plays an important part in the pathogenesis of gastric mucosal damage produced by ASA and these result was in accordance with those obtained by Aslam *et al.*(2013) and Mitra *et al.* (2014). Increased gastric level of MDA is possibly due to the generation of free radicals via auto-oxidation or through metal ion or superoxide catalyzed oxidation process and XO activity (Das and Roy, 2012). Xanthine oxidase (XO) is a major source of ROS generation that play an important role in the pathogenesis of peptic ulcer disease (Hajdu, *et al.,* 2014). In the present study, significant increase in XO activity of ASA treated group compared to normal control group may be indicate that there is a correlation between gastric ulcer and XO activity thus the measurement of xanthine oxidase activity may be used as a biochemical marker of acute gastric mucosal damage along with MDA concentration. This result was in line with Jainu *et al.* (2006).

The administration of ASA showed a significant decrease in gastric tissue SOD, GPX, GST activity ($P< 0.05$) (17.3±1.41 U/ml, 98.3 ± 16.21 U/L, 8.13 ± 0.88 U/L) respectively when compared with the normal control group (22.25 ± 1.25 U/ml, 197.5 ± 19.84 U/L, 10.48 ± 0.96 U/L) (Figure 3, 4 & 5). From the results of present study, activities of all free radical scavenging enzymes (SOD, GPX, GST) were found decreased by ASA. These findings were in consistence with several studies, which reported reduction in antioxidant enzyme activities in ASA treated groups (Pohle *et al.*, 2001; Jainu and Devi, 2004; Panda and Sonkamble, 2012 and Mitra *et al.,* 2014). The increased lipid peroxidation as expressed by increased level of MDA lead to inactivation of these enzymes by crosses linking with MDA; this will lead to accumulation of superoxide, H_2O_2 and hydroxyl radicals and consequently to an increase in lipid peroxidation (Nartey *et al.,* 2012). Also the decreased activities of these antioxidant enzyme may be due to rapid consumption and exhaustion of storage of this enzyme in catalyzing the overproduction of free radicals due to exposure to oxidative stress (Kamel *et al.,* 2014). Therefore, the greater the production of free radical, the greater the consumption of these antioxidant enzyme. In the same line (Panda *et al.,* 2012); Hussein *et al.* (2014) reported that SOD activity decreased significantly in the ethanol treated group of animals, which might be due to an excessive formation of superoxide anions.

The administration of omeprazole showed a significant decrease in gastric tissue MDA concentration ($P< 0.05$) (2.45 ± 0.45 µmol/l), (figure 1) and no significant decrease in gastric tissue XO activity ($P> 0.05$) (117.62 ± 11.66 U/L), (figure 2). In addition, the administration of omeprazole showed a significant increase in gastric tissue SOD, GPX, GST activity ($P< 0.05$) (21.89±1.01 U/ml, 193.3 ± 13.38 U/L, 12.28 ± 0.99 U/L) respectively when compared with the acetylsalicylic acid treated group (Figures 3, 4 and 5). The reduction in MDA levels after administration of omeprazole along with significant increased antioxidant enzyme activities strongly may be due to decreased lipid peroxidation and antioxidant activity of omeprazole. These results are in agreement with previous report that demonstrated a significant reduction in gastric tissue MDA level and increase in SOD activity in omeprazole pretreated group (Rahim *et al.,* 2014). Various studies has been confirmed the antioxidant gastroprotective properties of omeprazole independent of its proton-pump inhibitory potential but attributed to a decrease in

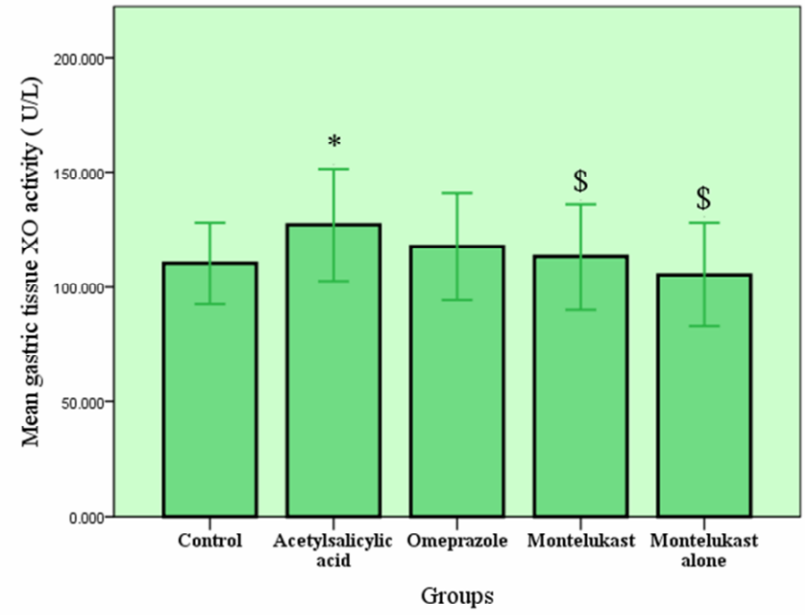
oxidative stress and an increase in antioxidants status (Abdul-Aziz, 2011; Morjan *et al.*, 2013 and Ittiyavirah and Shenika, 2014).

Figure.1 Effect of Treatments on MDA Concentration



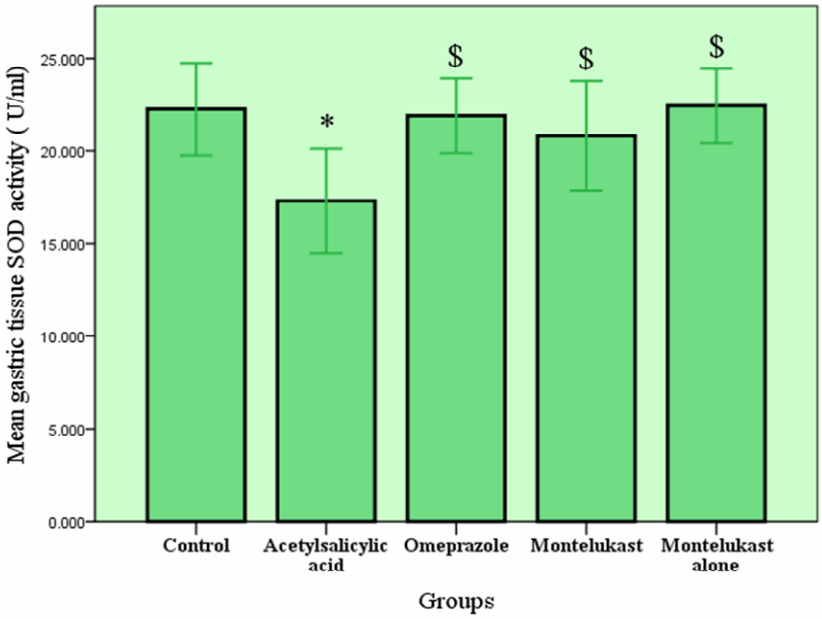
*: Different Significantly (P<0.05) from normal control group.
$: Different Significantly (P<0.05) from acetylsalicylic acid group.

Figure.2 Effect of Treatments on XO Activity



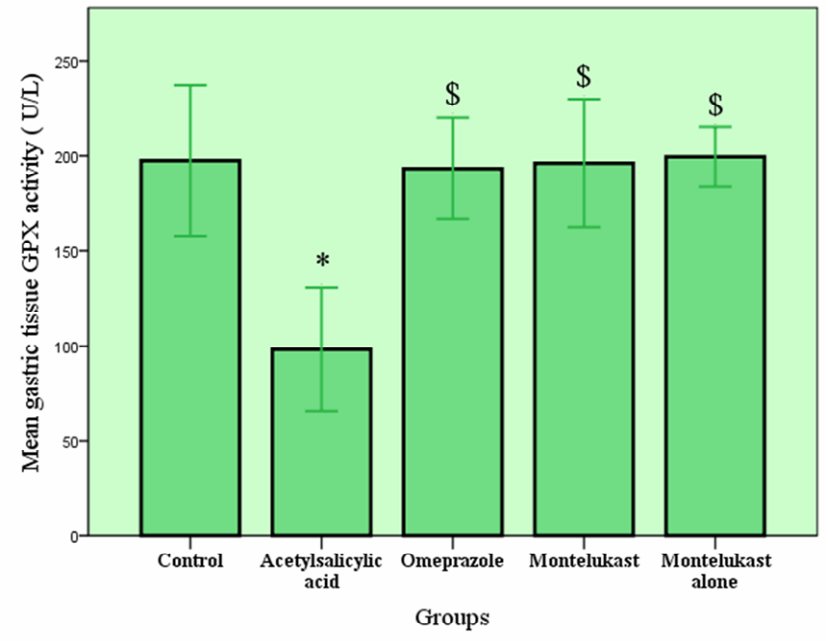
*: Different Significantly (P<0.05) from normal control group.
$: Different Significantly (P<0.05) from acetylsalicylic acid group.

Figure.3 Effect of Treatments on SOD Activity



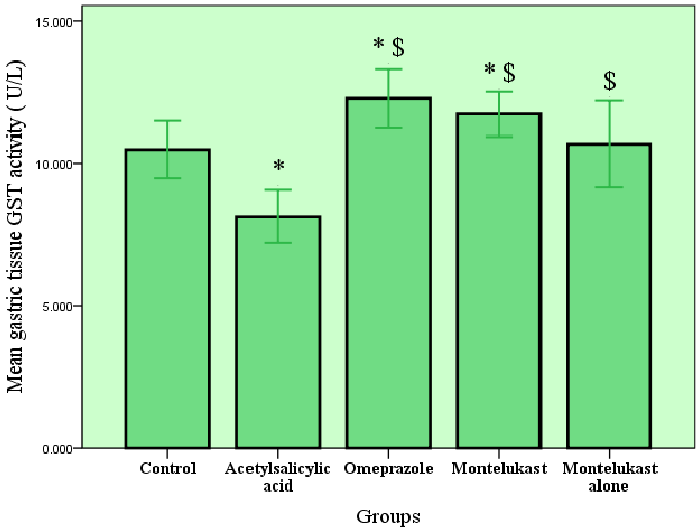
*: Different Significantly (P<0.05) from normal control group.
$: Different Significantly (P<0.05) from acetylsalicylic acid group.

Figure.4 Effect of Treatments on GPX Activity



*: Different Significantly (P<0.05) from normal control group.
$: Different Significantly (P<0.05) from acetylsalicylic acid group.

Figure.5 Effect of Treatments on GST activity



*: Different Significantly (P<0.05) from normal control group.
$: Different Significantly (P<0.05) from acetylsalicylic acid group.

The administration of montelukast significantly decreased gastric tissue MDA concentration and XO activity (P< 0.05) (2.15 ± 0.28 µmol/l) (113.13 ± 11.53 U/L) respectively, (Figures 1 and 2). In addition, the administration of montelukast showed a significant increase in gastric tissue SOD, GPX, GST activity (P< 0.05) (20.81±1.48 U/ml, 196.2 ± 16.88 U/L, 11.73 ± 0.76 U/L) respectively when compared with the acetylsalicylic acid treated group (Figure 3, 4 and 5). In this study, montelukast significantly reduce the gastric tissue MDA concentration and significantly elevates the SOD, GPX and GST activities as compared with ASA group. This can be attributed to improvement in the antioxidant status due to scavenging of free radicals (Kose *et al.*, 2012). This can provide a further support to the gastroprotective effect of montelukast. The significant decrease in gastric tissue MDA level was in agreement with other reports showing that montelukast could reduce MDA levels as antioxidant (Coskun *et al.*, 2011; Ocak *et al.*, 2014). From these observations believe that montelukast may exert its protective effect against ASA through inhibition of lipid peroxidation. Montelukast was found to increase the activities of antioxidant enzymes against gastric mucosal damage induced by several ways suggesting that scavenging of ROS may be due to its effective antioxidant activity, consequently resulting in reduced oxidative stress (Dengiz *et al.*, 2007; Abd-Allah and El-Debakey, 2009).

On the basis of the present results, it can be concluded that: Acetylsalicylic acid in dose of 500 mg/kg successfully induced ulcers in stomach. Oxidative stress plays an important role in ASA -induced gastric ulcer model. The gastroprotective effect of montelukast can be attributed to its ability to balance oxidant - antioxidant status.

References

Abd-Allah, O.M., El-Debakey, F. 2009. Prophylactic administration of Montelukast ameliorates the deleterious effect of dexamethasone on stress-induced gastric lesions in

rats. *Med. J. Cairo Univ.*, 77(4): 261–270.

Abdul-Aziz, K.K. 2011. Comparative evaluation of the anti-ulcer activity of curcumin and omeprazole during the acute phase of gastric ulcer. *FNS*, 2: 628–40.

Ackermann, E., Brill, A.S. 1974. Xanthine oxidase activity. In: Bergameyer H.U. (Ed). Method of enzymatic analysis, 2 nd edn. Academic Press Inc., USA. Pp. 521.

AlRashdi, A.S., Salama, S.M., Alkiyumi, S.S., Abdulla, M.A., Hadi, A.H., Abdelwahab, S.I., *et al.* 2012. Mechanisms of gastroprotective effects of ethanolic leaf extract of *Jasminum sambac* against HCl/Ethanol-induced gastricmucosal injury in rats. *Evid. Based Complement Alternat. Med.*, 2012: 1–15.

Amin, A. 2008. Chemopreventive effect of chlorella on the antioxidant system in DMBA-induced oxidative stress in liver. *Int. J. Pharmacol.*, 4(3): 169–76.

Arulkumaran, S., Ramprasath, V.R., Shanthi, P., Sachdanandam, P. 2007. Alteration of DMBA-induced oxidative stress by additive action of a modified indigenous preparation—Kalpaamruthaa. *Chem. Biol. Interact.*, 167(2): 99–106.

Aslam, B., Majeed, W., Javed, I., Muhammad, F., Khaliq, T., Ali, J. 2013. Gastroprotective effect of *Berberis vulgaris* (ZERESHK) seeds against gastric ulcer induced by aspirin in male adult albino mice. *Indo Am. J. Pharm. Res.*, 3(6): 4518–27.

Benninger, M.S., Waters, H. 2009. Montelukast: Pharmacology, Safety, Tolerability and Efficacy. *Clin. Med. Ther.*, 1: 1253–61.

Betteridge, D.J. 2000. What is oxidative stress? *Metabolism*, 49(2): 3–8.

Carl, A., Edward, R. 1999. Tietz text book of clinical Biochemistery , 3rd edn. Pp. 819–861, 1034–1054.

Coskun, A.K., Yigiter, M., Oral, A., Odabasoglu, F., Halici, Z., Mentes, O. 2011. The effects of montelukast on antioxidant enzymes and proinflammatory cytokines on the heart, liver, lungs, and kidneys in a rat model of cecal ligation and puncture–induced sepsis. *Sci. World J.*, 11: 1341–56.

Das, S.K., Roy, C. 2012. The protective role of *Aegle marmelos* on aspirin-induced gastro-duodenal ulceration in albino rat model: A possible involvement of antioxidants. *Saudi. J. Gastroenterol.*, 18(3): 188–194.

Dengiz, G.O., Odabasoglu, F., Halici, Z., Cadirci, E., Suleyman, H. 2007. Gastroprotective and antioxidant effects of montelukast on indomethacin-induced gastric ulcer in rats. *J. Pharmacol. Sci.*, 105: 94–102.

Ejam, A.H, Albayati, N.J., Ewadh, M.J., Al-Kaffaji, H.A. 2015. Histopathological assessment of antiulcerogenic effect of montelukast against acetylsalicylic acid induced gastric ulcer in male rabbit. *Med. J. Babylon*, 12 (2).

Habig, W.H., Pabst, M.J., Jakoby, W.B. 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, 249(22): 7130–9.

Hajdu, Z., Martins, A., Orbán-Gyapai, O., Forgo, P., Jedlinszki, N., Imre Máthé, I., *et al.* 2014. Xanthine oxidase-inhibitory activity and antioxidant properties of the methanol extract and flavonoids of

Artemisia asiatica. *Rec. Nat. Prod.*, 8(3): 299–302.

Heibashy, M.I., Mazen, G.M., Ibrahim, M.A. 2014. Efficacy and Safety of some Medical Herbs on Gastric Ulcer Induced by Aspirin in Rats. *JPBS*, 9(3): 19–27.

Hussein, S.A., El-Senosy, Y.A., Hassan, M.F. 2014. Gastro protective, antiapoptotic and anti-inflammatory effect of alpha-lipoic acid on ethanol induced gastric mucosal lesions in rats. *Am. J. Biochem. Mol. Biol.*, 4(2): 48–63.

Ittiyavirah, S.P., Shenika, M.S. 2014. Evaluation of antioxidant and anti-inflammatory activity of Omeprazole against experimentally induced colitis. *J. Sci. Innovat. Res.*, 3(3): 352–6.

Jainu, M., Devi, C.S. 2004. Antioxidant effect of methanolic extract of solanum nigrum berries on aspirin induced gastric mucosal injury. *Indian J. Clin. Biochem.*, 19(1): 57–61.

Jainu, M., Mohan, K.V., Devi, C.S. 2006. Protective effect of *Cissus quadrangularis* on neutrophil mediated tissue injury induced by aspirin in rats. *J. Ethnopharmacol.*, 104(3): 302–5.

Kamel, M.A., Hamza, R.Z., Abdel-Hamid, N.E., Mahmoud, F.A. 2014. Anti-ulcer and gastro protective effects of fenugreek, ginger and peppermint oils in experimentally induced gastric ulcer in rats. *J. Chem. Pharm. Res.*, 6(2): 451–68.

Konturek, J.W. 2003. Discovery by Jaworski of *HELICOBACTER PYLORI* and its pathogenetic role in peptic ulcer, gastritis and gastric cancer. *J. Physiol. Pharmacol.*, 54(3): 23–41.

Kose, E., Sapmaz, H.I., Sarihan, E., Vardi, N., Turkoz, Y., Ekinci, N. 2012. Beneficial effects of montelukast against methotrexate-induced liver toxicity: a biochemical and histological study. *Sci. World J.*, 2012: 1–6.

Marklund, S., Marklund, G. 1974. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469–74.

Mitra, P., Ghosh, T., Mitra, P.K. 2014. Anti gastric ulcer activity of *Amaranthus spinosus* Linn. leaves in aspirin induced gastric ulcer in rats and the underlying mechanism. *Sikkim Manipal Univ. Med. J.*, 1(2): 313–28.

Morjan, S., Al Laham, S., Atieh, R. 2013. Gastroprotective efficacy of folic acid and omeprazole in indomethacin-induced gastropathy in rats. *Int. J. Pharmacognosy Phytochem. Res.*, 5(2): 113–19.

Nartey, E.T., Ofosuhene, M., Agbale, C.M. 2012. Anti-ulcerogenic activity of the root bark extract of the African laburnum "*Cassia sieberiana*" and its effect on the anti-oxidant defence system in rats. *BMC Complement. Altern. Med.*, 12: 1–9.

Ocak, T., Duran, A., Özyalvacli, G., Ocak, Z., Terzi, E.H., Tosun, M. 2014. Protective effects of montelukast and *Hypericum perforatum* against intestinal ischemia-reperfusion injury in hamsters. *Turk. J. Med. Sci.*, 44: 381–6.

Panda, V., Ashar, H., Srinath, S. 2012. Antioxidant and hepatoprotective effect of *Garcinia indica* fruit rind in ethanol induced hepatic damage in rodents. *Interdiscip. Toxicol.*, 5(4): 207–13.

Panda, V., Sonkamble, M. 2012. Anti-ulcer activity of *Ipomoea batatas* tubers (sweet potato). *Funct. Foods Health Dis.,* 2(3): 48–61.

Pohle, T., Brzozowskia, T., Becker, J.C., Van der Voort, I.R., Markmann, A., Kontureka, S.J. 2001. Role of reactive oxygen metabolites in aspirin-induced gastric damage in humans: gastroprotection by vitamin C. *Aliment Pharmacol. Ther.,* 15: 677–87.

Rahim, N.A., Hassandarvish, P., Golbabapour, S., Ismail, S., Tayyab, S., Abdull, M.A. 2014. Gastroprotective effect of ethanolic extract of Curcuma xanthorrhiza leaf against ethanol-induced gastric mucosal lesions in Sprague-Dawley rats. *Biomed. Res. Int.,* 2014: 1–10.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., Hoekstra, W.G. 1973. Selenium: biochemical role as a component of glutathione peroxidase. *Science,* 179: 588–90.

Wallace, J.L. 2001. Pathogenesis of NSAID-induced gastroduodenal mucosal injury. *Best Pract. Res. Clin. Gastroenterol.,* 15(5): 691–703.