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Afr. J. Biomed. Res. Vol.19 (September, 2016); 235- 239

Full Length Research Paper

Antiulcer Effects of Melatonin in Wistar Rats –The Roles of Gastric Mucous, Antioxidants and Zinc

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ABSTRACT

Melatonin is known as a potent scavenger of reactive oxygen species. Zinc has also been reported to be involved in tissue regeneration. This activity has been suggested partly as its gastroprotective mechanism. The digestive system has been estimated to produce about 400 times of this neurohormone than the pineal gland. In this study, we report the role of gastric anti-oxidant enzymes, luminal mucosa zinc level and gastric mucus cell counts in the anti- ulcer effects of melatonin in rats. The experimental Wistar rats were divided into five (5) study groups. Each group was further divided into 5 subgroups (n=5) viz: /l Control, Melatonin (20 and 40mg/kg) pre-treated, Ranitidine pre-treated and Omeprazole pre-treated. Drugs were administered orally for 21 days. The effect of melatonin was investigated on indomethacin-induced gastric ulcer and gastric mucus cell counts in rats were measured. The luminal mucosa zinc levels, gastric antioxidant enzymes (SOD and Catalase), and oxidative biomarker for lipid peroxidation; Malondialdehyde (MDA) were determined. Our results showed suppression of oxidative stress (ROS) or its inhibition after 21 days of melatonin pre-treatment in rats. Catalase exhibited significantly stronger activity better than superoxide dismutase. Our study also shows enhanced gastric mucus cell counts in the pre-treated rats. The anti-ulcer mechanisms of melatonin may be due to stimulation of gastric mucus production via raised mucus cell counts, in addition to melatonin reactive oxygen species scavenging ability. It is however, not associated with changes in luminal mucosa zinc level.

Keywords: Melatonin, gastroprotection, antioxidant, indomethacin, zinc.

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Received: November 2015; Accepted: June, 2016

Abstracted by:

Bioline International, African Journals Online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION

Melatonin is an endogenous hormone synthesized and released by the pineal gland. This hormone is known to be a powerful direct scavenger of free radicals. Melatonin was reported to be more effective than some other antioxidants in protecting against oxidative damage (Reiter, 2000., Rogriguez *et al*, 2004), as well as showing potent anti-ulcer effect (Konturek *et al*, 1997; Bilici *et al*, 2002; Sener *et al*, 2006), and protecting against lipid peroxidation (Undeger *et al*, 2004). Melatonin and even its precursor, L-tryptophan have been shown to be highly gastroprotective against various models of mucosal injury (Cho *et al*, 1989; Delalastra *et al*, 1997; Kato *et al*, 1998; Konturek *et al*, 2006).

It was revealed that total amounts of melatonin in the digestive system may be about 400 times larger than in the pineal gland (Huether *et al*, 1992), and particularly in the stomach, ileum and colon of all species of animals tested including humans (Bubenik and Pang, 1997; Bubenik *et al*.1999; Messner *et al*, 2001) with high concentration in the gallbladder concentrated bile (Tan *et al*, 1999; Messner *et al*, 2001). Assuming gastrointestinal melatonin is involved in the local protection, it is expected that, exogenous melatonin and its precursor should be protective against the mucosal lesions even after pinealectomy. Pinealectomy was found to greatly reduce the basal plasma levels of melatonin and enhanced gastric ulcerogenicity of stress but failed to prevent the gastroprotective activity of melatonin and its precursor, tryptophan (Konturek *et al*, 2006).

Zinc has long been shown to play significant roles in the protection of the gastric epithelium against aggressive factors that may erode the mucosal wall to cause ulcers. Ogle and Cho (1978) first reported that Zinc sulphate dose-dependently antagonised the gastric actions of reserpine by preventing ulceration in the rat stomach, Cho *et al* (1987) also observed that zinc deficiency adversely affect rats by reducing the body weight gain and producing ulceration while it increases gastric secretory functions. Zinc sulphate also prevents histamine-mediated lesions produced by the alcohol, but not ulceration

due to PGE2 depletion by indomethacin (Cho *et al*, 1985). Apart from its protective effect in development of ulcers, zinc has also been reported to be necessary for ulcer healing. Watanabe *et al* (1995) reported that Zinc deficiency reduced cell proliferation by and delayed ulcer healing.

Although, the mechanisms by which melatonin exerts its gastroprotection activity have been attributed to the scavenging of reactive oxygen species (ROS) and its ability to attenuate lipid membrane peroxidation and damage (Sewerynek *et al*, 1995a; Sewerynek *et al*, 1995b; Konturek *et al*, 1997a; Konturek *et al*, 1997b), it may be necessary to investigate other modes of action on ulcer formation and healing. This study was designed to examine antioxidant enzymes, gastric mucus cell counts and serum zinc levels in rats pretreated with melatonin for 21days

MATERIALS AND METHODS

Animals and treatment

The experimental animals were acclimatized for two weeks and were fed on rats' pellets (Ladokun Feeds limited, Ibadan Nigeria) and water ad libitum. These animals were kept under standard laboratory conditions. After the period of acclimatization, twenty five animals were randomly divided into five groups (n=5) and were pre-treated for 21 days.

Group I served as control and received only water, while melatonin groups (II and III) received 20 and 40 mg/kg of the drug respectively. Groups IV and V were administered 50mg/kg Ranitidine and 20mg/kg Omeprazole respectively. All drugs were orally administered.

All procedures used in this study conformed to the guidelines of the care and use of animals in research and teaching (National Institute of Health (NIH) publication; 1996).

Indomethacin gastric ulcer induction

Experimental gastric ulceration was induced in the animals using the method previously described by Oluwole and Bolarinwa (1991).

Determination of lipid peroxidation:

This is based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA) which is the end-point of lipid peroxidation. Lipid peroxidation (LPO) was assayed as malondialdehyde (MDA) according to the method earlier described by Farombi *et al*, (2000).

Assay of catalase: Catalase activity was determined according to the method reported by Farombi *et al* (2000).

Assay of superoxide dismutase (SOD): SOD activity was measured by assessing the inhibition of autoxidation of adrenaline at 30^0C with the pH raised from 7.8 to 10.2 using the method described by Misra and Fridovich (1972)

Gastric mucus cell count: Gastric mucus cells were counted using an improvised calibrated microscope as was earlier described and reported by Oluwole *et al* (2008).

Determination of zinc in serum: Blood samples were obtained and placed in metal-free test tubes, capped with paraffin film. One (1) ml of serum was deproteinised with nine (9) ml of 10% trichloroacetic acid (TCA) in 0.1% Lanthanum solution for macro-element analysis. The resulting supernatant was diluted with 0.11% Lanthanum and was aspirated to the atomic absorption spectrophotometer (AAS). Diluted serum in a ratio of 1:4 of water was read in Perkin-Elmer atomic absorption spectrophotometer, model 703, and HGA 400 graphite furnace.

Drugs and chemicals used: Melatonin supplement (Naturemade Nutritional products, Missions Hills, CA), Ranitidine, Zantac (Glaxosmithkline, Cairo), Omeprazole (Medibios laboratories PVT, Limited, India), Indomethacin (Sigma), Sodium thiopental (Abbot laboratories), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Ellman reagent (5', 5' dithio-bis-2-nitrobenzoic acid), Sodium azide, 1-2,4-dinitrobenzene.

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). Means were compared using students't-test. Differences in means were considered statistically significant at $p<0.05$.

RESULTS

Effect of melatonin pre-treatment on indomethacin-induced gastric ulceration

The results are presented in Figure 1. Melatonin pre-treatment (20 and 40mg/kg) significantly reduced the degree of gastric ulceration assessed as mean ulcer score compared to the control. Mean ulcer score was significantly reduced in omeprazole (20mg/kg) pre-treated rats compared with control. Percentage inhibition in these pre-treated groups are melatonin (89.7; 20mg/kg), melatonin (75.9; 40mg/kg) and omeprazole (100; 20mg/kg).

Effect of melatonin pre-treatment on mean gastric mucus cell count

Figure 2 shows the results obtained as the mean gastric mucus cells counted per field of observation. The control has a mean count of 250.0 ± 49.7. Mean mucus cell counts in melatonin (40mg/kg) and omeprazole (20mg/kg) groups recorded significant increase when compared to control group ($p<0.05$). However, the low dose of melatonin (20mg/kg) and ranitidine (50mg/kg) groups were not significantly different from control.

Effect of melatonin pre-treatment on antioxidant enzymes Lipid peroxidation

The results of gastric mucosal Malondialdehyde (MDA) levels recorded in melatonin pre-treated animal groups are presented in figure 3.

Melatonin (20 and 40mg/kg) and omeprazole (20mg/kg) significantly reduced gastric lipid peroxidation as measured as malondialdehyde levels when compared to control ($p<0.05$). Superoxide dismutase (SOD) levels obtained from the study are presented in figure 4.

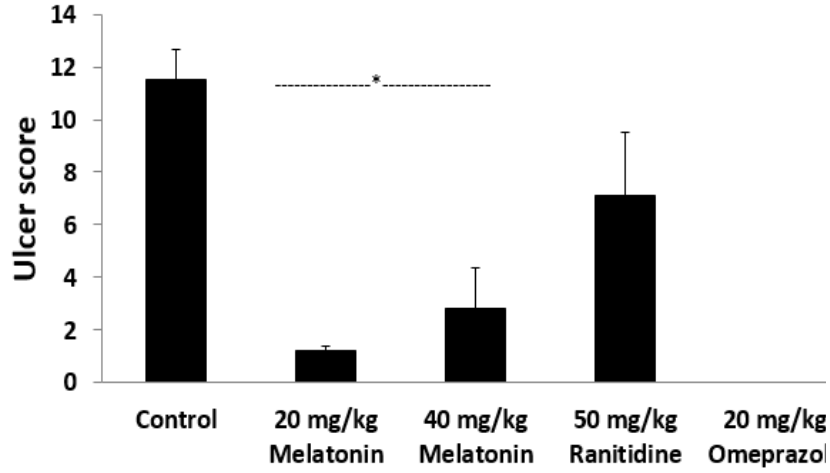

Figure 1:
Effect of melatonin pretreatment on mean ulcer score.

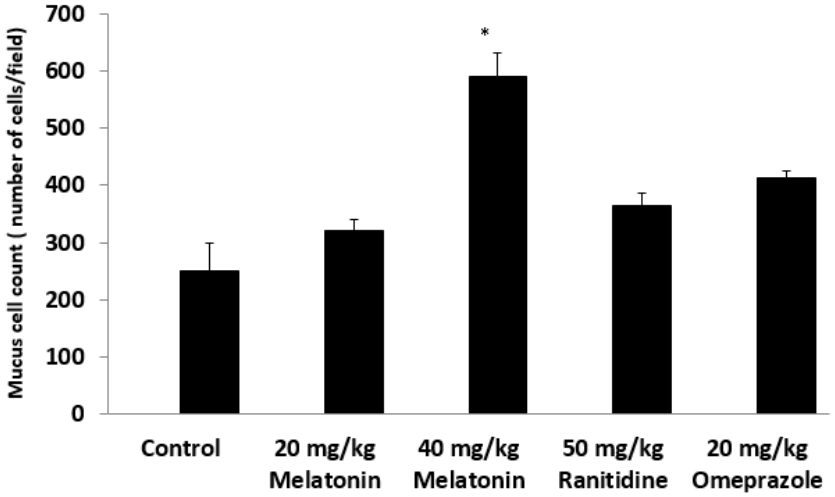

Figure 2:
Effect of melatonin pretreatment on gastric mucus cell counts

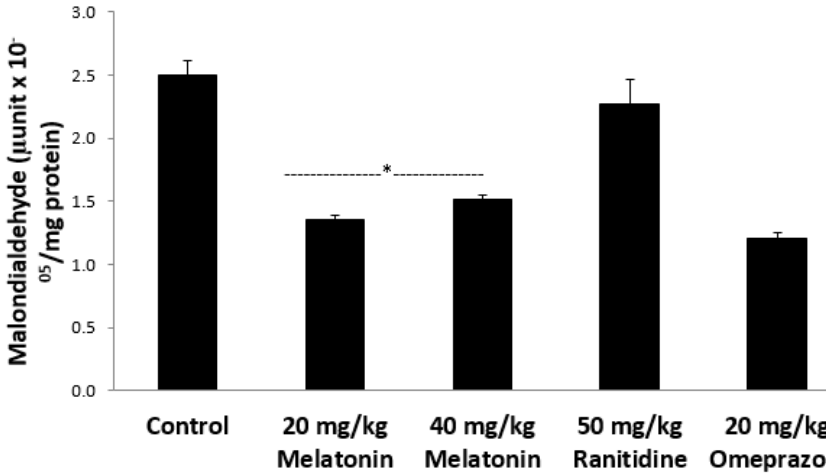

Figure 3:
Effect of melatonin pretreatment on malondialdehyde

Superoxide dismutase (SOD)

The control group had a value of 1048.0±36-6 (unit/mg protein). There was a significant increase in the SOD value of 1354.0±68.0 in 40mg/kg melatonin pre-treated group compared to the control ($p<0.05$). The other groups melatonin (20mg/kg), ranitidine (50mg/kg) and omeprazole (20mg/kg) showed no significant difference when compared with control group.

DISCUSSION

Melatonin at doses of 20 and 40mg/kg for 21 days significantly reduced gastric damage in indomethacin-induced ulceration in our investigation. This was in agreement with the report of Singh *et al* (2002) that, 20mg/kg melatonin significantly reduced mean ulcer score and mean ulcer index against indomethacin induced gastric injury. Also, in our investigation, omeprazole and the two doses of melatonin (20 and 40mg/kg) equally demonstrated 100%, 90% and 76% protection of gastric mucosa respectively. This supports previous work, that omeprazole significantly reduced the incidence of gastric mucosal injury induced by NSAIDs (Tuorkey and Abdul-Aziz, 2012). These results thus confirm that melatonin possesses significant anti-ulcerogenic properties.

The results on oxidative stress maker, malondialdehyde (MDA) showed that, pretreatment of rats with melatonin (20 and 40mg/kg), omeprazole (20mg/kg) significantly reduced lipid peroxidation measured as MDA content ($p<0.05$), while catalase activities in the melatonin pre-treated rats were significantly higher than the normal control (Fig 5) ($p<0.05$). The positive control groups (Ranitidine and omeprazole), also showed increased catalase activities compared to control ($p<0.05$). This agrees with the understanding that, induced oxidative stress give rise to elevated level of lipid peroxidation by-products (MDA), and reduction in SOD and catalase activities. The SOD levels were significantly increased in 40mg/kg melatonin group ($p<0.05$), but with no significant change in the noticed elevated value when compared with the control ($p<0.05$). Konturek *et al* (1997) asserted that any agent that can shield gastric mucosa from oxygen free radical (ROS) damage as in lipid peroxidation should serve as a

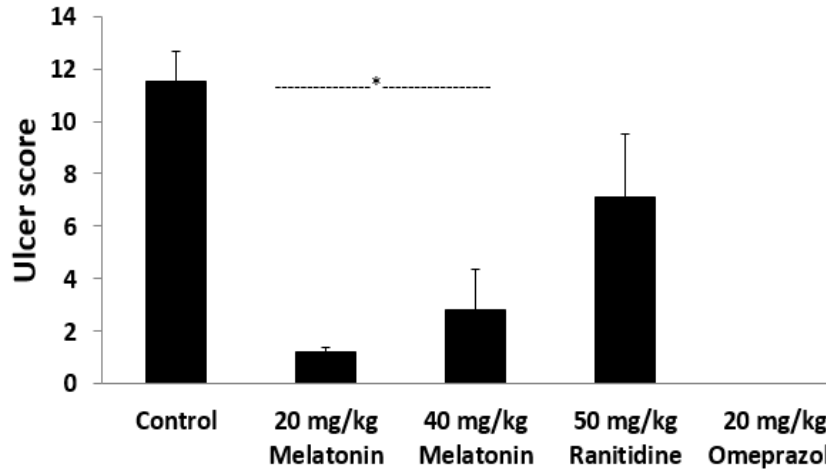

Figure 1:
Effect of melatonin pretreatment on mean ulcer score.

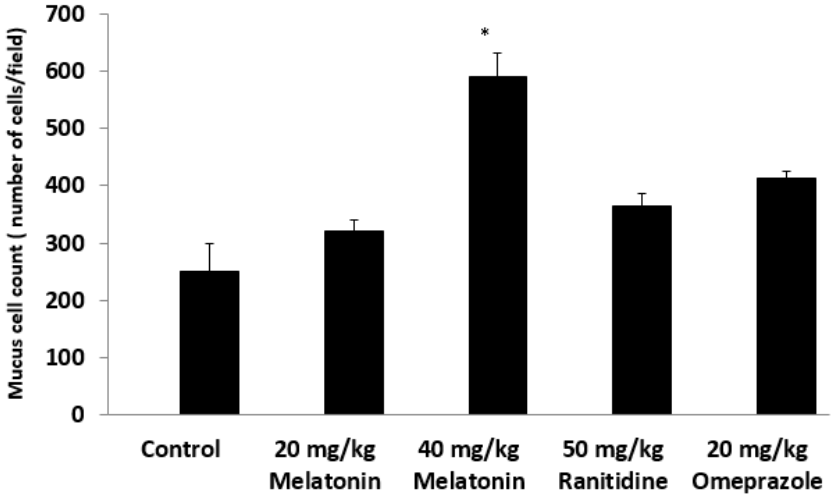

Figure 2:
Effect of melatonin pretreatment on gastric mucus cell counts

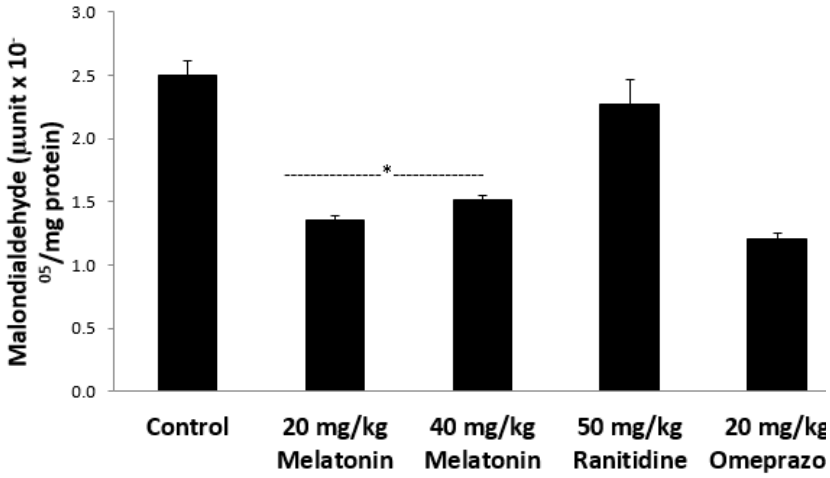

Figure 3:
Effect of melatonin pretreatment on malondialdehyde

gastroprotective agent. Melatonin and omeprazole in our study exhibited such properties. They significantly suppressed oxidative stress in the pre-treated rats.

Melatonin caused dose-dependent increase in mean mucus cell count; however the increase was significantly higher at 40mg/kg when compared with control ($p<0.05$). Similarly, both positive control groups i.e. ranitidine and omeprazole were observed to demonstrate increased in mucus cell counts.

In this study, Serum zinc levels in melatonin groups were not significantly different from the control group ($p<0.05$). No association between the possible involvement of zinc and the anti-ulcer mechanism of melatonin. The groups treated with the standard drugs; ranitidine (H_2-receptor blocker) and omeprazole (a proton pump inhibitor) however had increased serum zinc levels than both the normal control and melatonin pre-treated animals ($p<0.05$). The observation with melatonin is contrary to the report of Bediz *et al* (2002) who associated the increase in zinc levels to exogenous melatonin administration. It conflicts with the report of reduced zinc levels in gastritis, peptic ulcer, and gastric cancer. The question therefore is; could the anti-ulcer effect of melatonin be linked with changes in serum level? Definitely, our data did not support the hypothesis that anti-ulcer effect of melatonin requires upward regulation of zinc level.

We observed significantly higher gastric mucus cell counts in melatonin pre-treated animals when compared with the control animals. This implies stimulation of mucus cell growth and its proliferation. The bicarbonate-rich mucus secreted into the glands from mucus neck cells was suggested to be responsible for the first line of gastric mucosal barrier defence by forming thick mucus gel coating of the gastric surface (Engle *et al*, 1995). The biosynthesis of this mucin (MUC6) is said to be stimulated by epidermal growth factor (EGF) of the surface mucus cells in rats (Ichikawa *et al*, 1998; 2000). The improved gastric mucus cell counts noticed in this work may be due to direct activation of mucus producing cells by EGF.

In conclusion, melatonin produces its anti-ulcer effects through numerous mechanisms amongst which are; anti-oxidant activity but in particular inhibition of lipid peroxidation, and generation of

antioxidant enzyme, catalase and in addition the stimulation of gastric mucus cell growth. Exogenous melatonin may likely be a promising therapeutic agent for peptic ulcer

Acknowledgement

The assistance of Dr Olufadekemi T. Alabi in the area of graphical illustrations is gratefully acknowledged. We also want to thank Dr Chinedu Onwuchekwa for his technical advice and assistance in the preparation of the manuscript.

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