

## Efficacy and Safety of some Medical Herbs on Gastric Ulcer Induced by Aspirin in Rats

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**Abstract:** Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used drugs in inflammation treatment. NSAIDs are associated with several side effects especially on the stomach. Considering these limitations of NSAIDs side effect, alternate natural nontoxic antioxidant with potent antiulcer activity such as ginger or curcumin was needed. Thus, this study was conducted to evaluate the correction role of ranitidine alone or with ginger or/and curcumin on aspirin induced gastric ulcer in adult male albino rats. Gastric ulcer in rats was induced by administered aspirin (500mg/Kg body weight/day) for three successive days to the animals. The obtained data revealed that aspirin induced a significant ( $p < 0.05$ ) increase in macroscopic ulcer score, gastric acidity and gastric production of mucosal non-protein sulfhydryl group than those in control ones. The levels of proinflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$  & IL-8) were significantly ( $p < 0.05$ ) increase associated with remarkable elevation in the levels of total oxidant capacity and malondialdehyde (MDA) in ulcerogenic rats. On the other hand, aspirin caused significant ( $p < 0.05$ ) decrease in the gastric total anti-oxidant capacity, prostaglandine E2, cyclooxygenase and vascular endothelial cell growth factor (VEGF) levels as compared to control rats. These disturbances in all the pervious parameters were ameliorated after the ulcerogenic rats treated with ginger, curcumin or their mixture accompanied with ranitidine treatment dependent on the time of administration (1&2 weeks). These findings are consistent with the concept that curcumin and ginger are antioxidant agents. The underlying mechanisms of these effects were discussed with available recent researches.

**Keywords:** Aspirin, Curcumin, Ginger, Gastric Ulcer, Ranitidine.

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### I. Introduction

Gastric ulcer is the most prevalent gastrointestinal disorder and is known to develop due to imbalance between gastric offensive factors and protective factors. Several endogenous and exogenous factors are responsible for gastric ulceration. These include Helicobacter pylori infection, increase the production of gastric acids, pepsin and stomach juices, certain types of medicines, notably the non-steroidal anti-inflammatory drugs (NSAIDs) and even personal factors such as physical stresses and consumption of tobacco, alcohol and caffeine [1].

Non-steroidal anti-inflammatory drugs (NSAID) are commonly used medication with an expensive financial burden worldwide. Aspirin is one of NSAID that widely used for the treatment of rheumatoid arthritis and related diseases as well as the prevention of cardiovascular thrombotic diseases. Aspirin is associated with several side effects; it damages gastrointestinal mucosa by irritant action, causing alterations in mucosal permeability and/or suppression of prostaglandin synthesis [2]. Many other factors such as gastric acid and pepsin secretion, gastric microcirculation, proinflammatory cytokines interleukin (IL-1) and tumor necrosis factor (TNF- $\alpha$ ) play important roles in the genesis of gastric mucosal damage, and its subsequent development [3]. It has been reported that the increase in NO synthase (NOS) activity is involved in the gastrointestinal mucosal defense and also in the pathogenesis of mucosal damage [4].

Secretion of gastric acid is still recognized as a central component of gastric ulcer. Therefore, the main therapeutic target is the control of this secretion using acid blockers such as ranitidine [5]. It is available over the counter for oral administration or by prescription for parenteral administration for treatment of gastric ulcers, hypersecretory diseases and gastroesophageal reflux disease. Idiosyncratic ranitidine hepatotoxicity occurs in few people taking the drug [6]. Nowadays, gastric ulcer therapy faces a major drawback because most of the drugs currently available in the market show limited efficacy against gastric diseases and are often associated with severe side effects [7].

The use of herbal medicines for the prevention and treatment of different pathologies is in continuous expansion worldwide [8]. Ginger (Zingiber officinale) has played an important role in medicine as a folk remedy to treat many inflammatory conditions which associated pain [9]. The major pungent constituents of ginger, 6-gingerol and 6-shogaol have many interesting pharmacological effects, such as anti-oxidant, antitumor promoting and anti-inflammatory effects [10] [11] [12]. Moreover, Chandan et al. [13] found that ginger can block the effects of prostaglandins, thus it may help to protect stomach and prevent ulcer.

Curcumin, a yellow colouring ingredient of the spice turmeric obtained from the rhizome of *Curcuma longa*. It possesses a broad range of pharmacological activities, including antioxidant, anti-carcinogenic and anti-inflammatory effects [14] [15] [16]. Curcumin administration blocked gastric ulceration by preventing glutathione depletion, lipid peroxidation and protein oxidation that accelerate the healing process through re-epithelialization [17].

Therefore, the current study was designed to postulate the possible enhancement effect of the alternative natural substance curcumin, ginger or both of them associated with ranitidine against aspirin induced gastric mucosal damage in adult male rats.

## **II. Material And Methods**

Sixty adult male albino rats ( $150\pm 10$ g body weight) were employed in this study. They were obtained from the General Organization of Vaccine and Biological Preparations. Animals were allowed two weeks at the Animal House of the Nuclear Research Center, Inshas and kept under hygienic managerial and environmental conditions to adapt to the laboratory conditions. They were housed in metallic cages, maintained on constant environmental temperature ( $25\pm 3^\circ\text{C}$ ) and relative humidity (55-60%).

### **2.1 Experimental design:-**

Rats were fasted for overnight and only water was allowed ad libitum. Aspirin (500mg/Kg body weight/day) was given orally using oro-gastric cannula for successive three days [18]. At the end of induction period (The fourth day), five comparisons were made between control rats which received normal saline (0.9 % NaCl) orally using oro-gastric cannula and another five animal groups were suffered from gastric ulcer (Ten rats in each). The first one of them (10 rats) untreated for 1 and 2 weeks (5 rats in each interval) and served as recovery gastric ulcer rats group (RGU). The second gastric ulcer animals group treated orally with 2.5mg ranitidine / 100g b.wt weight as described by Jothi et al. [19] for 1 & 2 weeks (5 rats in each interval) and served as ranitidine (RAN) rats group. The third, fourth and fifth gastric ulcer animals groups were given ranitidine as described in the second GU animals group associated with administration of ginger powder (200mg/kg body weight) orally as a suspension in 3ml of 1% carboxymethyl cellulose in water according to Wang et al. [20], 30mg curcumin/100g b.wt./ kg b.wt/day orally as a suspension in 3ml of 1% carboxymethyl cellulose in water according to Mazen [21] and mixture of them (ginger and curcumin) and served as ginger (Ging), curcumin (Cur) and mixture (Mix) groups respectively for the same periods (1 & 2 weeks).

At the end of each experimental period, the blood was collected by heart puncture and serum was separated by centrifugation (3000 rpm at  $4^\circ\text{C}$  for 10 min) for estimated the biochemical parameters. As soon as possible, the stomach was removed to evaluate the changes in the physiological features (ulcer score, acidity of gastric juice and gastric mucosal non-protein sulfhydryl groups (NP-SH)).

### **2.2 Determination of physiological parameters:**

After sacrificed the animals, the stomach was immediately removed and cut along the greater curvature and dissected longitudinally, stretched on paraffin bed, washed with normal saline and distribution of bleeding spots were counted following the method of Szabo et al. [22].

Acidity of gastric juice was measured titrimetrically with 0.01N NaOH solutions by using 0.5% Topfer's reagent and 1% alcoholic phenolphthalein as an indicator [23]. Titratable acidity was calculated in meq/L. Total titratable acid output meq/L amount of NaOH that neutralize 100mg of gastric juice.

Gastric mucosal non-protein sulfhydryl groups (NP-SH) was measured according to the method of Sedlak & Lindsay [26]. The glandular part of the stomach was homogenized in ice-cold 0.02M ethylenediaminetetraacetic acid (EDTA). Aliquots of 5 ml of the homogenates were mixed in 15ml test tubes with 4 ml of distilled water and 1 ml of 50% trichloroacetic acid. The tubes were shaken intermittently for 10-15 minutes and centrifuged at 3000 g. Two ml of supernatant were mixed with 4ml of 0.4M Tris buffer, pH 8.9 and 0.1ml of 0.4% DTNB [5,5-dithio-bis-(2-nitrobenzoic acid)] was added and the sample was shaken. In this assay DTNB is reduced by non protein sulfhydryl groups present in TCA extract to 2-nitro-5-mercaptobenzoic acid having a characteristic yellow color. The absorbance was read within 5 minutes of addition of DTNB at 412 nm against a reagent blank with no homogenate. Protein content was assayed by the method of Lowry et al. [25].

### **2.3 Determination of biochemical parameters:**

Following a reported method of Cao et al. [26] and Flohe & Gunzler [27] for the determination of total antioxidant capacity (TAC) and total oxidant capacity (TOC) respectively and their manufacturer's instructions, the serum TAC (mmol/L) and TOC (mmol/L) were measured using Labor Diagnostika Nord GmbH & Co.; Nordhorn, Germany, commercial ELISA kits. Furthermore, malondialdehyde (MDA) concentration in the gastric mucosa was determined as an indicator of lipid peroxidation by thiobarbituric acid method as previously described by Pedeson et al. [28] by using ELISA technique using commercial kit (Oxis, USA).

The levels of rat interleukin-1 $\beta$  (rat IL-1 $\beta$ ), rat interleukin-8 (rat IL-8) were measured in serum using solid phase sandwich enzyme linked immunosorbent assay (ELISA) techniques according to Grassi et al. [29] and Baggiolini et al. [30] respectively. The kits were purchased from IBL Gesellschaft, Hamburg (Germany). Moreover, the levels of rat tumor necrosis factor- $\alpha$  (rat TNF- $\alpha$ ) in serum [31] were assayed by the aid of radioimmunoassay kits using solid phase component system (ICN Pharmaceuticals Inc, USA).

Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) level in the rat gastric mucosa was determined by enzyme-immunoassaying (Rat PGE<sub>2</sub> EIA kit; Cayman Chemicals, USA) according to Lee & Feldman [32]. The sequestration of neutrophils within the gastric mucosa was evaluated quantity by tissue myeloperoxidase (MPO) activity using ELISA technique. The MPO activity in the gastric mucosa was determined according to Olsen & Little [33]. This kit was purchased from GenWay Co. (USA). Cyclooxygenase activity was measured as the ability of tissue homogenates to metabolize arachidonic acid to PGE<sub>2</sub> according to the method described by Tomlinson et al. [34]. The kit was purchased from IBL Gesellschaft, Hamburg, Germany. The level of vascular endothelial growth factor (VEGF) in the gastric mucosa was determined at 492 nm by solid phase sandwich technique (ELISA) using commercial kits (Rat VEGF ELISA kit; IBL, Gesellschaft, Hamburg, Germany) according to Yao et al. [35].

Statistical differences between the means were assessed by analysis of variance (ANOVA) followed by Duncan's multiple range test according to Duncan [36] and Snedecor & Cochran [37] using a computer program (Costate). Values of P<0.05 were considered statistically significant.

### III. Results And Discussion

Gastric ulcer is one of the most common gastrointestinal tract diseases that affected humans for centuries. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin is widely used and is considered to be the major risk factor in gastric ulcers. In the current study, oral administration of aspirin to rats caused a significant increase in macroscopic ulcer score, gastric acidity and gastric production of mucosal non-protein sulfhydryl group (Table 1). The ulceration induced by aspirin may be attributed mainly to various processes, including generation of reactive oxygen species (ROS), initiation of lipid peroxidation and inhibition of prostaglandin synthesis. The decrease in the prostaglandin level impairs almost all aspects of gastroprotection and increases acid secretions associated with inhibition in mucus secretion, bicarbonate and phospholipid production which, in turn, aggravate the ulcer [38]. These results are in parallel with that obtained by Abdallah et al. [39]. The last authors reported that exposure of unprotected lumen of the stomach to accumulating acid could facilitate ulceration.

Table (1): Effects of curcumin, ginger and their mixture with ranitidine on gastric ulcer score, acidity and mucosal non-protein sulfhydryl group in aspirin induced gastric ulcer in rats.

Parameters		Groups	Normal Control	Gastric Ulcer	Ranitidine (RAN)			
					RAN	Ginger	Curcumin	Ginger + Curcumin
Macroscopic ulcer score (mm)	1 Week n = 5	Zero ± Zero <sup>A</sup> <sub>a</sub>	3.82 ± 0.023 <sup>B</sup> <sub>a</sub>	2.43 ± 0.015 <sup>C</sup> <sub>a</sub>	1.18 ± 0.008 <sup>D</sup> <sub>a</sub>	1.67 ± 0.011 <sup>E</sup> <sub>a</sub>	0.87 ± 0.006 <sup>F</sup> <sub>a</sub>	
	2 Week n = 5	Zero ± Zero <sup>A</sup> <sub>a</sub>	3.01 ± 0.019 <sup>B</sup> <sub>b</sub>	1.24 ± 0.009 <sup>C</sup> <sub>b</sub>	0.92 ± 0.007 <sup>D</sup> <sub>b</sub>	1.13 ± 0.007 <sup>E</sup> <sub>b</sub>	0.19 ± 0.003 <sup>F</sup> <sub>b</sub>	
Acidity (mEq/litre)	1 Week n = 5	43.11 ± 0.073 <sup>A</sup> <sub>a</sub>	88.29 ± 0.091 <sup>B</sup> <sub>a</sub>	62.58 ± 0.077 <sup>C</sup> <sub>a</sub>	53.26 ± 0.097 <sup>D</sup> <sub>a</sub>	56.85 ± 0.076 <sup>E</sup> <sub>a</sub>	47.33 ± 0.076 <sup>F</sup> <sub>a</sub>	
	2 Week n = 5	43.29 ± 0.076 <sup>A</sup> <sub>a</sub>	79.48 ± 0.086 <sup>B</sup> <sub>b</sub>	50.07 ± 0.072 <sup>C</sup> <sub>b</sub>	45.13 ± 0.089 <sup>D</sup> <sub>b</sub>	47.71 ± 0.072 <sup>E</sup> <sub>b</sub>	42.89 ± 0.072 <sup>A</sup> <sub>b</sub>	
NP-SH mol/g Protein tissue	1 Week n = 5	3.391 ± 0.049 <sup>A</sup> <sub>a</sub>	5.121 ± 0.072 <sup>B</sup> <sub>a</sub>	4.417 ± 0.069 <sup>C</sup> <sub>a</sub>	4.796 ± 0.078 <sup>D</sup> <sub>a</sub>	3.813 ± 0.057 <sup>E</sup> <sub>a</sub>	3.813 ± 0.057 <sup>E</sup> <sub>a</sub>	
	2 Week n = 5	3.397 ± 0.051 <sup>A</sup> <sub>a</sub>	4.404 ± 0.067 <sup>B</sup> <sub>b</sub>	3.638 ± 0.058 <sup>C</sup> <sub>b</sub>	4.109 ± 0.069 <sup>D</sup> <sub>b</sub>	3.406 ± 0.052 <sup>A</sup> <sub>b</sub>	3.406 ± 0.052 <sup>A</sup> <sub>b</sub>	

- Values are expressed as mean ± SE

<sup>A, B, C, D, E, F</sup> Means with a common superscript within a row are significantly different (P<0.05).

The mucus in the stomach is considered the first line of defense; which decreased due to suppress prostaglandin production and damage of the surface epithelial cells and mucus neck cells. The decrease in the mucus secretion allows hydrogen ions and pepsin to diffuse into the mucosa from the lumen. Back-diffusion of acid and pepsin into the tissues stimulate further acid and pepsin secretion to cause more damage, release histamine causing inflammation, lower in the mucosal blood flow and decrease gastric motility [40]. However, the increase of microvascular injury causes ischemia. Ischemia leads to gastric mucosal cells necrosis via many mechanisms as, low oxygen tension and the subsequent depletion of ATP generation; all affect sodium-potassium pump that leading to influx of sodium into the cell and osmotic gain of water. At the same time, the

intracellular calcium increases through influx from the extracellular fluid and its releases from intracellular stores. This activates phospholipases, protease and endonucleases enzymes which result in gastric cellular damage [41].

Free radicals production has been reported to play a fundamental role in the pathogenesis of NSAID induced gastric damage [42]. In the current work, serum total oxidant capacity (TOC) and gastric MDA levels were significantly ( $p < 0.05$ ) increased in rats subjected to aspirin compared to normal control rats. On the other hand, serum total antioxidant capacity (TAC) levels were significantly ( $p < 0.05$ ) decreased in rats throughout the whole experimental periods (1 & 2 weeks) as a result of aspirin administration (Table 2 & 3). These results seemed to be in complete accordance with studies made by Chakraborty et al. [43] and Uduak et al. [44]. They reported that generation of reactive oxygen species (ROS) plays a major role in the development of multiple pathologies, such as gastritis, peptic ulcerations or gastric adenocarcinoma. Also, free radicals lead to damage the cellular antioxidant enzymes which acting as the first line of cellular defense against oxidative injury. This might lead to aggravated tissue damage during gastric ulceration [45]. Moreover, Abdallah et al. [39] recorded that stomach ulceration induced by indomethacin was accompanied with a severe oxidative stress in gastric tissue causing damage to key biomolecules such as lipids and stimulation of lipid oxidation which leading to increase the accumulation of MDA as well as reduction in the gastric activity of catalase. MDA represents an end-product of the peroxidation of polyunsaturated fatty acids and related esters within cell membranes and is currently regarded as a reliable index of oxidative tissue damage.

Inflammation is also important in the pathogenesis of the gastric damage induced by NSAIDs [46]. As shown in table (2), the levels of cytokines profile (TNF- $\alpha$ , IL-1 $\beta$  and IL-8) were significantly ( $p < 0.05$ ) increased by aspirin administration in rats through the whole experimental periods (1 & 2 weeks) when compared to their corresponding normal control rats group. These data indicated that inflammation developed aggressively accompanied with aggravated gastric ulcer. These results are coincided with the finding of Jainu and Devi [47]. The authors reported that inflammation induced in the gastric mucosa by aspirin is accompanied with increasing TNF- $\alpha$  production which augments neutrophil-derived superoxide generation [48] and stimulates IL-1 $\beta$  production, leading to neutrophil accumulation [49]. Furthermore, Appleyard et al. [50] recorded that indomethacin up-regulated the synthesis of pro-inflammatory molecules like TNF- $\alpha$  contributing to mucosal injury. In study carried by Swarnakar et al. [51] indomethacin increased serum TNF- $\alpha$  and mucosal TBARS at the ulcer site seemed mostly to be responsible for ulcerogenesis.

Table (2): Effects of curcumin, ginger and their mixture with ranitidine on serum TAC, TOC and cytokines profile in aspirin induced gastric ulcer in rats.

Parameters	Groups	Normal Control	Gastric Ulcer	Ranitidine (RAN)			
				RAN	Ginger	Curcumin	Ginger + Curcumin
TAC (mmol/L)	1 Week n = 5	1.67 ± 0.017 <sup>A</sup> <sub>a</sub>	0.77 ± 0.008 <sup>B</sup> <sub>a</sub>	0.93 ± 0.011 <sup>C</sup> <sub>a</sub>	1.21 ± 0.013 <sup>D</sup> <sub>a</sub>	1.08 ± 0.012 <sup>E</sup> <sub>a</sub>	1.36 ± 0.014 <sup>F</sup> <sub>a</sub>
	2 Week n = 5	1.65 ± 0.016 <sup>A</sup> <sub>a</sub>	0.91 ± 0.010 <sup>B</sup> <sub>b</sub>	1.28 ± 0.014 <sup>C</sup> <sub>b</sub>	1.57 ± 0.015 <sup>D</sup> <sub>b</sub>	1.39 ± 0.013 <sup>E</sup> <sub>b</sub>	1.64 ± 0.016 <sup>A</sup> <sub>b</sub>
TOC (mmol/L)	1 Week n = 5	0.276 ± 0.0008 <sup>A</sup> <sub>a</sub>	1.089 ± 0.0042 <sup>B</sup> <sub>a</sub>	0.885 ± 0.0036 <sup>C</sup> <sub>a</sub>	0.717 ± 0.0029 <sup>D</sup> <sub>a</sub>	0.796 ± 0.0031 <sup>E</sup> <sub>a</sub>	0.664 ± 0.0025 <sup>F</sup> <sub>a</sub>
	2 Week n = 5	0.271 ± 0.0008 <sup>A</sup> <sub>a</sub>	0.954 ± 0.0039 <sup>B</sup> <sub>b</sub>	0.618 ± 0.0023 <sup>C</sup> <sub>b</sub>	0.492 ± 0.0014 <sup>D</sup> <sub>b</sub>	0.551 ± 0.0019 <sup>E</sup> <sub>b</sub>	0.337 ± 0.0011 <sup>F</sup> <sub>b</sub>
TNF- $\alpha$ (pg/ml)	1 Week n = 5	3.17 ± 0.229 <sup>A</sup> <sub>a</sub>	15.69 ± 0.827 <sup>B</sup> <sub>a</sub>	11.77 ± 0.106 <sup>C</sup> <sub>a</sub>	8.82 ± 0.096 <sup>D</sup> <sub>a</sub>	10.54 ± 0.101 <sup>E</sup> <sub>a</sub>	5.23 ± 0.101 <sup>E</sup> <sub>a</sub>
	2 Week n = 5	3.21 ± 0.235 <sup>A</sup> <sub>a</sub>	10.51 ± 0.708 <sup>B</sup> <sub>b</sub>	7.67 ± 0.124 <sup>C</sup> <sub>b</sub>	4.95 ± 0.117 <sup>D</sup> <sub>b</sub>	5.12 ± 0.138 <sup>D</sup> <sub>b</sub>	3.15 ± 0.138 <sup>A</sup> <sub>b</sub>
IL-1 $\beta$ (pg/ml)	1 Week n = 5	13.15 ± 0.161 <sup>A</sup> <sub>a</sub>	53.96 ± 0.891 <sup>B</sup> <sub>a</sub>	41.02 ± 0.787 <sup>C</sup> <sub>a</sub>	35.27 ± 0.809 <sup>D</sup> <sub>a</sub>	37.81 ± 0.756 <sup>E</sup> <sub>a</sub>	24.15 ± 0.404 <sup>F</sup> <sub>a</sub>
	2 Week n = 5	13.17 ± 0.158 <sup>A</sup> <sub>a</sub>	46.18 ± 0.846 <sup>B</sup> <sub>b</sub>	30.81 ± 0.562 <sup>C</sup> <sub>b</sub>	22.93 ± 0.329 <sup>D</sup> <sub>b</sub>	26.46 ± 0.671 <sup>E</sup> <sub>b</sub>	15.36 ± 0.264 <sup>F</sup> <sub>b</sub>
IL-8 (pg/ml)	1 Week n = 5	24.09 ± 0.649 <sup>A</sup> <sub>a</sub>	55.11 ± 1.272 <sup>B</sup> <sub>a</sub>	39.47 ± 0.889 <sup>C</sup> <sub>a</sub>	32.96 ± 0.758 <sup>D</sup> <sub>a</sub>	33.13 ± 0.767 <sup>D</sup> <sub>a</sub>	24.22 ± 0.651 <sup>A</sup> <sub>a</sub>
	2 Week n = 5	23.89 ± 0.651 <sup>A</sup> <sub>a</sub>	48.64 ± 1.187 <sup>B</sup> <sub>b</sub>	30.68 ± 0.708 <sup>C</sup> <sub>b</sub>	24.07 ± 0.662 <sup>A</sup> <sub>b</sub>	23.96 ± 0.659 <sup>A</sup> <sub>b</sub>	23.88 ± 0.653 <sup>A</sup> <sub>b</sub>

- Values are expressed as mean ± SE

<sup>A, B, C, D, E, F</sup> Means with a common superscript within a row are significantly different ( $P < 0.05$ ).

Polymorphonuclear migration is an early and critical event in the pathogenesis of gastric mucosal injury caused by NSAIDs. TNF- $\alpha$  was previously reported to be a proinflammatory cytokine that causes polymorphonuclear neutrophil migration, through up-regulating the expression of adhesion molecules in both

neutrophil and endothelial cells [52]. Probably, prostaglandins inhibition by NSAIDs is responsible for the TNF- $\alpha$  rise. NSAIDs drugs markedly reduce prostaglandin synthesis, which were known to be potent inhibitors of TNF- $\alpha$  release from macrophages and mast cells [53].

Neutrophil infiltration into the gastric mucosal tissues is also a critical process in the pathogenesis of variety of gastric ulcers. MPO level has been widely used as an index of neutrophil infiltration in various experimental gastric injuries and represent another indication to the degree of ulceration [54]. Table (3) showed a significant ( $p < 0.05$ ) increase in the gastric MPO activity following aspirin administration throughout the whole period of experiment (1 & 2 weeks). These results may suggested that the ulcers were initially severely inflamed and increased MPO activity result from neutrophil infiltration in the ulcer bases [45]. Moreover, Arakawa et al. [55] demonstrated that indomethacin treatment during the initial healing period of acetic acid both promotes persistent polymorphonuclear cell infiltration and increases the likelihood of ulcer recurrence.

Table (3): Effects of curcumin, ginger and their mixture with ranitidine on gastric MDA, PGE<sub>2</sub>, MPO, COX-2 and VEGF in aspirin induced gastric ulcer in rats.

Parameters	Groups	Normal Control	Gastric Ulcer	Ranitidine (RAN)			
				RAN	Ginger	Curcumin	Ginger + Curcumin
MDA (pmol/g gastric mucosa tissue)	1 Week n = 5	1.95 = 0.028 A <sub>a</sub>	7.76 = 0.117 B <sub>a</sub>	5.11 = 0.072 C <sub>a</sub>	4.21 = 0.058 D <sub>a</sub>	4.68 = 0.066 E <sub>a</sub>	3.57 = 0.048 F <sub>a</sub>
	2 Week n = 5	1.98 = 0.029 A <sub>a</sub>	5.82 = 0.098 B <sub>b</sub>	4.02 = 0.051 C <sub>b</sub>	3.09 = 0.044 D <sub>b</sub>	3.75 = 0.053 E <sub>b</sub>	2.34 = 0.039 F <sub>b</sub>
MPO activity (H <sub>2</sub> O <sub>2</sub> $\mu$ M/min/g protein of gastric mucosa tissue)	1 Week n = 5	7.45 = 0.238 A <sub>a</sub>	32.59 = 0.627 B <sub>a</sub>	19.68 = 0.476 C <sub>a</sub>	14.88 = 0.412 D <sub>a</sub>	17.29 = 0.437 E <sub>a</sub>	11.62 = 0.349 F <sub>a</sub>
	2 Week n = 5	7.51 = 0.242 A <sub>a</sub>	25.34 = 0.579 B <sub>b</sub>	13.79 = 0.391 C <sub>b</sub>	10.32 = 0.318 D <sub>b</sub>	12.96 = 0.365 E <sub>b</sub>	8.77 = 0.259 F <sub>b</sub>
PGE <sub>2</sub> (pg/mg protein of gastric mucosa tissue)	1 Week n = 5	2.68 = 0.047 A <sub>a</sub>	1.21 = 0.026 B <sub>a</sub>	1.46 = 0.034 C <sub>a</sub>	1.65 = 0.039 D <sub>a</sub>	1.61 = 0.038 D <sub>a</sub>	1.88 = 0.043 E <sub>a</sub>
	2 Week n = 5	2.71 = 0.048 A <sub>a</sub>	1.33 = 0.029 B <sub>b</sub>	1.51 = 0.033 C <sub>b</sub>	1.85 = 0.041 D <sub>b</sub>	1.90 = 0.045 D <sub>b</sub>	2.24 = 0.051 E <sub>b</sub>
COX-2 (ngPGE <sub>2</sub> /30min/mg protein of gastric mucosa tissue)	1 Week n = 5	4.68 = 0.147 A <sub>a</sub>	3.09 = 0.114 B <sub>a</sub>	3.38 = 0.123 C <sub>a</sub>	3.78 = 0.131 D <sub>a</sub>	3.61 = 0.129 E <sub>a</sub>	3.92 = 0.138 F <sub>a</sub>
	2 Week n = 5	4.72 = 0.151 A <sub>a</sub>	3.41 = 0.125 B <sub>b</sub>	3.69 = 0.134 C <sub>b</sub>	4.07 = 0.151 D <sub>b</sub>	3.88 = 0.143 E <sub>b</sub>	4.35 = 0.162 F <sub>b</sub>
VEGF (pg/mg gastric mucosa tissue)	1 Week n = 5	68.32 = 1.57 A <sub>a</sub>	39.51 = 0.83 B <sub>a</sub>	50.48 = 1.07 C <sub>a</sub>	56.27 = 1.15 D <sub>a</sub>	54.09 = 1.13 E <sub>a</sub>	59.53 = 1.24 F <sub>a</sub>
	2 Week n = 5	67.99 = 1.54 A <sub>a</sub>	28.76 = 0.78 B <sub>b</sub>	58.31 = 1.19 C <sub>b</sub>	63.37 = 1.36 D <sub>b</sub>	60.88 = 1.29 E <sub>b</sub>	67.87 = 1.55 A <sub>a</sub>

- Values are expressed as mean  $\pm$  SE

- A, B, C, D, E, F Means with a common superscript within a row are significantly different ( $P < 0.05$ ).

The suppression of gastric prostaglandin is the fundamental mechanism responsible for the gastrointestinal toxicity of NSAIDs. It stimulates the secretion of bicarbonate and mucus, maintains mucosal blood flow and regulates mucosal turn over and repair [56]. Prostaglandin synthesis depends upon the activity of cyclooxygenase (COX), a rate-limiting enzyme in the synthesis of eicosanoids. Two isoforms of COX were identified in many cells; a constitutive enzyme designated as COX-1 and inducible isoform known as COX-2. COX-1 appears to be responsible for the production of prostaglandin that is physiologically important for homeostatic functions, such as maintenance of the mucosal integrity and mucosal blood flow [57]. Prostaglandin-derived from COX-2 were implicated in the protective and ulcer healing activities of growth factors by the demonstration that COX-2 is upregulated in the edge of gastric ulcer and that this is significantly enhanced by the treatment with growth factors [58].

The observation in Table (3) recorded that aspirin administration to rats induced significant ( $p < 0.05$ ) reduction in the levels of gastric PGE<sub>2</sub>, COX-2 and VEGF through the experimental periods (1 & 2 weeks) as compared to control rats group. These results are in agreement with that obtained by Whittle [59]. The author reported that prostaglandins reduce the activation of neutrophils and the local release of reactive oxygen species. Therefore, NSAIDs can shift the mucosal balance toward the recruitment and endothelial adhesion of circulating neutrophils through the inhibition of prostaglandin biosynthesis.

Wallace et al. [60] investigated the functional roles of COX isoforms in the gastric mucosa, showing that COX-1-dependent prostaglandins are involved in the maintenance of mucus/bicarbonate secretion and blood flow, while COX-2 protects the mucosa from leucocyte endothelial adhesion and supports epithelial renewal. The authors also observed that selective COX-1 or COX-2 inhibitors did not damage the stomach when tested alone, while NSAIDs or the combined administration of COX-1 plus COX-2 selective inhibitors resulted in gastric erosions.

Furthermore, COX-2 induced in ulcerated gastric mucosa is involved in the defense and repairing mechanisms of the mucosa and that its inhibition by a selective COX-2 inhibitor delays ulcer healing. In human stomach, COX-2 is exclusively expressed in gastric mesenchymal cells such as fibroblasts and in inflammatory

cells of the ulcer bed and margins, suggesting that COX-2 expressed in mesenchymal cells at the ulcer margin plays a key role in the ulcer repair process [61].

Vascular endothelial growth factor (VEGF), is the most potent stimulator of angiogenesis. It is produced by a variety of cell types including macrophages, smooth muscle cells, fibroblasts, megakaryocytes and neoplastic cells [62]. There is a link among prostaglandin synthesis by the gastric mucosa, VEGF expression and angiogenesis. In addition, it has been known that cyclooxygenase-2 makes an important contribution to the healing of ulcers throughout the gastrointestinal tract, this may be linked to the role of VEGF in ulcer healing [63]. Furthermore, COX-2 and VEGF have been colocalized in fibroblasts in the ulcer bed. A selective COX-2 inhibitor suppressed VEGF release from human gastric fibroblasts, and this could be reversed by addition of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) to the culture medium [61].

The decrease in the level of VEGF may be due to suppress in the production of VEGF expression in the vascular smooth cells, epithelial cells and fibroblasts of stomach. The alteration in the VEGF expression may be due to the disturbances in the activity of phosphodiesterase, intracellular cyclic adenosine monophosphate (cAMP), von willebrand factor (vWF) level (as a reflection of angiogenesis) and p38 mitogen activated protein kinase (MAPK) activation [64].

Moreover, treatment with conventional nonsteroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors at doses that delay ulcer healing in rats was found to alter the ratios of pro- and antiangiogenic factors (VEGF and endostatin, respectively) in serum. COX-2-derived prostaglandins can stimulate the release of VEGF [61], whereas NSAID can interfere with downstream proangiogenic signaling of VEGF [65].

Ranitidine used as a standard antiulcer drug in this work and showed antiulcer activity by decreasing macroscopic ulcer score, gastric acid secretion and gastric production of mucosal non-protein sulfhydryl group (Table 1). Ranitidine has an ability to antagonize the binding of histamine to the H<sub>2</sub>-receptor on the parietal cells. Therefore, it can counter the effect of aspirin on acid secretion [66].

As shown in the current results (Tables 2 & 3) ranitidine treatment significantly ( $p < 0.05$ ) reverted the aspirin-induced changes in TOC, TAC and MDA along with MPO. This significant reduction in MDA levels along with significant increase in TAC level suggested the decrease in the level of lipid peroxidation and total oxidant capacity by the treatment with ranitidine. Moreover, ranitidine, an antisecretory drug, has often been reported to possess antioxidant and immunosuppressive actions, which may be responsible for its antiulcerogenic activity [67]. In addition, ranitidine caused significant ( $p < 0.05$ ) decrease in neutrophils infiltration which could represent a reduction in the source of ROS during the healing process.

The anti-ulcerative effects of ginger have previously been investigated in experimental gastric ulcer models [68]. According to data recorded in tables (1, 2 & 3), a marked correction was occurred in all studied parameters after the ulcerative rats were treated with ginger depending on the periods of treatment (1 & 2 weeks). These data may be attributed to the ability of ginger to increase the mucosal blood flow, stimulate secretion of mucus and bicarbonate in the stomach and potentiate the defense factors [3]. These results are in parallel with that obtained by Wang et al. [20]. The last authors reported that the administration of ginger to rats suffered from gastric ulcer led to improvement in the volume of gastric juice and acid production induced by aspirin and decrease the level of lipid peroxidation. The authors attributed these results to powerful antioxidant capacity and the pharmacodynamics as well as the pharmacokinetics properties of ginger oils.

Ginger has been shown to inhibit lipid peroxidation by scavenging superoxide and hydroxyl radicals which may be the probable mechanism for gastric mucosal protection [26]. The major constituents of ginger include volatile oils, oleoresin (gingerol), linoleic acid and trace elements such as magnesium, phosphorus and potassium. 6-Gingesulfonic acid is another compound identified in ginger extracts. By increasing the mucosal resistance or by potentiating defensive factors, 6-gingesulfonic acid has been shown to protect gastric mucosa from offensive factors which might have contributed to gastric mucosal protection [69].

In other studies, the pungent phenolic constituent of ginger, [6]-gingerol, inhibited lipopolysaccharide-induced iNOS expression and production of NO and other RNS in macrophages and blocked peroxynitrite-induced oxidation and nitration reactions in vitro [70]. These results suggested that [6]-gingerol is a potent inhibitor of NO synthesis and also an effective protector against peroxynitrite-mediated damage.

Anti-inflammatory activities of silica gel chromatography fractions of ginger have also been tested using an in vitro PGE<sub>2</sub> assay. Results of Yamahara & Huang [70] and Tyler [71] showed that most of the fractions containing gingerols and/or gingerol derivatives were excellent inhibitors of LPS-induced PGE<sub>2</sub> production.

Curcumin, a substance rich in phenolics, is known to possess antioxidant properties [72]. From tables (1-3), data in this investigation demonstrated that curcumin administration prevented the ulcerogenic effect of aspirin in rats depending on the period of treatment (1 & 2 weeks). It has been reported that curcumin can decrease gastric injury by preventing the peroxidase inactivation effect of indomethacin and scavenging reactive oxygen production by certain enzymes [73].

Furthermore, curcumin directly accelerates ulcer healing in a chronic gastric ulcer model induced by acetic acid in rats via inhibition of gastric acid secretion and its anti-inflammatory activity against prostaglandin production, iNOS and TNF- $\alpha$  formation [74]. In addition, curcumin acts as gastroprotectant against irritants by increasing mucin secretion in rabbits. It also decreases indomethacin induced increment in the luminal acid level, which is beneficial to prevent acid-induced aggravation of ulcer by its potent antioxidant activity through scavenging of reactive oxygen species (ROS) and protection of gastric peroxidase [75].

Curcumin can effectively block indomethacin-induced increased LPO, thiol depletion, peroxidase inactivation and overproduction of  $\cdot\text{OH}$  to prevent ROS-mediated gastric ulcers [72]. In another study, curcumin at doses 20, 40 and 80 mg/kg exerted its antiulcer activity not only by affecting oxidative stress and total antioxidant capacity, but also by inhibiting IL-6 secretion and preventing apoptosis in a pylorus-ligated model [76].

It was published that curcumin is an anti-inflammatory substance, with an inhibitory effect on transcription factor NF- $\kappa\text{B}$  activation. NF- $\kappa\text{B}$  is required for the expression of many genes linked with the host immune response, such as TNF- $\alpha$ , IL-1 $\beta$ , and iNOS [77]. Cytoplasmic NF- $\kappa\text{B}$  is complexed with its inhibitor I $\kappa\text{B}$  and is therefore, inactive. The cytokine-mediated activation of NF- $\kappa\text{B}$  requires activation of various kinases, which ultimately leads to the phosphorylation and degradation of I $\kappa\text{B}$ . Several beneficial effects of curcumin are consistent with its ability to inhibit the activity of NF- $\kappa\text{B}$  [78]. Moreover, Singh & Aggarwal [77] observed that curcumin inhibited NF- $\kappa\text{B}$  activation pathway after the convergence of various stimuli mediated by protein tyrosine kinase, protein kinase and ubiquitin conjugation enzymes, but before the phosphorylation and subsequent release of I $\kappa\text{B}$  complexed to NF- $\kappa\text{B}$ . Also, Jobin et al. [79] examined the modulatory potential of curcumin on NF- $\kappa\text{B}$  signaling pathways and found that curcumin prevented phosphorylation of I $\kappa\text{B}$  by inhibiting the activation of I $\kappa\text{B}$ -kinase (IKKs).

Co-administration of ginger and curcumin in the presence of ranitidine to the ulcerogenic rats provided a marked correction effects on all studied parameters. The mixture brought the levels of all parameters closer to normal levels, than observed in RAN. These results may be due to the synergistic effects of them to correct and repair the damage occur in gastric of rats.

In conclusion, gastric ulcer is a multifactorial disease that has become a real socio-economic burden and opposes a great challenge in its treatment. Usage of medications designed for treatment of gastric ulcer is faced by several drawback such as limited effectiveness, numerous side effects and the cost of gastric ulcer medications. Herbal compounds can provide an alternative preventive means for gastric ulcer as they are safer, cheaper with limited side effects. Thus, ginger or curcumin has a great potential to be used as a gastro-protective drug in combination with other drugs or alone. Its cytoprotective effect was due to its ability to maintain tissue and cellular integrity in the face of mucosal damage induced by aspirin. As they say prevention is better than cure. So, more investigations are definitely still needed.

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