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RESEARCH ARTICLE

The Protective Role of Bradykinin Potentiating Factor on Gastrointestinal Ulceration Induced by Indomethacin in Experimental Animals.

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Abstract

Although, indomethacin is used in the treatment of some diseases, it is associated with side effects. Therefore, this study evaluates the effect of bradykinin potentiating factor (BPF_7) as a natural products separated from the venom of jellyfish, *Cassiopia andromeda* on indomethacin-induced gastrointestinal ulceration and to examine the possibility of gastric ulcer healing by this natural peptides. The present study demonstrated that oral administration of indomethacin (10mg/kg b.w) is associated with several adverse effects on stomach and intestine tissues which involve gastric and peptic ulceration, inflammation and mucosal injury. These histological changes were diminished or oblished by BPF_7 treatment. In addition, indomethacin induced a significant increase in HcL, calcium, acid phosphatase and haemoglobin levels in gastric juice.While, the treatment with BPF_7 showed a significant improvement of these parameters as compared with the model ulcer animals. The deleterious effects of indomethacin in this study may be due to a direct action of these drugs as a cytotoxic effect or that increase the epithelial permeability and inhibition of endogenous prostaglandins synthesis. On the other hand, the study suggests that BPF_7 ameliorating the deleterious effects of indomethacin possibly by the direct effect of this factor which acts as Gastroprotective agent or indirect action through the stimulation of endogenous bradykinin which in turn enhances prostaglandins synthesis.

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are therapeutically useful as analgesic and anti-inflammatory agents. They are the most frequently prescribed drugs worldwide. Indomethacin is most popular NSAIDs; it was synthesized in 1963 for the treatment of rheumatoid arthritis, degenerative joint diseases, ankylosing spondylitis, gout, acute musculo-skeletal disorders, inflammation and edema following surgical technique and pain associated with primary dysmenorrheal (Shakeerabanu *et al.*, 2011).

The clinical use of NSAIDs including indomethacin is associated with potentially life-threatening deleterious effects causing gastrointestinal toxicity that result in damage to the gastroduodenal mucosa *via* several mechanisms (Wallace and Muscara, 2001).Indomethacin is known to induce gastric ulcer by inhibition of prostaglandins which are cytoprotective to gastric mucosa (Wallace, 2001), particularly due to the inhibition of cyclooxygenase pathway of arachidonic acid metabolism resulting in excessive production of leukotrienes and other products of 5-lipoxygenase pathway (Rainsford, 1987). NSAIDs are known to be aggressive agents for gastric ulcer development. In this respect, ulcer penetration as far as muscularis mucosa with coagulative and necrotic bed masses was reported in experimental animals (Mahendran *et al*., 2002; Ilahi *et al*., 2006; Lee *et al*.,2010). The authors concluded that the effect of indomethacin administration is more effective in stomach than

duodenal villi which show necrotic tips. In contrast, no lesion was observed in the stomach corpus of fed animals when indomethacin was given in toxic doses in either rats or mice (Satoh and Guth, 1981; Karatani *et al*., 1994; Anthony *et al*., 1996).

Gastric acid hypersecretion is one of the major pathogenic factors for the induction of gastric ulcer disease. The presence of acid in the lumen of the stomach is contributed to the pathogenesis of NSAID-induced ulcers and bleeding. Luminal acid interferes with the haemostasis and platelet aggregation and process of restitution, resulting in the conversion of superficial injury to deeper mucosal lesion and inactivates several growth factors that are important in mucosal defense and repair were reported (Wallace and Muscara, 2001).Administration of NSAIDs to experimental animals causes damage to vascular endothelium (Rainsford, 1983; Wallace *et al*., 1990). NSAIDs can delay the healing of pre-existing ulcers and promote their bleeding.This is related to their inhibitory effects on platelet aggregation (Prichard *et al*., 1989; Hawkey *et al*., 1991). The inhibition of platelet aggregation by NSAIDs occurs as a consequence of the inhibition of thromboxane synthesis which increases the risk of gastrointestinal bleeding (The salt collaborative group, 1991; Cryer *et al*., 1995).

The level of lysosomal enzymes as acid phosphatase in luminal content is used to evaluate the extent of tissue damage by many injurious agents (Szabo, 1987). Indomethacin is known to be aggressive agent and causes damage in the gastric mucosa (Moustafa *et al*., 2013). Also, Hemieda *et al*. (2004) demonstrated that indomethacin as a cytotoxic agent was found to produce marked elevation in the serum activity of acid phosphatase (ACP).Non-steroidal anti-inflammatory drugs are known to increase gastrointestinal permeability and may thus influence the absorption of calcium (Bijlsma and Rabelink, 1990).The concentration of intracellular Ca^{2+} regulates the state of acid-secreting membranes in the parietal cells. This may be due to its action on both the formation and the destruction of fusogenic lysophospholipids in these membranes (Olaisson *et al*., 1985). Therefore, calcium channel-blocking have the ability to depress gastric acid secretion and suppress stress-induced ulceration.

From the other hand, protection of gastric mucosa and inhibition of leucocytes infiltration of gastric wall in experimental animals by various natural extracts of plant and animal sources were recorded (Kobayashi *et al*. 2001; Swarnakar *et al*. 2005; Al-Radahe *et al*.2012; Abdel Galil and El-Awdan, 2012; Sankar *et al*., 2013).The sponges, *Haliclona petrosia* and *Discodemia* produced powerful anti-cancer and anti-inflammatory agents (Blunt *et al*., 2004). The marine compound manoalide isolated from the sponge *Luffariella variabilis*, inhibited inflammation (Glaser and Jacobs, 1986, 1987) and novel sesterterpenes type anti-inflammatory drugs were isolated and characterized in corals (Shin *et al*., 1991) and sponge (Pastor *et al*., 1999). In higher animal also melittin which is the principle toxic peptide of bee venom has a strong anti-inflammatory aging and used as traditional medicine for treatment of different types of diseases including gastrointestinal tract (Abdu and Alahmari, 2013). Bradykinin potentiating factors (BPFs), which potentiate the effects of bradykinin (BK) both in *vivo* and in *vitro*, have been reported to be found in some toxic marine and terrestrial animals (Larson *et al*., 1991; Glasgow *et al*., 1997; Abu-amra, 2001). Accordingly, the present study aims to investigate the effects of bradykinin potentiating factor (BPF_7) as a natural peptide separated from jellyfish, *Cassiopia andromeda* on indomethacin- induced gastric and peptic ulceration.

Materials AND Methods

Indomethacin

Indomethacin was obtained commercially from Khaira Pharm. Chem. IND. CO. Cairo, Egypt.

Bradykinin-potentiating factor (BPF_7)

Jellyfish, *Cassiopia andromeda*, is distributed in the Red Sea and it was reported as a venomous species. In this study, jelly fish, *Cassiopia andromeda*, was collected from two shallow water locations at 60 km and 70 km northern and southern of Quasar city, Egypt. Aqueous extracts were centrifuged. The supernatant was frozen. The BPF_7 separated from jelly fish was isolated and purified according to the method of Ferreria (1965).

Animals

60 healthy adult male albino mice (25 - 30) from the breeding unit, department of Zoology, faculty of Science, SohagUniversity were used. The animals were housed under normal conditions in wire cages throughout the experimental period (15 days).

Animal grouping

Animals were divided into six groups each composed of 10 animals. The first group served as a control group (G1). The mice of the second and the third groups (G2, G3) received repeated oral doses (10 mg/kg b.w.) daily or day after the other, respectively, during 15 days in order to induceulcers (Davies, 1998; Abdel Galil and El-Awdan, 2012). The fourth group was injected intraperitonally (i.p.) daily with BPF_7(10µg/gm b.w) for 15 days. The fifth and sixth groups were also induced for gastric ulcer with commercial indomethacin orally as described previously in group two and group three, respectively, in addition these groups treated with BPF_7as used in treating the fourth group .

Processing

At day 15, all animals of each group were sacrificed and dissected. From each animal stomach and intestine were taken quickly. Parts of these organs were taken for histological and histochemical examination. Each stomach was opened and the gastric juice were collected in tubes containing saline solution (NaCl 0.9N) and centrifuged at 3000 r.p.m for 10 minutes. Supernatant was separated and stored at -20 C° until used for the biochemical assay of calcium, HcL, acid phosphatase and haemoglobin.For histological studystomach and intestine were excised and fixed in carnoy fixative for half an hour, then dehydrated in absolute alcohol. Specimens were cleared in methyle benzoate followed by toluene and then infiltrated with melted paraffin. Infiltrated specimens were impregnated and then sectioned. Sections of 5µm were obtained and stained with haematoxylin and eosin (H&E) stain for standard histological examination, PAS for polysaccharides and acridine orange/ethidium-bromide stain for cell viability (Drury and Wallington, 1980). Haematoxylin & Eosin and those of PAS-stained sections were examined with light microscope while those of acridine orange/ethidium bromide-stained sections for cell viability were examined with fluorescence microscope. Sections were photographed and processed as required.Gastric acid, haemoglobin, calcium and acid phosphatase in gastric juice were determined according to Vogel (1987), Titz (1976), Gindler and King (1972) and Kind and King (1954), respectively.

Results

Gastric juice analysis

Analysis of gastric juice of male mice treatment with indomethacin daily (G2) and day after the other (alternative) (G3) is shown in (Tables. 1, 2). The present results revealed that there was a significant increase in HCL, calcium, acid phosphatase and haemoglobin in either the daily or day after the other of indomethacin administration compared to those of the normal control group (G1). While, there was no significant difference in previous parameters when the normal animals injected with BPF_7 (G4) compared with the corresponding control group (G1). On the other hand, the results revealed that the treatment of ulceration groups (G5 and G6) with BPF_7 showed a gradual significant -improvement in these parameters as compared to the control group (G1). While, there was a significant decreasing effect in these parameters when compared to the two groups with ulceration (G2 and G3) as shown in Tables (1, 2).

Table (1): Effect of BPF_7 on HcL and calcium in gastric juice of male mice treated with indomethacin daily and day after the other for 15 days in different groups.

parameters		(G1) Control	(G2) INDO daily	(G3) INDO alternative	(G4) BPF_7	(G5) INDO+ BPF_7 daily	(G6) INDO+ BPF_7 alternative
HcL meq/L	Mean ± SE	12.5±1.11	46.6±4.21	41.6±4.7	15±1.29	20±2.58	10.8±0.83
	Significance(1)		p< 0.001	p< 0.005	P>0.05	P<0.05	p>0.05
	Significance(2)			P>0.05	p< 0.001	p< 0.005	p< 0.001
	Significance(3)				p< 0.005	p< 0.05	p< 0.005
	% of change(1)		+272.8	+232.8	+20	+60	-13.6
	% of change(2)			-10.7	-67.8	-57	-76.8
	% of change(3)				-63.9	-51.9	-74
Calcium mg/dL	Mean ±SE	0.55±0.06	1.96±0.123	1.24±0.209	0.54±0.109	0.61±0.135	0.47±0.08
	Significance(1)		p< 0.001	p< 0.05	P>0.05	P>0.05	P>0.05
	Significance(2)			p< 0.05	p<0.001	p<0.001	p< 0.001
	Significance(3)				p< 0.05	p<0.05	p< 0.05
	% of change(1)		+256	+125	-1.8	+10.9	-14.5
	% of change(2)			-36.7	-72.4	-68.8	-76
	% of change(3)				-56	-50.8	-62

Non – significantp>0.05, significant p< 0.05, highly significant p<0.001.
Significance (1): from G1.Significance (2): from G2.Significance (3): from G3.
% of change (1): different from G1 .% of change (2): different from G2. % of change (3): different from G3.

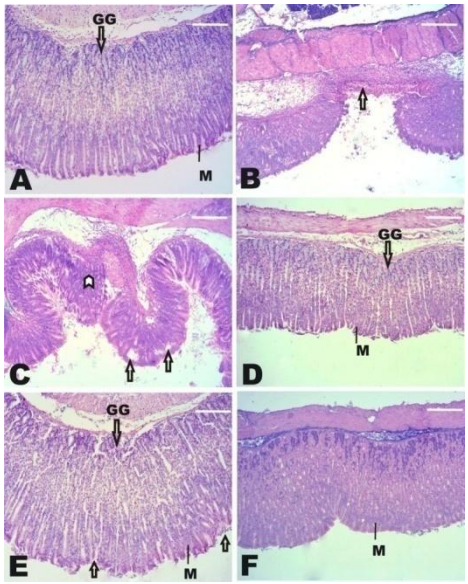
Table (2): Effect of BPF_7 on acid phosphatase and haemoglobin concentration in gastric juice of male mice treated with indomethacin daily and day after the other for 15 days in different groups.

parameters		(G1) Control	(G2) INDO daily	(G3) INDO alternative	(G4) BPF_7	(G5) INDO+ BPF_7 daily	(G6) INDO+ BPF_7 alternative
Acid phosphatase u/L	Mean ± SE	0.44±0.04	1.82±0.20	1.05±0.13	0.58±0.04	0.66±0.08	0.47±0.05
	Significance(1)		p< 0.001	p< 0.01	p>0.05	p< 0.05	P>0.05
	Significance(2)			p< 0.05	p< 0.005	P<0.005	p< 0.001
	Significance(3)				p< 0.05	P<0.05	p< 0.05
	% of change (1)		+313.6	+138.6	+31.8	+50	+6.8
	% of change (2)			-42	-68	-63.7	-74
	% of change (3)				-44.7	-37	-55.2
Hb g/dL	Mean ±SE	0.00±0.00	1.45±0.11	0.36±0.03	0.00±0.00	0.044±0.03	0.04±0.038
	Significance(1)		p< 0.001	p< 0.001	*	p>0.05	p>0.05
	Significance(2)			p< 0.001	p< 0.001	p< 0.001	p< 0.001
	Significance(3)				p< 0.001	p< 0.005	p< 0.005
	% of change (1)		+145	+36	*	+4.4	+4
	% of change (2)			-75	-100	-96.9	-97.2
	% of change (3)				-100	-87.7	-88.8

Non – significantp>0.05, significant p< 0.05, highly significant p<0.001.
Significance (1): from G1. Significance (2): from G2. Significance (3): from G3.
% of change (1): different from G1. % of change (2): different from G2. % of change (3): different from G3.

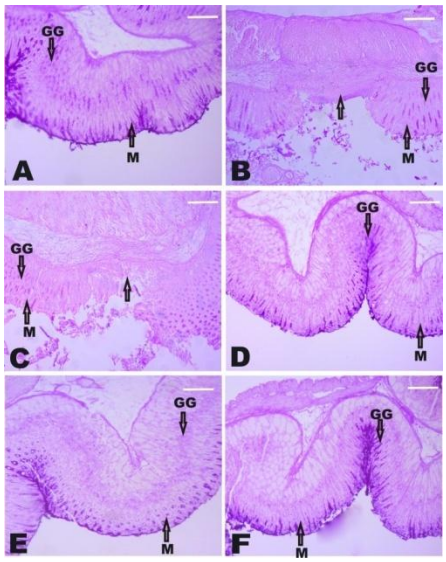
Histological study
Stomach

In indomethacin- administrated groups, perforation and several points of inflammation with diminished gastric glands (Pl. 1 B, C) were observed in daily- or day after the other of administration compared to both the control (Pl. 1 A) and the bradykinin potentiating factor- treated animals (Pl. 1 D). Daily co-administration of indomethacin and bradykinin potentiating factor results in a superficial points of ulceration with prominent gastric glands (Pl. 1E). While in bradykinin co-administration with indomethacin day after the other, intact mucosa with intensely-stained nuclei of gastric glands were observed (Pl. 1F). Also, PAS-stained sections revealed a decrease in the adherent mucus in indomethacin-administrated groups (Pl. 2B, C) as compared to both the control (Pl. 2A) and the bradykinin potentiating factor treated animals (Pl. 2D). Intense adherent mucous was observed in co-administrated animals with bradykinin potentiating factor either in the daily (Pl. 2E) or in the day after the other of indomethacin- administration (Pl. 2F). In addition, acridine orange/ethidium bromide-stained sections, showing depressed fluorescence in indomethacin- administration (Pl. 3B, C) as compared to the control (Pl. 3A) and the bradykinin potentiating factor treated animals (Pl. 3D). Recovered fluorescence was noted in co-administrated animals either in the daily (Pl. 3E) or day after the other (Pl. 3 F) of indomethacin administration.



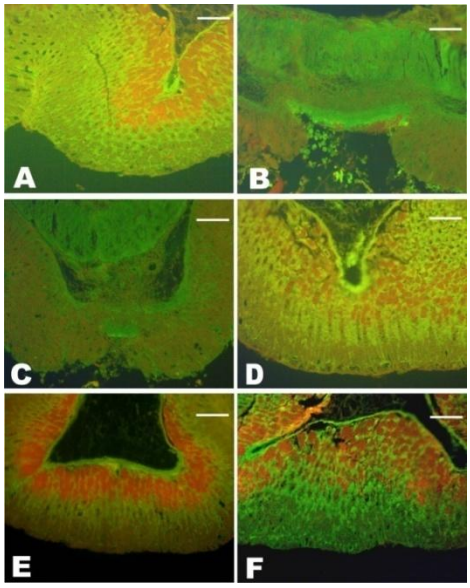
Pl. 1: Photomicrographs of histological sections of stomach showing mucosal perforation (arrow)in daily (B), points of ulceration (arrows) and inflammation (arrow head) in day after the other (C) of indomethacinadministration against intact mucosa (M) with prominent gastric glands (GG) in control (A) and bradykinin potentiating factor-treated animals(D). Recovered mucosa with little ulcerative points (arrows), gastric glands (GG) and intact mucosa (M) were noted in co-administration in the daily (E) and day after the other (F) of indomethacin-administration.

H&E stain, scale bar 20 µm.



Pl. 2: Photomicrographs of stomach showing decrease of adherent mucous of the mucosa in indomethacin of daily (B) or day after the other (C) of administration (arrows) compared to control (A) and the bradykinin potentiating factor-treated animals (D). Intense adherent mucous was noted in daily or day after the other of bradykinin potentiating factor of co-administration (E, F), respectively. (M, mucosa; GG, gastric gland)

PAS stain, scale bar 20µm.

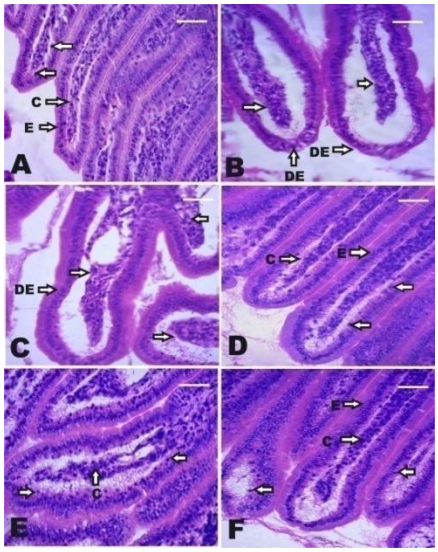


Pl. 3: Photomicrographs of stomach showing depressed fluorescence of mucosa in daily (B) and day after the other (C) of indomethacin administration compared to control (A) and bradykinin potentiating factor-treated animals (D), recovered fluorescence was noted in daily (E) or day after the other (F) of co-administration with bradykinin potentiating factor. Acridine orange ethidium bromide stain, scale bar 20µm.

Intestine

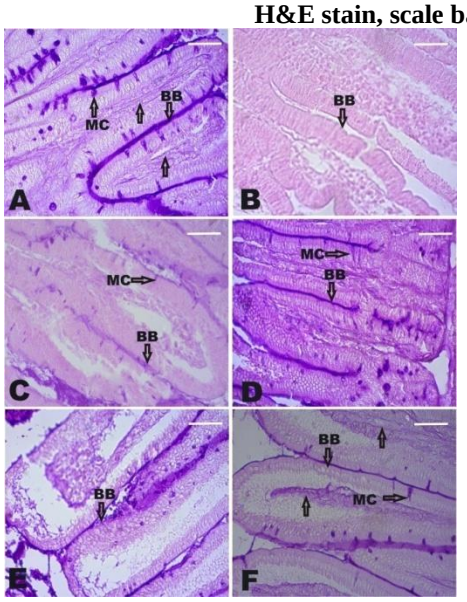
Histological study of the intestine revealed the idealized villus structure. Simple columnar epithelia, connective tissue core and complementary lymphocytes that relatively penetrate the basement membrane were detected in control sections (Pl. 4A). In either daily – or day after the other of indomethacin- administration, squamation and detachment of epithelia at the villus tips that accompanied with shrinkage of the connective tissue core were the most observed symptoms (Pl. 4B, C), respectively, compared to control. In bradykinin potentiating factor-treated animals, intensely-stained nuclei of both the epithelia and those of the connective tissue core was noted (Pl. 4D) as compared to control. Also, increased lymphocytes were noted closely associated to the basement membrane. In co-administration of indomethacin, in either daily (Pl. 4E) or day after theother (Pl. 4F) with bradykinin potentiating factor, epithelial detachment and epithelial squamation were not observed as in case of indomethacin administration. Also increased basophilia of both the epithelial cells and those of the connective tissue core with concomitant increase of lymphocytes were noted. In PAS-stained sections of control, intestinsely-stained mucous secreting cells, brush border of epithelia and reticular fibers were noted (Pl. 5A). In indomethacin of daily administrated animals, these components are negatively stained (Pl. 5B). In contrast, little stained brush border and mucous secreting cells were noted in indomethacin-administrated day after the other with the absence of reticular fibers of the connective tissue core (Pl. 5C) compared to control and those of daily administrated indomethacin. In bradykinin potentiating factor-treated animals, increased stainability of the apical portion of epithelial cells and reticular fibers of connective tissue core accompanied with similar stainability of both the mucous secreting cells and the brush border were noted (Pl. 5D) compared to control. In co-administration of indomethacin and bradykinin potentiating factor of daily or day after the other (Pl. 5E, F), improvement of stainability of mucous secreting cells and the brush border were noted, compared to indomethacin administrated animals. Reticular fibers of connective tissue core are best detected in day after the other of co-administration (Pl. 5F) than in daily administrated animals.

Acridine orange/ethidium bromide-stained sections of intestine revealed inhibition of fluorescence in either daily - or day after the other– of indomethacin administrated animals (Pl. 6B, C), respectively, as compared to control (Pl. 6 A) or those of bradykinin-treated animals (Pl. 6D). In co-administrated animals for both indomethacin and bradykinin potentiating factor, recovery of fluorescence was best detected in animals those exposed to indomethacin and bradykinin potentiating factor either those of daily or day after the other of administration (Pls. E, F), respectively.



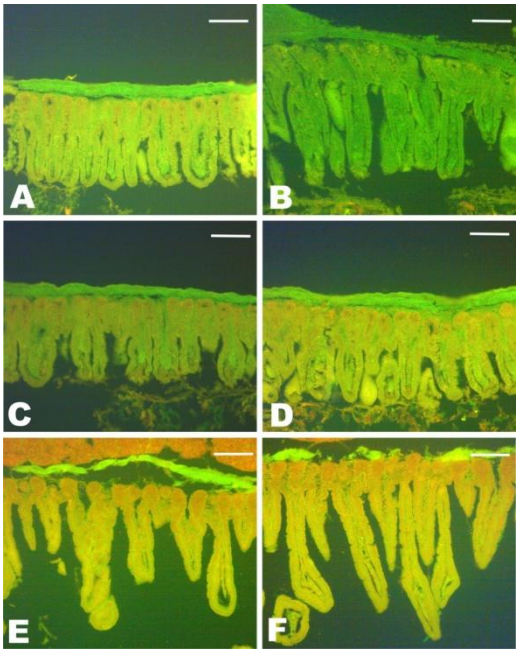
PL. 4: Photomicrographs of intestine showing the idealized villus structure of control (A). Squamation, epithelial detachement (DE), shrinkage of connective tissue core (arrows) intestine in indomethacin-administrated animals of daily and day after the other, respectively (B, C). Increased basophilia with associated lymphocytes (arrows) in bradykinin potentiating factor-treated animals (D). Increased basophilia and complementary lymphocytes with little epithelial detachment (arrows) or squamation compared to indomethacin in indomethacin combined with bradykinin potentiating factor treatment were noted (E, F).

H&E stain, scale bar 20µm.



PL. 5: Photomicrographs of intestinal villi of control (A) showing intensely-stained components. Stained less and faintly stained components in indomethacin administration (B, C) against a well stained in bradykinin potentiating factor treated animals (D) that recovered in combined administration (E, F). Mucous secreting cells (MC), brush border (B.B) and reticular fibers (arrows).

PAS stain, scale bar 20µm.



PL. 6: Photomicrographs of acridine orange-ethidium bromide-stained intestinal sections showing depressed fluorescence in indomethacin of either the daily - (B) or day after the other - (C) administrated animals compared to both the control (A) and the bradykinin potentiating factor treatment (D). Recovery of fluorescence is best detected co-administration of indomethacin and bradykinin potentiating factor (Pls; E, F**). Acridine orange-ethidium bromide-stain, scale bar20µm.**

Discussion

In the present study, administration of indomethacin daily or alternative for 15 days to adult male mice induce histological changes and deleterious effects on stomach and intestine which involve gastric and peptic ulceration, inflammation in the gastrointestinal tract, mucosal perforation and shrinkage of the villus connective tissue core. These results are parallel with those obtained by many investigators (Clive and Stoff, 1984; Davies, 1998; Wolfe *et al*., 1999; Silva *et al*., 2012). The abnormalities induced by indomethacin in the stomach and intestine in this study may be due to a direct action of this drug as a cytotoxic agent (Gürbüz *et al*.,1999).In support of this assumption, Shakeerabanu *et al*. (2011) reported that the indomethacin induced peptic ulcer disease and gastrointestinal. Moreover, gastric and peptic ulcers caused by many factors like aggressive factors, stress and drugs. The aggressive factors such as NSAIDs have been found to produce gastroduodenal ulcers often with bleeding, perforation (Sivri, 2004) and rise in epithelial permeability (Allen *et al*., 1991 and Gürbüz *et al*., 1999). Accordingly, it is suggested that the observed effects by indomethacinin this study can be attributed to permeability changes. These results are consistent with the data of Duffey *et al*. (1981) and Kurtel *et al*. (1992). The authors found that alteration in mucosal integrity in some tissues caused by indomethacin is due to the increased epithelial permeability that results and may be lead to inflammation, perforation and ulceration in stomach and small intestine (Silva *et al*., 2012).

Another factors in indomethacin- induced deleterious effect on stomach and intestine are inhibition of cytoprotective prostaglandin synthesis, *via* the inhibition of cyclooxygenase enzyme (Seibert *et al*., 1994), release reactive oxygen species (Pihan*et al*., 1987), mitochondrial dysfunction (Basivireddy *et al*., 2002) and reduce mucosal blood flow (Kubes *et al*., 1991; Wallace *et al*., 1991). In support of this, some investigators using non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin have reported similar side effects (Wallace *et al*., 1990; Hatazawa *et al*., 2006; Kim, 2008; Takeuchi *et al*., 2010). Moreover, surface mucosal blood flow has been found to play an important role in the ulcer healing process (Spechler, 2002;Cyires, 2005). Therefore a reduced blood flow leads to gastric damage (Santos *et al*., 2005).

The data obtained from the present investigation revealed that the treatment with indomethacin daily or alternative induced a significant increase in the HcL, calcium, acid phosphatase and haemoglobin levels in gastric juice as compared to the normal control group. The increase in these parameters in gastric juice is utilized as a marker of gastric mucosal damage and gastric ulcers (Abd El-Kader *et al*., 2011). With regard to the treatment of ulcerated animals with BPF_7, the ulcerated groups treated with BPF_7 showed a significant improvement of these previous parameters as compared with the model ulcer animals. These improvements are attributed to the effect of endogenous bradykinin which is potentiated by BPF_7 may stimulate the release of prostaglandins (PGs) in a

variety of animal tissues (Levant *et al*., 2006), which in turn inhibits acid secretion that facilitate ulcer healing. This assumption is supported by Aly (1987) who indicated that gastroduodenal protection by prostaglandins is due to increase the mucosal resistance as well as the decrease in aggressive factor mainly acid and pepsin. Also, Terez *et al*. (1990) found that PGs play a role in the control of gastric acid secretion and prevent the damage to gastrointestinal tract, in response to indomethacin (Hatazawa *et al*., 2006; Takeuchi *et al*., 2010). On the other hand, the reducing of gastric acid secretion may be due to the effector of PGE_2 and related PGs likewise which suppress isolated rat stomach enterochromaffin-like cell (ECL) and histamine secretions which located in and adjacent to oxyntic glands (Lindstorm *et al*., 2001). Also, Soll (1986) reported that prostaglandins exert inhibition effects on parietal cells, so the inhibition of their synthesis by indomethacin can result in an increase of gastric acid secretion (Ligumsky *et al*., 1983). A significant increase in gastric juice haemoglobin content after treatment with indomethacin may be due to the damage of the vascular endothelium following the administration of indomethacin to experimental animals (Rainsford, 1983; Wallace *et al*., 1990).

Also, Prichard *et al*. (1989) and Hawkey *et al*. (1991) reported that the ability of indomethacin to promote the bleeding of pre-existing ulcers is most probably related to their inhibitory effects on platelet aggregation which occurs as a consequence of the inhibition of thromboxane synthesis. On the other hand, the obtained results may be attributed to the ability of indomethacin to reduce gastric mucosal blood flow (Ashley *et al*., 1985; Gana *et al*., 1987).As shown in the present results, BPF_7treatment significantly ameliorates the indomethacin-induced changes in the gastric juice haemoglobin content. Also, this significant reduction may be due tothe endogenous bradykinin which in turn stimulated blood flow and prevented congestion and capillary damage. Bradykinin is known to enhance gastric mucosal blood flow *via* activation of B2-recceptors (Petho *et al*., 1994). On the other hand, prostaglandin, a key molecule that stimulates the complex array of ulcer healing and acts as a cytoprotective against mucosal damage and regulates mucosal turn over and repair (Hayllar and Bjarnason, 1995; Hiruma- lima *et al*., 2006).

The data obtained from the present investigation revealed that the significant increase in the gastric juice and acid phosphatase by indomethacin were mitigated and improved by BPF_7. This result may be due to the activation PG synthesis by BPF_7. This suggestion is in harmony with previous studies which indicated that gastric ulceration, vasoconstriction and lysosomal enzymes released were significantly reduced by PGE_2 (Terez *et al*., 1990). PG stabilizes the lysosomal membrane and inhibits the release of lysosomal acid phosphatase which causes mucosal damage. Furthermore, Whittle (1981) reported that PGs have a preferential action on the lysosomal membrane acid phosphatase.

In the present study, the treatment with indomethacin caused significant increase in Ca^{2+} luminal efflux as compared with the control animals. Since disturbances in intracellular sequestration or sudden influxes of extracellular Ca^{2+} have both been implicated in cellular injury (Halliwell, 1987), the treatment of ulcer groups with BPF_7 shows a significant reduction and improvement of Ca^{2+} luminal efflux as compared with the model ulcer animals. This result may be due to the increase of PGs synthesis. The increase of the endogenous PGs synthesis inhibited calcium mobilization that confirms the anti-ulcerogenic activity. In agreement with this result, EL-Bayer *et al*. (1995) found that PGE2, PGE, and 6-Keto PGF1α are effectively inhibit adherence, aggregation, calcium mobilization, superoxide generation and lysosomal enzyme release from the stimulated neutrophils.

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