Original Article

Possible Protective Effect of Capsaicin against Indomethacin-induced damage in Jejunum of adult male albino rats (Histological and Histochemical study)

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ABSTRACT

Background and Objectives: Indomethacin is widely used in treatment of many rheumatic conditions. This use is limited by its damaging effect on gastrointestinal mucosa. Capsaicin is the main pungent and active principal ingredient in hot chili peppers. It has been used widely in the field of medicine. This study aimed to evaluate the protective effect of capsaicin against indomethacin-induced jejunal damage.

Materials and Methods: Seventy adult male albino rats, used in this study, were divided equally into 7 groups. Group I (control) received distilled water. Group II received the solvent of capsaicin. Group III received 15 mg/kg BW of indomethacin. Group IV received low dose of capsaicin prior to indomethacin. Group V received high dose of capsaicin prior to indomethacin. Group VI received low dose of capsaicin. Group VII received high dose of capsaicin. All solutions were given intragastrically. All animals were sacrificed 24 hours after ingestion of solutions. Jejunal specimens were processed to perform histological (H&E and Masson's trichrome) and histochemical stains (combined alcian blue and PAS) and were examined under light microscope.

Results: Giving indomethacin caused: decrease in the height of villi and thickening of the brush border, increase in width of villi and mitotic index of crypt cells, haemorrhage, and inflammatory cellular infiltration. Ingestion of low dose of capsaicin prior to indomethacin prevented the effect of indomethacin on jejunal mucosa more than high dose.

Conclusion: Giving low dose of capsaicin prior to indomethacin prevented the effects of indomethacin but high dose did not produce the same effect.

Key Words: Capsaicin, jejunum, indomethacin.

Revised: 23 June 2020, **Accepted:** 24 June 2020.

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ISSN:2536-9172, June 2020, Vol. 4, No. 1

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs in the community for treatment of rheumatologic as well as non-rheumatologic conditions[1]. Indomethacin causes ulceration in the rat small intestine, mainly the jejunum along the mesenteric margin. This site was reported to be susceptible to indomethacin induced injury as it possesses vascular compromised sites[2].

Capsaicin is hot peppers that used by humans since prehistorical time[3]. Capsaicin is the main pungent and active principal ingredient in hot chili peppers, which elicits burning pain by activating specific receptors on sensory nerve endings[4]. Capsaicin is an odorless and white crystal. One part in 100,000 can be detected by tasting. It is slightly soluble in carbon disulfide, insoluble in water, freely soluble in alcohol, ether, benzene, and chloroform. It is fairly resistant to acids and alkali solutions at room temperatures. Chemically it is a fat-soluble phenolic compound[5]. Capsaicin affects the histophysiologies of numerous systems in the organism, mainly the cardiovascular, gastrointestinal, and respiratory systems. It has started to be used widely in the field of medicine and in the pharmaceutical industry[6].

Several pharmacological, physiological and biochemical studies were done to investigate the protective effects of capsaicin on the stomach, duodenal and jejunal ulcers induced by indomethacin; however, up to our knowledge there are no available histological or histochemical studies that confirm those previous studies.

Therefore, the present work is designed to study the effects of indomethacin and capsaicin on the jejunum of adult male albino rats, and to elucidate the possible protective role of capsaicin against indomethacin induced jejunal damage, using histological and histochemical techniques.

 DOI: 10.21608/jmh.2020.32344.1076

MATERIALS AND METHODS

Drugs and chemicals:

- Carboxymethyl cellulose sodium low viscosity is the solvent for capsaicin [Biochemika Diagnostika und Pharmazeutika, Germany].
- Indomethacin capsules [Nile Co. under Chiesi licence farmaceutici, Italy].
- Capsaicin powder [Fluka, sigma-Aldrich, Germany].

Preparation of Indomethacin and Capsaicin:

- Capsule of 50 mg of indomethacin sodium was dissolved in one ml propylene glycol and made up to 20 ml with distilled water i.e. (2.5 mg/ml).
- Capsaicin powder was dissolved in carboxymethyl cellulose sodium low viscosity as 1 mg/20 ml (50 µg/ml).

Animals:

Seventy adult male albino rats, weighing 150-180 grams, were randomized into 7 groups, each of 10 rats. Animals were housed under standardized conditions away from any stressful stimuli with 12 hours day/night cycle for light. The animals were kept with free access to standard pellet animal diet and tap water for an acclimatizing period of one week. Each group was put in a cage with wide mesh bottom to prevent them from eating their faeces or hairs. Experimental procedures were approved by the research ethics committee at Faculty of Medicine, Suez Canal University.

Experimental protocol:

Twenty-four hours before the experiment, animals were deprived of food, but allowed free access to tap water. Animals received the following different calculated doses according to their respective weights through orogastric intubation.

Group I (control group): Animals received a single dose of 1 ml distilled water, then received 1 ml distilled water after half an hour.

Group II (solvent group): Animals received a single dose of carboxymethyl cellulose sodium 10 mg/ml in water, then received distilled water after half an hour[7].

Group III (Indomethacin group): Animals received a single dose of 15 mg/kg BW of indomethacin then received distilled water after half an hour[7,8].

Group IV (Low dose of capsaicin prior to indomethacin group): Animals received a single dose of capsaicin 100 µg/kg BW then received a single dose of indomethacin 15 mg/kg BW after half an hour[9].

Group V (High dose of capsaicin prior to indomethacin group): Animals received a single dose of 1 mg/kg BW of capsaicin then received 15 mg/kg BW of indomethacin after half an hour[9].

Group VI (Low dose of capsaicin group)

Group VII (High dose of capsaicin group)

All animals in each group were sacrificed by cervical dislocation under ether anaesthesia 24 hours after the end of the experiment. 10 cm long specimen was taken from the mid part of the jejunum and was divided into 2 segments. One segment of them was fixed in 10% neutral buffered formaldehyde, processed for 5 µm paraffin sections, and subjected to the H&E and Masson's trichrome stains. The other segment was fixed in Bouin's fixative, processed, and prepared for combined alcian blue and periodic acid Schiff's reagent (PAS) technique for acidic and neutral mucins[10].

Morphometric analysis:

- Quantitative analysis of histopathological changes in the jejunum was done using image analyzer (Super eye-Heidi Software Co., Cairo, Egypt) to determine the following parameters: height of the villi, width of the villi, colour area percentage of the greenish collagen fibers in the lamina propria and submucosa; and optical density of the PAS positive material (magenta colour) in the brush border of the villi.

- Quantitative analysis of the mitotic index (MI) of cells in the crypts of Lieberkühn was done as following: the number of both; cells in mitosis (mitotic figures) and cells in interphase (None-dividing cells) were counted manually. Then the mitotic index percentage was calculated by dividing the number of cells in mitosis by the total number of cells multiplied in 100.

- The frequency distribution of histopathological changes (haemorrhage & heavy cellular infiltration) in the jejunum was calculated for each change in each group of animals[2].

All parameters were measured in ten non-overlapping high-power fields (×40) from three different sections of different rats of each group.

Statistical analysis:

The mean and standard deviation were calculated for each group of the animals of treated groups, then, in each treated group mean was evaluated by pair wise comparison to the control group and to the indomethacin group means using t-test. Analysis of the frequency distribution (in percentage) of the histopathological changes was done using Chi-square test. The level of significance was set at $P<0.05$. Data were analyzed using SPSS program version 11, (Chicago, IL, USA).

RESULTS

Histological results:

Light microscopic examination of the jejunal sections stained with H&E, in group I, revealed the normal structure of the four basic layers of jejunal wall. Mucosa has thrown into finger like projections (Villi). The villus consists of a core of loose connective tissue (CT) covered by a simple columnar epithelium (enterocytes). Crypts of Lieberkühn, open onto the luminal surface of the jejunum at the bases of the villi. These glands are lined by a simple columnar epithelium that is continuous with that of the villi. Goblet cells appeared empty and are scattered in the epithelium of both villi and crypts. Muscularis mucosa consists of smooth muscle cells. Submucosa consists of a dense CT. Muscularis externa consists of smooth muscle cells. Serosa consists of simple squamous epithelium, and a small amount of underlying CT (Fig. 1 [I]). The jejunal sections in group II showed the same histological structure as that of the control group (Fig. 1 [II]).

On the other hand, the examination of jejunal sections stained with H&E of the treated groups (III, IV, V, VI and VII) revealed the appearance of histopathological changes. In group III, because of ingestion of indomethacin, abnormalities were detected in the villi in the form of shortening with blunted apices. Also, their surface epithelium was discontinuous with patchy erosions, especially at the tips of the villi. In the intact areas, the columnar cells covering the surface of the villi showed pleomorphic cells (various forms of tall and short cells). In addition, degenerate-looking cells appeared with deeply eosinophilic cytoplasm and pyknotic nuclei. Heavy inflammatory cellular infiltration was also observed. There was also separation of the basement membrane of the surface epithelium from the villus core by inflammatory edema. Goblet cells number were apparently increased. Interstitial hemorrhage was detected in the core of villi. In the crypts mitotic figures appeared (Fig. 1 [III]). In group IV, as a result of ingestion of low dose of capsaicin half an hour before indomethacin, most of villi appeared nearly normal in shape; meanwhile few of them were short as compared to jejunal sections in control group. Most of the covering epithelial cells in this group were normal. Few foci with mild inflammatory cellular infiltration were detected in the core of the villi. Mitotic figures appeared in the crypts (Fig. 2 [IV]). In group V, which received high dose of capsaicin half an hour before indomethacin, there were most of villi are found to be short but few of them are normal in shape (decrease in number of the normal villi), separation of the basement membrane of the surface epithelium from the core of villi, multiple foci of inflammatory cellular infiltration in the core of the villi and appearance of areas of interstitial hemorrhage. In the crypts mitotic figures were seen (Fig. 2 [V]). In groups VI & VII which received only low & high doses of capsaicin respectively, the layers of the jejunal sections appeared approximately like that of the control group (Fig. 2 [VI & VII]). However, in group VII, there were few of goblet cells were apparently depleted and there were multiple foci of inflammatory cellular infiltration in the core of the villi (Fig. 2 [VII]).

Light microscopic examination of the jejunal sections in control group stained with Masson's trichrome showed few collagen fibers in the core of the villi and lamina propria between the crypts were stained green, while the submucosa showed more collagen fibers (Fig. 3 [I]). All other groups showed collagen fibers distribution that is nearly like that of the control group (Figs, 3 [II, III, IV, V, VI, & VII]).

Histochemical results:

Light microscopic examination of the jejunal sections stained with combined alcian blue & PAS technique for acid and neutral mucins in group I revealed positive PAS reaction in the brush border of enterocytes. Basement membrane of villous epithelium showed mild positive PAS reaction. Some goblet cells were stained blue by alcian blue, while others stained pink by PAS. Third group of goblet cells have mixed mucins and stained violet (Fig. 4 [I]). Likewise, the jejunal sections in group II have similar findings (Fig. 4 [II]).

However, because of ingestion of indomethacin in group III, there was a weak positive PAS reaction in the brush border of enterocytes and even loss of PAS reaction in the eroded areas. There is apparently increase in goblet cells, with acid mucins, along the entire surface of the villi, while absence of goblet cells with mixed mucins. Appearance of sloughed blue stained mucus in the lumen of the jejunum. Furthermore, depleted goblet cells are apparently increased (Fig. 4 [III]). In contrast, to indomethacin group, ingestion of low dose of capsaicin prior to indomethacin (Group IV) resulted in strong positive PAS reaction in the brush border of enterocytes which is nearly more than control group. The villous epithelium rests on a basement membrane stained pink with a mild positive PAS reaction. Also, goblet cells with acid mucins (stained blue with alcian blue), goblet cells with neutral mucins (stained pink with PAS) and goblet cells that show violet staining (have a mixture of mucins) are approximately like those of control group. Depleted goblet cells are apparently increased. The lumen of jejunum has sloughed blue stained mucus (Fig. 4 [IV]). However, ingestion of high dose of capsaicin prior to indomethacin (Group V); as well as ingestion of low dose of capsaicin only (Group VI) produced alcian blue and PAS reaction similar to that of control group except the presence of depleted goblet cells (Fig. 4 [V, VI]). On the other hand, ingestion of high dose of capsaicin only (Group VII) resulted in alcian blue and PAS reaction similar to that of group III, given that the PAS reaction in brush border is not as weak as that of group III and presence of goblet cells with mixed mucins in the epithelium of group VII (Fig. 4 [VII]).

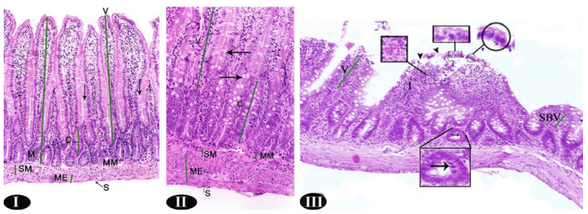


Fig. 1: Photomicrographs of the jejunum of rats in both control group (I) and other experimental groups (II, III) showing: In groups I & II the jejunal wall appeared to has four basic layers: mucosa (M), submucosa (SM), muscularis externa (ME) and serosa (S). The mucosa shows long slender villi (V). Crypts of Lieberkühn (C) are present in between the bases of the villi. Muscularis mucosa (MM) also seen beneath the crypts. Goblet cells (arrows) appeared empty in-between the enterocytes that cover the villi. In group III there is apparent decrease in number of villi (V) and abnormality in their shapes. Some villi appear short and broad (SBV) with blunted tips. The surface epithelium of the villi has pleomorphic cells (rectangle inset), degenerate-looking cells (circle inset) and patchy erosions (arrowheads). Numerous foci of inflammatory cellular infiltration (I) are shown in the core of villi. Separation (↕) of the basement membrane of the surface epithelium from the villus core, and interstitial hemorrhage is also seen (square inset). A mitotic figure (Arrow) appeared in the inset (H&E, I, III, x100; II x160; All insets x100).

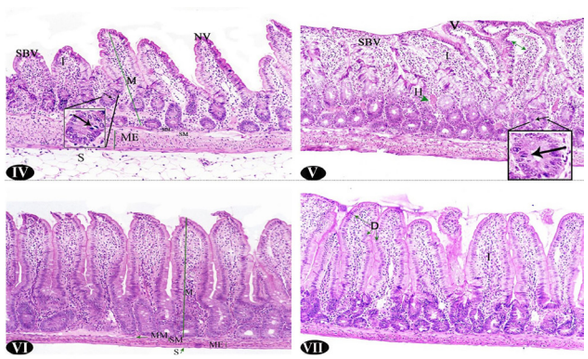


Fig. 2: Photomicrographs of the jejunum of rats in experimental groups (IV, V, VI, & VII) showing: In group IV the structure of the mucosa (M) appeared like that of the control group. Most of villi appeared normal (NV), while some villi are short and broad (SBV). The villus surface epithelium is intact. Few foci of inflammatory cellular infiltration (I) are shown. Muscularis mucosa (MM), submucosa (SM), muscularis externa (ME) & serosa (S) are also seen similar to control group. A mitotic figure (Arrow) is seen in the inset. In group V there is apparent decrease in number of the normal villi (V). Some villi appear short and broad (SBV) with blunted tips. There are multiple foci of inflammatory cellular infiltration (I) and areas of interstitial hemorrhage (H). Separation (↕) of the basement membrane of the surface epithelium from the villus core is also shown. A mitotic figure (Arrow) is showed in the inset In group VI the mucosa (M), muscularis mucosa (MM), submucosa (SM), muscularis externa (ME), and serosa (S), appeared like control group. In group VII the normal structure of the mucosa has few depleted goblet cells (D) and a few foci of inflammatory cellular infiltration (I) are also seen. (H&E, IV, V, VI, & VII x100; All insets x100).

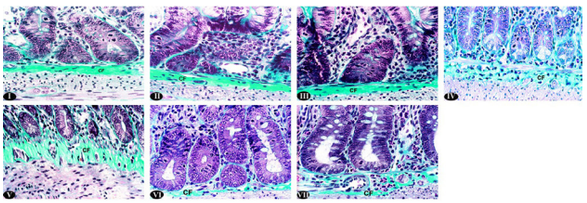


Fig. 3: Photomicrographs of sections from the jejunum of rats in both control and other experimental groups showing that all groups (I, II, III, IV, V, VI & VII) have collagen fibers (CF) stained green, in the lamina propria in-between the crypts of Lieberkühn and submucosa. (Masson's trichrome, I, II, III, IV, V, VI, & VII x400)

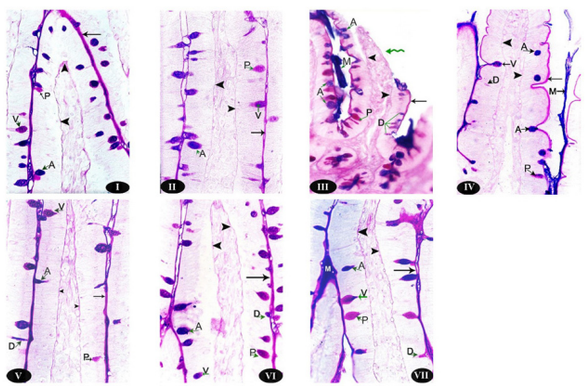


Fig. 4: Photomicrographs of sections from the jejunum of rats in both control group (I) and other experimental groups (II, III, IV, V, VI & VII): showing positive PAS reaction (arrow) in the brush border of enterocytes in all groups. However, it appeared weak or disappeared in group III, but appeared strong in group IV. The basement membrane of the villous epithelium has a mild positive PAS reaction (arrowheads) in all groups (I, II, III, IV, V, VI, & VII). Goblet cells either have positive alcian blue (A) or positive PAS reaction (P) are seen in all groups (I, II, III, IV, V, VI, & VII). The goblet cells that have positive alcian blue (A) are apparently increased in group III. The goblet cells show violet staining (V) are not detected in group III but appeared in all other groups (I, II, IV, V, VI and VII). Depleted goblet (D) cells are seen in groups III, IV, V, VI, and VII. Sloughed mucus (M) appeared blue in the jejunal lumen in groups III, IV and VII. (Combined Alcian blue & PAS, I, II, III, IV, V, VI, & VII x400)

Morphometric & statistical results:

The mean height of villi (µm) showed a significant reduction in groups III, IV and V as compared to control group. In contrast there is a significant increase in groups II, IV, V, VI and VII as compared to indomethacin group III (Table 1) (Histogram 1).

The mean width of the villi (µm) showed a significant increase in groups III and V as compared to control group. However, the width of the villi (µm) is increased in groups II, IV, VI and VII but with non-significant difference when compared to control group but there is a significant difference when compared to group III (Table 1) (Histogram 2).

The mean colour area percentage of collagen fibers showed non-significant difference for groups II, III, IV, V, VI and VII as compared to the control group (Table 1).

The mean of optical density of PAS reaction in the brush border of the villi showed a significant decrease in group III as compared to control group. In contrast, there was a significant increase in groups II, IV, V, VI and VII as compared to group III. The most important finding in this group was the marked significant increase of optical density of PAS material in group IV as compared to both control group and group III (Table 1) (Histogram 3)

The mean of mitotic index percentage of the stem cells in the crypts of Lieberkühn showed a significant increase in groups III, IV and V as compared to control group. In contrast there is a significant decrease in groups II, VI and VII as compared to group III (Table 1) (Histogram 4).

The frequency distribution of haemorrhage in animals of different groups showed a significant increase in groups III, V and VII, as compared to control group. Conversely, there was a significant decrease in groups IV, VI and VII as compared to group III (Table 2).

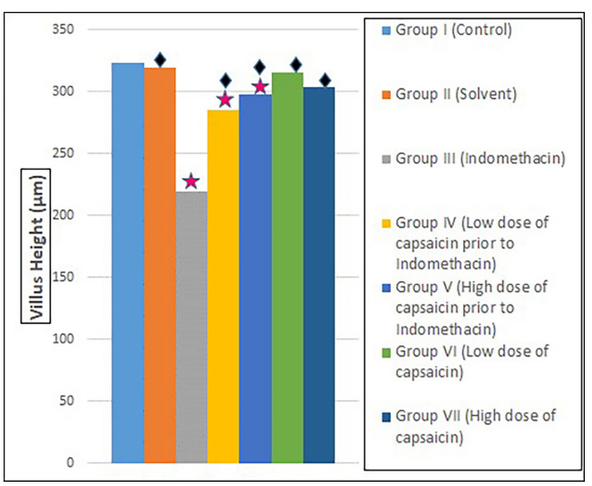
The frequency distribution of heavy cellular infiltration in the core of jejunal villi of animals of different groups showed a significant increase in groups III and V as compared to control group. Conversely, there was a significant decrease in groups IV, V, VI and VII, as compared to group III (Table 2).

Table 1: The mean±SD of villus height (µm), villus width (µm), colour area % of collagen fibers, optical density of PAS +ve reaction in the brush border of the villi and mitotic index % of the stem cells in the crypts of Lieberkühn in the control and experimental groups

Groups	Villus height (µm)	Villus width (µm)	Colour area % of collagen fibers	Optical density of PAS +ve reaction in brush border of villi	Mitotic index % of the stem cells in crypts of Lieberkühn
Group I (Control)	323.41±7.88	87.60±28.05	4.97±0.48	0.130±0.002	6.9 ± 0.5
Group II (Solvent)	319.57±6.38♦	89.58±25.1♦	4.95±0.38	0.131±0.004♦	6.88±1.4♦
Group III (Indomethacin)	219.19±6.12*	134.81±59.82*	4.89±1.12	0.101±0.003*	13.36±3.5*
Group IV (Low dose of capsaicin prior to Indomethacin)	284.86±8.94♦*	98.62±25.68♦	4.79±0.04	0.159±0.002*♦	11.09±2.4*
Group V (High dose of capsaicin prior to Indomethacin)	297.76±6.24♦*	111.07±30.83*	4.80±0.04	0.137±0.002♦	11.36±1.3*
Group VI (Low dose of capsaicin)	315.11±5.88♦	98.96±24.06♦	4.88±0.36	0.130±0.003♦	8.86±1.6♦
Group VII (High dose of capsaicin)	303.33±5.78♦	95.77±25.52♦	4.87±0.03	0.127±0.003♦	8.97±0.2♦

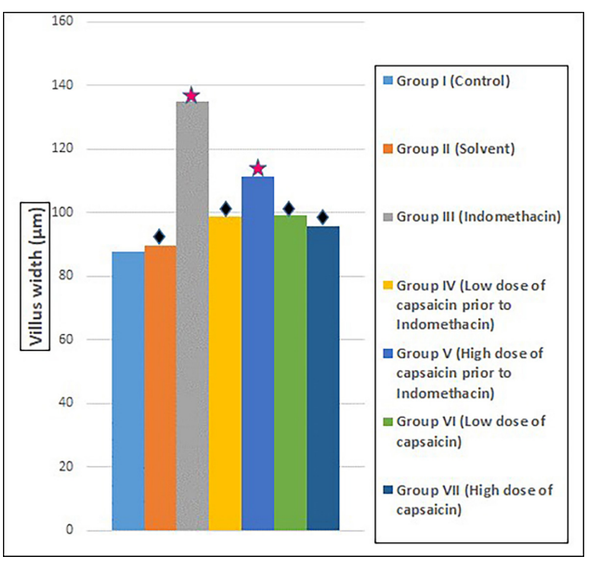
*Significant compared to control;♦Significant compared to indomethacin group; SD=Standard deviation

Histogram 1: Villus height (µm) in all tested groups.



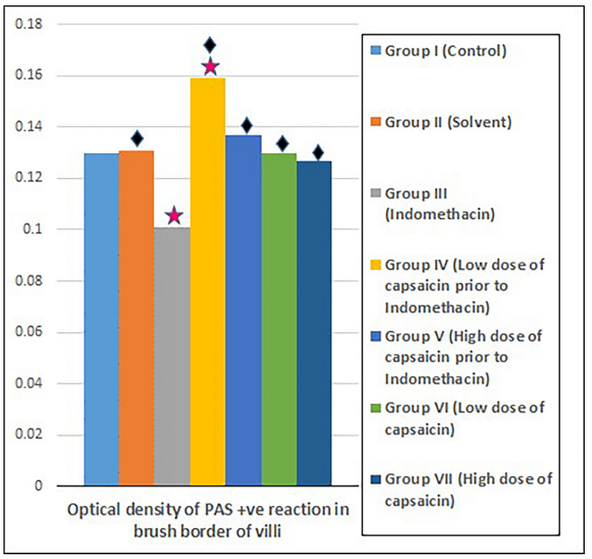
*Significant compared to control; ♦Significant compared to indomethacin group ($P < 0.05$)

Histogram 2: Villus width (µm) in all tested groups.



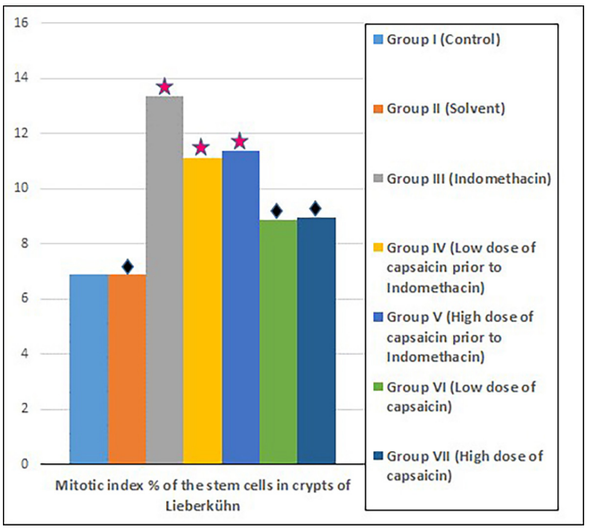
*Significant compared to control; ♦Significant compared to indomethacin group ($P < 0.05$)

Histogram 3: Optical density of PAS +ve reaction in brush border of villi in all tested groups.



*Significant compared to control; ♦Significant compared to indomethacin group ($P < 0.05$)

Histogram 4: Mitotic index % of the stem cells in crypts of Lieberkühn in all tested groups.



*Significant compared to control; ♦Significant compared to indomethacin group ($P < 0.05$)

Table 2: The frequency distribution (in percentage) of histopathological changes in the jejunum of animals in the control and experimental groups

	Histopathological changes	
	Interstitial Hemorrhage	Inflammatory cellular infiltration
Groups	Affected animals (%)	Affected animals (%)
Group I (Control)	0	0
Group II (Solvent)	0	0
Group III (Indomethacin)	100*	100*
Group IV (Low dose of capsaicin prior to Indomethacin)	30♦	30♦
Group V (High dose of capsaicin prior to Indomethacin)	80*	50*
Group VI (Low dose of capsaicin)	0♦	0♦
Group VII (High dose of capsaicin)	40*♦	20♦

*significant compared to control; ♦ significant compared to indomethacin group

DISCUSSION

The use of indomethacin causes intestinal damage and jejunal mucosal lesions[11]. Jejunal ulceration induced by a single oral dose of the indomethacin to rats is a chemically induced experimental model of inflammatory bowel disease[12]. It was found that capsaicin can inhibit acute gastric mucosal lesions caused by several injurious factors such as hydrochloric acid, ethanol, aspirin, and indomethacin[13]. Capsaicin protective effect was reported to be through various mechanisms such as increased gastric mucosal blood flow and gastric mucus secretion and facilitated gastric epithelial restitution[14].

In the present work, indomethacin ingestion in group III induced decrease in the height and increase in the width of the villi, as compared to the control group. This was in accordance with the results of Nygard *et al*[15] who found that two hours after oral indomethacin, the jejunal mucosa showed short broad villi and they attributed that to smooth muscle contraction. Likewise, in group III indomethacin treatment also resulted in changes in the epithelial surface of the villi, in the form of tall and short cells (pleomorphic cells) and necrotic cells. The pleomorphism can be explained by reprogramming of stem cells into a new pathway which can be caused by generating signals from cytokines, growth factors and extracellular matrix components in the cell environment[13]. The necrotic cells found in the intestinal epithelium can be explained by the presence of ischemia caused by indomethacin intake[14]. This ischemia resulted in decrease in oxygen tension within the cells which led to failure of sodium pump, influx of sodium and water,

resulting in cell swelling; endoplasmic reticulum and mitochondrial swelling and clumping of chromatin and this finally produced necrosis in these cells[15]. Moreover, in group III the loss of the apical parts of epithelial cells can be due to intestinal ulceration and degeneration of villi with indomethacin treatment which could be attributed to reduction in jejunal blood flow, which lead to ischaemia that is sufficient to cause macroscopic damage along the mesenteric margin[2].

In the present work also group III showed haemorrhage. Indomethacin was found to produce focal slowing of villous blood flow as well as acute endothelial damage that lead to vascular leakage and hemorrhage which may explain the present findings[16].

In the current work indomethacin in group III induced infiltration of the lamina propria by inflammatory cells. Similarly, Anthony *et al*[2] reported that three hours, after dosing with oral indomethacin, infiltration of the mucosa by inflammatory cells was apparent. They explained it by the exposure of the mucosa to luminal bacteria, food and bile acids which caused mucosal inflammation. Additionally, indomethacin ingestion in group III produced significant decrease in the positive PAS reaction in the brush border of enterocytes and confirmed by the decrease in the optical density of PAS reaction in the brush border. This is caused by reduction in the sugar contents of the brush border membrane which attributed to the oxidative stress caused by indomethacin[17]. Moreover, in indomethacin group III, the alcian blue stained (acidic mucopolysaccharides) goblet cells were found to be apparently more than the PAS stained (neutral mucopolysaccharides) goblet cells. This can be attributed to the presence of bacterial colonization caused by indomethacin[11]. This bacterial colonization stimulates the secretion of acidic mucopolysaccharides which act in resisting the enzymatic degradation caused by bacteria[18].

Concerning cellular turnover in crypts of Lieberkühn, a statistically significant increase in the mitotic index was found in group III. This could be clarified by the work of Kelly *et al*[19] who suggested that the increase in cell losses from the epithelial surface, caused by indomethacin, triggered a compensatory trophic reaction. Additionally, this study clarified that mitotic index had statistically significant increase in crypts of Lieberkühn, in groups IV & V. It was proven that capsaicin facilitates the repair of the mucosa of crypts of Lieberkühn induced by endotoxins[20].

Giving a low dose of capsaicin alone (100 µg/kg in group IV) half an hour prior to indomethacin eliminated the harmful effect of indomethacin on the jejunal structure. So, capsaicin produced a significant protective effect against indomethacin. The structure of the mucosa was nearly like control group. The percentage of haemorrhage and inflammatory infiltration in the jejunum were significantly decreased comparing to group III. This was in accordance with previous studies which used subcutaneous capsaicin ten days prior to nonlethal doses of endotoxin and found prevention of the endotoxin-induced damage of the intestinal mucosa[20,21,22]. Another study also showed that capsaicin reduced significantly the gastric mucosal damage induced by ethanol, which is known to be a potent injurious substance to gastric mucosa[23]. This was confirmed by Denaro *et al*.[24] who stated that capsaicin in low doses provide repair of the gastrointestinal mucosal injury. Furthermore, Low dose of capsaicin protects gastric mucosa indomethacin-induced injuries[25]. Additionally, in some studies on in vitro human cell culture, it was found that capsaicin reduced the bacterial growth which is considered as a cause of mucosal inflammation[26,27]. The explanation is low doses of capsaicin improve gastric blood flow to the mucosa, increase submucosal blood perfusion, and consequently minimize mucosal lesions produced by ethanol[28]. Capsaicin provide protection to gastric mucosa through suppression of proinflammatory cytokines[29].

Different mechanisms were suggested for the protective effect of small doses (100-800 µg/kg) of capsaicin. Some investigators reported that capsaicin decreased gastric acid secretion, increased duodenal HCO3 output by stimulation of capsaicin-sensitive sensory neurons, increased mucosal blood flow and increased PG generation[22,30,31]. Others stated that capsaicin produced relaxation of smooth muscles of small intestine due to decrease acetylcholine release in the small intestine. Acetylcholine acts through muscarinic receptor M2 & M3 on the smooth muscle leading to increase in the motility of the intestine[11]. Moreover, in group IV the interstitial hemorrhage and inflammatory cellular infiltration was significantly decreased. It was found that capsaicin prevented gastric mucosa micro-bleeding after intake of indomethacin[32]. AS well capsaicin has anti-inflammatory effects on H. pylori-induced chronic gastritis through the suppression of inflammatory factors such as Tnf-α[14]. Toyoda *et al*.[33] considered that capsaicin is an effective antioxidant which abolish any oxidative stress[34].

One of the striking results is the statistically significant increase in the PAS positive material in the brush border of enterocytes of group IV compared to the control and indomethacin groups indicates less damage of the epithelium. This can be explained by the intestinal secretion caused by capsaicin, is probably due to the anti-muscarinic action of capsaicin, mobilization of mucocytes in superficial epithelium of the jejunum and increased secretion of mucopolysaccharides[2,35].

Additionally, in group IV some of goblet cells were found to be depleted. This would reflect one of the possible protective mechanisms to directly overcome the damaging effect of indomethacin through increased mucus secretion.

The goblet cells accumulate secretory product, discharge it, and then refill. This cycle may be repeated many times in the life span of the intestinal goblet cells which is only four to six days[36]. In support of this explanation, capsaicin is considered a good stimulant to produce mucus from gastric mucosa[37].

Giving capsaicin in high doses (1mg/kg) in group V, half an hour prior to indomethacin produced a limited protective effect against indomethacin. compared to group IV. The percentage of inflammatory cell infiltration in group V was significantly less within the connective tissue core of the villi, compared to indomethacin group but more than that in group IV. Decrease in mean height and increase in mean width of villi. However, there was separation of the basement membrane of the surface epithelium from the connective tissue core of villi. This dose of capsaicin increased the anti-inflammatory IL-10 levels and attenuated the increases in proinflammatory cytokines. Therefore, this could explain the reduction of the inflammatory response, yet not stopped completely, as evidenced by the presence of cellular infiltration and oedema which most probably caused the separation of the basement membrane of surface epithelium from the lamina propria[9]. Combined alcian blue & PAS staining, in (group V) showed that almost several goblet cells appeared evacuated. This means that the combined injurious effects of high dose of capsaicin and indomethacin stimulated most of goblet cells to evacuate their content to produce a layer of mucous acting as a protective barrier. This can be explained by the study of Kuo *et al*.[38] who found that goblet cells increased the secretion of neuropeptides substance P (SP) and neurokinin A (NKA) in a dose-dependent manner. These neuropeptides consequently directly stimulate goblet cells secretion.

Giving a higher dose of capsaicin only (1mg/kg in group VII) intragastrically produced some histopathological changes in the form of inflammatory cell infiltration and haemorrhage. Also, in this group, there was PAS positive material in the brush border of enterocytes which was nearly like that of the control group. However, a few numbers of goblet cells evacuated their content. Capsaicin in this dose may act as an irritant to the mucosal lining and it stimulated the goblet cells to evacuate its content to produce a protective mucous layer. The explanation of mucosal damage produced by large dose of capsaicin is through increase in cytosolic Ca++ that lead to mitochondrial damage and neuronal death. This causes mucosal desensitization which increases intestinal motility and decreases mucosal blood flow causing ischemia, interstitial haemorrhage, and inflammatory cellular infiltration[39,40].

CONCLUSION

In conclusion: the results of the present work provided evidence that capsaicin when given prior to indomethacin in a low dose (100 µg/kg BW) is more protective to jejunal mucosa against injury by indomethacin than the high dose (1 mg/kg BW).

CONFLICT OF INTEREST

There are no conflicts of interest.

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الملخص العربى

التأثير الوقائى المحتمل لمادة الكابسياسين ضد تأثيرات الإندوميثاثين على الصائم في الأمعاء الدقيقة فى ذكور الفئران البيضاء (دراسة هستولوجية وهستوكيميائية)

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الخلفية و الأهداف: الاندوميثاسين واسع الانتشار في علاج العديد من الحالات الروماتيزمية. ويحد من استخدامه، التلف الذي يسببه في الغشاء المخاطي للأمعاء. الكابسايسين هو العنصر الرئيسي اللاذع والنشط في الفلفل الحار. ويتم استخدام الكابسياسين على نطاق واسع في مجال الطب. وتهدف هذه الدراسة إلى تقييم الدور الوقائى المُحتَمل للكابسياسين ضد تأثيرات الإندوميثاثين على الصائم في الأمعاء الدقيقة.

المواد وطرق البحث: تم تنفيذ هذه الدراسة على ٧٠ من ذكور الفئران البيضاء البالغة وتم تقسيم الفئران إلى ٧ مجموعات متساوية. المجموعة الأولى (الضابطة(، أُعطيت الماء المقطر. المجموعة الثانية أعطيت مذيب الكابسياسين. المجموعة الثالثة أُعطيت ١٥ ملجم/كجم من وزن الجسم من الاندوميثاسين. المجموعة الرابعة أُعطيت جرعة صغيرة من الكابسياسين قبل الاندوميثاسين. المجموعة الخامسة أُعطيت جرعة كبيرة من الكابسياسين قبل الاندوميثاسين. المجموعة السادسة أُعطيت جرعة صغيرة من الكابسياسين. المجموعة السابعة أُعطيت جرعة كبيرة من الكابسياسين. وقد أُعطيت كل المحاليل عن طريق الفم. ثم بعد مرور ٢٤ ساعة من بداية التجربة تم قتل الفئران فى كل مجموعة واستخرجت عينات من الصائم، وتم تحضيرها لعمل الصبغات الهستولوجية (الهيماتوكسيلين والايوسين، ثلاثي الألوان لماسون) ، والهستوكيميائية (كاشف شيف البيرايودي وأزرق ألسيان) لتفحص بالميكروسكوب الضوئي.

النتائج: تسبب إعطاء عقار الإندوميثاسين انخفاضاً في: ارتفاع الخملات، وسمك الغشاء الذي يغطيها وزيادةً في: عرض الخملات، ومؤشر إنقسام الخلايا في السراديب والنزيف ونفاذ الخلايا الالتهابية. وقد أدى تناول جرعة صغيرة من الكابسياسين قبل الاندوميثاسين الحفاظ على تركيب بطانة الصائم من تأثير الاندوميثاسين اكثر مما فعل تناول الجرعة الكبيرة.

الخلاصه: إعطاء جرعة صغيرة من الكابسياسين قبل الاندوميثاسين منع آثار الإندوميثاسين أفضل من إعطاء جرعة كبيرة من الكابسياسين قبل الاندوميثاسين.