JOURNAL OF INTERNATIONAL ACADEMIC RESEARCH FOR MULTIDISCIPLINARY
Impact Factor 1.625, ISSN: 2320-5083, Volume 3, Issue 5, June 2015

INFLUENCE OF BRADYKININ POTENTIATING FACTOR ON PROTECTING THE LIVER AND KIDNEY AGAINST THE TOXICITY OF INDOMETHACIN IN MALE MICE

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ABSTRACT

Recently, the attention was focused on bioactive peptides obtained from natural sources and physiological significance of these substances in some diseases characterized by chronic inflammation and tissue damage. Therefore, this experiment was conducted to evaluate the role of bradykinin potentiating factor (BPF_7) separated from jellyfish, *Cassiopia andromeda*, as a natural peptide on liver and kidney dysfunction resulting from indomethacin in model ulcer animals. 60 male mice weighing (25-30 gm.) were used. They were divided into six equal groups. The first group served as control. The second and third groups treated orally with indomethacin (10mg/kg b.w) once day or day after the other respectively during 15 days. The fourth, fifth and sixth groups treated with BPF_7 either alone or in combination with indomethacin throughout the period of the experiment. Treatment with indomethacin induced histological changes and several adverse effects on hepatic and renal tissues which involve inflammation, cellular hypertrophy and glomerular shrinkage paralleled by a significant increase in plasma ALT, AST, urea, uric acid and creatinine levels. These effects were attributed to the hepatocellular and renal damage which in turn declined liver and kidney functions. In contrast, BPF_7 was a significantly ameliorates the deleterious effects induced by indomethacin on these tested parameters without any histopathological changes in hepatic or renal tissues. This improvement in studied parameters may be due to protein biosynthesis and stimulation of glomerular filtration rate. Moreover, the activation of BK and inhibition of angiotensin converting enzyme (ACE) by BPF_7could produce protective effects on renal and liver functions.

KEYWORDS: Bradykinin Potentiating Factor (BPF7), Indomethacin, ALT, AST.

INTRO|DUCTION

The toxicity of some NSAIDs such as piroxicam, indomethacin and aspirin has been evaluated in experimental animals (Davies, 1998; Wolfe*et al*., 1999; Abatan *et al*., 2006).

Also, abnormal liver functions, acute renal failure and nephrotic syndrome in patients after using NSAIDs have been detected by some investigators (Domenyget *et al*., 1984; Paulus and Furst, 1987; Magdalou *et al*., 1990). Moreover similar findings on the effect of NSAIDs in addition to hepatocyte hypertrophy, nuclear pyknosis, hydropic degeneration and organellar damage of liver tissue were also reported (Aydin *et al*., 2003; Hummdi and Habashi, 2012; El-Kordy and Makhlouf, 2014). Since earlier in general, NSAIDs are well known to induce hepatic and renal injury. Tubular lesions and intertubular inflammatory cell invasion with glomerular dilation were noted as the most effects of indomethacin, piroxicam, diclofenac and mefenamic acid (El-Banhawy *et al*., 1994; Ebaid *et al*., 2007; El-Kordy and Makhlouf, 2014;Somchit *et al*., 2014).

Several authors observed that the indomethacin causes alterations in biochemistry of plasma and histological changes in liver, kidney and bone marrow of experimental animals (Rabinovitz and Thiel,1992; Tomic *et al*., 2008; Shakeerabanu *et al*., 2011; Silva *et al*., 2012). They concluded that these adverse effects contributed to oxidative stress induced by the drug and inhibition of prostaglandin synthesis. Moreover, the fact that indomethacin caused greater damage to the animals is further confirmed by the histopathological lesions produced. Administration of indomethacin caused periportal hepatic necrosis and Kupffer's cells proliferation. These are all signs of acute hepatoxicity (Hodgson and Levi, 1985; Smith *et al*., 1986; Klaassen, 2001). Some studies have shown that indomethacin, aspirin and salicylates produce acute tubular necrosis, renal necrosis and enhancement of vasopressin activity in animals. These effects lead to water retention and give credence to the acute renal failure (Aranold *et al*., 1973; Prescott, 1982; Clive and Stoff, 1984). Furthermore, at last three different types of nephrotoxicity have been associated with NSAIDs administration (Bach, 1997; Tarloff, 1997). These include acute renal failure, analgesic nephropathy and interstitial nephritis which are characterized by a diffuse interstitial edema with infiltration of inflammatory cells (Whelton and Watson, 1998).

An increase of urea and creatinine concentrations in serum is a good indicator for kidney diseases and may indicate renal damage (Abdel Aziz, 2001). In addition, venoms of *cnidarian* animals were found to be of medical importance (Rich and Cheras, 2009). The toxins of the box jellyfish (*Chirone fleckeri*)*;* irukandji(*Carukia barnesi*) and blue jellyfish (*Catostylus mosaicus*) have been detected to include bradykinin and related polypeptides which have a kinin-like action (Burnett and Calton, 1974). Also, the jelly fish, *Cassiopia andromeda* crude extract was found to ameliorate the effect of zearalenone mycotoxin on the

liver of male mice (Al-Seeni *et al.,* 2011). Bradykinin (BK) is one of the main effectors of the kallikerin-kinin system. It attenuates liver damage and fibrosis development in a rat model of chronic liver injury (Pau *et al*., 2007), mediates the beneficial effects of renin-angiotensin system inhibitors which have effective role in liver regeneration (Ramalho *et al*., 2002; Campbell *et al*., 2004), enhanced prostaglandin synthesis (Levant *et al*., 2006) which is a key molecule that stimulates the complex array of ulcer healing mechanism (Abdallah*et al*., 2011), induced vascular permeability and mitogenesis (Wu *et al*., 2002) and stimulates the synthesis of prolactin and growth hormone (Chihara *et al*., 1982). The growth hormone and the growth factors increase protein synthesis and stimulate the proliferation of mammalian cells (Montogomery*et al*., 1980).Therefore, this experiment was conducted to evaluate the role of bradykinin potentiating factor (BPF_7) separated from jellyfish, *Cassiopia andromeda*as a natural peptide on liver and kidney dysfunction resulting from indomethacin in model ulcer animals.

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, 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, Sohag University 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 (alternative), respectively during 15 days in order to induce ulcers (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_7 as used in treating the fourth group .

Processing

At day 15, all animals of each group were sacrificed and dissected. The blood samples were taken from the heart in plastic tubes containing EDTA as anticoagulant and the samples were centrifuged at 3000 r.p.m for 20 minutes to obtain clear plasma that stored at -20Ċ until used for studies. From each animal, liver and kidney were taken quickly. Parts of these organs were taken for histological and histochemical examination

Histological assessment

For histological study, liver, kidney, stomach and intestine were excised and fixed in carnoy fixative for half an hour and 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-stained 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.

Plasma analysis

The Plasma level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined spectrophotometrically using transaminases kits according to the method described by Henary *et al*. (1960). Uric acid, urea and creatinine were measured according to Barham and Trinder (1972); Fawcett and Soctt (1960) and Bartles *et al*. (1972), respectively.

Statistical analysis:-

Results were expressed as mean ± S.E and statistically processed using t-test for comparison between each experimental group and the control. Statistical significance was acceptable at a level of $p<0.05$.

RESULTS

Analysis of serum constituents has proved to be useful in the detection and diagnosis of metabolic disturbance and disease. The present results showed highly significant difference in the urea, uric acid, creatinine, ALT and AST of male mice treated with indomethacin daily and alternative (G2 and G3) as compared to normal group (G1) (Tables 1 – 3). Also, both the normal male mice (G4) and ulceration groups (G5 and G6) treated with BPF_7 showed a significant increase in the plasma levels of AST, ALT and urea without apparent of significant changes in plasma uric acid and creatinine levels as compared to normal control group (G1). Moreover, the results revealed that there are a significant decreasing effect and improvement in these parameters when compared the BPF_7 group (G4) and ulceration groups treated with BPF_7 (G5 and G6) with the groups of indomethacin daily (G2) and alternative only (G3) as shown in Tables (1, 2, 3).

Table (1): Effect of BPF_7 on urea and uric acid concentration in blood plasma 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_7daily	(G6) INDO+ BPF_7 alternative
Urea mg/dL	Mean ±SE	30.2±1.7	87.5±2.4	70.1±3.0	48.3±2.07	52.9±3.2	37.6±1.56
	Significance(1)		p< 0.001	p< 0.001	p< 0.001	p< 0.005	p< 0.05
	Significance(2)			p< 0.01	p< 0.001	p< 0.001	p< 0.001
	Significance(3)				p< 0.005	p< 0.05	p< 0.001
	% of change(1)		+189.7	+132	+59.9	+75	+24.5
	% of change (2)			-19.8	-44.8	-39.5	-57
	% of change (3)				-31	-24.5	-46.3
Uric acid mg/dL	Mean ±SE	3.68±0.25	6.11±0.34	5.57±0.36	3.74±0.42	4.03±0.38	3.6±0.30
	Significance(1)		p< 0.005	p< 0.01	p>0.05	p>0.05	P> 0.05
	Significance(2)			P>0.05	p< 0.01	p< 0.01	p< 0.005
	Significance(3)				p< 0.05	p< 0.05	p< 0.05
	% of change (1)		+66	+51	+1.6	+9.5	-2.17
	% of change (2)			-8.8	-38.7	-34	-41
	% of change (3)				-32.8	-27.6	-35.36

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 fromG3.

Table (2): Effect of BPF_7 on creatinine concentration in blood plasma 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
Creatinine mg/dL	Mean ±SE	0.39±0.019	1.059±0.14	0.96±0.11	0.459±0.037	0.46±0.51	0.426±0.037
	Significance(1)		p< 0.005	p< 0.005	P>0.05	P>0.05	P>0.05
	Significance(2)			P>0.05	p< 0.01	p< 0.01	p< 0.005
	Significance(3)				p< 0.005	p< 0.01	p< 0.005
	% of change (1)		+171.5	+146	+17.6	+17.9	+9.2
	% of change (2)			-9.3	-56.6	-56.5	-59.7
	% of change (3)				-52.18	-51.9	-55.6

Non – significant p>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 fromG3.

Table (3): Effect of BPF_7 on ALT and AST concentration in blood plasma of male mice treated with indomethacindaily and day after the other for 15days 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
ALTu/L	Mean±SE	26±1.6	90.38±2.8	59.01±2.22	33.06±1.75	39.4±1.99	38.8±2.77
	Significance(1)		p< 0.001	p< 0.001	p< 0.05	p< 0.005	p< 0.01
	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)		+247.6	+126.9	+27.15	+51.5	+49.2
	% of change (2)			-34.7	-63.4	-56.4	-57
	% of change (3)				-43.9	-33.2	-34
ASTu/L	Mean ±SE	46.16±2.3	201.83±4.2	174.83±3.6	85.16±2.7	78.83±2.4	57.83±2.08
	Significance(1)		p< 0.001	p< 0.001	p< 0.001	p< 0.001	P<0.01
	Significance(2)			p< 0.005	p< 0.001	p< 0.001	p< 0.001
	Significance(3)				p< 0.001	p< 0.001	p< 0.001
	% of change (1)		+337	+278	+84.4	+70.7	+25
	% of change (2)			-13.3	-57.8	-60.9	-71
	% of change (3)				-51	-574.9	-66.9

Non – significant p>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 fromG3.

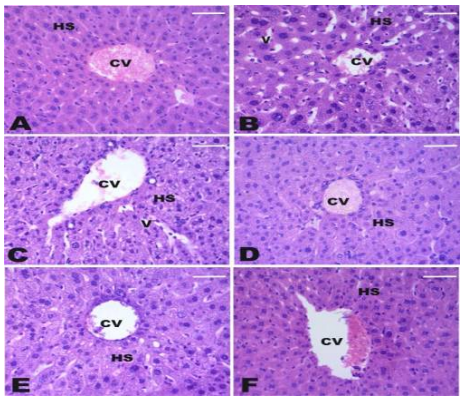
Liver

Normal hepatic paranchyma consisting of hepatic cords interspersed with blood sinusoids was noted in both the control (Pl. 1A) and the bradykinin potentiating factor–treated animals (Pl. 1D). In indomethacin–administrated animals of daily administration, cellular hypertrophy

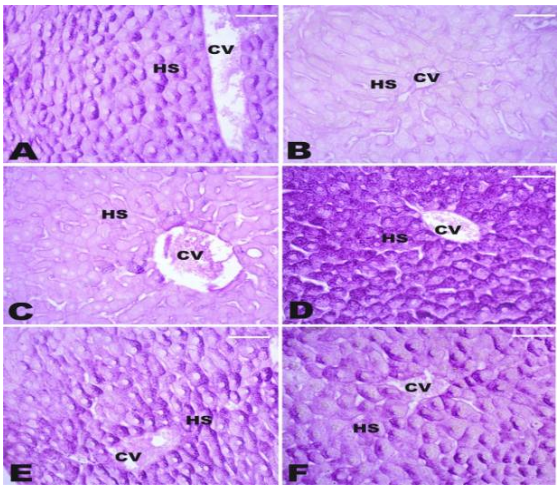
and dilated sinusoids with inflammatory cells were noted (Pl. 1B). In day after the other of indomethacin administrated animals, disorganized parenchyma was noted (Pl. 1C) as compared to that of control or bradykinin potentiating factor treated animals. Tissue recovery was best observed in co-administration of indomethacin and bradykinin potentiating factor in day after the other (Pl. 1F) than in those of daily co-administration which showing little hypertrophy (Pl. 1E) as compared to those of daily exposed indomethacin.

PAS-stained liver sections revealed a negative content of polysaccharides either in the daily- or the day after the other-administrated indomethacin (Pl. 2B, C) as compared to control (Pl. 2A). In bradykinin potentiating factor–treated animals, increase of carbohydrate content was noted (Pl. 2D). Recovery of carbohydrate content of the liver tissue was noted in co-administration either daily or day after the other (Pl. 2E, F) as compared to those of indomethacin administration.

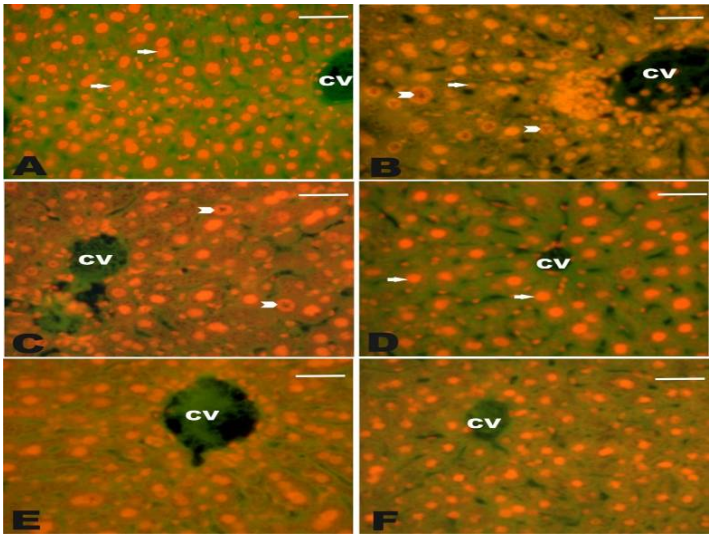
Acridine orange/ethidium bromidestained liver sections also revealed the damage at the central region of cell nuclei of indomethacin in daily administrated animals (Pl. 3B) than in the day after the other of administration (Pl. 3C). The effect of indomethacin is reflected by the stained inflammatory cells and the pale fluorescence of the central region of the cell nuclei compared to their peripheral region in daily administrated animals. Recovered fluorescence of nuclei in co-administrated animals was detected (Pl. 3E, F) as compared to either the indomethacin administration (Pl. 3B, C) or both of the control (Pl. 9A) and bradykinin potentiating factor treatment (Pl. 3D).



PL. 1: Photomicrographs of liver tissue showing cellular hypertrophy and disorganization in indomethacin of daily (B) or day after the other (C) of administration compared to the control (A) or bradykinin potentiating factor treatment (D). Recovered tissue was noted in daily (E) or day after the other (F) of co-administration than in the corresponding of indomethacin administration. Hs, hepatic cords; Cv, central vein. H&E stain, scale bar 20µm.



PL. 2: Photomicrographs of liver tissue showing negative contents of carbohydrates in daily (B) or day after the other (C) of indomethacin administration compared to the control (A) or bradykinin potentiating factor treatment (D). Recovered carbohydrate content was noted in co-administration of both the indomethacin and bradykinin potentiating factor of daily (E) or day after the other (F). Hs, hepatic cords; Cv, central vein. PAS stain, scale bar 20µm.

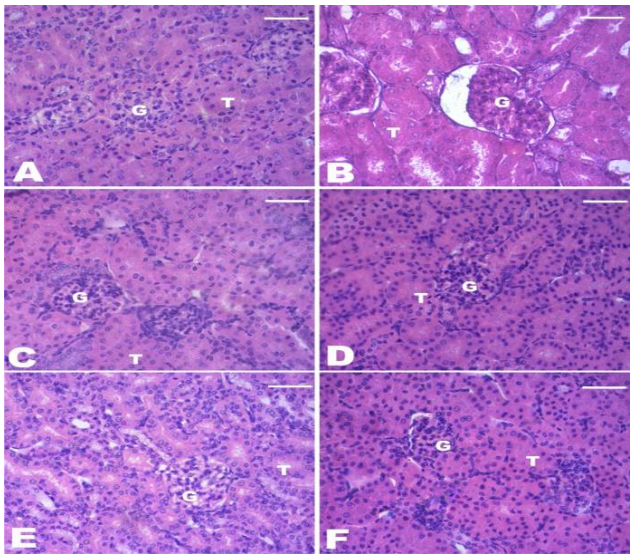


PL. 3: Photomicrographs of acridine orange/ethidium bromide-stained liver sections showing bright–stained inflammatory cells (arrows) and negative–stained central region of hepatocyte nuclei (arrow head) of daily administrated indomethacin (B) compared to bright–stained nuclei of control (A), day after the other of indomethacin administration (C) and bradykinin potentiating factor treatment (D). Recovered stained nuclei, and decrease of inflammatory cells were noted in daily and day after the other of co-administration (Pl. E,F). Acridineorange-ethidium bromide stain, scale bar 20µm.

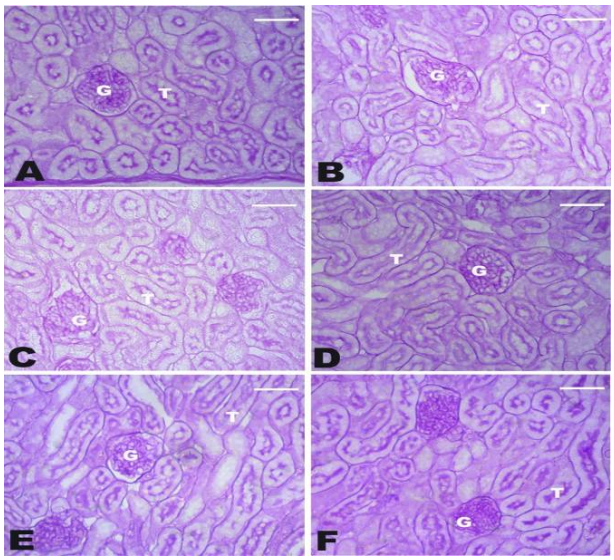
Kidney

In kidney cortex, the most observed effect was noted in daily administrated indomethacin among the glomerular shrinkage (Pl. 4B) compared to the day after the other of indomethacin administration (Pl. 4C), control (Pl. 4A), and the bradykinin potentiating factor treated animals (Pl. 4D). Co-administration of bradykinin and indomethacin ameliorate the glomerular shrinkage in daily administration (Pl. 4E) while it increase the nuclear stainability throughout the cortical tissue (Pl. 4F) similar to that observed in bradykinin potentiating factor-treated animals (Pl. 4D).

In PAS-stained sections, the effect of indomethacin in either the daily – or the day after the other – administrated animals is clearly obvious than in haematoxylin and eosin-stained sections. Inhibition of mesangial and brush border stainability of the glomeruli and kidney tubules was noted (Pl. 5B, C) as compared to the stainability of both in control (Pl. 5A) and bradykinin potentiating factor-treated animals (Pl. 5D). In co-administration of daily or day after the other of administrated animals, recovered stainability of mesangial cells and the brush border of kidney tubules was noted (Pl. 5E, F)**.**



PL.4:Photomicrographs of histological sections through the kidney cortex showing shrinked glomerulus of daily administrated indomethacin (B) compared to control (A), day after the other of indomethacin administration (C) and bradykinin potentiating factor treatment (D). Amelioration of glomerular shrinkage in daily co-administration (E) and intense-stained nuclei in day after the other of co-administrated (F) were noted. G, glomeruli; T, Tubules. H & E stain, scale bar 20 µm.



PL. 5:Photomicrographs of histological sections through the kidney cortex showing depressed stained mesangial and brush border of glomeruli (G) and kidney tubules of daily (B) and day after the other (C) of indomethacin administration compared to control (A) and bradykinin potentiating factor treatment (D). Recovered stainability is shown in co-administration either in the daily (E) or day after the other (F). Glomeruli (G),Kidney tubules (T). PAS-stain, scale bar 20µm.

DISCUSSION

In the present study, administration of indomethacin induced histological changes and deleterious effects on liver and kidney which involve inflammation, cellular hypertrophy and glomerular shrinkage. The abnormalities induced by indomethacin in the liver and kidney in this study were attributed to a direct action of this drug as a cytotoxic agent (Gürbüz *et al*.,1999).On the other hand, the results revealed that the ulceration groups treated with bradykinin potentiating factor (BPF_7) showed an improvement of histological changes. Therefore, the study suggests that BPF_7 has the potential of ameliorating the toxic effects of indomethacin, possibly by the direct effect of this factor which acts as a cytoprotective agent or indirect action through the stimulation of endogenous bradykinin which in turn, enhances prostaglandins synthesis (Larson *et al*., 1991; Glasgow *et al*., 1997 and Abu-amra, 2001).In support of this, the toxicity of indomethacin is mainly attributed to inhibition of cyclooxygenase (Cox) which leads to depletion and block of endogenous prostaglandins (PGs) that could be a major factor in the pathogenesis of these lesions (Ajeigbe *et al*., 2011; Shakeerabanu *et al*., 2011). Also, prostaglandins supplementation prevents the damage to

gastrointestinal tract in response to indomethacin (Hatazawa *et al*., 2006; Takeuchi *et al*., 2010).

In the present study, indomethacin daily or alternative treatment caused a significant increase in plasma ALT, AST, urea, uric acid and creatinine levels compared to normal control group. Similar results were observed by many investigators (Kaneko and Cornelius, 1985; Bush, 1991; Duncan *et al*., 1994; Klaassen, 2001; Abatan *et al*., 2006; Silva *et al*., 2012). These parameters used as a good indicator for diagnosis of renal and liver diseases (Sturgill and Lambart, 1997; Abdel-Aziz, 2001). In addition, NSAIDs such as indomethacin, piroxicam and diclofenac induced hepatic injury, renal damage and enhanced vasopressin activity which lead to water retention and give credence to the acute renal failure (Aranold *et al*., 1973; Prescott, 1982; Clive and Stoff, 1984; Bush, 1991; Ebaid *et al*., 2007; El-Kordy and Makhlouf, 2014; Somchit *et al*., 2014). Therefore, these effects were due to the toxic effect of the drug which leads to hepatocellular and renal damage. In support of this, several reports have concluded that the administration of indomethacin caused a decline in liver and kidney functions, associated with increased plasma or serum level of AST, ALT, urea, uric acid and creatinine (Bush, 1991; Abatan *et al*., 2006).

The present data demonstrated that both the normal male mice (G4) and the ulceration groups (G5, G6) treated with BPF_7 showed a significant increase in the plasma levels of AST, ALT and urea without apparent significant changes in plasma uric acid and creatinine levels as compared to control group. Also, the results indicated that the enhancement of the tested parameters (AST, ALT and urea) were noted without any histopathological changes in hepatic and renal tissues and the effect of this factor (BPF_7) on these parameters were less significant than the effect of indomethacin daily or alternative. The marked increase in hepatic enzymes by this factor may be due to an enhancement of protein biosynthesis, changes of cellular permeability and release of cyclic AMP. In support of this, a significant enhancement in cyclic AMP was observed in many cells with bradykinin which stimulated by this factor (Enjalbert *et al*., 1980; Etgen and Browning, 1983; Abu-Amra, 2000; Abu-Amra and Abd El-Rehim, 1992). Moreover, the increase in plasma urea by BPF_7 may be attributed to increased ammonia production which is converted to urea by the liver or may be due to stimulation of the kidney glomerular filtration rate by this factor as previously reported (Abd El-Rehim, 1995; Abu-Amra, 2001).

Regarding the treated groups, the results showed that the BPF_7 was a significantly minimizes and ameliorates the deleterious effects induced by indomethacin on tested

parameters as compared with ulceration groups. This amelioration may be attributed to a direct effect of this extracted factor which contains many amino acids (16 amino acids) (Seleem, 2003) that play an important role in modulating numerous physiological functions (Chilaka *et al*., 2006). Also, the protective effect of BPF_7 may be resulting from the activation of endogenous bradykinin (BK) and inhibition of angiotensin converting enzyme (ACE) (Faria-Ferreira *et al*., 1999 and Seleem, 2003) which play an important role in attenuating the suppressed liver and kidney function (Dean *et al*., 1997; Maclaughlin *et al*., 1998; Andersen *et al*., 2000; Knigge*et al*., 2000; Taal and Brenner, 2000; Pau *et al*., 2007), reducing liver and kidney injury and promoting liver regeneration (Katsutashi *et al*., 2006; Print, 2008; Padrissa *et al*., 2009) resulting in minimizing the hazardous effects of indomethacin on the liver and renal function. Moreover, BK has a protective effect on the progression of renal failure (Knigge *et al*., 2000). Therefore these findings suggest that activation of BK and inhibition of ACE by BPF_7 could produce improving effects on renal and liver function (Wang *et al*., 2000).

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