Abstract. – Objectives: The current study was carried out to examine the influence of aspirin (400 mg/kg of body weight) and gum acacia (one g/day) and their combination on pancreatic, intestinal mucosal enzymes, intestinal tissue iron and zinc after 21 days of treatment on experimental rats.

Materials and Methods: The treated rats were sacrificed and the pancreatic and intestinal lipase and amylase were measured photometrically. Moreover, zinc and iron level were determined using atomic absorption spectrometry. Intestinal sections were stained with hematoxylin and eosin.

Results: The results showed that treatment with aspirin caused a marked decrease in pancreatic lipase and amylase compared with that of the control group. This decrease in aspirin treated group was accompanied by significant increase in the intestinal amylase, lipase, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) compared with the control group. On the other hand, gum combined with aspirin caused a significant increase in pancreatic and intestinal lipase, amylase accompanied with significant increase in intestinal ALP and LDH compared with that of the control group. Concentration of Zn in wet weight of intestine showed a significant decrease in aspirin and aspirin combined with gum groups but it was increased significantly in gum acacia treated group. Histological analysis revealed intestinal epithelial damage in aspirin treated rats, this damage was not noticed with gum acacia treatment. Co-administration of gum in combination with aspirin indicated some changes of denuded intestinal mucosal cells compared with that of the control.

Conclusions: Gum acacia exhibited a protective property that can ameliorate the alterations induced hazardous effect of aspirin treatment.

Key Words: Aspirin, Gum acacia, Digestive Enzymes, Iron, Zinc.

Introduction

Salicylic acid, the active substance in plants used for thousands of years as medicaments, was well known and showed that it was effective in rheumatic fever since 1876. More recently sodium salicylate was also in use as a treatment for chronic rheumatoid arthritis and gout as well as an antiseptic compound. Many clinical trials have shown that aspirin given once a day in doses as low as 75 mg will help to prevent heart attacks or strokes. Smith and Willis showed that aspirin prevented the release of prostaglandins from aggregating human platelets and Ferreira et al. demonstrated that aspirin-like drugs blocked prostaglandin release from the perfused, isolated spleen of the dog. Vane proposed that all non-steroidal anti-inflammatory drugs (NSAIDs) act by inhibiting cyclooxygenases (COX), thereby reducing prostaglandin formation, providing a unifying explanation for their therapeutic actions and their side effects. One suggestion is that uncoupling of oxidative phosphorylation may be the biochemical mechanism underlying the "topical" toxicity of NSAIDs. Uncoupling of oxidative phosphorylation has been documented in vitro with some NSAIDs and in vivo in the case of aspirin.

Guar gum has been used to treat diabetes to curb the appetite. It also, has the ability to carry toxins out of the body. It should not be used by those who have had gastrointestinal surgery or colon disorder. Five grams per day of guar gum thrice daily with water at meal time for three weeks reduced total plasma cholesterol 26 mg/dl from 244 mg/dl, and reduced low density lipoprotein cholesterol by 25 mg/dl. Effect on high density lipoprotein cholesterol and very low density lipoprotein cholesterol was insignificant. Studies have shown that soluble fiber can enhance the in-
intestinal immune function (fiber, arabinogalactan). A large portion of the body’s immune system is localized to the gastrointestinal (GI) wall and in mesenteric lymph nodes. Bacteria form a protective layer and help regulate inflammation and immunity. Elimination of bacteria from the mouse GI tract by antibiotics results in significant immune response suppression, suggesting that intestinal bacteria play an important role in host defense. In an animal study, consumption of gum acacia stimulated intestinal and splenic immune system function. The effects of gum acacia consumption on cholesterol levels have been equivocal. Generally, the rabbit was the most sensitive species and the rat and mouse the least sensitive.

Intake of three grams of aspirin per day has been shown to decrease blood levels of zinc. Aspirin appeared to increase loss of zinc in the urine in this study, and the effect was noted beginning three days after starting aspirin. Therefore, it is important to identify and evaluate dietary factors that affect zinc absorption. With such knowledge, better advice may be given with regard to avoiding or limiting components with inhibitory effects on zinc absorption and choosing foods or dietary components that enhance zinc absorption. Furthermore, agriculture and food processing methods that reduce the content of inhibitors of zinc absorption may be developed and put into common use. In the present work we aimed to identify some of changes associated with aspirin administration in experimental animals and how the gum acacia can modify effectively these changes.

**Materials and Methods**

**Animals**

Twenty male Sprague-Dawley rats (four groups) weighing 200 g were used throughout these studies. Some rats were placed on diets containing 400 mg/kg body weight of aspirin per day as (first group), gum acacia one g per day as (second group) and aspirin followed by gum in the same doses (third group) for period of 21 days. Control rats were placed on normal diet and water ad libitum (fourth group). The local Ethical Committee approved the present study.

**Enzyme Determination**

Rats received aspirin (400 mg/kg), gum or their combination were decapitated after 24 hours of last treatment. Pancreas and jejunum (five cm long segment after duodenum) were immediately excised. Jejunum were flushed with ice cold 0.9% saline. The jejunal segments were then cut open longitudinally and mucosal scraped with microscopic slide. The pancreas and mucosal scrapings were homogenized in 0.9% saline. Homogenates were centrifuged at 3000 rpm for 10 minutes and the supernatant was used for enzymatic assays. Organellar specific marker enzymes were carried out as described previously. Alkaline phosphatase and lactate dehydrogenase enzymes activity are expressed in mmol h⁻¹ litre⁻¹. Amylase and lipase enzymes (BioMérieux, France) were measured photometrically using Beckman DU spectrophotometer (Beckman Coulter, CA, USA).

**Metals Determination**

Intestine slices were wet-ashed by HCl₂-HNO₃ method. The Zn and Fe (µg/g. tissue weight) were determined by atomic absorption spectrometry using Hitachi Z-8000 (Hitachi, Tokyo, Japan).

**Histological Analysis**

Jejunum was removed, fixed in aqueous Bouin’s solution for 24 hours; dehydrated in ascending series of ethyl alcohol (70%, 80%, 90% and 100%), then cleared in terpineol and embedded in paraffin wax. Paraffin sections (4-6 um thick) were prepared and stained with Hematoxylin and Eosin.

**Statistical Analysis**

Quantitative data were expressed as mean ± standard deviation (SD) and the results were tabulated and statistically analyzed using PASW 18 program for data management and calculations (SPSS, Chicago, IL, USA). Mann-Whitney test was for comparison purposes between two groups, whereas Kruskal-Wallis test was used to compare more than two groups. P values ≤ 0.05 were considered statistically significant.

**Results**

In the current study, the treatment of aspirin (400 mg/kg of body weight), gum (g/day) and gum in combination with aspirin for 21 days in male albino rats are recorded in Table I and Figures 1-6. Aspirin treatment led to significant de-
crease ($P < 0.001$) in pancreatic lipase and amylase enzymes. Pancreatic lipase and amylase in gum treated group and in aspirin combined with gum treated group revealed a significant increase ($P < 0.001$) compared with that of the control rats. Intestinal lipase, amylase, ALP and LDH recorded a significant increase ($P < 0.001$) in aspirin treated group and aspirin combined with gum treated group while they revealed a significant decrease ($P < 0.001$) in gum treated group compared to that of the control group. Zn concentration in wet weight tissue recorded a significant decrease ($P < 0.001$) in aspirin treated group and in aspirin combined with gum treated group but it increased significantly ($P < 0.001$) in case of gum treated group compared with that of the control group (Table I and Figures 1 to 3). On the other hand, the iron concentration was decreased significantly in aspirin treated group and increased significantly in gum treated group but it was increased insignificantly in aspirin combined with gum group versus the control group ($P < 0.001$, $P < 0.001$, and $P > 0.05$ respectively).

Light microscopy examination showed inflammatory changes in intestinal mucosa after 21 days from aspirin administration. However, aspirin in combination with gum induced a minor polymorphic infiltrate which became intense.

Table I. The results of different investigated enzymes in male albino rats in different groups treated with aspirin, gum and both compared to a control group. Results are expressed as means ± SD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Aspirin</th>
<th>Gum</th>
<th>Aspirin plus Gum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic lipase (U/L)</td>
<td>21.96 ± 0.929</td>
<td>15.34 ± 0.992</td>
<td>37.00 ± 2.149</td>
<td>28.02 ± 1.627</td>
</tr>
<tr>
<td>Pancreatic amylase (U/L)</td>
<td>44.1 ± 2.077</td>
<td>40.46 ± 0.709</td>
<td>51.2 ± 1.977</td>
<td>46.08 ± 2.04</td>
</tr>
<tr>
<td>Intestinal mucosal lipase (U/L)</td>
<td>35.6 ± 1.517</td>
<td>47.38 ± 2.013</td>
<td>26.56 ± 3.445</td>
<td>48.32 ± 6.071</td>
</tr>
<tr>
<td>Intestinal mucosal amylase (U/L)</td>
<td>11.2 ± 0.644</td>
<td>15.68 ± 0.55</td>
<td>8.46 ± 1.122</td>
<td>16.94 ± 1.479</td>
</tr>
<tr>
<td>ALP (mmol h-1 litre-1)</td>
<td>0.52 ± 0.068</td>
<td>1.9 ± 0.3</td>
<td>0.32 ± 0.057</td>
<td>0.74 ± 0.108</td>
</tr>
<tr>
<td>LDH (mmol h-1 litre-1)</td>
<td>57.8 ± 1.789</td>
<td>96.6 ± 4.393</td>
<td>38.6 ± 2.302</td>
<td>68.6 ± 2.302</td>
</tr>
<tr>
<td>Intestinal Zn (µg/g)</td>
<td>12.76 ± 0.913</td>
<td>7.42 ± 0.811</td>
<td>18.2 ± 1.681</td>
<td>9.4 ± 0.762</td>
</tr>
<tr>
<td>Intestinal Fe (µg/g)</td>
<td>8.34 ± 0.853</td>
<td>4.58 ± 0.661</td>
<td>12.06 ± 1.214</td>
<td>9.18 ± 0.581</td>
</tr>
</tbody>
</table>

Figure 1. Levels of pancreatic and intestinal enzymes in male albino rats in different groups treated with aspirin, gum and both compared to a control group.
around the focal erosions. Whereas animals receiving gum showed histological profile comparable to that of the control (Figures 4 to 6).

**Discussion**

Here, we showed directly by enzyme technique that intestinal mucosa and brush border are affected within 21 days of aspirin administration. We expand previous *in vitro* studies of aspirin on mitochondrial energy production, showing that the uncoupling of oxidative phosphorylation and/or inhibition of electron transport is related to the acidic nature of aspirin and that rendering aspirin non-acidic and ineffective as proton translocators coincides with their improved gastrointestinal tolerability. After aspirin (400 mg/kg) ingestion, the most noticeable changes in subcellular organelle marker enzyme activity...
were found in the jejunum\textsuperscript{27}. There was a significant increase in the activities of the brush border marker enzymes after 21 days of treatment\textsuperscript{28,29}.

Although the pathogenic events of aspirin-induced gastrointestinal damage are controversial, there is a consensus that there is an important "topical" component of the damage both in the stomach and small intestine which is independent of aspirin action to inhibit cyclooxygenases\textsuperscript{30}. The precise nature of the "topical" phase of damage is uncertain. Firstly, one suggestion is that NSAIDs have a detergent-like action which disrupts mucus gel\textsuperscript{31} and/or cell membrane integrity\textsuperscript{32}. The decreased activity of the brush border enzyme alkaline phosphatase is consistent with this suggestion. Secondly, acidic NSAIDs may concentrate in the mucosa\textsuperscript{33} but how this causes damage is uncertain. The subcellular organelle enzyme studies suggest that mitochondria are affected following oral indomethacin. Mitochondrial enzyme activities were all significantly increased, which is, however, an unusual response to damage and not readily explained. One possibility is that the substrate access to the membrane bound enzymes is facilitated via increased mitochondrial membrane permeability caused by the effect of indomethacin on mitochondrial energy production. The light microscopy studies showed dose dependent \textit{in vivo} mitochondrial changes following indomethacin administration which are suggestive of uncoupling and/or inhibition of electron transport. The \textit{in vitro} studies show that all the acidic aspirin uncoupled oxidative phosphorylation at concentrations which are likely to be achieved within intestinal epithelial cells following ingestion of aspirin, whereas paracetamol, an analgesic without gastrointestinal toxicity, and the non-acidic aspirin which have an improved gastrointestinal tolerability profile, did not. These findings expand on earlier studies which demonstrated that some aspirin uncoupled oxidative phosphorylation\textsuperscript{34} and indirect evidence that suggested that uncoupling of oxidative phosphorylation or inhibition of electron transport may play a role in their intestinal toxicity\textsuperscript{35}. Furthermore, other studies showed that the uncoupling property of NSAIDs resides within their carboxylic or enolic acid groups (along with their lipid solubility characteristics). However, the doses of aspirin used in this study, although an order of magnitude greater than that required to inhibit cyclooxygenases\textsuperscript{36} are within the range conventionally used to investigate the pathogenesis of aspirin induced gastrointestinal toxicity.
The “topical” effect of aspirin is most pronounced at the site of drug absorption following ingestion. Absorbed aspirin are largely (over 99%) bound to albumin, so that an effective concentration for mitochondrial uncoupling may not be reached in other tissues. It was suggested that the basis for reduced toxicity was due to the release of soluble fibers come from the storage materials of the gum used as pectins, mucilages. These soluble fibers attract water and turns to gel during digestion, rendering the molecule from the activity of oxidative phosphorylation. Also, in this context it is interesting that the esterification of aspirin is associated with strikingly improved short term gastric tolerability in the rat. Collectively, these findings support an important pathogenic role of the “topical” action of aspirin in intestinal damage.

Administration of naproxen (250 mg tid) in ten healthy volunteers for either seven or fourteen days resulted in a 35% increase in urinary zinc excretion but serum zinc levels remained unchanged. However, another report indicates that serum zinc levels were altered by NSAID therapy and decreased to 10.47 mmol/L. Clinically, signs and symptoms of zinc deficiency include alopecia, dermatitis, diarrhea, growth retardation, increased susceptibility to infection, and loss of appetite or sense of taste. Severe zinc deficiency further impacts dermatologic, gastrointestinal, immune, nervous, reproductive, respiratory, and skeletal systems.

NSAIDs can damage the stomach as well as the small and large intestines, causing ulceration, chronic bleeding, and eventually iron deficiency. Also, iron deficiency may be associated with oxidative DNA damage. In children, iron deficiency leads to cognitive dysfunction. Other pathologies associated with depleted levels of iron include anemia and compromised immune function. Symptoms include dizziness, fatigue, shortness of breath, pallor, and tachycardia.

Spiller et al. studied the effect of guar gum and an oat fiber source on plasma lipoproteins and cholesterol in hypercholesterolemic adults. Also, studies have shown that soluble fiber of the gum can enhance intestinal immune function (fiber, arabinogalactan). A large portion of the body’s immune system is localized to the gastrointestinal wall and in mesenteric lymph nodes, where consumption of gum acacia stimulated intestinal and splenic immune system function. Gum resins is applied to the milky juices of certain plants which consist of gum soluble in water, resin and essential oil soluble in alcohol, other vegetable matter and a small amount of mineral matter. They are generally opaque and solid, and often brittle. When finely powdered and rubbed down with water they form emulsions, the undissolved resin being suspended in the gum solution. Their chief uses are in medicine. Gums are a high-energy food source composed mainly of water, complex polysaccharides, calcium, and trace minerals (iron, aluminum, silicon, potassium, magnesium, and sodium).

Gum arabic has the property to bind cations, especially divalent cations as calcium and magnesium. Due to this effect the amount of calcium and magnesium in the caecum rises considerably. The result is a supply of these cations in the large bowel, where they are efficiently absorbed. Furthermore, the Arabic gum (Acacia gum) enhances the absorption of minerals from the diet.

Dikshith et al. investigated the effect of increment of gum in the diet of rats for 52 weeks. Parameters investigated included growth excretion levels of nitrogen in urine, hematological values, organs weight. At the end of treatment, organs such as heart, liver, spleen, kidneys, and adrenals were weighed and preserved for histopathological observations. No compound related effects were observed. Gum was found to be transformed to a gelatinous state at a higher level in the intestine and to be transported more rapidly through the intestinal tract. Adding refined fibers to the basal diet did not significantly affect apparent mineral balance of calcium, magnesium, manganese, iron, copper or zinc, with the exception of a negative mineral balance for manganese with carboxymethylcellulose. Karaya gum had a mean positive balance for all minerals tested. Karaya gum does not disintegrate or decompose appreciably in the alimentary tract. In a study of 10 dogs, 95% of the orally administered gum was recovered in the faeces. It absorbs a large quantity of water and therefore acts as a mechanical laxative. It tends to increase faecal nitrogen excretion, does not affect starch digestion in the dog and does not inhibit the utilization of vitamin A in rats.

In conclusion, the current study illustrates the functional and biochemical changes associated with histopathological alterations induced by aspirin treatment for 21 days. It revealed a significant role of gum extract for maintaining the balance of the pancreatic, intestinal enzymes and the
intestinal content of iron and zinc in aspirin treated rats. Moreover, the gum acacia showed a potential as anti-ulcer activity by virtue of its various effects on mucosal offensive and defensive factors.

References


