PHYSIOLOGICAL IMPACTS OF CHITOSAN SINGLY OR IN CONJUGATION WITH H2 RECEPTOR ANTAGONIST DRUG DURING NSAID THERAPY IN RATS

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ABSTRACT
Aspirin is a potent nonsteroidal anti-inflammatory drug (NSAID) that is used for the treatment of rheumatoid arthritis and related diseases as well as the prevention of cardiovascular thrombotic diseases. The aim of the present study is to explore how Chitosan alleviates gastric inflammation induced by Aspirin in rats and investigate whether Chitosan singly or in-conjugated with Ranitidine could be more effective. Fifty four healthy female rats were divided into nine groups. Aspirin by two doses (7 or 27 mg/ kg) combined administration with Ranitidine (27 mg/kg) as reference drug or Chitosan 2% or conjugate of Chitosan with Ranitidine by the same concentration via oral route for ten days. Chitosan singly produced significant effects on gastric acidity, ulcer index, Mucosal IL-1β, TNF-α, NO, MPO, PSH (P>0.05) and some serum parameters against Aspirin treatments. As well as, conjugated treatments showed the highest degrees of normalization against Aspirin or Ranitidine treatments in most tested parameters. So, the current study give the evidence that Chitosan could alleviate gastric inflammation in rats and strongly suggested to be a potential antiulcer drug equivalent to Ranitidine. Collectively, Therapy with conjugation of Chitosan and Ranitidine is the better choice than monotherapy with Ranitidine or Chitosan against the selected doses of Aspirin.

KEYWORDS: Chitosan – Ranitidine- gastroprotective parameters-immuno modulatory cytokines-mucosal myeloperoxidase.
INTRODUCTION
Chitosan, the linear polymer of D-glucosamine and N-acetyl-D-glucosamine in \( \beta \) linkage, is obtained by deacetylation of chitin. It has been applied in food, specific physiochemical and biocompatible properties.\(^{1,2}\) Chitosan has attracted increasing attention due to its non-toxicity and superior biocompatibility. Several reports have described the effects of Chitosan on growth\(^{3,4}\), immunity\(^{5}\), oxidative stress\(^{6}\), antimicrobial\(^{7}\) hypolipidemic\(^{8}\), and accelerated wound healing effect\(^{9}\). Also, it has been particularly anti-inflammatory activities both in vitro\(^{10,11}\) and in vivo.\(^{12-14}\) It has been shown to have specific immunomodulatory effects: i.e Chitosan has a significant enhancing effect on the cellular and humoral immune function\(^{15}\) and could stimulate macrophages in the rats and increase the nitric oxide (NO), interleukin-1\(\beta\) (IL-1\(\beta\)) and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) secretion.\(^{16}\) Recently, much interest has been focused on the gastroprotective and ulcer healing promoting action of Chitosan. The balance between proinflammatory and anti-inflammatory cytokines plays an important role in various diseases. An imbalance in favour of the proinflammatory cytokines has been implicated in the pathogenesis of different diseases including ulcerative colitis.\(^{17}\) Non-steroidal anti-inflammatory drugs play important roles in the genesis of gastric mucosal damage, and its subsequent development.\(^{18,19}\) It has been reported that many factors such as gastric acid and pepsin secretion, gastric microcirculation, prostaglandin E2 (PGE2) content\(^{20}\), proinflammatory cytokines interleukin I L-1 and tumor necrosis factor (TNF-\(\alpha\))\(^{21}\) as well as NO levels are involved in the gastrointestinal mucosal defense and contributed to the pathogenesis of mucosal damage.\(^{22}\) Aspirin is a potent nonsteroidal anti-inflammatory drug (NSAID) that is used for the treatment of rheumatoid arthritis and related diseases as well as the prevention of cardiovascular thrombotic diseases. Ranitidine hydrochloride is H2 receptors antagonist that used for the treatment of peptic ulcer diseases.\(^{23}\) The present study was aimed to investigate whether treatment with Chitosan singly or in-conjugate with Ranitidine could reduce gastric inflammation induced by Aspirin and to explore how Chitosan alleviates gastric inflammation in rats via testing some anti-inflammatory cytokines, some physiological and biochemical related parameters.

MATERIALS AND METHODS

**Drugs**

All materials and reagents were obtained from Sigma (Sigma Aldrich, Germany). Chitosan (Chito.) \( \geq 85\% \) deacetylated, molwt \( \sim 600000 \) molwt; \( \sim 400 \) m Pa.s, 1 % in acetic acid at 20 \( {^\circ} \)C). Ranitidine (Ranit.) was used as a reference antiulcer drug and Aspirin (Asp.;
Acetyl salicylic acid) was chosen as NSAIDs, they were obtained from the local pharmacy. The drugs were suspended in dist. water and orally administered to the rats at a dosage of 300 mg/kg body weight/day calculated as 5.4 mg/200 g body weight rat for Ranitidine\textsuperscript{[24]}, while Aspirin was administered daily at therapeutic doses of 75 & 300 mg as recommended doses for human equivalent to 1.4 & 5.4 mg/200 g body weight.\textsuperscript{[25]} Chitosan was orally administered at 2\%.\textsuperscript{[26]}

**Animals**

Healthy adult female white rats (6–8 weeks old), each weight between 200–220 g, were obtained from the animal house at the National Organization for Drug Control and Research (NODCAR). They were housed in wire cages with natural ventilation and illumination and allowed free water and standard diet for 10 days before beginning of the experiment. The rats were deprived of food for 24 h before the experiment and only allowed access to water 2 h prior to the experiment. They were randomly divided into nine groups, six rats per group, and individually housed in cages with a wide-mesh wire bottom to prevent coprophagy and dominancy. One hour before treatments start single dose of absolute ethanol (1 ml/rat) was orally administered to all groups. Group 1 serves as control. Groups 2, 4, 6, 8 received oral daily low dose of Aspirin (7 mg/kg.p.o.) for ten days, while groups 3, 5, 7, 9 received daily high dose of Aspirin (27 mg/body weight) combined administration with Ranitidine (27 mg/kg) as reference drug or Chitosan 2\% or conjugate of Chitosan and Ranitidine by the same concentration for ten days. Throughout the experiment, all procedures, animals and the experimental protocols were approved by the Institutional Ethics Committee at NODCAR; and were carried out according to the criteria outlined in the “guide for the care and use of laboratory animals”.

**Visual gastric lesions and gastric lesion score**

At the end of experimental period, all animals were sacrificed and stomachs were excised along its greater curvature. After rinsing with normal saline, the mucosa was examined for the presence of petechiae or frank hemorrhage lesions. Lesions were scored according to their length (a score of 5 for lesions with length between 1 and 3 mm; a score of 10 for lesions greater than 3 mm). The sums of total scores were used for comparison. All treatment groups were coded to prevent measurement bias.
**Measurement of Gastric Juice Acidity**

The animals were sacrificed, and their stomachs were removed. The stomach contents were collected, measured, centrifuged, and subjected to analysis for titratable acidity against 0.01 N NaOH to pH 7.

**Measurement of mucosal TNF-α, IL-1β and PGE2 levels**

One hundred milligrams of scraped mucosa was homogenized in 1.0 mL of ice-cold potassium phosphate buffer (pH 7.4). Aliquots of homogenate supernatants in phosphate buffer solution were obtained by centrifugation at 10 000 g for 10 min. Concentration of TNF-α, IL-1β and PGE2 in the supernatant of mucosal homogenates was determined by ELISA according to the manufacturer’s instructions using eBioscience kit, while Prostaglandin E2 was determined by ELISA kit from R&D Systems. The concentration of TNF-α, IL-1β and PGE2 was expressed as pictogram per milligram protein.

**Measurement of mucosal Nitric oxide level (NO)**

The nitrite/nitrate content, indicative of NO production by iNOS, was monitored by the Greiss reagent assay\[27\], Gastric mucosal tissue 10 % in phosphate buffer pH 7.4 were mixed with Greiss reagent, consisting of 1% sulfanilamide in 5% phosphoric acid and 0.1% N-(1-napthyl) ethylenediaminedihydrochloride (1:1 ratio [vol/vol]), and incubated for 20 min. The change in absorbance was monitored at 562 nm (standard curve, 0 to 200μmol sodium nitrite).

**Determination of mucosal Myeloperoxidase activity (MPO)**

Gastric mucosal myeloperoxidase concentration was assayed to quantify the degree of neutrophil infiltration. MPO activity was measured according to the modified method of Bradley et al.\[28]\ The homogenized samples were frozen and thawed three times and centrifuged at 1500 × g for 10 min at 4°C. MPO activity in the supernatant was determined by adding 100 μl of the supernatant to 1.9 ml of 10 mM phosphate buffer (pH 6.0) and 1 ml of 1.5 mM O-dianisidine hydrochloride containing 0.0005% (wt/vol) hydrogen peroxide. The changes in absorbance at 460 nm of each sample were recorded in a UV–vis spectrophotometer. One unit of MPO activity is defined as the amount that degrades 1 μmol of peroxide per minute at 25°C. The concentration of MPO was expressed as unit per milligram protein.
**Determination of mucosal Thiol groups (PSH) and Glutathione STransferase (GST):**

The protein thiols (PSH) content was measured by the methods described by Koster, et al.$^{[29]}$

Protein SH-group reacts with 5, 5 dithiobis (2-nitrobenzoic acid) DTNB-Ellman's reagent) at pH7.4 to give a colored product 5-thio-2-nitrobenzoic acid which was measured colorimetrically at 412 nm. Glutathione-S-transferase (GST) activity was determined by the method described by Habig et al.$^{[30]}$ 1-Chloro-2, 4-dinitrobenzene (CDNB)–GSH conjugate formed at 37°C was spectrophotometrically assayed at 340 nm.

**Determination of serum TNF-α, IL-1β, PGE2 and PSH levels:**

Blood samples vials were centrifuged at 1000×g for 10 min at 4°C. The levels of serum IL-1β and TNF-α for rats were determined quantitatively by enzyme-linked immunosorbent assay (ELISA) using eBioscience kit. While Prostaglandin E2 was determined by ELISA kit from R&D Systems.

**Statistical analysis**

All values are reported as the mean ± S.E. and were analyzed by one-way ANOVA followed by post-hoc test for multiple comparisons (Duncan and Dunnett t (2-sided)), using the Statistical Package for the Social Sciences software (SPSS 17). The differences between means were considered statistically significant when the P value was less than 0.05.

**RESULTS**

Administration of Aspirin at 7mg/kg didn't induce any visual observations more than that seen among control rats, while administration of 27 mg resulted in the appearance of linear and dotted erosions in the gastric mucosa of treated rats. Pretreatment by Ranitidine; Chitosan, or the conjugate were significantly (P<0.05) decrease the ulcer index by 50.9%; 58.19% and 74.55% respectively versus the corresponding Aspirin group (Table 1).

**Table 1: Effect of oral repeated dose of Chitosan, Ranitidine and Their conjugated on ulcer index and total acidity against Aspirin therapy.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total acidity</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31.33±0.49</td>
<td>1.667±0.67</td>
</tr>
<tr>
<td>Asp-low</td>
<td>38.70±1.23</td>
<td>1.667±0.67</td>
</tr>
<tr>
<td>Asp-high</td>
<td>206.67±5.58</td>
<td>9.167±0.83</td>
</tr>
<tr>
<td>Ranit+Asp-low</td>
<td>28.60±0.73</td>
<td>1.667±0.67</td>
</tr>
<tr>
<td>Ranit+Asp-high</td>
<td>84.56±2.32</td>
<td>4.500±1.36</td>
</tr>
<tr>
<td>Chito+Asp-low</td>
<td>26.81±0.61</td>
<td>1.667±0.67</td>
</tr>
</tbody>
</table>
Results were expressed as mean ±SE for each 6 rats.

* Significant difference against control at P<0.05
Groups have the same letter mean non significant at P < 0.05
Groups have different letters means significant at P < 0.05

Slight changes in total acidy output reflecting non-significant changes among all the treated groups in response to repeated daily dose of Aspirin (7mg/kg), while high dose(27mg/kg) induced significant increase in total acidy out put more than six folded comparing to control at P<0.05. Pretreatment with ranitidine, chitosan or their conjugation attenuated this increase, where the maximum reduction were observed with the conjugation by 62.93% from the corresponding Aspirin dose (Table1).

Marked significant reduction in mucosal PGE2 levels were observed to both Aspirin doses comparing to control one. Pretreatment with Chitosan, Ranitidine showed slight to moderate significant improvement in PGE2 level. Their conjugate showed the highest improvement about three folds than the low dose Aspirin at P<0.05. On the other hand, these supplementation failed to produce any significant change in mucosal PGE2 levels post high dose Aspirin. In contrast, Aspirin administration induced significant increase in serum PGE2 levels. Graduated significant improvement in its level were recorded by pretreatment with Chitosan, Ranitidine and their conjugate as shown in Figure (1) and Table (2).

Table 2: Effect of Ranitidine, Chitosan or Their conjugate on serum TNF-α, IL-1β, and PGE2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum TNF-α</th>
<th>Serum IL-1β</th>
<th>Serum PGE2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pg/ml</td>
<td>Pg/ml</td>
<td>Pg/ml</td>
</tr>
<tr>
<td>Control</td>
<td>412.83±25.539</td>
<td>217.167±8.167</td>
<td>190.82±11.731</td>
</tr>
<tr>
<td>Asp-low</td>
<td>1459.33±89.905</td>
<td>1789.00±40.171</td>
<td>860.67±19.243</td>
</tr>
<tr>
<td>Asp-high</td>
<td>1945.67±30.494</td>
<td>2301.33±132.655</td>
<td>1084.33±46.456</td>
</tr>
<tr>
<td>Ranit+Asp-low</td>
<td>778.00±21.320</td>
<td>905.33±17.074</td>
<td>579.67±7.953</td>
</tr>
<tr>
<td>Ranit+Asp-high</td>
<td>1702.00±27.425</td>
<td>1256.00±24.503</td>
<td>498.67±23.293</td>
</tr>
<tr>
<td>Chito+Asp-low</td>
<td>833.00±19.047</td>
<td>1021.33±34.92</td>
<td>784.00±7.127</td>
</tr>
<tr>
<td>Chito +Asp-high</td>
<td>1548.67±34.443</td>
<td>1526.33±44.423</td>
<td>715.00±25.941</td>
</tr>
<tr>
<td>Ranit+ Chito+Asp-low</td>
<td>301.33±16.461</td>
<td>741.67±29.29</td>
<td>192.33±5.937</td>
</tr>
<tr>
<td>Ranit+ Chito+ Asp-high</td>
<td>655.33±18.491</td>
<td>945.00±34.155</td>
<td>341.33±20.958</td>
</tr>
</tbody>
</table>
Results were expressed as mean ±SE for each 6 rats

* Significant difference against control at P<0.0
Groups have the same letter mean non-significant at P < 0.05
Groups have different letters mean significant at P < 0.05

**Figure 1:** Effect of Ranitidine, Chitosan or Their conjugate on mucosal PGE2

In Fig (2&3), mucosal concentrations of TNF-α, IL-1β were significantly increased after administration of low and high doses of Aspirin versus the control respectively. The increment in TNF-α, IL-1β were suppressed by pretreatment with Ranitidine; Chitosan and their conjugate, reflecting different patterns of significant changes versus the control and the corresponding Aspirin ones at P<0.05. Ranitidine conjugate with Chitosan revealed the best gastroprotective treatments against the selected Aspirin doses on TNF-α and IL-1β at P<0.05. Also, Serum TNF-α and IL-1β level showed the same patterns of changes estimated by the mucosal one as shown in Table (2).

**Figure 2:** Effect of Ranitidine, Chitosan or Their conjugate on mucosal IL-1β.
Administration of Aspirin at 7 and 27 mg/kg for ten days resulted in significant elevation in NO levels among treated animals against untreated ones at P<0.05. Pretreatment with Chitosan singly or in-conjugated with Ranitidine could restore its level to the control one bearing non-significant signs at P<0.05. (Fig 4). Significant induction in mucosal MPO activity was estimated after treatment by the selected doses of Aspirin by 52.76% and 55.85% than the control value at P<0.05. These inductions were suppressed significantly under the control value by pretreatment with Ranitidine, Chitosan and their conjugate (Fig 5).
While administration of Aspirin at 7mg/kg didn’t induce any significant change in mucosal protein thiol level in treated animals; administration of 27 mg significantly lowered its level by 53.23% from the control rats. Pretreatment by Ranitidine induced slight non-significant increase in protein thiol level against the control and low dose of Aspirin at P<0.05. Significant enhancement in mucosal thiol level were estimated after pretreatment by Chitosan singly or in-conjugation with Ranitidine(Fig 6).

On the other hand, the selected doses of Aspirin induced marked significant elevation in serum thiol levels. Meanwhile pretreatment with Ranitidine restored it to the normal level; pretreatment by Chitosan singly or in-conjugation with Ranitidine could restore that increase among Aspirin low dose groups only comparing to control untreated group(Table3).Marked significant inhibition in mucosal and serum GST activities by low and high Aspirin doses.
were observed comparing to control one. Pretreatment with Ranitidine; Chitosan or their conjugate showed some trials for improvement but couldn't normalized GST activities. Fig(7) & Table (3).

Table 3: Effect of Ranitidine, Chitosan or Their conjugate on serum TP, PSH and GST

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum TP</th>
<th>Serum PSH</th>
<th>Serum GST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.831&lt;sup&gt;cd&lt;/sup&gt;±0.106</td>
<td>2371.568&lt;sup&gt;a&lt;/sup&gt;±167.314</td>
<td>255.204&lt;sup&gt;sc&lt;/sup&gt;±1.904</td>
</tr>
<tr>
<td>Asp-low</td>
<td>6.375&lt;sup&gt;ab&lt;/sup&gt;±0.147</td>
<td>3906.862&lt;sup&gt;sc&lt;/sup&gt;±203.493</td>
<td>179.688&lt;sup&gt;sc&lt;/sup&gt;±8.862</td>
</tr>
<tr>
<td>Asp-high</td>
<td>5.858&lt;sup&gt;a&lt;/sup&gt;±0.385</td>
<td>3629.901&lt;sup&gt;a&lt;/sup&gt;±68.994</td>
<td>158.626&lt;sup&gt;bc&lt;/sup&gt;±10.695</td>
</tr>
<tr>
<td>Ranit+Asp-low</td>
<td>6.141&lt;sup&gt;ab&lt;/sup&gt;±0.255</td>
<td>2632.348&lt;sup&gt;c&lt;/sup&gt;±119.318</td>
<td>219.679&lt;sup&gt;d&lt;/sup&gt;±14.053</td>
</tr>
<tr>
<td>Ranit+Asp-high</td>
<td>8.657&lt;sup&gt;d&lt;/sup&gt;±0.195</td>
<td>2709.545&lt;sup&gt;ab&lt;/sup&gt;±80.588</td>
<td>173.053&lt;sup&gt;c&lt;/sup&gt;±17.087</td>
</tr>
<tr>
<td>Chito+Asp-low</td>
<td>6.603&lt;sup&gt;ab&lt;/sup&gt;±0.157</td>
<td>2492.156&lt;sup&gt;c&lt;/sup&gt;±90.335</td>
<td>140.620&lt;sup&gt;ab&lt;/sup&gt;±6.99</td>
</tr>
<tr>
<td>Chito+Asp-high</td>
<td>8.545&lt;sup&gt;c&lt;/sup&gt;±0.237</td>
<td>3624.999&lt;sup&gt;c&lt;/sup&gt;±67.551</td>
<td>120.757&lt;sup&gt;d&lt;/sup&gt;±5.738</td>
</tr>
<tr>
<td>Ranit+ Chito+Asp-low</td>
<td>7.080&lt;sup&gt;bc&lt;/sup&gt;±0.607</td>
<td>2377.451&lt;sup&gt;a&lt;/sup&gt;±145.639</td>
<td>166.666&lt;sup&gt;ab&lt;/sup&gt;±9.317</td>
</tr>
<tr>
<td>Ranit+ Chito+Asp-high</td>
<td>7.091&lt;sup&gt;bc&lt;/sup&gt;±0.58</td>
<td>3066.176&lt;sup&gt;b&lt;/sup&gt;±139.616</td>
<td>156.250&lt;sup&gt;bc&lt;/sup&gt;±6.658</td>
</tr>
</tbody>
</table>

Results were expressed as mean ±SE for each 6 rats

* Significant difference against control at P<0.0

Groups have the same letter mean non-significant at P < 0.05

Groups have different letters means significant at P < 0.05

![Mucosal GST](image)

**Figure 7: Effect of Ranitidine, Chitosan or Their conjugate on mucosal GST**

Significant reduction in serum total protein content was estimated after treatment by Aspirin doses by -18.59% and -25.19% than the control value at P<0.05. While supplementations with Ranitidine and Chitosan singly induced moderate enhancement in total protein content
against Aspirin high dose (10.54% and 9.12% at P<0.05; respectively); it couldn't normalized total protein content induced by low Aspirin dose as shown in Table (3).

DISCUSSION

The link between non-steroidal anti-inflammatory drugs (NSAIDs) and the presence of upper gastrointestinal complications has been established.[31] Currently, the use of NSAIDs is the second most common cause of ulcers and the rate of NSAIDs induced ulcers is increasing, which presumably cause ulcers by disrupting mucosal defense and repair mechanisms.[32]

NSAIDs interfere with the cyclooxygenase (COX) pathways which lead to the production of prostanoids (prostaglandins, prostacycline, and thromboxane). Cyclooxygenase (COX) catalyzes the conversion of arachidonic acid to prostaglandin H2, the precursor of a variety of biological active mediators such as prostaglandin E2, prostacyclin, and thromboxane A2.[33]

Two isoforms of COX have been identified: COX-1 and COX-2. COX-1 is constitutively present in most tissues and seems to catalyze the synthesis of prostaglandins for normal physiological functions.[34] High amount of PGE2 derived from COX- 2 induced by many pro-inflammatory mediators including tumor necrosis factor-α (TNF-α) interleukin-1β (IL-1β), and lipopolysaccharide (LPS) has been implicated in the pathogenesis of sepsis and inflammation.[35,36] It has been reported that PGE2 prevents gastric mucosal damage by Aspirin in human beings and animals.[37] The present protective effect of Chitosan singly or in-conjugation has shown to be dose dependent as it couldn't countered the inhibition in mucosal prostaglandin synthesis induce by high dose of Aspirin. Therefore, inhibition of mucosal lesions by Chitosan can be attributed to prostaglandin synthesis against low doses of Aspirin only.

In current experiment, local gastric injury is dose dependent, since administration of Aspirin (7 mg/kg b.wt) showed no visual gastric lesions and not significantly changed gastric acidity output. Ranitidine is commonly recommended for the treatment of peptic ulcer diseases.[38] So, we hypothesize that Chitosan singly or in conjugation with Ranitidine could attenuate Aspirin induce gastric mucosal injury. The antiulcer effect can be attributed to the mucoadhesive properties of the conjugation of Chitosan with Ranitidine as reported in our previous work using the same conjugation against ethanol induced gastric ulcer. Chitosan is a basic polysaccharide and it is easy dissolved in stomach by gastric acid, forming viscous gel like those of mucus glycoprotein which may protect gastric mucosa form acid (H+), also Chitosan having an amino group that may neutralize H+ in gastric juice and H+ back-
diffused into the mucosa\textsuperscript{[39]}, or might be recognized by the immune system, which in turn activate the immune response pathways and enhanced cell mediated immune response especially proinflammatory cytokines TNF-\(\alpha\) and IL-1\(\beta\).\textsuperscript{[40]} Chou et al.\textsuperscript{[41]} supported our hypothesis and reported that chitosan could inhibit pro-inflammatory cytokines including TNF-\(\alpha\) and IL-1\(\beta\) but increase the inflammatory cytokines IL-10 generation resulting in an attenuation of the pro-inflammatory/anti-inflammatory cytokines ratio. Also, Yoon et al.\textsuperscript{[42]} confirmed that the recovery effect of chitosan on IL-6 and NO secretion might be induced via stimulus of TNF \(\alpha\) in lipopolysaccharide (LPS)-treated RAW 264.7 macrophage. These were in agreement with our results where TNF-\(\alpha\) and IL-1\(\beta\) showed significant decrease after administration of Chitosan alone or conjugated with Ranitidine. TNF-\(\alpha\) is a pro-inflammatory cytokine and has recently been shown to be a crucial mediator of NSAIDs-induced gastric mucosal injury.\textsuperscript{[43]} Also, TNF-\(\alpha\) is a cytokine that strongly stimulates neutrophil adherence by inducing synthesis and expression of adhesion molecules on endothelial cells and neutrophil.\textsuperscript{[44]} TNF-\(\alpha\) augments neutrophil-derived superoxide generation and upregulates the expression of adhesion molecules on neutrophil and endothelium, and stimulates production of IL-1\(\beta\), leading to neutrophil accumulation.\textsuperscript{[44]} Furthermore, studies on experimental models have shown that intravenous administration of TNF-\(\alpha\) produces extensive neutrophil infiltration within the microvasculature of the digestive tract. It is well known that, NO plays an important role in regulating acid secretion and maintaining the integrity of gastric mucosa against hyperacidity or exposure to ulcerogens.\textsuperscript{[45,46]}

The ulcerogenic effects of Aspirin is accompanied by impaired gastric blood flow and vascular injury as well as increased production of NO due to the overexpression of iNOS.\textsuperscript{[47]} NO has been recognize as a basic mediator in the regulation of gastric mucosal microcirculation.\textsuperscript{[48,49]} It has been demonstrated that NO generated from endothelial NO synthase (eNOS) plays an important role in gastrointestinal defense, whereas NO generated from inducible NO synthase (iNOS) participates in ulcer formation through the production of oxygen derived free radical and their cytotoxic action.\textsuperscript{[47,48]} We observed that Chitosan conjugate with Ranitidine was more potent than Ranitidine in reducing acid secretion and improving gastric NO levels. This may be explained by considering the effects of Chitosan in increasing eNOS and inhibiting iNOS for optimal regulation of NO production.\textsuperscript{[50]} Protein SH (PSH) are important for the maintenance of protein structure and functions in the gastric mucosa, e.g., their modifications via oxidation, alkylation and conjugation may change the functions of mucosal parenchymal or endothelial cells.\textsuperscript{[51]} Concerning the role of PSH in the
pathogenesis of Aspirin induce gastric mucosal damage, the current data clearly illustrated depletion of mucosal total thiols after Aspirin high dose administrating and complete recovery by combined treatments which could be contributed to the antioxidant properties of Ranitidine and Chitosan as mentioned before.\(^{[52]}\)

Although glutathione S transferase is known as an important detoxication enzyme acting on endogenous and exogenous compounds.\(^{[53]}\) The current experimental data showed unexpected impacts for this enzyme since administration of Aspirin doses singly significantly reduced its activity which could contributed to its substrate specificities. However, a study by Wu and Mathews\(^{[54]}\) support our explanation and stated that Glutathione transferase is among the enzymes that have been found to be inhibited by indomethacin. Also, Oakley\(^{[53]}\) recommended to register changes in the levels of GST isoenzymes, which have different though partly overlapping and its isoenzymes have a tissue specific distribution. These results are in line with previous reports that demonstrated decrease in GST following acute administration of alcohol to rats.\(^{[55,56]}\)

Significantly decrease serum total protein that could be due to liver function impairment and decreased hepatic protein synthesis as supported by previous studies on ethanol induced gastric ulcer.\(^{[57,58]}\) However, increased in serum protein level among rats administrated ranitidine or Chitosan singly against high dose Aspirin would refer to the immunomodulatory and-inflammatory properties of both Ranitidine and Chitosan. Collectively, the current study give the evidence that chitosan could alleviate gastric inflammation in rats by its capability to increase gastro-protective parameters and reduced the aggressive factors optimizing the balance between them and strongly suggested Chitosan as a potential antiulcer drug equivalent to Ranitidine. Also, therapy by conjugation of Chitosan with Ranitidine is the better choice than monotherapy with Ranitidine or Chitosan against the selected doses of Aspirin.

**REFERENCE**


