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Protective Effect of Ginger Extract against Aspirin Induced Acute Gastric, Renal and Hepatic Damage in Rats

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ABSTRACT

The present study aimed to investigate the role of ginger in the prevention of aspirin-induced gastric and hepato-renal injury. Forty rats were distributed into 4 groups: normal control, aspirin, ginger control, and ginger plus aspirin. The normal control group was gavaged with saline for 7 days. The second group similarly treated with control except it was administered about 150 mg/kg aspirin on the 7th day. The animal of third group was treated with ginger orally for 7 days. The forth group was similarly treated as the previous group except it was administrated aspirin on the 7th day. The results of the biochemical analysis reported that ginger supplement preserved liver and kidney function serum markers close to normal. Also, histopathological findings demonstrated that pretreatment with ginger reversed aspirin-induced gastric, renal and hepatic tissues damage. Additionally, assessment of oxidative stress in tissues homogenates revealed that pretreatment with ginger attenuates significantly ($p<0.001$) lipid peroxidation marker malondialdehyde (MDA) and preserved the anti-oxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in the three investigated organs. It could be concluded that ginger pretreatment attenuates the aspirin-induced gastric and hepato-renal lesions via inhibiting the resultant oxidative stress.

Introduction

Aspirin, also known as acetylsalicylic acid (ASA), is a salicylate medication, worldwide popular used painkiller, antipyretic and anti-inflammatory. Aspirin is part of a group of nonsteroidal anti-inflammatory drugs (NSAIDs). Unlike most of NSAIDS, Aspirin irreversibly inhibits the cyclooxygenase1 (COX-1) platelets variant more than the COX-2 inflammatory variant of the enzyme. Therefore, Aspirin is the "gold standard" long antiplatelet agent, at low daily doses, to help prevent heart attacks, strokes, and thrombi formation in people at high risk of developing blood clots [1]. However, the main side effects of aspirin are gastrointestinal ulcers and stomach bleeding particularly with higher doses. Though daily aspirin therapy can help prevent a clot-related stroke, the risk of a bleeding stroke is increased [2]. In the meantime, excessive administration of combined analgesics contained aspirin or other NSAIDs leads to kidney injury. The specific kidney injuries induced by analgesics are renal papillary necrosis and chronic interstitial nephritis [3].

Interestingly, NSAIDs, in addition to antimicrobial agents, are the most common causes of drug induced liver injury (DILI) [4-6]. Moreover, NSAIDs-induced acute combined hepato-nephrotoxicity has been reported [7, 8]. Accordingly, the adverse effect of aspirin and its related NSAIDs triggered the research for traditional medicinal plants.

Ginger (Zingiber officinale Roscoe, family: Zingiberaceae) originated in South-East Asia and is the most common spice, used all over the world [9]. The main constituents of ginger are zingerone, shogaols, and gingerols. Ginger pharmacological properties are varied including antioxidant [10], anti-inflammatory [11], anticancer [12], and antimicrobial activities. The current study was constructed to evaluate the comprehensive ameliorative effect of ginger against aspirin induced lesions on stomach, kidney and liver.

Materials and methods

Animals and Study design

Forty male Wistar albino rats (150-200 g) were obtained from experimental animal farm within Kafrelsheikh

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University. For 7 days of adaptation, all animals were fed on standard laboratory diet and water *ad libitum* and kept in cages at a temperature of 20 ºC with a 12-hour (h) dark/light cycle before and during experiments. This study was approved by Kaferelsheikh University, Faculty of Veterinary Medicine Ethical Committee.

After acclimatization, animals were divided into four groups, each of 10 animals, with different pre-treatments for 7 days, as follows: normal control group (C) and aspirin group (A) were left on normal water and food without any treatment. Ginger dried powder was purchased from local market of Herbs and Medicinal plants. Authentication of the plant was confirmed by staff members of Botany Department, Faculty of Science, Kafrelsheikh University, Kafr Elsheikh, Egypt. Ginger control group (G) and ginger pretreated group (A+G) were given ginger (100 mg/kg body weight daily) by gavages [13]. On day 7, 12 hours before aspirin administration (150 mg/kg) [13], rats were deprived of food and water while coprophagy was avoided but had free access to water 2 h before aspirin administration. Then, gastric, kidney and liver lesions were induced by administration of aspirin (150 mg/kg) [13] in the aspirin and ginger plus aspirin groups. Three hours after aspirin administration, all animals were anaesthetized by inhalation of ether. Blood samples were collected from the lateral canthus of the eye. Then, animals were sacrificed and stomach, kidney and liver were cleanly excised.

Serum samples

Serum samples were obtained and biochemical parameters were determined spectrophotometericaly using the available commercial kits based on the specific method for every parameters: AST and ALT according to Schumann and Klauke [14], creatinine according to Sabbagh *et al.,* [15], urea based on method of Tabacco *et al.,* [16], uric acid according to Fossoti *et al.,* [17].

Tissue samples

The stomach, liver as well as kidney were immediately excised, washed several times from blood by ice-cold isotonic saline. They were dissected from any adhering connective tissue weighed and shock-freeze in liquid nitrogen (-170°C) and stored at -80°C. The specimens were homogenized individually with tissue homogenizer to make 10% of homogenate. The homogenates were prepared for analysis by centrifugation at 18000 rpm (4°C) for 30 min and the supernatant was kept at -20°C for analysis of oxidative stress markers including Lipid Peroxidation (LP), Superoxide Dismutase (SOD) and catalase (CAT).

Analysis of oxidative stress

The amount of malondialdehyde (MDA) in tissue homogenate of stomach, liver and kidney as an indicator of LP was determined according to Ohkawa *et al.,* [18] based on the reaction with thiobarbituric acid. SOD activity was estimated as described by Marklund *et al.,* [19] based on inhibiting pyrogallol autoxidation by SOD. The activity of SOD in tissue is directly proportional to the inhibition rate. CAT was assayed based on Aebei's method [20].

Histopathology

Specimens from stomach, liver and kidney were collected from all experimental and control groups and fixed in 10% neutral buffered formalin, dehydrated in ascending concentrations of ethyl alcohol (70–100%) and then prepared using standard procedures for Hematoxylin and Eosin (H&A) stain as described by Bancroft *et la.,* [21].

Statistical analysis

Statistical analysis was done by Graphpad prism V5. Statistical analysis was performed using one-way analysis of variance (ANOVA) to compare the inter- and intra-group findings. The values depicting $p < 0.05$ were considered as statistically significant.

Results

Biochemical findings

The results presented in **Table (1)** showed that serum transaminases (ALT and AST) as well as kidney function biomarkers (creatinine, urea and uric acid) have a highly significant increase ($p \leq 0.001$) in aspirin intoxicated group compared to control group. On the other hand, pretreatment with ginger decreased significantly ($p \leq 0.001$) these biomarkers compared to aspirin intoxicated group and maintained them close to normal levels recorded from the control group. **Figure 1** demonstrated significant ($p \leq 0.001$) reduction in the level of the anti-oxidant enzymes (SOD and CAT) but significantly increased (MDA) assayed in homogenates of stomach, kidney and liver taken from aspirin intoxicated animals compared to control group. Conversely, administration of ginger prior to aspirin preserved the anti-oxidant capacity reflected by a highly significant ($p \leq 0.001$) increase in the levels of SOD and CAT accompanied by reduction in MDA concentrationn in the three organs.

Histopathological findings

The light microscope findings are demonstrated in **Figure 2**, organs are top horizontal and groups are left vertical. In rats treated with aspirin group (A), Glandular stomach showed diffuse widespread ulcer with very clear damage to the mucosal surface and surrounded by overhanging gastric mucosal margins. The base of the lesion is completely denuded of epithelium, and even the underlying muscularis mucosae; the sub-mucosa is completely exposed with marked congestion of the sub-mucosal blood vessels in addition to infiltration of eosinophils and mast cells. Additionally, Kidney's section of the same group showed congestion of glomerular tufts as well as congestion of peritubular blood capillaries and proliferation of the intimal layers of the renal blood vessels with prominent endothelial cells. Lining epithelium of the renal tubules demonstrated different degenerative changes as papillary necrosis in the proximal convoluted tubules and vacuolar degeneration. Vacuolation of lining epithelium was consistent with hydropic or fatty change. Also, Liver tissue from (A) group revealed congestion of the central vein and blood sinusoids. Hepatocytes within the centro-

lobular areas showed round clear vacuoles consistent to fatty changes. Pretreatment of the rats with orally administered ginger extract (100 mg/kg/day) in (A+G) group significantly reduced gastric lesion developed due to aspirin administration and ameliorated several pathological changes in the above-mentioned gastric mucosal lesions, surface mucosa showed erosion as well as disappearance of gastric pits. Sections from livers and kidneys of rats pretreated with ginger (A+G) showed corrective changes similar the normal limits in (C) group. Tissue sections of the three organs taken from animals given ginger alone in group (G) showed similar to normal celluar architecture.

Table 1: Effect of ginger on liver and kidney function markers of control and experimental rats.

parameter \ Group	Control	Aspirin	Ginger	Aspirin + Ginger
Liver function biomarkers				
AST(IU/L)	76.41±5.95	113.2±7.61***	75.3±6.4$^{###}$	86.3±5.85$^{###}$
ALT(IU/L)	26.1±2.1	65.71±4.3***	27.8±1.89$^{###}$	33.3±1.88$^{###}$
Kidney function biomarkers				
Creatinine (mg/dl)	0.67±0.04	1.71±0.12***	0.70±0.04$^{##}$	0.88±0.55$^{###}$
Urea (mg/dl)	28.2±1.99	53.97±3.3***	27.01±2.1$^{##}$	30.9±2.2$^{###}$
Uric acid (mg/dl)	1.56±0.1	4.65±0.33***	1.65±0.09$^{###}$	2.08±0.14$^{###}$

All values are expressed as mean ± standard deviation (SD), n=10 for each treatment group. $^{***}p<0.001$ is highly significant different from control group; $^{###}p<0.001$ is highly significant different from aspirin treated group.

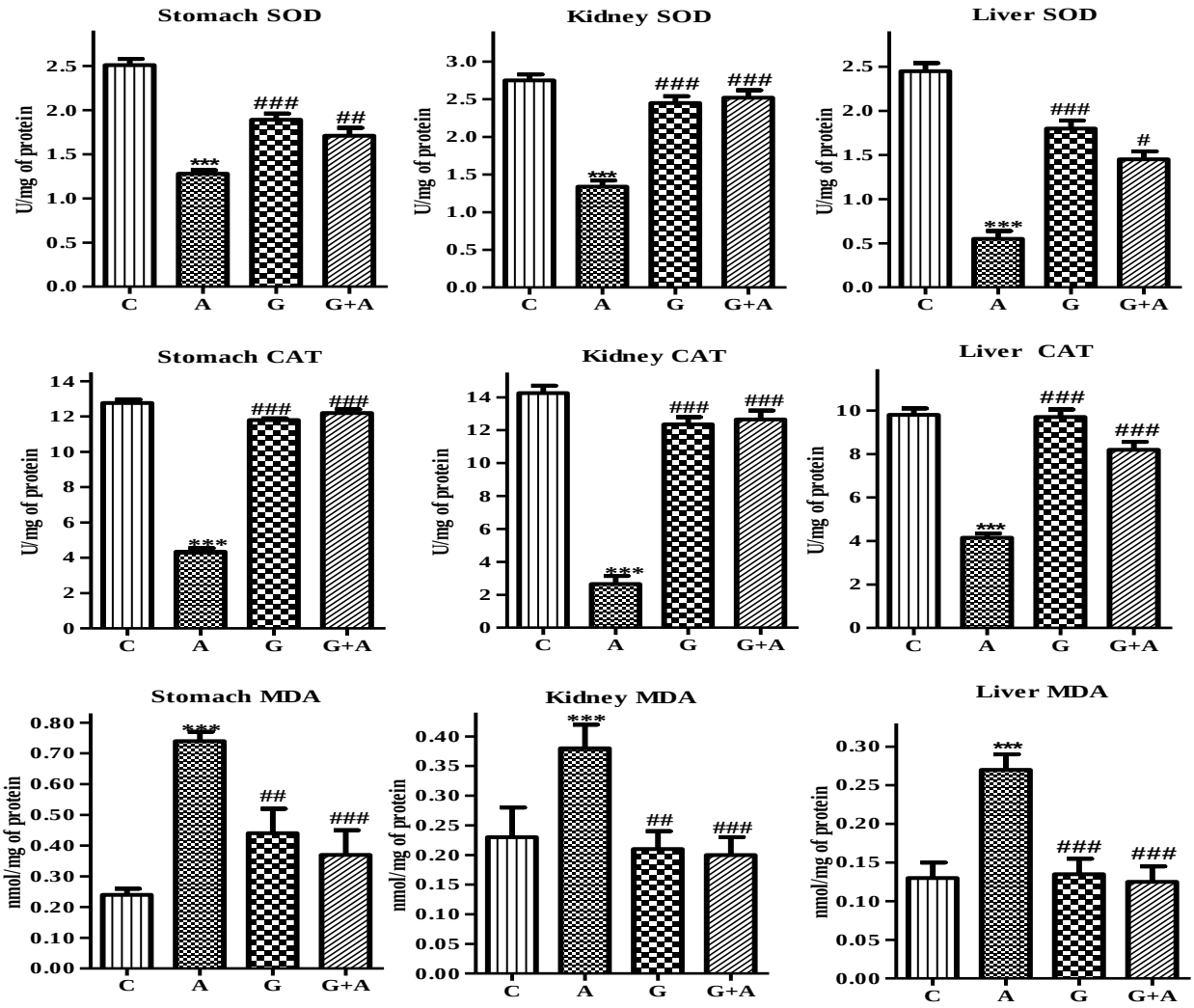

Fig. 1: Activities of SOD and CAT and MDA level in homogenates of stomach liver and kidney in control (C), Aspirin treated (A), Ginger treated (G) and Aspirin and Ginger co-treated (G+A). Values are the mean of 8 measurements ± SD. ***p ≤0.001 compared to control group (C), ##p ≤0.01 compared to treated group (A), ###p ≤0.001 compared to treated group (A).

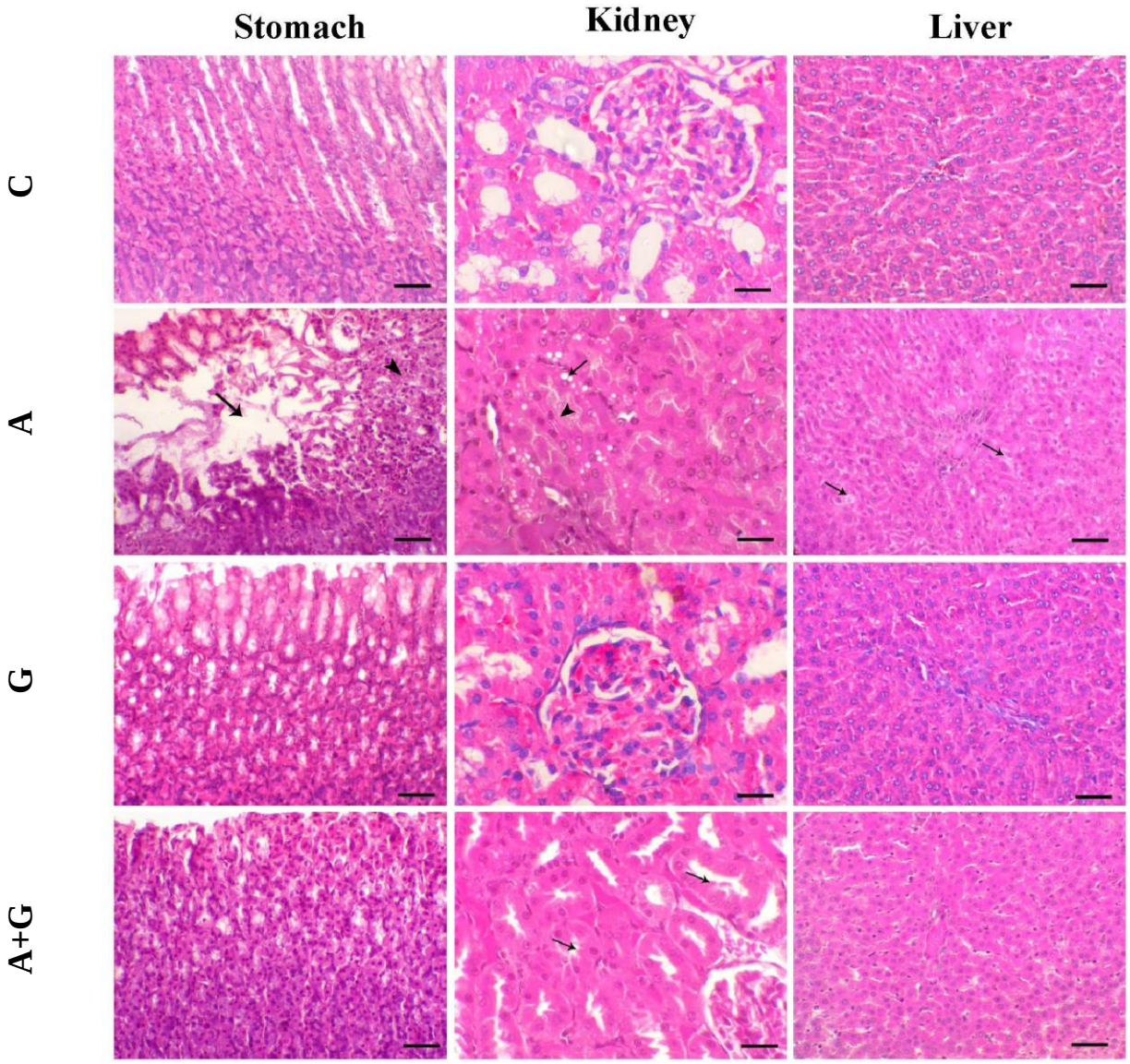

Fig. 2: Histopathological observations in homogenates of stomach liver and kidney in control **(C)**, Aspirin treated **(A)**, Ginger treated **(G)** and Aspirin and Ginger co-treated **(A+G)**. The 1st upper lane of the photomicrographs obtained from control animal showing normal gastric mucosa, kidney and liver of control animal. The 2nd lane of the photomicrographs obtained from aspirin-treated animal showing the ulcerated gastric mucosa (arrow) accompanied with severe degeneration of the gland, renal papillary necrosis (arrowhead) and fatty changes within the renal tubular epithelial lining and marked diffuse hepatic vacuolation. The 3rd lane of control animal treated with ginger showing normal gastric, renal and hepatic structures. The 4th lane of animal treated with A+ G revealing mild degeneration of the gastric glands and renal and hepatic tissues were within normal limits. H&E stain, Bar = 100 µm.

Discussion:

The aim of the present study is to assess the potential protective effect of ginger on aspirin adverse effects on stomach, kidney and liver. Based on the data obtainedfrom the current study, there is a significant increase in serum renal function biomarkers, creatinine, urea and uric acid, which is in agreement with previous records [22-24]. Interestingly, the pathomechanism of aspirin induced nephropathy seems similar to its gastropathy and is due to direct toxicity [25,26] or to ischemia resulting from prostaglandin (PGE2) inhibitory effect of aspirin [27]. Similar to stomach, PGE2 produced by the kidney is important to sustain optimum blood flow to the kidney. Decreased PGE2 concentration by aspirin causes reduction in blood flow. Since the renal cortex (outside) is the first destination of blood and renal medulla (inside) is the second one, the deeper structures of the kidney are most affected by reduced blood flow. Consequently, the innermost structures of the kidney, the renal papillae, are especially dependent on PGE2 synthesis to maintain adequate blood flow. Inhibition of PGE2 production therefore rather selectively damages the renal papillae, increasing the risk of renal papillary necrosis [28,29].

Our results of renal function tests carried out for co-treated group (Aspirin and ginger) showed significant

reduction in creatinine, urea and uric acid levels compared with those of aspirin treated animals. The results demonstrated close to normal kidney functions, which were consistent with Mehrdad *et al.,* [30] who stated that ginger has a beneficial effect for removal of urea and creatinine from plasma and considered as a therapeutic herb to manage renal function. The corrective histopathological findings after treatment with ginger extracts give an additional support that ginger mops up free radicals generation by aspirin, reduces inflammation, improves kidney function, and induces healthy state of renal cells, suggesting its significant role as renal protective agent. This was attributed to their enormous concentrations of natural antioxidants: flavonoids, sterols, and alkaloids. In harmony with our results, ginger showed a potential defensive role against renal ischemia [31], renal damage induced by alcohol intoxication [32], and renal toxicity induced by doxorubicin [33].

The data obtained from liver function tests of aspirin intoxicated animals revealed significant elevation of serum transaminases (ALT, AST) in consistency with previous reports [34] indicating damage of hepatic tissue. Consequently, Aspirin hepatotoxicity is demonstrated in light microscopic findings showing degenerative changes of patchy necrosis with substantial periportal infiltration of inflammatory cells including eosinophil, mast cells and lymphocytes typically as described by Prescott [35]. Additionally, the investigation of hepatic tissue homogenate showed increased lipid peroxidation marker (MDA) but decreased antioxidant enzymes (SOD and CAT) in agreement with previous records [36] indicating severe oxidative stress as a result of the acute hepatitis. On the other hand, serum samples from ginger and aspirin co-treated animals revealed close to normal values of transaminases (ALT and AST) confirmed by corrective changes on hepatic tissue sections of the same animals when examined by light microscope. The reported results seem to be similar to those recorded by [37,38]. Also, the anti-oxidant capacity of ginger was demonstrated as recovery of hepatic redox parameters.

Surprisingly, ginger has prostaglandin inhibitory effect the same like aspirin [39,40]. However, it will not be a surprise if downstream effects of both aspirin and ginger are explained. While aspirin chemopreventive effect is mediated through inhibition of cyclooxygenases increasing the susceptibility of cancer cells to apoptosis [41] aspirin showed a cyclooxygenase-independent mechanism during apoptosis signaling [42]. Furthermore, proapooptotic role along with antitumor effect of aspirin are mediated through activation of NF-κB and induction of oxidative stress [43]. As already reported, apoptosis is initiated through the phenomenon of mitochondrial permeability transition (MPT) that can induce loss of mitochondrial membrane potential, oxidative stress, and a decrease in ATP production upon pathological activation [44]. Battaglia *et al.,* [45] demonstrated that induction of MPT by salicylate is the result of oxidative stress. On the other side, ginger demonstrated whole body protection against oxidative stress [46] including inhibition of submolecular pathways such as NF-κB [46,47].

The histopathological results of the current work demonstrated that aspirin oral administration led to remarkable ulcers in the glandular area of the stomach excised from aspirin treated rats compared with other animals. The observations are in harmony with previous study demonstrating that aspirin can produce marked ulcers in rats [13]. Underlying mechanism of aspirin induced gastric ulcer, like other NSAIDs, looks complicated and multifactorial. Nevertheless, Aspirin effects could be classified to systemic and topic. The classical systemic mechanism stated that aspirin induces ulcer through reduction of synthesis of PGE2s [48,49] and increasing NO levels [50]. The optimum gastric blood flow is mainly maintained by normal levels of PGE2s and NO [51,52], which supports the integrity of the gastric mucosa and its adaptation for chronic aspirin intake [53]. Moreover, aspirin injured mucosal tissue appeared with significant granulocytes infiltration. Accumulation of mast cells and eosinophils were observed before with aspirin induced gastric ulcer model [54]. Mast cells aggregation is due to aspirin reduced PGE2 levels. Additionally, Mast cells produce high levels of histamine that initiates gastric mucosal injury [55]. Also, observation of high count of neutrophil is supported by previous workers [56,57], which signified the neutrophil dependent microvascular injury in initiation of aspirin induced gastric ulcer. Furthermore, aspirin induced gastropathy could be attenuated by inhibition of neutrophil accumulation [56,58]. What is more, administration of TNF-α inhibitors attenuates aspirin and other NSAIDs induced gastric ulcer [56,59]. TNF-α increases neutrophil-derived superoxide generation [60] and stimulates IL-1 production, leading to neutrophil infiltrations [61,62]. By this means, reactive oxygen species (ROS) play an important role in pathomechanism of aspirin induced gastric ulcer [63]. The results of the present study revealed significant reduction in the gastric antioxidant enzymes, SOD and CAT similar to previous records [38]. In contrast, MDA, the indicator of lipid peroxidation showed significant high levels. As suggested before, gastric ulcer could be due to attenuation of mucosal antioxidant mechanisms and augmentation of lipid peroxidation [60]. Surprisingly, aspirin has the capacity to induce gastric ulcer if it is administered orally unlike other NSAIDS such as indomethacin which cause injury regardless of route of administration emphasizing the importance of topical injury in aspirin-induced ulcers [64]. In Co-treated animals (ginger and aspirin) the histopatholgical findings revealed gastric mucosa with close to normal architectures. The anti-ulcerative effects of ginger have previously been investigated in experimental gastric ulcer models [65-67]. However, the mechanism underlying the protective effects of ginger against gastric damage is unclear. From our results, ginger powder is likely to protect the stomach against aspirin induced ulcer by improving mucosal blood flow [13]

reducing iNOS activity in the gastric mucosa [13,50] and inflammatory cytokines (TNF and IL-1) expression [68,69]. These effects of ginger powder could to be attributable to the main ingredients of ginger, gingerol and shogaol [69]. In the meantime, results of oxidative stress markers were reversed after oral administration of ginger extract with aspirin. Ginger enhanced SOD and activity, and reduced MDA level, which seems in agreement with previous studies [70-72].

In the present study, light microscopic lesion of renal tissues collected from aspirin treated animals demonstrated the well-defined lesions of papillary necrosis and cortical interstitial nephritis, the typical lesion associated with abuse of aspirin [73].

Conclusion

Based on the data obtained from the current study, it could be suggested that oral administration of ginger powder has antioxidant capacity to protect stomach, kidney and liver against aspirin induced injury mainly by boosting antioxidant enzymes and inhibiting lipid peroxidation. Taken together, ginger could be the supplement when aspirin is the drug of choice as anti-platelet aggregation.

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