

# MELATONIN PROTECTS THE INTEGRITY OF GASTRIC STRUCTURE FROM A STERILE TISSUE INJURY AND AUGMENTS BOTH MONONUCLEAR AND POLYMORPHONUCLEAR PERIPHERAL BLOOD CELLS INDUCED BY THE INJURY IN WISTAR ALBINO RATS

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## ABSTRACT

Peptic ulcer is a common upper gastrointestinal disease that remains a major public health problem. Gastric ulceration caused by Nonsteroidal Anti-Inflammatory Drugs (NSAID), stress and alcohol are the common causes of gastric ulcer formation in humans following helicobacter pylori bacterial infection of the stomach. The neurohormone, melatonin was reported to protect against NSAID- and stress-induced gastric lesions. We sought to determine whether melatonin, which is known to have antioxidant effects and induces systemic leukocyte mobilization, can protect the gastric structure from a sterile tissue injury. Equally divided melatonin or vehicle pre-treated Albino rats (N = 20) were subjected to sterile tissue injury of gastric ulceration using hypertonic sodium chloride solution. Melatonin treatment significantly protected the animals from gastric lesions induced by hypertonic salt compared to control vehicle-treated animals that show formation of gastric lesions in all examined rats. In addition, melatonin treatment significantly increased sterile tissue injury induction of both mononuclear and polymorphonuclear peripheral blood cells. We conclude that melatonin protects sterile tissue injury-induced gastric lesions and augments white blood cell populations in response to this type of tissue injury.

**Keywords:** Melatonin, Sterile Tissue Injury, Gastric Ulcer, PBMC, PMN, Antioxidant

## 1. INTRODUCTION

Gastric ulcer is a necrosis or eroded areas in the mucosa of the stomach due to the deleterious effects of the secreted hydrochloric acid and peptic juice (Yuan *et al.*, 2006; Desia *et al.*, 1997). The most common cause of gastric ulcer is a curved bacillus bacterium called helicobacter pylori that is commonly found in the stomach (Marshall and Warren, 1984; McColl, 2010; Chey and Wong, 2007). Other causes of gastric ulcers are the use of NSAID (Wallace, 2000; 2008), alcohol consumption (Bujanda, 2000; Muralidhar *et al.*, 2009) and stress (Levenstein, 1998; Khalefa *et al.*, 2010).

Melatonin is a hormone produced primarily by the pineal gland in the brain. It is derived from the

neurotransmitter, serotonin (Cardinali and Pevet, 1998) and plays an important role in controlling the body's circadian rhythms, sleep-wake cycle (Tan *et al.*, 2003; Silva *et al.*, 2013) and regulates other hormones such as insulin and insulin growth factor receptor and thyroid hormone. Melatonin is formed not only by the pineal gland, but also in the retina, kidneys and digestive tract (Jaworek *et al.*, 2005). Melatonin exerts most of its physiological effects by binding to the melatonin receptors MT1 and MT2, which are G protein-coupled receptors located in the brain and some peripheral organs (Dubocovich *et al.*, 2000; McArthur *et al.*, 1997; Li *et al.*, 2013). However, the strong antioxidant effects of melatonin are produced via a receptor-independent method that may help strengthen and modulates the

immune system (Reiter *et al.*, 2004; Vico *et al.*, 2013). Additionally it was found that human peripheral blood mononuclear cells synthesize biologically relevant amounts of melatonin (Vico *et al.*, 2004) and melatonin receptors have been identified in human lymphocytes and monocytes (Maurino *et al.*, 2000).

The antioxidant effect of melatonin was attributed to the ability of the substance to protect against NSAID (Lastra *et al.*, 1999; Ganguly *et al.*, 2005; Ganguly and Swarnakar, 2009), stress (Konturek *et al.*, 2006; Bandyopadhyay *et al.*, 2000) and alcohol (Swarnakar *et al.*, 2007) induced gastric ulcer and lesions. In addition, we recently found that melatonin increased blood leukocyte counts before and after sterile tissue injuries (Sakr and Al-Ani, 2013). Therefore, the aim of the present study is to investigate whether melatonin is able to protect the gastric tissue from an induced sterile tissue injury and if it is accompanied by up-regulation of Peripheral Blood Mononuclear Cells (PBMC) and Polymorphonuclear (PMN) cells.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

All reagents and chemicals were obtained from Sigma Aldrich (St Louis, USA) unless otherwise stated. A stock solution of melatonin was prepared freshly every 3 or 4 days containing 348 mg of melatonin dissolved in 10 mL of 96% ethanol and stored at -20°C. The final concentration of melatonin (20 µg mL<sup>-1</sup>) was administered in tap water for a period of 6 weeks. Water bottles were covered with aluminium foil to protect against light.

### 2.2. Animal Protocol

Male Albino rats 7-8 weeks of age (150-200g) were obtained from the experimental animal care center of the college of medicine of King Khalid University. The rats were provided Purina chow diet ad libitum and were kept individually in well ventilated cages under standard conditions of humidity (55±5%) temperature (25°C) and light (12/12hr light-dark cycles). All experiments performed on laboratory animals in this study followed the "Principles of laboratory animal care" (NIH Publication No. 85, Rev, 1985). This study has been approved by the ethical committee of College of Medicine, King Khalid University, KSA. After acclimatization for two weeks, rats were randomly assigned to two groups, each containing 10 rats. Group 1, rats with gastric ulcer treated with melatonin and group

2, rats with gastric ulcer only. Treatment with melatonin (20 µg mL<sup>-1</sup>) was initiated 4 weeks before induction of injury and two weeks post-injury induction. Data collected before melatonin treatment were regarded as the baseline levels (control) for group 1 and data collected before the induction of injury in group 2 were regarded as the baseline levels (control). Baseline levels of the white blood cell (total leucocyte, lymphocyte, monocyte, neutrophils and eosinophils) counts were recorded at the start of melatonin treatment. Counts were recorded again on day 1 of the 5th week at 1-2 hr before induction of the injury. Post injury counts were performed on day 4 and 7 of the 5th week and on day 4 of the 6th week. Blood was drawn from the retro-orbital plexus of veins using heparinized capillary tubes. The samples were then immediately analyzed using an automated closed tube hematology system (ADVIA 60, Bayer Corp NY) that yields a report on levels of all White Blood Cell (WBC) types present.

### 2.3. Induction of Gastric Lesions

Gastric ulcers were induced as described previously (Sakr and Al-Ani, 2013). Briefly, following an overnight fast, rats were anesthetized and then gavaged with 10 mL kg<sup>-1</sup> of 25 % (w/v) aqueous NaCl solution. To assure that there was successful induction of an ulcer, a set of three (fasted) naïve rats were gavaged in parallel. 2 h later, these rats were dissected under anesthesia by a single Intraperitoneal (I.P) administration of pentobarbital sodium (150 mg kg<sup>-1</sup>) and their stomachs excised and opened through the greater curvature. After washing with saline, the extent of the gastric lesion (s) that was induced was assessed using a binocular magnifier; degeneration of the gastric mucosa was qualified by direct examination.

### 2.4. Tissue Preparation and Histological Analysis

After the collection of blood, stomach and other organs were collected and fixed with 10% formaline for 12 h prior to dehydration with alcohols and paraffin embedding using standard methods. Stomach 5 µm paraffin sections were stained with Hematoxylin and Eosin (H&E) and analyzed for gastric lesions caused by the injury and potential gastric protection by melatonin.

### 2.5. Statistical Analysis

All data is expressed as the mean ±SD. Statistical analysis was performed with ANOVA using Stat Plus (Analysis Soft, USA) to evaluate the main effects and interactions of Group and time on each white blood cell type simultaneously. All statistical analyses were conducted using SPSS, general linear model.

### 3. RESULTS

#### 3.1. Melatonin Protects Sterile Tissue Injury-Induced Gastric Lesions

To test the hypothesis that melatonin can protect the stomach from lesions induced by sterile tissue injury, hypertonic sodium chloride solution was used as an agent to induce the gastric ulceration in Albino rats. We then compared its effects with animals pre-treated with melatonin ( $20 \mu\text{g mL}^{-1}$ ) four weeks prior to injury induction and continued until the sacrifice day. Hypertonic sodium chloride solution induced severe gastric lesions as indicated by mucosal erosions and destruction of gastric epithelial tight junctions, interrupted muscularis mucosae and submucosal injuries (**Fig. 1A**). Whereas, in animals that received melatonin, the integrity of their gastric tissue was substantially protected from the salt-induced lesions (**Fig. 1B**) and was almost comparable to non-injured rats (**Fig. 1C**).

#### 3.2. Melatonin Augments Sterile Tissue Injury-Induced Up-Regulation of PBMC and PMN

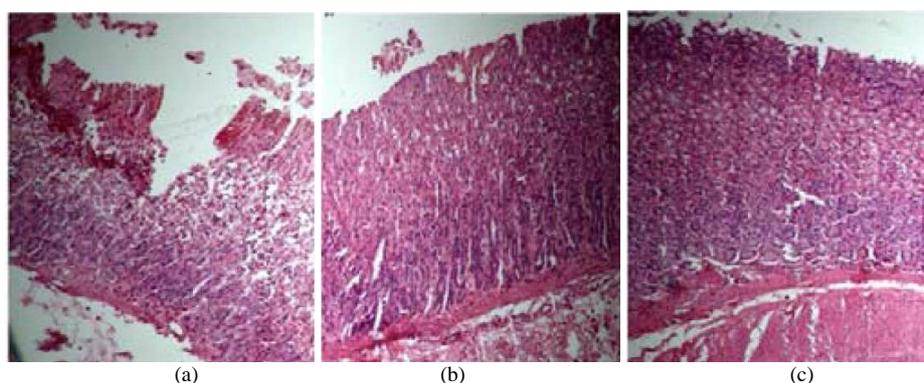
We recently demonstrated that melatonin augmented sterile tissue injuries induced total leukocytes in blood circulation (Sakr and Al-Ani, 2013). Here, we further analyzed the differential cell counts and found a significant increase in both PBMC (lymphocytes and monocytes) and PMN (neutrophils and eosinophils) white blood cells in response to gastric sterile injury by hypertonic sodium chloride solution (**Fig. 2 A and B**). The analyzed leukocytes peaked four days post-injury, then lymphocytes declined in number until returning to the basal level after seven days post-injury time (**Fig. 2A**). Whereas, the number of monocytes, neutrophils and eosinophils remained significantly high compared to controls for eleven days following sterile gastric injury (**Fig. 2A and B**). Melatonin ( $20 \mu\text{g mL}^{-1}$ ) pre-treatment for four weeks prior to gastric injury induction was able to mimic hypertonic sodium chloride effects in increasing PBMC and PMN cell counts (**Fig. 3A**). The effect of melatonin was further monitored post-injury time and administration of the drug was continued for further two weeks. The numbers of PBMC and PMN cells, eleven days post-injury induction, were significantly higher in injured rats that received melatonin compared to controls, vehicle- 'treated' injured rats (**Fig. 3B**).

### 4. DISCUSSION

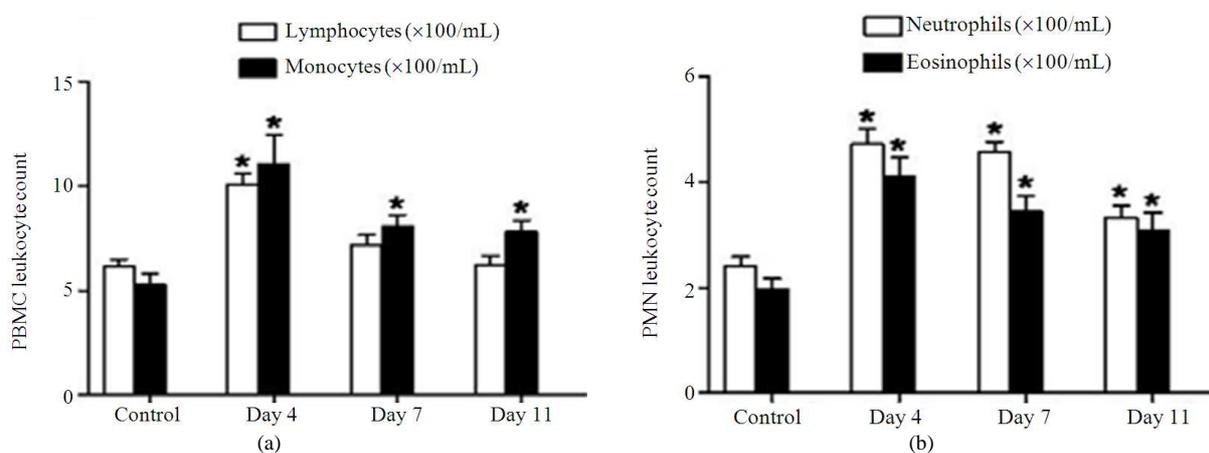
The main finding of this study using a sterile gastric injury animal model was that the potent antioxidant neurohormone, melatonin protected the gastric structure from a sterile tissue injury induced by hypertonic sodium chloride solution. Furthermore, this study shows that melatonin mobilized PBMC and PMN cells into blood circulation and augmented sterile tissue injury-induced PBMC and PMN blood cell counts. These conclusions were supported by the data indicating that hypertonic sodium chloride solution caused severe erosions of the gastric mucosa, destruction of gastric epithelial tight junctions and interruption to muscularis mucosae and submucosa of Albino rats. Administration of melatonin before and after the gastric injury for a period of six weeks clearly protected the integrity of gastric structure from the deleterious effects of hypertonic salt (**Fig. 1**). In addition, both hypertonic sodium chloride solution and melatonin increased the levels of lymphocytes and monocytes (PBMC) and neutrophils and eosinophils (PMN) in the blood (**Fig. 2 and 3**). However, the maximum levels of PBMC and PMN were achieved when melatonin was given to injured rats compared to melatonin or injury alone (**Fig. 2 and 3**) and (data not shown).

As outlined above, our results with melatonin protecting rats against gastric lesions induced by a sterile tissue injury are in agreement with previous studies that showed melatonin protected rats from stress-induced gastric lesions and the maximum protection was achieved during the night when melatonin secretion is the highest compared to the day time (Brzozowski *et al.*, 2007). In addition, removal of the pineal gland that produces melatonin augmented stress-induced gastric lesions in rats (Brzozowski *et al.*, 2007). Furthermore, gastric lesions in rats induced by NSAID such as piroxicam and indomethacin were protected (>90%) by melatonin (Bandyopadhyay *et al.*, 2004; Basu *et al.*, 2013).

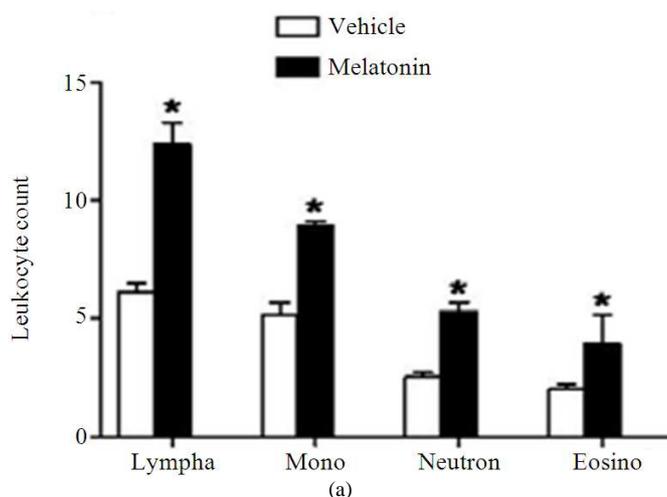
Our data with the induction of PBMC and PMN by melatonin using gastric sterile tissue injury model (**Fig. 2 and 3**) supports a role for melatonin in leukocyte recruitment to the injury sites (Ley *et al.*, 2007; Petri *et al.*, 2008). Neutrophils are recruited to the site of sterile and non-sterile injuries and involved in the healing process as neutrophil depletion markedly impaired tissue healing (Gong and Koho, 2010; Phillipson and Kubers, 2011).

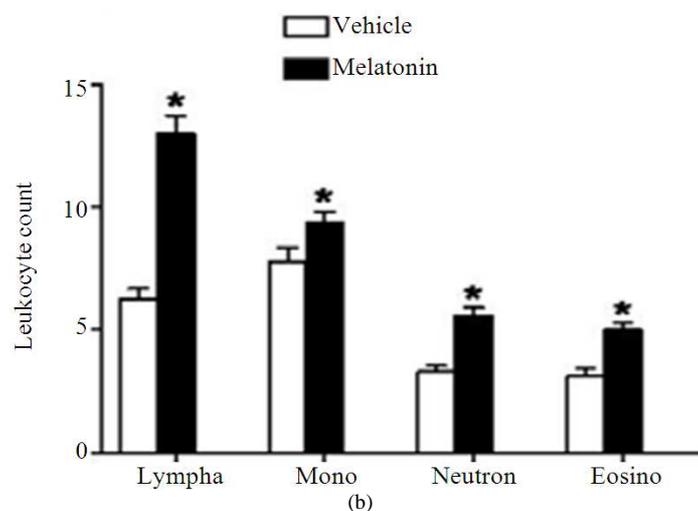


**Fig. 1.** Melatonin protects induction of sterile gastric lesions. H&E stained sections (200×) of stomach from rats injured with hypertonic sodium chloride solution (A) or injured stomach pre-treated with melatonin (B) compared with stomach section from uninjured vehicle-treated animals (C)



**Fig. 2.** White blood cells count in response to sterile gastric injury. A, PBMC, lymphocytes and monocytes and (B) PMN, neutrophils and eosinophils cells count at 4, 7 and 11 days post sterile gastric injury were measured. Results represent the mean (±S.D.); n = 10. \*: p<0.05 Vs control, uninjured rats please note: Lymphocytes and neutrophils (×1000/mL)





**Fig. 3.** White blood cells count in response to melatonin treatment. A, Lymphocytes ( $\times 1000/\text{mL}$ ), monocytes ( $\times 100/\text{mL}$ ), neutrophils ( $\times 1000/\text{mL}$ ) and eosinophils ( $\times 100/\text{mL}$ ) cells count in response to four weeks melatonin treatment before the induction of the sterile gastric injury were measured. B, Cell numbers were evaluated eleven days post injury time in animals treated with melatonin and compared it with injured but vehicle-treated rats for the same period. Results represent the mean ( $\pm$ S.D.);  $n = 10$ . \*:  $p < 0.05$  Vs day 0 (A) and Vs controls (B)

In addition, subpopulations of neutrophil, similar to monocytes, with pro-inflammatory and anti-inflammatory properties are recently proposed (Christoffersson *et al.*, 2012; Kolackowska and Kubes, 2012). Our findings can be compared with data obtained by others using animal experiments. For example, mice which received exogenous melatonin for two weeks showed an increase in number of monocytes in the bone marrow (Currier *et al.*, 2000) and the anti-apoptotic effects of melatonin on lymphocytes and neutrophils was reported on rats injected with HL-60 leukemia cells (Delgado *et al.*, 2011) that increased animals immunity. Therefore, our approach is applicable to study the induction of sterile injuries in animal models.

## 5. CONCLUSION

In summary, using a sterile gastric tissue injury rat model, we have demonstrated that melatonin is a protector of gastric tissue against hypertonic sodium chloride-induced gastric lesions and melatonin is acting as an inducer of PBMC and PMN cells. Our findings would suggest that melatonin may play a role in the pathophysiology of both sterile and non sterile tissue injuries.

### 5.1. Disclosures

We declare no competing financial interests.

## 6. ACKNOWLEDGMENT

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## 7. REFERENCES

- Bandyopadhyay, D., G. Ghosh, A. Bandyopadhyay and R.J. Reiter, 2004. Melatonin protects against piroxicam-induced gastric ulceration. *J. Pineal Res.*, 36: 195-203. DOI: 10.1111/j.1600-079X.2004.00118.x
- Bandyopadhyay, D., K. Biswas and U. Bandyopadhyay, 2000. Melatonin protects against stress-induced gastric lesions by scavenging the hydroxyl radical. *J. Pineal Res.*, 29: 143-151. DOI: 10.1034/j.1600-079X.2000.290303.x
- Basu, A., A.K. Ghosh, M. Basu, E. Mitra and D. Mukherjee *et al.*, 2013. Melatonin and ranitidine in combination protects against piroxicam-induced gastric ulceration in rats through antioxidant mechanism (s). *J. Cell Tissue Res.* 13: 3779-3798.
- Brzozowski, T., K.Z. Korczala, P.C. Konturek and S.J. Konturek *et al.*, 2007. Role of circadian rhythm and endogenous melatonin in pathogenesis of acute gastric bleeding erosion induced by stress. *J. Physiol. Pharmacol.*, 58: 53-64. PMID: 18212400

- Bujanda, L., 2000. The effects of alcohol consumption upon the gastrointestinal tract effects of alcohol upon the GI tract. *Am. J. Gastroenterol*, 95: 3374-3382. DOI: 10.1111/j.1572-0241.2000.03347.x
- Cardinali, D.P. and P. Pevet, 1998. Basic aspects of melatonin action. *Sleep Med. Rev.*, 2: 175-190. PMID: 15310500
- Chey, W.D. and B.C. Wong, 2007. Management of helicobacter pylori infection. *Am. J. Gastroenterol.*, 102: 1808-1825
- Christoffersson, G., E. Vagesjo, J. Vandooren, M. Liden and S. Massena *et al.*, 2012. VEGF-A recruits a proangiogenic MMP-9- delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissues. *Blood*, 120: 4653-4662. DOI: 10.1182/blood-2012-04-421040, PMID: 22966168
- Currier, N.L., L.Z. Sun and S.C. Miller, 2000. Exogenous melatonin: Quantitative enhancement *in vivo* of cells mediating non-specific immunity. *J. Neuroimmunol.*, 104: 101-108. DOI: 10.1016/S0165-5728(99)00271-4
- Delgado, J., M.D.P. Terron, V.G. Martinez, C.L. Sanchez and C. Barriga *et al.*, 2011. Pariete JA, Rodriguez AB. Oral melatonin administration and programmed cell death of neutrophils, lymphocytes and other cell types from rats injected with HL-60 cells. *J. Applied Biomed.*, 9: 197-207. DOI 10.2478/v10136-011-0006-3
- Desia, J.K., R.K. Goyal and N.S. Palmar, 1997. Pathogenesis of peptic ulcer disease and current trends in therapy. *Ind. J. Physiol. Pharmacol.*, 41: 3-15. PMID: 10225026
- Dubocovich, M.L., D.P. Cardinali, P. Delagrance, D.N. Krause and D. Strosberg *et al.*, 2000. Melatonin Receptors. In: *The IUPHAR Compendium of Receptor Characterization and Classification*. IUPHAR (Ed.), London: IUPHAR Media, pp: 271-277.
- Ganguly, K. and S. Swarnakar, 2009. Induction of matrix metalloproteinase-9 and -3 in nonsteroidal antiinflammatory drug-induced acute gastric ulcers in mice: regulation by melatonin. *J. Pineal Res.*, 47: 43-55. DOI: 10.1111/j.1600079X.2009.00687.x
- Ganguly, K., P. Maity, R.J. Reiter and S. Swarnakar, 2005. Effect of melatonin on secreted and induced matrix metalloproteinase-9 and -2 activity during prevention of indomethacin-induced gastric ulcer. *J. Pineal Res.*, 39: 307-315. PMID: 16150113
- Gong, Y. and D.R. Koho, 2010. Neutrophils promote inflammatory angiogenesis via release of performed VEGF in an *in vivo* corneal model. *Cell Tissue Res.*, 339: 437-448. DOI: 10.1007/s00441-009-0908-5
- Jaworek, J., T. Brzozowski and S.J. Konturek, 2005. Melatonin as an organoprotector in the stomach and the pancreas. *J. Pineal Res.*, 38: 73-83. DOI: 10.1111/j.1600-079X.2004.00179.x
- Khalefa, A.A., D.I. Abd-Alaleem and K.I. Attiaa, 2010. The protective effects of ghrelin and leptin against stress-induced gastric ulcer in rats. *Arab J. Gastroenterol.*, 11: 74-78. DOI: 10.1016/j.ajg.2010.04.005
- Kolackowska, E. and P. Kubes, 2012. Angiogenic neutrophils: A novel subpopulation paradigm. *Blood*, 120: 4455-4457. DOI: 10.1182/blood-2012-09-457226
- Konturek, S.J., P.C. Konturek and T. Brzozowski, 2006. Melatonin in gastroprotection against stress-induced acute gastric lesions and in healing of chronic gastric ulcers. *J. Physiol. Pharmacol.*, 57: 51-66. PMID: 17233075
- Lastra, A.D.L.C., V. Motilva, M.J. Martin, A. Nieto and M.D. Barranco *et al.*, 1999. Protective effect of melatonin on indomethacin-induced gastric injury in rats. *J. Pineal Res.*, 26: 101-107. PMID: 10100736
- Levenstein, S., 1998. Stress and peptic ulcer: Life beyond helicobacter. *BMJ*, 316: 538-541.
- Ley, K., C. Laudanna, M.I. Cybulsky and S. Nourshargh, 2007. Getting to the site of inflammation: The leukocyte adhesion cascade updated. *Nature Rev. Immunol.*, 7: 678-689. DOI: 10.1038/nri2156
- Li, D.Y., D.G. Smith, R. Hardeland, M.Y. Yang and H.L., Xu *et al.*, 2013. Melatonin receptor genes in vertebrates. *Int. J. Mol. Sci.*, 14: 11208-11223. DOI: 10.3390/ijms140611208
- Marshall, B.J. and J.R. Warren, 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet*, 1: 1311-1315. DOI: 10.1016/S0140-6736(84)91816-6
- Maurino, S.G., D. Pozo, J.R. Calvo and J.M. Guerrero, 2000. Correlation between nuclear melatonin receptor expression and enhanced cytokine production in human lymphocytic and monocytic cell lines. *J. Pineal Res.*, 29: 129-137. DOI:10.1034/j.1600-079X.2000.290301.x
- McArthur, A.J., A.E. Hunt and M.U. Gillette, 1997. Melatonin action and signal transduction in the rat suprachiasmatic circadian clock: Activation of protein kinase C at dusk and dawn. *Endocrinology*, 138: 627-634. PMID: 9002996
- McCull, K.E., 2010. Helicobacter pylori infection. *NEJM*, 362: 1597-1604. DOI: 10.1056/NEJMcpl001110

- Muralidhar, T.S., A. Balaji, C. Bandopadhyay and S.L. Shantha, 2009. Cytoprotective effect of ulgen, a polyherbal formulation against physical and chemical factor induced gastric ulcers in wister Albino rats. *Am. J. Pharmacol. Toxicol.*, 4: 80-84. DOI: 10.3844/ajptsp.2009.80.84
- Petri, P., M. Phillipson and P. Kubes, 2008. The physiology of leukocyte recruitment: An *in vivo* prospective. *J. Immunol.*, 180: 6439-6446. PMID: 18453558
- Phillipson, M. and P. Kubes, 2011. The neutrophil in vascular inflammation. *Nat. Med.*, 17: 1381-1390. DOI: 10.1038/nm.2514
- Reiter, R.J., D.X. Tan and M.A. Pappolla, 2004. Melatonin relieves the neural oxidative burden that contributes to dementias. *Annals New York Acad. Sci.*, 1035: 179-196. DOI: 10.1196/annals.1332.012
- Sakr, H.F. and B. Al-Ani, 2013. Modulation of proinflammatory cytokines and leukocyte mobilization by melatonin in response to sterile tissue injury in wistar Albino rats. *Saudi Med. J.*, 34: 470-476. PMID: 23677262
- Silva, A., A. Cristina, Honorio-Franca, F.R. Giachini, L. Mores and E.G.D. Souza *et al.*, 2013. Bioactive factors of colostrum and human milk exhibits a daynight variation. *Am. J. Immunol.*, 9: 68-74. DOI: 10.3844/ajisp.2013.68.74
- Swarnakar, S., A. Mishra, K. Ganguly and A.V. Sharma, 2007. Matrix metalloproteinase-9 activity and expression is reduced by melatonin during prevention of ethanol-induced gastric ulcer in mice. *J. Pineal Res.*, 43: 56-64. PMID: 17614836
- Tan, D.X., L.C. Manchester, R. Hardeland, S.L. Burillo and J.C. Mayo *et al.*, 2003. Melatonin: A hormone, a tissue factor, an autocoid, a paracoid and an antioxidant vitamin. *J. Pineal Res.*, 34: 75-8. DOI: 10.1034/j.1600-079X.2003.02111.x
- Vico, A.C., P.J. Lardone, N.A. Sanchez, A.R. Rodriguez and J.M. Guerrero, 2013. Melatonin: Buffering the immune system. *Int. J. Moleculer Sci.*, 14: 8638-8683. DOI: 10.3390/ijms14048638
- Vico, C.E.C.A., J.R. Calvo and P. Abreu, 2004. Evidence of melatonin synthesis by human lymphocytes and its physiological significance: Possible role as intracrine, autocrine and/or paracrine substance. *FASEB J.*, 18: 537-539. PMID: 14715696
- Wallace, J.L., 2008. Prostaglandins, NSAIDs and gastric mucosal protection: Why doesn't the stomach digest itself? *Physiol. Rev.*, 88: 1547-1565. DOI: 10.1152/physrev.00004.2008
- Wallace, J.L., 2000. How do NSAIDs cause ulcer disease? *Best Pract. Res. Clin. Gastroenterol.*, 14: 147-159. DOI: 10.1053/bega.1999.0065
- Yuan, Y., Hunt, R.H., T. Ireneus and I.T. Padol, 2006. Peptic ulcer disease today. *Nature Clinical Practice Gastroenterol. Hepatol.*, 3: 80-85. DOI: 10.1038/ncpgasthep0393