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ORIGINAL ARTICLE

Modulation of the pharmacological properties of meloxicam by octreotide in rats

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Inflammation;
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Abstract The purpose of this study was to investigate the anti-inflammatory, analgesic, antipyretic and antiulcer properties of somatostatin analogue octreotide (10 and 100 μg/kg) and its influence on the effect of NSAID meloxicam (1 and 2 mg/kg) in rats. Carrageenan-induced rat paw oedema was used as an acute anti-inflammatory model as well as adjuvant-induced arthritis as a chronic model. Hot plate test on rats and acetic acid (0.6%) writhing test were used as acute analgesic models while the plantar test using an infrared beam directed to the paw of arthritic rats was used as a chronic analgesic model. Antipyretic effect was evaluated using Brewer's yeast (44%) induced hyperthermia in rats while pylorus ligation was used as a model to evaluate the ulcerogenic effects. Meloxicam, octreotide and their combinations administered subcutaneously showed anti-inflammatory effects in both acute and chronic models. Only the high doses of meloxicam and octreotide showed significant analgesic effect in the hot plate test, while all doses showed significant analgesic effects in the acetic acid-induced writhing test and in the plantar test. In yeast-induced hyperthermia, only meloxicam has an antipyretic effect. Meloxicam resulted in profound gastric lesions and exerted deleterious effects on the gastric mucosa in pyloric-ligated rats. Octreotide did not cause any harmful effect on the gastric mucosa, besides; octreotide attenuated the harmful ulcerogenic effects of

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meloxicam when administered in combination with it. Both meloxicam and octreotide and their combination significantly decreased the malondialdehyde (MDA) content in the arthritic rats indicating their antioxidant effects.

1. Introduction

Inflammation is a sequence of defensive reactions of the vascularized tissues to the pathogenic insult of another origin. Rheumatoid arthritis (RA) is a chronic, systemic, relapsing, debilitating inflammatory autoimmune disorder that presents as a symmetric polyarthritis associated with swelling and pain in multiple joints (Sweeney and Firestein, 2004).

Most classical nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin, indomethacin or ibuprofen, show little specificity of inhibition towards COX isforms (Vane and Botting, 1995; Lora et al., 1997). Studies with compounds such as meloxicam (Dequeker et al., 1998), nimesulide (Lapeyre-Mestre et al., 2011), celecoxib (Louder et al., 2011) have demonstrated that selective COX-2 inhibitors retain the anti-inflammatory effects characteristic of NSAIDs with a marked increase in gastrointestinal tolerability as compared to classical non selective ones. Meloxicam is a member of the enolic acid group of NSAIDs. It is a COX-2 preferential with less gastrointestinal toxicity than nonselective NSAIDs (Beubler, 2003).

Somatostain is a neuropeptide that is widely distributed in the stomach, the gastrointestinal tract and the pancreas. Various actions of somatostatin are mediated through specific membrane receptors, which have been demonstrated in various regions of the brain. Furthermore, receptors are identified in the anterior pituitary, endocrine and exocrine pancreas, the mucosa of the gastrointestinal tract, as well as in cells of the immune system (Sreenivasan et al., 2011).

Somatostatin functions as a neurotransmitter with generally inhibitory actions in many regions of the central nervous system. Synthetic, metabolically stable analogues have been developed for clinical use. However, only two of these, namely octreotide and lanreotide, were introduced for routine therapies in humans (Strosberg et al., 2011).

Somatostatin plays an important role in inflammation. The expression of somatostatin has been found in the inflammatory foci, and this neurohormone is considered to be a local anti-inflammatory factor (Ohno et al., 1990). Activation of peripheral somatostatin receptors reduces both the inflammatory pain and the activity of sensitized receptors (Carlton et al., 2001).

It has been shown that somatostatin may restrict the inflammatory process in several ways: It down modulates a number of immune functions such as lymphocyte proliferation, immunoglobulin production and the release of the proinflammatory cytokines such as interferon-γ (INF-γ) (Ten Bokum et al., 2000). It acts antagonistically to substance P, the main neuroendocrine proinflammatory mediator (Ten Bokum et al., 2000). It also inhibits plasma and leucocyte extravasation (Wiedermann et al., 1993) and induces vasoconstriction and inhibits angiogenesis (Reubi et al., 1994; Heppelmann and Pawlak, 1997).

Taken together, the aim of the present work is to study the possible modulation of the pharmacological properties of meloxicam by the combined therapy with octreotide.

2. Material and methods

2.1. Animals

Adult male albino Sprague–Dawley rats weighing 130–150 g and adult male Swiss albino mice weighing 20–25 g were used in the present study; they were obtained from the animal house colony in the National Research Center (Giza, Egypt).

2.2. Drugs

1. Octreotide acetate was obtained from Novartis Pharma (Cairo, Egypt), and the used ampoule was 100 μg/ml. Two doses were used: 10 and 100 μg/kg.
2. Meloxicam was generously gifted from Amoun Pharmaceutical Company (Cairo, Egypt) and used in two doses: 1 and 2 mg/kg.

3. Methods

3.1. The carrageenan-induced rat paw oedema

Paw oedema was induced by subplantar injection of 100 μl of 1% sterile carrageenan in saline into the right hind paw (Winter et al., 1962). The rats received vehicle or drugs 1 h before carrageenan injection. The hind paw volume was measured immediately before carrageenan injection and at selected times thereafter by water displacement method using 7410, plythesmometer, Ugo Basile, Comerio, Italy (Fig. VI) (Chattopadhyay et al., 2002).

3.2. The adjuvant-induced arthritis

Adjuvant arthritis was induced by subplantar injection of 0.1 ml Freund's complete adjuvant (FCA) in the right hind paw (Pearson, 1956). Paw volume was duplicately measured just prior to adjuvant injection and at intervals of three days for 30 days after adjuvant injection using a water displacement plethysmometer and the mean values were recorded (Chakraborty et al., 2004).

3.3. The hot plate test

For three consecutive days preceding the experiment, rats were adapted on the hot plate by placing them on a plate maintained at room temperature for 15 min each day. Each animal was then placed gently onto a 50 °C hot plate to perform the test. Latency to exhibit nociceptive responses, such as licking paws or jumping off the hot plate was determined 30, 60, 120 min after administration of test substances or the saline (Laviola and Alleva, 1990).

3.4. The writhing test

The vehicle and the drugs were administered subcutaneously 30 min before the intraperitoneal injection of 0.6% acetic acid in distilled water (10 ml/kg). The stretching reaction was

Table 1 Effect of meloxicam, octreotide and their combinations on the carrageenan-induced paw oedema in rats.

Groups	Oedema volume (ml)				
	0 h	1 h	2 h	3 h	4 h
Control	0.36 ± 0.012	0.55 ± 0.011*	0.59 ± 0.015*	0.62 ± 0.012*	0.64 ± 0.011*
Mel. 0.5 mg/kg	0.34 ± 0.009	0.43 ± 0.017*,@	0.47 ± 0.015*,@	0.50 ± 0.014*,@	0.52 ± 0.015*,@
Mel. 1 mg/kg	0.36 ± 0.026	0.45 ± 0.014*,@	0.47 ± 0.015*,@	0.50 ± 0.009*,@	0.52 ± 0.009*,@
Mel. 2 mg/kg	0.34 ± 0.007	0.41 ± 0.008*,@	0.44 ± 0.011*,@	0.47 ± 0.012*,@	0.49 ± 0.011*,@
Oct. 10 μg/kg	0.31 ± 0.013	0.43*@ ± 0.011	0.49*@ ± 0.010	0.52*@ ± 0.008	0.53 ± 0.020 *@
Oct. 25 μg/kg	0.34 ± 0.006	0.44*@ ± 0.008	0.50*@ ± 0.013	0.53*@ ± 0.011	0.55 ± 0.010 *@
Oct. 100 μg/kg	0.33 ± 0.005	0.43*@ ± 0.009	0.48*@ ± 0.012	0.51*@ ± 0.010	0.52 ± 0.010 *@
Mel. 1 mg/kg + Oct 10 μg/kg	0.35 ± 0.005	0.45 ± 0.009*,@	0.47 ± 0.007*,@	0.50 ± 0.007*,@	0.51 ± 0.009*,@
Mel. 1 mg/kg + Oct. 100 μg/kg	0.33 ± 0.006	0.39 ± 0.005*,@	0.42 ± 0.009*,@	0.46 ± 0.011*,@	0.49 ± 0.011*,@

The data represent the mean ± standard error of the mean (n = 6–10).
* Statistically significant from zero time: $P < 0.05$.
@ Statistically significant from the control group at the corresponding time: $P < 0.05$.

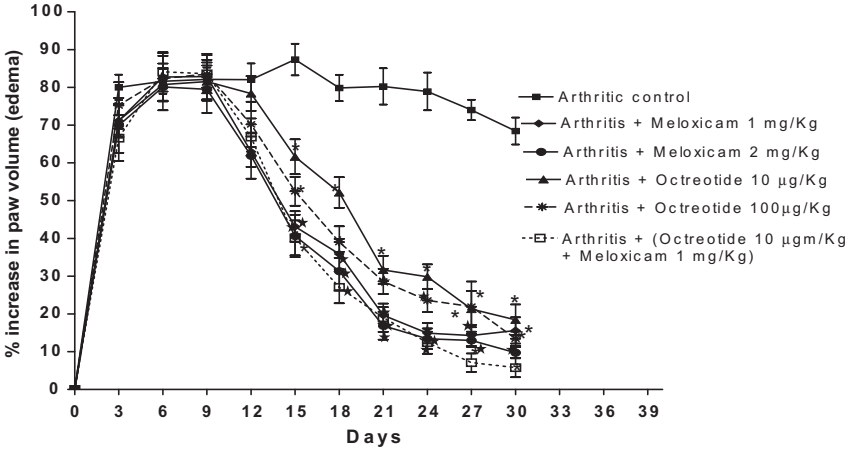

Figure 1 Effect of meloxicam (1 and 2 mg/kg), octreotide (10 and 100 μg/kg) and their combination (meloxicam 1 mg/kg + octreotide 10 μg/kg) on arthritic rat paw oedema. The data represent the mean ± standard error of the mean (n = 6–10). *Statistically significant from control arthritic group at the corresponding time point: $P < 0.05$. Other significances' signs were omitted for simplicity.

Table 2 Effect of meloxicam, octreotide and their combination on the perception of thermal pain in hot plate test in rats.

Groups	Reaction time (s)			
	Zero time	30 min	60 min	120 min
Control	8.30 ± 0.63	7.89 ± 0.93	9.56 ± 0.67	9.9 ± 0.60
Mel. 1 mg/kg	7.55 ± 0.79	15.26*,@ ± 2.26	12.53 ± 0.84	12.46 ± 1.43
Mel. 2 mg/kg	8.28 ± 0.78	16.41*,@ ± 0.94	16.64*,@ ± 2.82	17.10*,@ ± 1.96
Oct. 10 μg/kg	7.55 ± 0.79	7.77 ± 1.00	11.92 ± 1.16	12.93 ± 2.78
Oct. 100 μg/kg	8.20 ± 0.93	14.35 ± 0.60	16.64*,@ ± 1.70	16.55*,@ ± 1.20
Mel. 1 mg/kg + Oct. 10 μg/kg	8.06 ± 0.90	10.58 ± 1.90	13.76 ± 1.50	15.01*,@ ± 1.31

The data represents the mean ± standard error of the mean (n = 8).
* Statistically significant from control arthritic group at the corresponding time point: $P < 0.05$.
@ Statistically significant from zero time: $P < 0.05$.

observed (Koster et al., 1959). Number of writhes (muscular contractions) was counted for 30 min immediately after the acetic acid injection and expressed as writhing numbers (Chakraborty et al., 2004).

3.5. The plantar test

Animals were allowed to accommodate in 10 cm × 17 cm enclosure of 7371-plantar test, Ugo Basile, Comerio, Italy. An infrared beam, (Halogen 64,607 OSRAM, 8 V-50 W-IR movable bulb, Ugo Basile, Comerio, Italy) was applied through the transparent surface of the enclosure to the plantar surface of the right hind paw of each animal. Time required for the animal to withdraw its paw was recorded. Pain threshold assessment was performed in the following days: day 0 (before arthritic induction), 5, 9, 13, 17, 21, 25 and 29 (Kwon et al., 2002; Chillingworth and Donaldson, 2003).

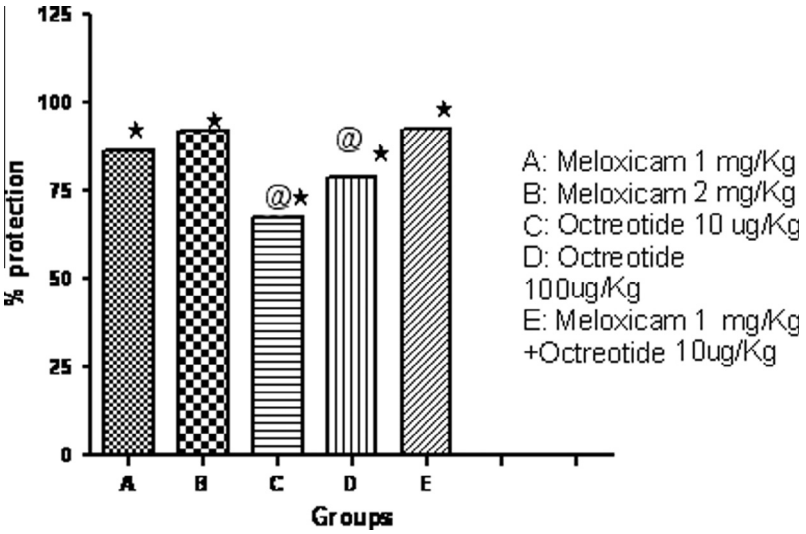

Figure 2 Effect of meloxicam (1 and 2 mg/kg), octreotide (10 and 100 μg/kg) and their combination (meloxicam 1 mg/kg + octreotide 10 μg/kg) on acetic acid-induced writhing response in mice. The data represent the mean ± standard error of the mean (n = 6–10). *Significantly different from the control group $P < 0.05$. @Statistically significant from combination (octreotide 10 μg/kg + meloxicam 1 mg/kg): $P < 0.05$.

3.6. Antipyretic effects

One millilitre per hundred grams body weight of 44% yeast suspension was administered by an intramuscular injection into each animal of all the tested groups. Before yeast injection rectal temperature was recorded for all groups. The rectal temperature measured 18 h following the yeast injection serves as the basic line of the elevated body temperature. Rectal temperature was recorded by a multichannel electric thermometer (TMP 812 Digital Thermometer, Ugo Basile, Comerio, Italy) 1 and 2 h after drugs' administration (Roszkowski et al., 1971).

3.7. Evaluation of gastric ulcerogenic potential

Studies were performed in pyloric ligated rats (Shay et al., 1945). Under ether anaesthesia a midline abdominal incision was made. The pylorus was ligated, care being exercised that neither damage to the blood supply nor traction on the pylorus occurs. The test compounds were injected subcutaneously immediately after pylorus ligation. After 4 h, the animals were sacrificed, the abdomen was opened and a ligature was placed around the oesophagus close to the diaphragm. The stomach was removed, and the contents were drained in a centrifuge tube. The mucosa was examined for mucosal necrotic lesions, red streaks and red erosions and photos were taken with a digital camera. The total lesion number was counted as well as the severity of lesions was calculated (Mózsik et al., 1982). The volume of the gastric juice was carefully collected in graduated tubes. After centrifugation the gastric acid output was determined by titration to pH 7.0 with 0.1 N NaOH and H^+ output was expressed as μEq/4 h (Isenberg, 1978). The gastric mucosa was subjected to a histological examination.

After macroscopic evaluation of mucosal lesions, the stomachs were pinned flat on cardboard and immersed in 10% formalin solution and later embedded in paraffin. Haematoxylin and eosin sections were evaluated qualitatively under light microscopy (Drury and Wallington, 1980).

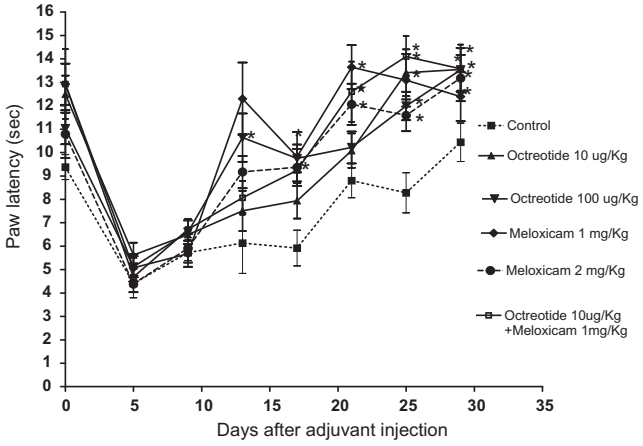

Figure 3 Effect of meloxicam, octreotide and their combination on the perception of thermal pain in arthritic rats (plantar test). The data represent the mean paw latency time ± standard error of the mean of eight rats. *Statistically significant from control arthritic group at the corresponding time point: $P < 0.05$.

4. Statistical analysis

Values were expressed as means ± S.E. mean. Comparisons between means were carried out using different statistical tests according to the nature of the determined parameter. Results of ulcer numbers and severity were analysed using Kruskal–Wallis nonparametric test followed by Dunn's multiple comparisons test.

Data of carrageenan-induced rat paw oedema, adjuvant-induced arthritis, hotplate test, plantar test, antipyretic effects which involved measuring a repeated parameter on time intervals, were analysed using repeated measures 2-way ANOVA followed by Tukey HSD test for multiple comparisons. Results of the experiments other than those mentioned were analysed using one way ANOVA followed by Tukey HSD test for multiple comparisons.

Table 3 Effect of meloxicam, octreotide and their combination on yeast-induced hyperpyrexia in rats.

Group	Rectal temperature (°C)			
	Pre-yeast	Pre-treatment	Post-drug 60 min	Post-drug 120 min
Control (hyperthermic rats)	37.20 ± 0.17	38.90* ± 0.13	39.08* ± 0.19	39.21* ± 0.16
Mel. 1 mg/kg	37.00 ± 0.12	38.80* ± 0.11	37.32@ ± 0.34	37.20@ ± 0.30
Mel. 2 mg/kg	37.12 ± 0.14	38.66* ± 0.18	37.40@ ± 0.22	37.13@ ± 1.02
Oct. 10 μg/kg	37.06 ± 0.25	38.60* ± 0.21	38.48* ± 0.25	39.11* ± 0.18
Oct. 100 μg/kg	37.57 ± 0.15	39.10* ± 0.14	38.23 ± 0.38	39.33* ± 0.26
Mel. 1 mg/kg + Oct. 10 μg/kg	36.80 ± 0.18	38.52* ± 0.17	37.30@ ± 0.15	38.25* ± 0.21

The data represents the mean ± standard error of the mean ($n = 8$).
* Statistically significant from zero time: $P < 0.05$.
@ Statistically significant from the hyperthermic control group at the corresponding time: $P < 0.05$.

Table 4 Effect of meloxicam, octreotide and their combination on the gastric mucosa in pylorus ligated rats using indomethacin as a standard drug.

Parameters	Groups						
	Normal	Indomethacin 20 mg/kg	Meloxicam 1 mg/kg	Meloxicam 2 mg/kg	Octreotide 10μ/kg	Octreotide 100μ/kg	Meloxicam 1 mg/kg + Octreotide 10 μg/kg
Number of lesions per rat	–	15.00 ± 4.01*,†	10.20* ± 4.46	14.20 ± 3.60*,†	–	–	5.60 ± 1.24
Severity of lesions per rat	–	29.70 ± 7.18*,†	19.00* ± 4.98	26.40 ± 6.68*,†	–	–	11.40 ± 2.10
Gastric acid volume (ml/4 h)	6.43 ± 0.48	8.35 ± 0.58	7.5 ± 0.65	7.65 ± 0.46	5.38 ± 0.58	7.20 ± 0.69	6.90 ± 0.61
Gastric acid output (μEq/4 h)	654.3 ± 74.1	1066.3 ± 107.8*,†	981* ± 91.70	985.3 ± 97.90*,†	642@47.50	562.8 ± 42.20@,†	866.8 ± 22.31

The data represents the mean of 6–8 animals ± standard error of the mean.
* Statistically significant from the normal group: $P < 0.05$.
@ Statistically significant from ulcer induced indomethacin group: $P < 0.05$.
† Statistically significant from combination (meloxicam 1 mg/kg + octreotide 10 μg/kg): $P < 0.05$.

A probability level of less than 0.05 was accepted as being significant in all types of statistical tests. SPSS software (version 10) was used to carry out all statistical tests except for the 2-way ANOVA which was performed using Statistical software, version 17.

5. Results

5.1. The carrageenan-induced rat paw oedema

Results are presented in Table 1. Pretreatment with meloxicam significantly decreased the carrageenan-induced oedema. The inhibitory effect of 0.5, 1 and 2 mg/kg meloxicam was 35.7%, 42.9% and 46.4%, respectively, at the fourth hour. Similarly, pretreatment with octreotide significantly decreased the carrageenan-induced oedema. The inhibitory effect of 10, 25 and 100 μg/kg octreotide was 21.4%, 25% and 32.1%, respectively, at the fourth hour. Pretreatment with combinations of meloxicam 1 mg/kg and octreotide 10 μg/kg or meloxicam 1 mg/kg and octreotide 100 μg/kg significantly decreased the carrageenan-induced oedema with an inhibitory effect of 42.9% at the fourth hour of the experiment.

5.2. The adjuvant-induced arthritis

Results are graphically illustrated in Fig. 1.

5.3. The hot plate test

Results are presented in Table 2. Meloxicam 1 mg/kg did not significantly increase the reaction time allover the experiment except after 30 min of drug administration when compared to the control group. However, meloxicam 2 mg/kg showed a significant increase in the reaction time at 30, 60 and 120 min following drug administration as compared with the control animals. Octreotide 100 μg/kg resulted in a significant increase in the reaction time after 60 and 120 min following drug administration.

5.4. The writhing test

Results are graphically illustrated in Fig. 2. Administration of meloxicam (1 and 2 mg/kg), octreotide (10 and 100 μg/kg) or their combination meloxicam (1 mg/kg) and octreotide (10 μg/kg) before inducing writhing resulted in a significant decrease in the number of writhes in mice.

5.5. The plantar test

Results are graphically illustrated in Fig. 3.

5.6. Antipyretic effects

There was a statistical significant effect for meloxicam 1 and 2 mg/kg and the combination of meloxicam (1 mg/kg) and

octreotide (10 μg/kg), where they produced a significant decrease in the elevated body temperature reaching normal values when compared with those of the control group. Results are presented in Table 3.

5.7. Evaluation of gastric ulcerogenic potential

Results are presented in Table 4. Meloxicam (1 and 2 mg/kg) resulted in a number of lesions of 10.2 and 14.2 with a severity of 19 and 26.4, respectively. In addition, acid output was increased by 50% and 50.5%, respectively, as compared to that of the pyloric ligated normal group. Octreotide (10 and 100 μg/kg) did not cause lesions and did not significantly affect the normal gastric volume. Acid output was not significantly changed from the normal group. Treatment of the rats with the combination of meloxicam (1 mg/kg) and octreotide (10 μg/kg) resulted in normal gastric acid volume and normal acid output. Results are presented in Table 4.

5.8. Histological examination of the gastric mucosa

The normal picture of the gastric mucosa was demonstrated after examination of sections obtained from the normal group receiving saline (Fig. 4). Gastric mucosa of rats which received indomethacin was highly affected showing a big area of ulceration occupying about two-thirds of the thickness of the mucosa (Fig. 5). The tissue architecture is more or less preserved. Gastric mucosa of rats that received meloxicam 1 mg/kg showed that the normal architecture of the glands is distorted with the presence of many dilated blood vessels in between the muscle fibres of the muscularis mucosa, which is thickened. Lymphocytic infiltration is seen in the muscularis mucosa and in the bases of the glands (Fig. 6). Gastric mucosa of rats that received meloxicam 2 mg/kg showed some deformities of the pits of the glands with abnormally dilated blood vessels in between and at the bases of the pits (Fig. 7).

Gastric mucosa of rats injected with octreotide 10 μg/kg shows more or less normal architecture of the gastric glands. Slight lymphocytic infiltration is seen in the lamina propria between the bases of the glands (Fig. 8). Gastric mucosa of rats injected with octreotide 100 μg/kg showed that the structural architecture is still preserved, although there are unstained areas in the lumen of the glands due to necrosis and degeneration of many cells in the glands (Fig. 9).

Gastric mucosa of rats injected with a combination of octreotide 10 μg/kg and meloxicam 1 mg/kg shows nearly normal architecture of the mucosal glands in spite of the presence of oedema in the necks of some glands. Area of congestion is seen at the base of the glands with thickening of the muscularis mucosa (Fig. 10).

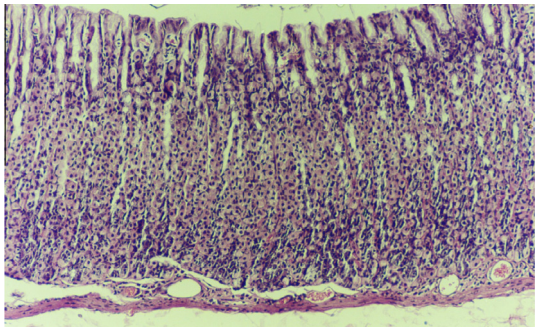

Figure 4 A photomicrograph of the gastric mucosa of the control rat.

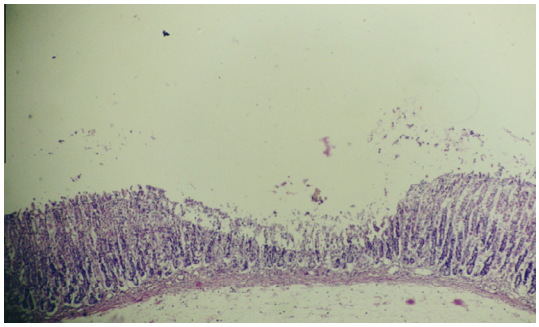

Figure 5 A photomicrograph of the gastric mucosa after indomethacin (20 mg/kg).

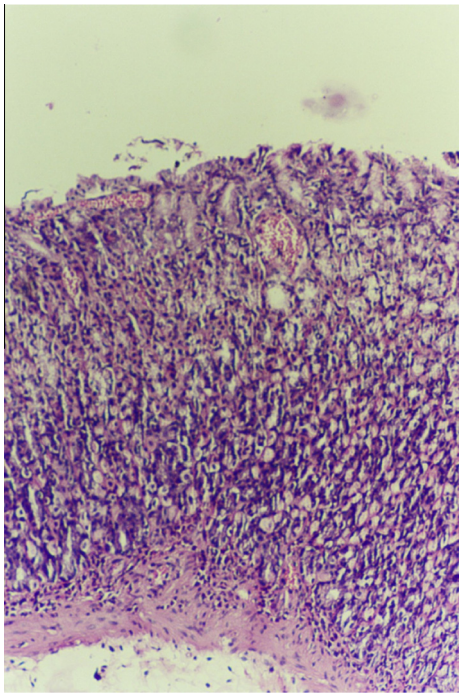

Figure 6 A photomicrograph of the gastric mucosa after administration of meloxicam (1 mg/kg) and meloxicam (2 mg/kg), respectively.

6. Discussion

The carrageenan-induced rat paw oedema in the present experiments revealed visible redness and pronounced swelling that were well developed by 4 h. The same finding was observed by other authors (Panda and Chowdary, 2008; Li et al., 2003). Currently, meloxicam showed significant anti-inflammatory effect. These results are in agreement with those of many studies showing the anti-inflammatory effect of meloxicam (Engelhardt et al., 1995; Corbett et al., 2010). The anti-inflammatory effect of meloxicam was evident starting from the first hour of carrageenan-induced oedema, suggesting interference with the inflammatory mediators implicated in

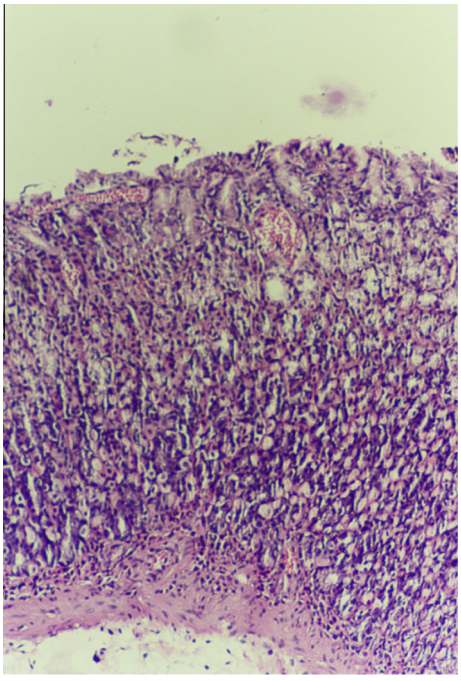

Figure 7 A photomicrograph of the gastric mucosa after administration of meloxicam (1 mg/kg) and meloxicam (2 mg/kg), respectively.

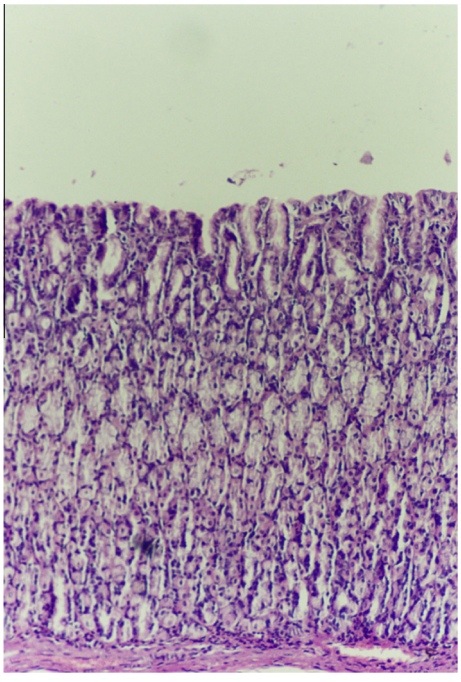

Figure 8 A photomicrograph of the gastric mucosa after administration of octreotide (10 μg/kg) and octreotide (100 μg/kg).

the early phase and possibly the cyclooxygenase products. The anti-inflammatory effect of meloxicam indicates its extended effect in the late phase of carrageenan-induced oedema, hence, affecting further release of prostaglandins as well as the production of neutrophil-derived free radicals such as hydrogen peroxide, superoxide and other neutrophil derived mediators (Peskar et al., 1991; Warner et al., 1999).

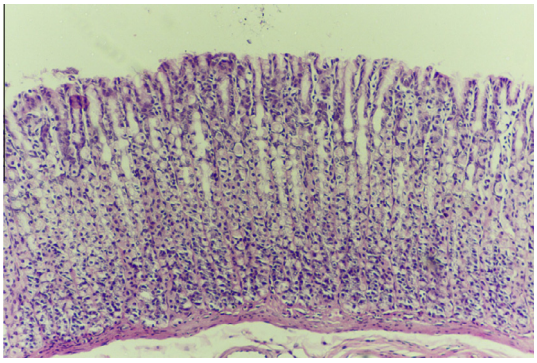

Figure 9 A photomicrograph of the gastric mucosa after administration of octreotide (10 μg/kg) and octreotide (100 μg/kg).

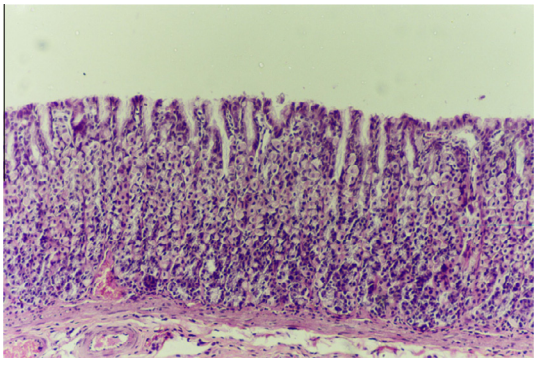

Figure 10 A photomicrograph of the gastric mucosa after meloxicam (1 mg/kg) + octreotide (10 μg/kg) adminstration.

Octreotide administered 1 h before the injection of carrageenan inhibited formation of oedema. The effect started after the first hour and persisted till the fourth hour. The acute anti-inflammatory effect of octreotide elicited in the present study is in accordance with what was observed by many authors (Heppelmann and Pawlak, 1997; Kurnatowska and Pawlikowski, 2001; Imhof et al., 2011).

It has been suggested that somatostatin may act as an anti-inflammatory agent by inhibiting plasma and leukocyte extravasation. Somatostatin acts antagonistically to substance P, the main neuroendocrine proinflammatory mediator (Ten Bokum et al., 2000). It induces vasoconstriction and inhibits angiogenesis (Szolcsányi et al., 1998).

Combinations of meloxicam and octreotide in the present study significantly decreased the carrageenan-induced oedema in a similar way like that of each of them alone. It is worth pointing out that using the combination of the drugs in acute experiments produced similar effects like using the single drugs.

Results of the present study revealed that oedema was produced in Freund's-induced inflammation (Donaldson et al., 1993). The biphasic nature of the disease is particularly pronounced in the rat polyarthritis model (Calvino et al., 1987). As a preferential COX-2 inhibitor, the significant decrease of

the paw oedema formed in Freund's adjuvant-induced arthritis by meloxicam was in accordance with results of many researchers (Engelhardt et al., 1995; Agha et al., 1999; Tsubouchi et al., 2000).

Octreotide in the present study significantly decreased the inflammatory response in the paw oedema volume in rats starting from day 15 till the end of the experiment. Octreotide results were consistent with the data concluded by others (Kurnatowska and Pawlikowski, 2000). The authors examined the effect of somatostatin analogue octreotide on the adjuvant arthritis in rats. It was suggested that somatostatin down-modulates a number of immune functions such as lymphocytes proliferation, immunoglobulin production and the release of the proinflammatory cytokines such as interferon-γ (INF-γ) (Ten Bokum et al., 2000), which may aid in the anti-inflammatory effect in chronic models like arthritis. Furthermore, the inhibition of plasma and leukocyte extravasation (Wiedermann et al., 1993), inhibition of inflammation promoting factors such as substance P (Lotz et al., 1987) and the decreased proliferation of synovial cells (Tannenbaum and Patel, 1986) could be other possible mechanisms by which somatostatin and its analogues exert the anti-inflammatory effect in chronic inflammatory models.

One way in which somatostatin may reduce arthritic symptoms through peripheral mechanisms is through the inhibition of inflammation promoting factors such as substance P (Lotz et al., 1987). Furthermore, it has been pointed out that somatostatin has been shown to inhibit immunoglobulin production by B lymphocytes and proliferation of human T lymphocytes (Levine et al., 1984).

The current study indicated that meloxicam octreotide have both peripheral and central analgesic properties. The hot plate test has been used by many investigators and has been found to be suitable for evaluation of centrally but not peripherally acting analgesics (Woolfe and McDonald, 1944). In order to distinguish between the central and peripheral analgesic actions of the tested drugs, their peripheral analgesic activity was investigated by their inhibitory effects on chemical-induced nociceptive stimuli (Zakaria et al., 2001).

Both meloxicam and octreotide may exert an analgesic effect probably by inhibiting synthesis or action of prostaglandins. Results of meloxicam in the writhing test of mice are in accordance with what other studies revealed (Warner and Mitchell, 2004). Inhibition of prostaglandin synthesis in the CNS appears to be a property of all NSAIDs (Cain et al., 1997).

Results of the present experiments indicated that meloxicam, octreotide as well as their combination produced a significant rise in the time required for the rats to respond to the thermal stimulation. These results are in accordance with those of other authors who investigated the analgesic effects of both meloxicam (Engelhardt et al., 1995) and octreotide (Sebastian and Belchetz, 1990). The suggested mode of the antinociceptive action of NSAIDs was considered to be related to their anti-inflammatory action and was thought to be due to the inhibition of prostaglandin production at the site of inflammation (Ferreira, 1979). The analgesic effect of octreotide shown as inhibition of nociceptive stimuli can be explained on the basis of blocking the release of substance P (Lembeck et al., 1982). The present results showed that meloxicam produced a decrease in the elevated body temperature. However no effect was produced by octreotide 10 or 100 μg/kg. The major mechanism of antipyretics involves lowering PGE_2 by directly inhibiting COX activity (Flower and Vane, 1972).

In the pyloric ligated rats in the current study, it has been shown that indomethacin significantly resulted in a lesion number with a high severity. Furthermore, a significant increase in the acid output compared to that of the pyloric ligated normal group was produced. These results are consistent with other previous experiments (Halici et al., 2005). Anti-inflammatory agents such as indomethacin reduce gastric cyclooxygenase activity, decrease endogenous prostaglandin levels (Konturek et al., 1984) and increase acid secretion (Sairam et al., 2002). Results of the present study showed that meloxicam induced lesions in rats and significantly increased acid output as compared to that of the pyloric ligated normal group indicating that it could induce gastrointestinal lesions. These results lie on line with previous reports (Villegas et al., 2001) but such results did not fully support other reported results that showed that meloxicam is superior to conventional NSAIDs in terms of its anti-inflammatory potency and showed particularly good gastric tolerance (Engelhardt et al., 1995).

Meloxicam has demonstrated a preferential inhibition for COX-2 in a large number of biological assay models. Nevertheless, this selectivity is not free of toxic effects on gastric mucosa, and the use of it is still associated with gastrointestinal side effects (McKenna, 1999). In low doses, meloxicam may be safer than other NSAIDs, but its toxicity profile may not be much better than that of non-selective COX inhibitors (Churchill et al., 1996).

In the present study, octreotide caused no lesions and significantly lowered the acid output in the stomach indicating that this effect could be involved in its gastroprotection. More interestingly, treatment of the rats with the combination of meloxicam and octreotide resulted in normal gastric acid volume and normal acid output. Somatostatin downregulates exocrine secretion of pancreatic enzymes and gastric acid (Schusdziarra, 1996) which may explain its gastroprotective effect. The decrease in acidity in pylorus-ligated rats suggests that an anti-secretory action occurred and may explain how somatostatin works.

In rats with ulcer, the gastrin level in the plasma, gastric juice and the mucosal tissue increase, while the somatostatin level declines (Sun et al., 2002). The combination of the anti-inflammatory drug (meloxicam) and gastroprotective effect of the somatostatin analogue (octreotide) is favourable when taken to account the serious limitations of a large number of anti-inflammatory agents that show a tendency to produce gastric irritation, bleeding and mucosal cellular damage (Lanza, 1984, Nassar et al., 2011).

Summing up, the data presented above indicate that the octreotide possesses anti-inflammatory, analgesic effects together with a favourable gastrointestinal profile. Octreotide is proposed as a lead molecule for a new class of anti-inflammatory and analgesic agents especially with patients suffering from other chronic diseases and already taking octreotide as a drug. The search for other somatostatin analogues exhibiting higher effects is needed since they represent a new promising group of anti-inflammatory drugs.

References

Agha, A.M., El-Khatib, A.S., Al-Zuhair, H., 1999. Modulation of oxidant status by meloxicam in experimentally induced arthritis. Pharmacol. Res. 40, 385–392.

Beubler, E., 2003. Pharmacology of cyclooxygenase 2 inhibition. Wien. Med. Wochenschr. 153 (5–6), 95–99.

Cain, C.K., Francis, J.M., Plone, M.A., Emerich, D.F., Lindner, M.D., 1997. Pain-related disability and effects of chronic morphine in the adjuvant-induced arthritis model of chronic pain. Physiol. Behav. 62, 199–205.

Calvino, B., Crepon-Bernard, M.O., Le Bars, D., 1987. Parallel clinical and behavioral studies of adjuvant-induced arthritis in the rat: possible relationship with chronic pain. Behav. Brain Res. 24, 11–29.

Carlton, S.M., Du, J., Davidson, E., Zhou, S., Coggeshall, R.E., 2001. Somatostatin receptors on peripheral primary afferent terminals: inhibition of sensitized nociceptors. Pain 90, 233–244.

Chakraborty, A., Devi, R.K., Rita, S., Sharatchandra, K., Singh, T.I., 2004. Preliminary studies on anti-inflammatory and analgesic activities of *Spilanthes acmella* in experimental animal models. Indian J. Pharmacol. 36, 148–150.

Chattopadhyay, D., Arunachalam, G., Mandal, A.B., Sur, T.K., Mandal, S.C., Bhattacharya, S.K., 2002. Antimicrobial and anti-inflammatory activity of folklore: *Mallotus peltatus* leaf extract. J. Ethnopharmacol. 82, 229–237.

Chillingworth, N.L., Donaldson, L.F., 2003. Characterisation of a Freund's complete adjuvant-induced model of chronic arthritis in mice. J. Neurosci. Methods 128, 45–52.

Churchill, L., Graham, A.G., Shih, C.K., Pauletti, D., Farina, P.R., Grob, P.M., 1996. Selective inhibition of human cyclooxygenase-2 by meloxicam. Inflammopharmacology 4, 125–135.

Corbett, S., Daniel, J., Drayton, R., Field, M., Steinhardt, R., Garrett, N., 2010. Evaluation of the anti-inflammatory effects of ellagic acid. J. Perianesth. Nurs. 4, 214–220.

Dequeker, J., Hawkey, C., Kahan, A., Steinbruck, K., Alegre, C., Baumelon, E., Begaud, B., Isomaki, H., Littlejohn, G., Mau, J., Papazoglou, S., 1998. Improvement in gastrointestinal tolerability of the selective cyclooxygenase (COX)-2 inhibitor, meloxicam, compared with piroxicam: results of the Safety and Efficacy Large-scale Evaluation of COX-inhibiting Therapies (SELECT) trial in osteoarthritis. Br. J. Rheumatol. 37, 946–951.

Donaldson, L.F., Seckl, J.R., McQueen, D.S., 1993. A discrete adjuvant-induced monoarthritis in the rat: effects of adjuvant dose. J. Neurosci. Methods 49, 5–10.

Drury, R.A.B., Wallington, E.A., 1980. Carleton's Histological Technique, fourth ed. Oxford University Press, Oxford, New York, Toronto.

Engelhardt, G., Homma, D., Schlegel, K., Utzmann, R., Schnitzler, C., 1995. Anti-inflammatory, analgesic, antipyretic and related properties of meloxicam, a new non-steroidal anti-inflammatory agent with favourable gastrointestinal tolerance. Inflamm. Res. 44, 423–433.

Ferreira, S.H., 1979. In: Houck, J.C. (Ed.), Chemical Messengers of the Inflammatory Process. Elsevier/North-Holland Biomedical Press, Amsterdam, p. 113 (Chapter 3).

Flower, R.J., Vane, J.R., 1972. Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). Nature 240, 410–411.

Halici, M., Odabasoglu, F., Suleyman, H., Cakir, A., Aslan, A., Bayir, Y., 2005. Effects of water extract of *Usnea longissima* on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. Phytomedicine 12, 656–662.

Heppelmann, B., Pawlak, M., 1997. Inhibitory effect of somatostatin on the mechanosensitivity of articular afferents in normal and inflamed knee joints of the rat. Pain 73, 377–382.

Imhof, A.K., Glück, L., Gajda, M., Lupp, A., Bräuer, R., Schaible, H.G., Schulz, S., 2011. Differential antiinflammatory and antinociceptive effects of the somatostatin analogs octreotide and pasireotide in a mouse model of immune-mediated arthritis. Arthritis Rheum. 8, 2352–2362.

Isenberg, J.I., 1978. Gastric secretory testing. In: Sleisenger, M.H., Fordtran, J.S. (Eds.), Gastrointestinal Disease: Pathophysiology, Diagnosis, Management. Saunders, W.B, pp. 714–732.

Konturek, S.J., Obtulowiecz, W., Kwiecieu, N., Oleksy, J., 1984. Generation of prostaglandin in gastric mucosa of patients with peptic ulcer disease: effect of nonsteroidal anti-inflammatory compounds. Scand. J. Gastroenterol. 19, 75–77.

Koster, R., Anderson, M., de Beer, E.J., 1959. Acetic acid for analgesic screening. Feder. Proc. 18, 412–416.

Kurnatowska, I., Pawlikowski, M., 2000. Effect of somatostatin analog-octreotide on adjuvant arthritis in rat. Neuro Endocrinol. Lett. 21, 121–126.

Kurnatowska, I., Pawlikowski, M., 2001. Anti-inflammatory effects of somatostatin analogs on zymosan-induced earlobe inflammation in mice: comparison with dexamethazone and ketoprofen. Neuroimmunomodulation 9, 119–124.

Kwon, Y.B., Lee, H.J., Han, H.J., Mar, W.C., Kang, S.K., Yoon, O.B., Beitz, A.J., Lee, J.H., 2002. The water-soluble fraction of bee venom produces antinociceptive and anti-inflammatory effects on rheumatoid arthritis in rats. Life Sci. 71, 191–204.

Lapeyre-Mestre, M., Grolleau, S., Montastruc, J.L., 2011. Adverse drug reactions associated with the use of NSAIDs: a case/noncase analysis of spontaneous reports from the French pharmacovigilance database 2002–2006. Fundam. Clin. Pharmacol. 2, 1472–8206.

Laviola, G., Alleva, E., 1990. Ontogeny of muscimol effects on locomotor activity, habituation and pain reactivity in mice. Psychopharmacology 102, 41–48.

Lembeck, F., Donnerer, J., Bartho, L., 1982. Inhibition of neurogenic vasodilatation ad plasma extravasation by substance P antagonists, somatostatin and [D-Met2, Pro5] enkephalinamide. Eur. J. Pharmacol. 85, 171–176.

Levine, J.D., Clark, R., Devor, M., Helms, C., Moskowitz, M.A., Basbaum, A.I., 1984. Intraneuronal substance P contributes to the severity of experimental arthritis. Science 226, 547–549.

Li da, W., Hyun, J.E., Jeong, C.S., Kim, Y.S., Lee, E.B., 2003. Antiinflammatory activity of alpha-hederin methyl ester from the alkaline hydrolysate of the butanol fraction of *Kalopanax pictus* bark extract. Biol. Pharm. Bull. 26, 429–433.

Lora, M., Morisset, S., Menard, H.A., Leduc, R., de Brum-Fernandes, A.J., 1997. Expression of recombinant human cyclooxygenase isoenzymes in transfected COS-7 cells in vitro and inhibition by tenoxicam, indomethacin and aspirin. Prostaglandins Leukot. Essent. Fatty Acids 56, 361–367.

Lotz, M., Vaughan, J.H., Carson, D.A., 1987. Substance P activation of rheumatoid synoviocytes: neural pathway in pathogenesis of arthritis. Science 235, 893–895.

Louder, A.M., Joshi, A.V., Ball, A.T., Cappelleri, J.C., 2011. Impact of Celecoxib restrictions in medicare beneficiaries with arthritis. Am. J. Manag. Care 17 (7), 503–512.

McKenna, F., 1999. COX-2: separating myth from reality. Scand. J. Rheumatol. 28 (Suppl. 109), 19–29.

Mózsik, G.Y., Móron, F., Jávor, T., 1982. Cellular mechanisms of the development of gastric mucosal damage and of gastrocytoprotection induced by prostacyclin in rats. A pharmacological study. Prostaglandins Leukot. Med. 9, 71–84.

Nassar, N.N., Schaalan, M.F., Zaki, H.F., Abdallah, D.M., 2011. Octreotide ameliorates gastric lesions in chronically mild stressed rats. World J. Gastroenterol. 9, 1135–1142.

Ohno, H., Kuraishi, Y., Nanayama, T., Minami, M., Kawamura, M., Satoh, M., 1990. Somatostatin is increased in the dorsal root ganglia of adjuvant-inflamed rat. Neurosci. Res. 8, 179–188.

Panda, S.S., Chowdary, P.V., 2008. Synthesis of novel indolyl-pyrimidine antiinflammatory, antioxidant and antibacterial agents. Indian J. Pharm. Sci. 70 (2), 208–215.

Pearson, C.M., 1956. Development of arthritis, periarthritis and periostitis in rats given adjuvants. Proc. Soc. Exp. Biol. Med. 91, 95–101.

Peskar, B.M., Trautmann, M., Nowak, P., Peskar, B.A., 1991. Release of 15-hydroxy-5,8,11,13-eicosatetraenoic acid and cysteinyl-leukotrienes

in carrageenan-induced inflammation: effect of non-steroidal anti-inflammatory drugs. Agents Actions 33, 240–246.

Reubi, J.C., Waser, B., Markusse, H.M., Krenning, E.P., VanHagen, M., Laissue, J.A., 1994. Vascular somatostatin receptors in synovium from patients with rheumatoid arthritis. Eur. J. Pharmacol. 271, 371–378.

Roszkowski, A.P., Rooks, W.H., Tomolonis, S.A.J., Miller, L.M., 1971. Anti-inflammatory and analgesic properties of d-2-(6-methoxy-2-naphthyl)-propionic acid (naproxen). J. Pharmacol. Exp. Ther. 179, 114–123.

Sairam, K., Rao, Ch.V., Babu, M.D., Vijay Kumar, K.V., Agarwal, V.K., Goel, R.K., 2002. Antiulcerogenic effect of ethanolic extract of *Emblica officinalis*: an experimental study. J. Ethanopharmacol. 82, 1–9.

Schusdziarra, V., 1996. Somatostatin: biological actions and pathophysiology. In: Scarpignato, C. (Ed.), Octreotide: From Basic Science to Clinical Medicine, Progress in Basic and Clinical Pharmacology, vol. 10. S. Karger AG, Basel, pp. 35–53.

Sebastian, J.P., Belchetz, P.E., 1990. Pain relief in acromegalic patients treated with octreotide. J. Endocr. (Suppl. 124) (Abs. 172).

Shay, H., Komarow, S.A., Fels, S.S., Meranze, D., Gruenstein, M., Siplet, H., 1945. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology 5, 43–61.

Sreenivasan, V.K., Stremovskiy, O.A., Kelf, T.A., Heblinski, M., Goodchild, A.K., Connor, M., Deyev, S.M., Zvyagin, A.V., 2011. Pharmacological characterization of a recombinant, fluorescent somatostatin receptor agonist. Bioconjug. Chem. 22 (9), 1768–1775.

Strosberg, J.R., Cheema, A., Kvols, L.K., 2011. A review of systemic and liver-directed therapies for metastatic neuroendocrine tumors of the gastroenteropancreatic tract. Cancer Control 2, 127–137.

Sun, F.P., Song, Y.G., Cheng, W., Zhao, T., Yao, Y.L., 2002. Gastrin, somatostatin, G and D cells of gastric ulcer in rats. World J. Gastroenterol. 8, 375–378.

Sweeney, S.E., Firestein, G.S., 2004. Rheumatoid arthritis: regulation of synovial inflammation. Int. J. Biochem. Cell Biol. 36, 372–378.

Szolcsányi, J., Pintér, E., Helyes, Z.S., Oroszi, G., Németh, J., 1998. Systemic anti-inflammatory effect induced by counter-irritation through a local release of somatostatin from nociceptors. Br. J. Pharmacol. 125, 916–922.

Tannenbaum, G.S., Patel, Y.C., 1986. On the fate of centrally administered somatostatin in the rat: massive hypersomatostatinemia resulting from leakage into the peripheral circulation has effects on growth hormone secretion and glucoregulation. Endocrinology 118, 2137–2143.

Ten Bokum, A.M., Hofland, L.J., van Hagen, P.M., 2000. Somatostatin and somatostatin receptors in the immune system: a review. Eur. Cytokine Netw. 2, 161–176.

Tsubouchi, Y., Sano, H., Yamada, R., Hashiramoto, A., Kohno, M., Kusaka, Y., Kondo, M., 2000. Preferential inhibition of cyclooxygenase-2 by meloxicam in human rheumatoid synoviocytes. Eur. J. Pharmacol. 395, 255–263.

Vane, J.R., Botting, R.M., 1995. New insights into the mode of action of anti-inflammatory drugs. Inflamm. Res. 44, 1–10.

Villegas, I., Alarcón de la Lastra, C., La Casa, C., Motilva, V., Martn, M.J., 2001. Effects of food intake and oxidative stress on intestinal lesions caused by meloxicam and piroxicam in rats. Eur. J. Pharmacol. 414, 79–86.

Warner, T.D., Mitchell, J.A., 2004. Cyclooxygenase: new forms, new inhibitors, and lessons from the clinic. FASEB J. 18, 790–804.

Warner, T.D., Giuliano, F., Vojnovic, L., 1999. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. Proc. Natl. Acad. Sci. USA 96, 7563–7568.

Wiedermann, C.J., Reinisch, N., Braunsteiner, H., 1993. Stimulation of monocyte chemotaxis by human growth hormone and its deactivation by somatostatin. Blood 82, 954–960.

Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenin-induced oedema in hind paw of the rat as an assay for antiinflammatory drugs. Proc. Soc. Exp. Biol. Med. 111, 544–547.

Woolfe, G., McDonald, A.D., 1944. The evaluation of the analgesic action of pethidine hydrochloride (DEMEROL). J. Pharmacol. Exp. Ther. 80, 300–307.

Zakaria, M.N.M., Isalm, M.W., Radhakrishnan, R., Chen, H.B., Kamil, M., Al-Gifri, A.N., Chan, K., Al-Attas, A., 2001. Antinociceptive and anti-inflammatory properties of *Caralluma arabica*. J. Ethnopharmacol. 76, 155–158.