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ORIGINAL ARTICLE

Effect of melittin on mice stomach

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Abstract Melittin, the main bee venom component, has many positive biological effects and a relatively low toxicity in various cell types. However, there is no evidence of the effect of melittin on gastrointestinal cells. In the present study, we investigated the histological and immunohistochemical effects of melittin on mice stomach. Adult male mice (Albino Swiss) were randomly divided into two groups (7 mice for each group): control group and melittin only treated group (10 and 40 μg/kg). These mice were sacrificed, then samples from the stomach were collected and prepared for histopathological studies by using alcian blue stain and immunohistochemical studies by using smooth muscle actin (SMA) antibody. Treatment with melittin alone do not cause any harmful effect on the stomach tissue where the microscopic examination of Alcian blue stained section showed the normal distribution of the mucous secreting cells of the stomach tissues. On other hand, no changes were observed on smooth muscle cells. This study demonstrated the safety of using melittin on gastrointestinal tissues if used in definite dose and for suitable duration, which offers an opportunity for its use as a treatment for many diseases of the gastrointestinal tract.

1. Introduction

Melittin has many positive biological effects and a relatively low toxicity (Raghuraman and Chattopadhyay, 2004). In the central and peripheral nervous system, it stimulates many peripheral chemoreceptors, blocks transmission of the vegetative synapse and the polysynaptic neuronal paths, and increases brain blood circulation, influence of behavior patterns, pain-soothing and has cholinolytic action (against acetylcholine) (Shkenderov and Ivanov, 1983; Karimi et al., 2011). In the cardiovascular system, melittin increases coronary and peripheral blood circulation, improves the microcirculation of blood in the tissues, slows down heart at lower doses and stimulates it at higher ones and lowers blood pressure. Melittin acts against blood coagulation (fibrinolytic), stimulates the building of erythrocytes and improves the regeneration of leucocytes and erythrocytes. Also it is antiarrhythmic (Yalcin et al., 2009). In the endocrine system, melittin increases secretion of thyroid, hypophysis and the hypothalamus hormones, plasma adrenaline, noradrenaline, vasopressin

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levels and renin activity (Ortmann et al., 1992; Yalcin and Savci, 2007). In the immune system, melittin has an immunosuppressive and immunoactivating action(Son et al., 2007). Melittin also has antibiotic, fungicidal, antiviral and bactericidal action against different pathogens such as *Candida albicans*, and inactivation of Herpes, Leukemia and HIV viruses (Conlon et al., 2003). In addition, melittin has metabolic effects, where it increases the protein and nucleotide metabolism (Conlon et al., 2003).

In contrast, high concentrations of melittin exhibited a variety of toxic effects such as lysis of the eukaryotic cells (Klocek and Seelig, 2008) through increasing cell membrane permeability to ions and stimulating the activity of phospholipase A2 (Mollay and Kreil, 1974), inhibition of (H^+, K^+) ATPase (Cuppoletti et al., 1989; Cuppoletti and Abbott, 1990) and inhibition and aggregation of the membrane proteins (Clague and Cherry, 1988; Mahaney and Thomas, 1991). It also inhibited mitochondrial respiration due to disturbances of membrane and organized respiration complexes (Shkinev et al., 1978). Injection of melittin also induced spontaneous pain, increased blood flow (neurogenic inflammation) as well as the appearance of regions of hyperalgesia around the site of injection (Sumikura et al., 2003).

In the gastrointestinal GI tract, melittin is a potential permeability enhancer and transiently increases intestinal permeability. It also exhibits necrotic cytotoxicity in the gastrointestinal cells (Maher et al., 2007). In the gastric mucosa, melittin decreased the nutrient membrane's partial conductance of Cl^-, K^+, and Na^+, as well as the secretory membrane partial conductance of Cl^-, without affecting the secretory partial conductance of Na^+ or K^+ (Carrasquer et al., 1998). Low concentrations of melittin were not cytotoxic in both cell and tissue structures, although it caused an increase in paracellular permeability in the mucosal epithelium (Maher et al., 2007). It has also been reported that melittin did not affect the cellular functions such as mucus secretion (Maher and McClean, 2006). Therefore, low doses of melittin may be used in therapeutic delivery of oral preparations (Maher et al., 2007). In contrast, high concentrations of melittin have multiple mechanisms of toxicity including cytolytic activity, where it may cause a necrotic change and loss of plasma membrane integrity (Masquelier et al., 2004). Melittin can also increase gastrointestinal permeability, being especially effective in the colon (Maher et al., 2007). There are several reports on the interaction of melittin with the epithelial cell structure and function including pathways which are linked with paracellular permeability (Maher et al., 2007).

Long term melittin treatment resulted in a loss of microvilli, an increase in cell debris and destruction of intestinal tight junctions and cell–cell adhesion (Tosteson et al., 1985). In intestinal enterocytes and goblet cells, melittin led to a reduction in cell viability associated with necrotic cell death, and increased mucus secretions (Maher et al., 2007; Maher and

Table 1 Alcian blue stainability in tissues of the body of the stomach in control and experimental groups.

	Control group	Melittin groups					
		10 μg/kg			40 μg/kg		
		3D	5D	10D	3D	5D	10D
Gastric pits	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+
Gastric glands	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+

(+ + + +): Highly intense stainability.
(+ + +): Intense stainability.
(+ +): Moderate stainability.
(+): Weak stainability.

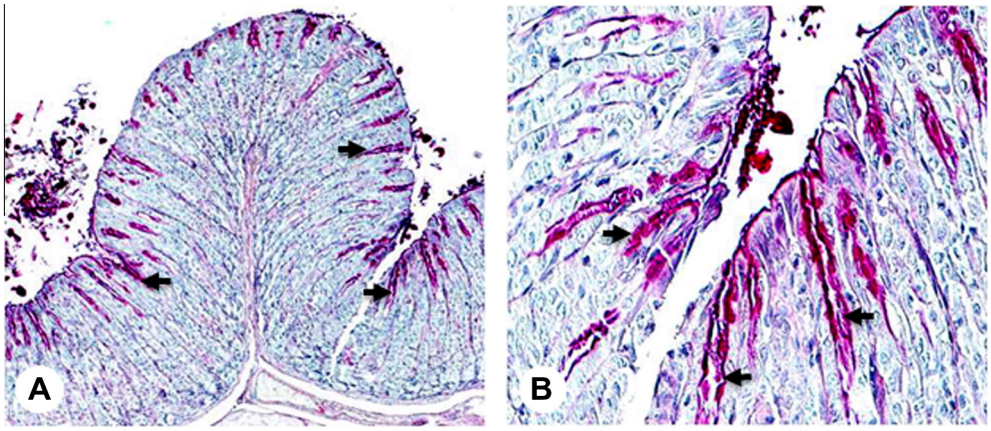

Figure 1 Transverse section in the body of the stomach of a control mouse (A) showing the normal distribution of the mucous secreting cells of the gastric pits (arrows) that positively reacted with Alcian blue (Alcian blue, X100); (B) showing high magnification of the previous figure in which the mucous secreting cells of the gastric pits (arrows) display positive reactivity with Alcian blue (Alcian blue, X400).

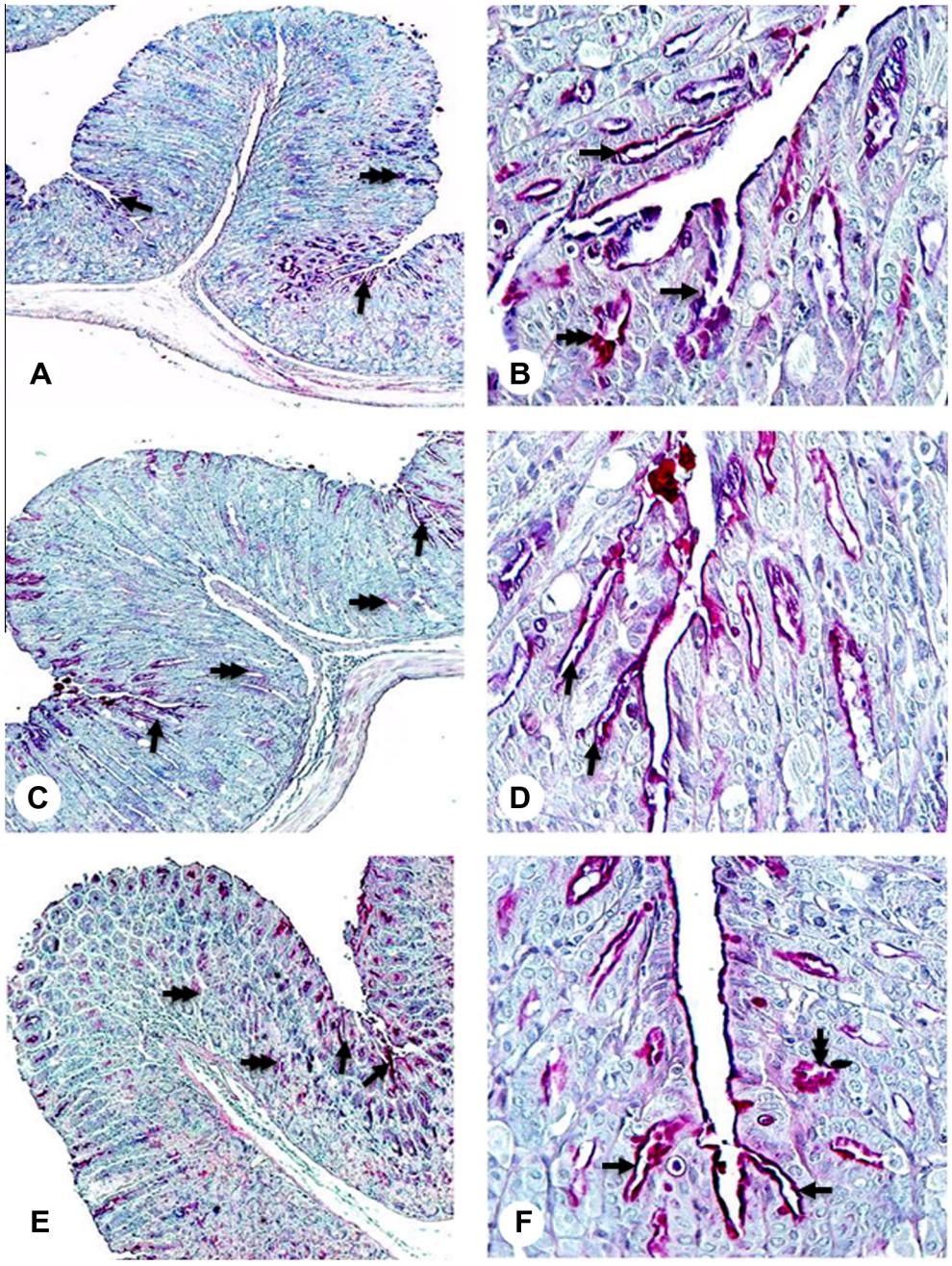

Figure 2 Effect of melittin (10 μg/kg) on the stomach body. (A) Transverse section in the body of the stomach of a mouse treated with melittin (10 μg/kg) for 3 days showing more or less normal presence of the positively reacted mucous secreting cells of gastric pits (arrows) and a few gastric glands (double arrows) (Alcian blue, X100). (B) Transverse section in the body of the stomach of a mouse treated with melittin (10 μg/kg) for 3 days showing mucous secreting cells of the gastric pits (arrows) and the gastric glands (double arrows) which are positively reacted with Alcian blue (Alcian blue, X400). (C) Transverse section in the body of the stomach of a mouse treated with melittin (10 μg/kg) for 5 days showing positive reactivity with Alcian blue at the mucous secreting cells of a number of gastric pits (arrows) and gastric glands (double arrows) (Alcian blue, X100). (D) Transverse section in the body of the stomach of a mouse treated with melittin (10 μg/kg) for 5 days showing enlarged part of the previous figure in which the gastric pits (arrows) display more or less normal presence of the mucous secreting cells that react with Alcian blue (Alcian blue, X400). (E) Transverse section in the body of the stomach of a mouse treated with melittin (10 μg/kg) for 10 days showing gastric pits (arrows) and gastric glands (double arrows) that contain mucous secreting cells positively reacted with Alcian blue (Alcian blue, X100). (F) Transverse section in the body of the stomach of a mouse treated with melittin (10 μg/kg) for 10 days showing a higher magnification of gastric pits (arrows) lined by mucous secreting cells that positively reacted with Alcian blue. Double arrow: Positively reacted gastric glands (Alcian blue, X400).

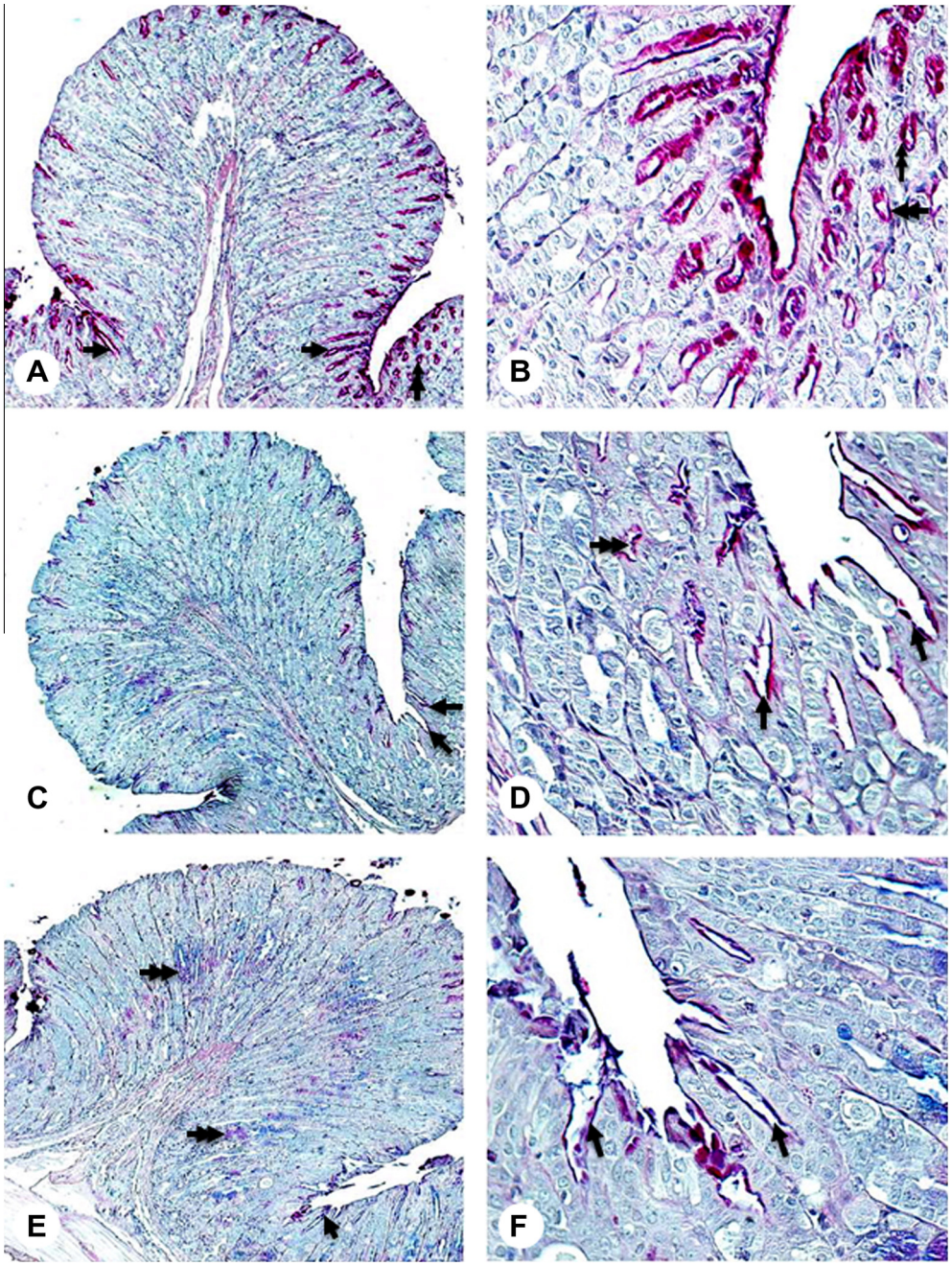

Figure 3 Effect of melittin (40 μg/kg) on the stomach body. (A) Transverse section in the body of the stomach of a mouse treated with melittin (40 μg/kg) for 3 days showing positive reactivity with Alcian blue at the mucous secreting cells of the gastric pits (arrows) and gastric glands (double arrows) (Alcian blue, X100). (B) Transverse section in the body of the stomach of a mouse treated with melittin (40 μg/kg) for 3 days showing an enlarged part of the previous figure that displays positively reacted mucous secreting cells of the gastric glands (double arrows) (Alcian blue, X400). (C) Transverse section in the body of the stomach of a mouse treated with melittin (40 μg/kg) for 5 days showing less Alcian blue reactivity of the mucous secreting cells of gastric pits (arrows) compared to those of the control sections (Alcian blue, X100). (D) Transverse section in the body of the stomach of a mouse treated with melittin (40 μg/kg) for 5 days showing weak reactivity of the mucous secreting cells at the gastric pits (arrows) and the gastric glands (double arrows) (Alcian blue, X400). (E) Transverse section in the body of the stomach of a mouse treated with melittin (40 μg/kg) for 10 days showing weak reactivity with Alcian blue at a few mucous secreting cells within the gastric glands (double arrows) and a few numbers of gastric pits (arrows) (Alcian blue, X100). (F) Transverse section in the body of the stomach of a mouse treated with melittin (40 μg/kg) for 10 days showing an enlarged part of the previous figure which displays weak reactivity of the mucous secreting cells at a few gastric pits (arrows) (Alcian blue, X400).

McClean, 2008). This production of mucus in the GI tract may potentially reduce the effects of melittin on permeation enhancement (Behrens et al., 2002). These aspects of melittin activity and its ability to disrupt the integrity of the cell

membrane may be involved in the peptide cytotoxicity which is considered as a limiting factor in the use of melittin in infectious disease (Maher and McClean, 2006).

2. Materials and methods

2.1. Melittin

Melittin (the principle hemolytic component of honeybee venom) was obtained from Sigma Chemical Company in the form of a powder. 0.135 g of melittin was dissolved in 100 ml of distilled water. Melittin solution was divided into small aliquots that were kept frozen (−20 °C) until the time of use. The solution was diluted to prepare the required concentrations (10 and 40 μg/kg body weight).

2.2. Experimental animals

Adult male Albino mice (25 ± 5 g) were kindly supplied by The Animal House of King Fahd Medical Research Center, King Abdulaziz University, Jeddah. The mice were transferred to wire-bottomed cages at the animal house of King Fahd Medical Research Center. The animals were kept at an ambient temperature and fed on a special rodent diet supplied by Medical Professions for the Veterinary Products and Fodders Additions Company (MUVCO). The mice were given fresh water through glass bottles with a capillary dropper fixed to the wall of the cage in a position to be available for the mice. Water was changed and the cages were cleaned every day. The mice were weighted just before the beginning of each experiment.

2.3. Experimental groups

2.3.1. Control group

The control group included seven adult male Albino mice. Each mouse was treated by using the stomach feeding tube with a daily dose of 1 ml distilled water for ten days.

2.3.2. Melittin group

Forty-two mice were divided into six subgroups (7 mice each) and treated by using the stomach feeding tube as follows: The first three subgroups were treated daily with a melittin (10 μg/kg body weight) for 3, 5 or 10 days, while the others were treated daily with a single dose of melittin (40 μg/kg body weight) for 3, 5 or 10 days. Melittin doses have been selected on the basis of preliminary experiments in order to find out the safe dose to be used in the stomach tissue.

Table 2 Immunohistochemical reactivity of the smooth muscle actin (SMA) antigen at tissues of the stomach body.

Group	Muscularis mucosa	Muscularis externa
Control group	+ + +	+ + +
Melittin group (10 μg/kg) for 3 days	+ + +	+ + +
Melittin group (10 μg/kg) for 5 days	+ + +	+ + +
Melittin group (10 μg/kg) for 10 days	+ + +	+ + +
Melittin group (40 μg/kg) for 3 days	+ + +	+ + +
Melittin group (40 μg/kg) for 5 days	+ + +	+ + +
Melittin group (40 μg/kg) for 10 days	+ + +	+ + +

(+) Weak reactivity.
(+ ±) Moderate to weak reactivity.
(+ +) Moderate reactivity.
(+ + +) Intense reactivity.

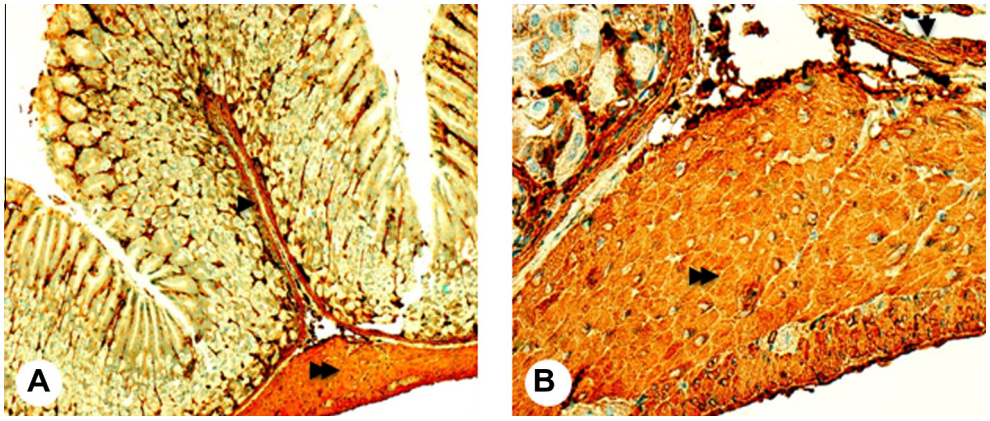

Figure 4 Transverse section in the body of the stomach of a control mouse (A) showing intense smooth muscle actin (SMA) reactivity at the muscularis mucosa (arrow head) and the muscularis externa (double arrow heads) (SMA immunohistochemistry, X100); (B) showing a higher magnification of the muscularis externa (double arrow heads) and muscularis mucosa (arrow head) that displays intense SMA reactivity (SMA immunohistochemistry, X400).

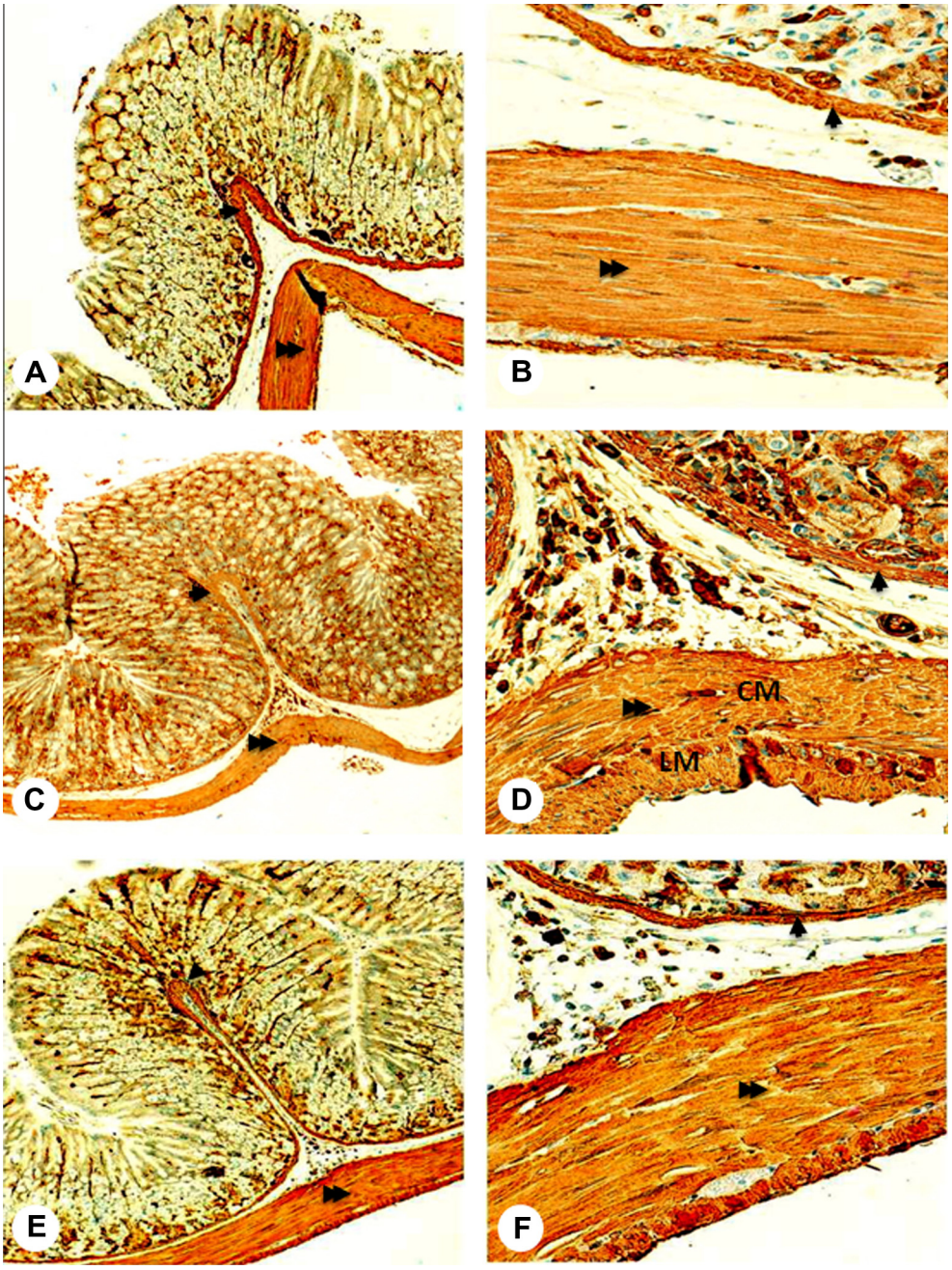

Figure 5 Effect of melittin (10 μg/kg) on the stomach body. (A) Transverse section in the body of the stomach of a mouse treated with melittin (10 μg/kg) for 3 days showing intense reactivity of SMA at the muscularis mucosa (arrow head) and muscularis externa (double arrow heads) (SMA immunohistochemistry, X100). (B) Transverse section in the body of the stomach of a mouse treated with melittin (10 μg/kg) for 3 days showing a high magnification of the muscularis mucosa (arrow head) and muscularis externa (double arrow heads) which displays intense reactivity with SMA (SMA immunohistochemistry, X400). (C) Transverse section in the body of the stomach of a mouse treated with melittin (10 μg/kg) for 5 days showing intense reactivity of SMA at the muscularis mucosa (arrow head) and the muscularis externa (double arrow heads) (SMA immunohistochemistry, X100). (D) Transverse section in the body of the stomach of a mouse treated with melittin (10 μg/kg) for 5 days showing an enlarged part of the previous figure in which the muscularis externa (double arrow heads) reveals intense SMA reactivity. LM: Longitudinal muscles; CM: Circular muscles (SMA immunohistochemistry, X400). (E) Transverse section in the body of the stomach of a mouse treated with melittin (10 μg/kg) for 10 days showing muscularis mucosa (arrow head) and muscularis externa (double arrow heads) that intensely reacted with SMA (SMA immunohistochemistry, X100). (F) Transverse section in the body of the stomach of a mouse treated with melittin (10 μg/kg) for 10 days showing an enlarged part of the muscularis externa (double arrow heads) and the muscular mucosa (arrow head) that intensely reacted with SMA (SMA immunohistochemistry, X400).

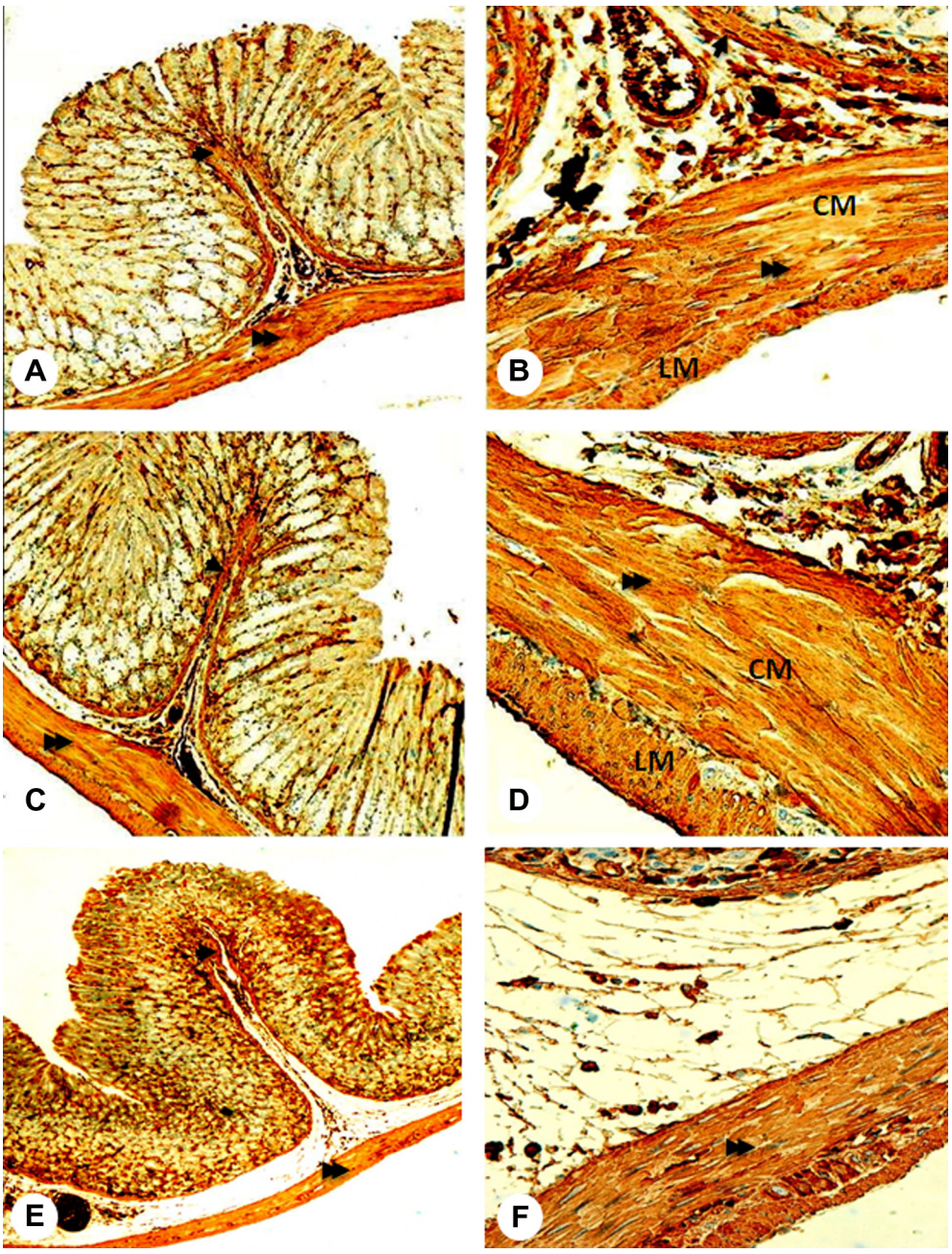

Figure 6 Effect of melittin (40 μg/kg) on the stomach body. (A) Transverse section in the body of the stomach of a mouse treated with melittin (40 μg/kg) for 3 days showing intense reactivity with SMA at the muscularis mucosa (arrow head) and the muscularis externa (double arrow heads) (SMA immunohistochemistry, X100). (B) Transverse section in the body of the stomach of a mouse treated with melittin (40 μg/kg) for 3 days showing an enlarged part of the muscularis externa (double arrow heads) that displays intense SMA reactivity. Note intensely reacted muscularis mucosa (arrow head). LM: Longitudinal muscles; CM: Circular muscles (SMA immunohistochemistry, X400). (C) Transverse section in the body of the stomach of a mouse treated with melittin (40 μg/kg) for 5 days showing the muscularis mucosa (arrow head) and the muscularis externa (double arrow heads) with intense SMA reactivity (SMA immunohistochemistry, X100). (D) Transverse section in the body of the stomach of a mouse treated with melittin (40 μg/kg) for 5 days showing an enlarged part of the muscularis externa (double arrow heads) with intense SMA reactivity. LM: Longitudinal muscles; CM: Circular muscles (SMA immunohistochemistry, X400). (E) Transverse section in the body of the stomach of a mouse treated with melittin (40 μg/kg) for 10 days showing intense reactivity with SMA at the muscularis mucosa (arrow head) and the muscularis externa (double arrow heads) (SMA immunohistochemistry, X100). (F) Transverse section in the body of the stomach of a mouse treated with melittin (40 μg/kg) for 10 days showing an enlarged part of the previous figure which displays intense SMA reactivity at the muscularis externa (double arrow heads) (SMA immunohistochemistry, X400).

After 24 h from each treatment, mice from all groups were sacrificed under light ether anesthesia. Samples from the stomach body collected from all animals were prepared for histological and immunohistochemical studies.

On scarification, samples from the stomach were immediately removed from each animal and then washed within a physiological saline solution (0.85% NaCl) for the removal of the blood or food remnants, which might obstruct the process of fixation. Small pieces (about 4 mm in diameter) from each sample were obtained by using a sharp blade. Tissue samples were allowed to remain in the fixative (10% neutral buffered formalin) for 24 h. The fixed samples were washed overnight under running water, then dehydrated through ascending series of ethyl alcohol (30%, 50%, 70%, 80%, 90%, 95%, and 2 changes of 100%) for 2 h each.

Clearing was next by moving the tissues into a mixture of absolute ethanol and toluene (1:1) for 2 h, then in two changes of pure toluene (2 h each). Tissue samples were then placed into a mixture of toluene and paraffin (1:1) in the oven. The tissues were then infiltrated in pure paraffin and embedded in paraffin block by using **Paraffin Embedding Machine (**LS-100; Bio-Equip Company). The blocks were allowed to cool slowly in a water bath (20–25 °C).

Paraffin blocks were trimmed for removing excess paraffin around the tissue samples by using a sharp blade. The paraffin blocks were sectioned at a thickness of five microns by using a rotating microtome (Bright instrument LTD, England) at the Histology Unit of Anatomy Department, Faculty of Medicine, King Abdul-Aziz University. The paraffin sections were floated over a warm water bath and picked up by clean glass microscopic slides, which contained glycerin Mayer's adhesive media (egg albumin + glycerin + sodium salicylate). The slides were placed in a warm oven at 25 °C for about 15 min.

2.4. Techniques

The paraffin sections were used in the following techniques:

2.4.1. Alcian blue technique

Alcian blue staining technique was used to detect acid mucosubstances and acetic mucins in different mucous secreting cells of the stomach (Sheehan and Hrapchak, 1980; Bancroft and Stevens, 1982).

2.4.2. Immunohistochemical (IHC) techniques

Immunohistochemical staining is a valuable tool for detecting specific antigens in tissues (Cuello, 1993). The Smooth Muscle Actin (SMA) (obtained from Ventana Company) was used in the present study as a primary antibody to detect Muscle Actin marker mouse monoclonal antibody.

3. Results

3.1. Alcian blue-stained sections

Alcian blue stain was applied for the detection of the mucous secreting cells in the gastric tissues of the control and experimental groups (Table 1).

3.1.1. Control group

In sections of the stomach body of control mice, the mucous secreting cells of the gastric pits and the gastric glands showed a strong stainability with Alcian blue indicated by blue coloration at these cells (Fig. 1A and B).

3.1.2. Melittin treated group

Treatment with 10 μg/kg melittin for 3 days (Fig. 2A and B), 5 days (Fig. 2C and D) or 10 days (Fig. 2E and F) showed more or less normal presence of positively stained mucous secreting cells of the gastric pits and the gastric glands similar to those of the control group.

After treatment with 40 μg/kg melittin for 3 days, the body of the stomach showed positive stainability with Alcian blue at the mucous secreting cells of the gastric pits and the gastric glands (Fig. 3A and B). However, after treatment with the same dose of melittin for 5 days less Alcian blue stainability was displayed by the mucous secreting cells of the gastric pits and the gastric glands compared to those of the control group (Fig. 3C and D). Weak stainability with Alcian blue at a few mucous secreting cells within the gastric glands and a few numbers of gastric pits were observed after 10 days of treatment with the same dose of melittin (Fig. 3E and F).

3.2. Immunohistochemical reactivity of the smooth muscle actin (SMA) antigen

The immunohistochemical reactivity of the smooth muscle actin (SMA) antigen, the specific antigen of the smooth muscle fibers, was indicated by a brown coloration at the smooth muscles of the muscularis mucosa and the muscularis externa of the stomach body tissues of control and treated mice. SMA reactivity of different smooth muscles is summarized in Table 2.

3.2.1. Control group

Microscopic examination of sections of the stomach body from control mice showed intense SMA reactivity at the muscularis mucosa and the muscularis externa (Fig. 4A and B).

3.2.2. Melittin treated group

The stomach body of mice treated with 10 μg/kg melittin for 3 days showed intense SMA reactivity at the muscularis mucosa and muscularis externa (Fig. 5A and B). Similar results were recorded in the muscularis mucosa and muscularis externa of the stomach body in mice treated with the same dose of melittin for 5 days (Fig. 5C and D) or 10 days (Fig. 5E and F).

Treatment of mice with 40 μg/kg melittin also showed intense SMA reactivity at the muscularis mucosa and the muscularis externa after 3 days (Fig. 6A and B), 5 days (Fig. 6C and D) or 10 days (Fig. 6E and F) of treatment.

4. Discussion

In this research we investigated the histological and immunohistochemical effects of melittin on mice stomach, where there is no evidence of the effect of melittin on gastrointestinal cells. The microscopic examination of Alcian blue stained section showed the normal histological features of the four layers, and the distribution of the mucous secreting cells of the stomach tissues. From previous results, it is obvious that both doses of melittin (10 and 40 μg/kg) have no harmful effects on the histological structure of the stomach tissues. These findings agree with those of Maher et al. (2007) who informed that melittin at low concentrations did not induce any cytotoxicity in the intestinal tissues of rats and did not affect their cellular functions such as mucus secretion. Our finding is also similar to Yun et al.

(2011) who showed that the pancreas of mice treated with melittin (10 and 50 μg/kg) displayed typical histological structure, and that these doses were safe on the tissue. In this study, the immunohistochemical detection of Smooth Muscle Actin (SMA) antigen was used to determine the effect of melittin on smooth muscle fibers of the muscularis mucosa and the muscularis externa (properia) of stomach tissues, where the SMA plays an important role in the regulation of Ca^{2+} sensitivity since it induced an increase in the intracellular calcium (Bitar, 2003). Prakash et al. (2010) added that SMA is related to the contractile and the cytoskeletal functions in various types of cells, where it serves to facilitate cell contraction and migration. The results of the present study showed that no changes were observed on the reactivity of SMA in mice tissues treated with melittin confirmed the safety effects and the protective role of melittin. Furthermore, Son et al. (2006) and Yun et al. (2011) supported our finding by explaining the protective effect of melittin against the abnormal cases of smooth muscles including ***increasing the growth of smooth muscle cells besides the significant inhibition on the proliferations of the smooth muscle cells through inducing apoptosis to the proliferating cells, thus lead to regulation of the smooth muscle contractility.

5. Conclusion

This project concluded that the use of melittin in a specific dose and for certain duration is safe on gastrointestinal tissues and does not have any cytotoxic effects on the tissues and cellular function such as mucus production of the living organism, These finding push the studies one step further toward the therapeutic effect of melittin as a treatment for many diseases of the gastrointestinal tract.

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References

Bancroft, J., Stevens, A., 1982. Theory and Practice of Histological Techniques, second ed. Churchill Livingstone, N.Y.

Behrens, I., Pena, A.I., Alonso, M.J., Kissel, T., 2002. Comparative uptake studies of bioadhesive and non-bioadhesive nanoparticles in human intestinal cell lines and rats: the effect of mucus on particle adsorption and transport. Pharm. Res. 19 (8), 1185–1193.

Bitar, K., 2003. Function of gastrointestinal smooth muscle: from signaling to contractile proteins. Am. J. Med. 115 (3), 15–23.

Carrasquer, G., Ming, L., Yang, S., Schwartz, M., 1998. Effect of melittin on PD, resistance and short-circuit current in the frog gastric mucosa. Biochim. Biophys. Acta 1369 (2), 346–354.

Clague, M.J., Cherry, R.J., 1988. Comparison of p25 presequence peptide and melittin. Red blood cell haemolysis and band 3 aggregations. Biochem. J. 252 (3), 791–794.

Conlon, J.M., Sonnevend, A., Patel, M., Camasamudram, V., Nowotny, N., Zilahi, E., Iwamuro, S., Nielsen, P.F., Pál, T., 2003. A melittin-related peptide from the skin of the Japanese frog, Rana tagoi, with antimicrobial and cytolytic properties. Biochem. Biophys. Res. Commun. 306 (2), 496–500.

Cuello, A.C., 1993. Immunohistochemistry II. Wiley, USA, Chap. 1, pp. 23–25.

Cuppoletti, J., Abbott, A.J., 1990. Interaction of melittin with the ($Na^n + K^+$) ATPase: evidence for a melittin-induced conformational change. Arch. Biochem. Biophys. J. 283 (2), 249–257.

Cuppoletti, J., Blumenthal, K.M., Malinowska, D.H., 1989. Melittin inhibition of the gastric ($H^+ + K^+$) ATPase and photoaffinity labeling with [125I] azidosalicylyl melittin. Arch. Biochem. Biophys. 275 (1), 263–270.

Karimi, A., Parivar, K., Nabiuni, M., Haghighi, S., Imani, S., Afrouzi, H., 2011. Effect of honey bee venom on Lewis rats with experimental allergic encephalomyelitis as regards changes of GABA and glutamate. J. Am. Sci. 7 (1), 295–300.

Klocek, G., Seelig, J., 2008. Melittin interaction with sulfated cell surface sugars. J. Biochem. 47 (9), 2841–2849.

Mahaney, J.E., Thomas, D.D., 1991. Effects of melittin on molecular dynamics and Ca-ATPase activity in sarcoplasmic reticulum membranes: electron paramagnetic resonance. Biochemistry 30 (29), 7171–7180.

Maher, S., Mcclean, S., 2006. Investigation of the cytotoxicity of eukaryotic and prokaryotic antimicrobial peptides in intestinal epithelial cells in vitro. Biochem. Pharmacol. 71 (9), 1289–1298.

Maher, S., Mcclean, S., 2008. Melittin exhibits necrotic cytotoxicity in gastrointestinal cells which is attenuated by cholesterol. Biochem. Pharmacol. 75 (5), 1104–1114.

Maher, S., Feighery, L., Brayden, D., Mcclean, S., 2007. Melittin as a permeability enhancer II: *in vitro* investigations in human mucus secreting intestinal monolayers and rat colonic mucosa. Pharm. Res. 24 (7), 1346–1356.

Masquelier, M., Zhou, Q.F., Gruber, A., Vitols, S., 2004. Relationship between daunorubicin concentration and apoptosis induction in leukemic cells. Biochem. Pharmacol. 67 (6), 1047–1056.

Mollay, C., Kreil, G., 1974. Enhancement of bee venom phospholipase A2 activity by melittin, direct lytic factor from cobra venom and polymyxin B. FEBS Lett. 46 (1), 141–144.

Ortmann, O., Johannsen, K., Knuppen, R., Emons, G., 1992. Acute effects of oestradiol and progesterone on melittin- and gonadotrophin-releasing hormone-induced LH secretion. J. Endocrinol. 132 (2), 251–259.

Prakash, S., LeMaire, S., Guo, D., Russell, L., Regalado, E., Golabbakhsh, H., Johnson, R., Safi, H., Estrera, A., Coselli, J., Bray, M., Leal, S., Milewicz, D., Belmont, J., 2010. Rare copy number variants disrupt genes regulating vascular smooth muscle cell adhesion and contractility in sporadic thoracic aortic aneurysms and dissections. Am. J. Hum. Genet. 87 (6), 743–756.

Raghuraman, H., Chattopadhyay, A., 2004. Effect of micellar charge on the conformation and dynamics of melittin. Biosci. Rep. 33 (7), 611–622.

Sheehan, D., Hrapchak, B., 1980. Theory and Practice of Histotechnology, second ed. Battelle Press, Ohio.

Shkenderov, S., Ivanov, T., 1983. The Bee Products, first ed. Springer, USA.

Shkinev, A.V., Gagel'gans, A.I., Tashmukhamedov, B.A., Miroshnikov, A.I., 1978. Effect of melitten from bee venom on mitochondrial functions. Biokhimiia 43 (8), 1452–1457.

Son, D.J., Ha, S.J., Song, H.S., Lim, Y., Yun, Y.P., Lee, J.W., Moon, D.C., Park, Y.H., Park, B.S., Song, M.J., Hong, J.T., 2006. Melittin inhibits vascular smooth muscle cell proliferation through induction of apoptosis *via* suppression of nuclear factor-kappaB and Akt activation and enhancement of apoptotic protein expression. J. Pharmacol. Exp. Ther. 317 (2), 627–634.

Son, D.J., Lee, J.W., Lee, Y.H., Song, H.S., Lee, C.K., Hong, J.T., 2007. Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. Pharmacol. Ther. 115 (2), 246–270.

Sumikura, H., Andersen, O.K., Drewes, A.M., Arendt-Nielsen, L., 2003. A comparison of hyperalgesia and neurogenic inflammation induced by melittin and capsaicin in humans. Neurosci. Lett. 337 (3), 147–150.

Tosteson, M.T., Holmes, S.J., Razin, M., Tosteson, D.C., 1985. Melittin lysis of red cells. J. Membr. Biol. 87 (1), 35–44.

Yalcin, M., Savci, V., 2007. Cardiovascular effects of centrally injected melittin in hemorrhaged hypotensive rats: the investigation of peripheral mechanisms. Neuropeptides 41 (6), 465–475.

Yalcin, M., Aydin, C., Savci, V., 2009. Cardiovascular effect of peripheral injected melittin in normotensive conscious rats: mediation of the central cholinergic system. Prostag Leukotr Ess. 81 (5), 341–347.

Yun, S., Bae, G., Kim, M., Park, K., Koo, B., Kim, B., Kim, T., Seo, S., Shin, Y., Lee, S., Song, H., Park, S., 2011. Melittin inhibit sceruleinduced acute pancreatitis *via* inhibition of the JNK pathway. Int. Immunopharmacol. 11 (12), 2062–2072.