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The Protective Role of Melatonin on Small Intestine after Thioacetamide Intoxication in Rats

التأثير الوقائي للميلاتونين على الأمعاء الدقيقة في الجرذان بعد تسممها بمركب ثيوأسيتاميد

Maisaa Mohamed Al-Rawi

ميساء محمد الراوي

Department of Biology, Girls College, Makkah, Saudi Arabia.

P.O. box 10122 Makkah 21955

E-mail: mrawi5@hotmail.com

ABSTRACT: The present study deals with the effect of melatonin (MT), a very potent and efficient endogenous free radical scavenger, against the toxic effect induced by thioacetamide (TAA) on structure of small intestine in rats. Melatonin acts as a primary nonenzymatic antioxidant and protects the tissue from oxidative damage. Thioacetamide, which was originally used as a fungicide, is proposed hepatotoxin commonly used to induce liver cirrhosis in rats and other species. However, very little attention has been dealt with the effects of TAA on the intestinal cells and there functions. It was therefore of interest to study the aging variation in the sequenced responses of injury caused by TAA and regeneration capacity of recovery after using melatonin. For this purpose male Wistar rats aging 1, 3 and 12 months were arranged in three main groups (15 rats for each group). Each main group then divided into: (1) control group, (2) intoxicated group (a singe high dose 300 mg/kg/body weight of TAA) and (3) treated group, administered the same dose of thioacetamide with melatonin (5 mg/kg/body weight) daily injected orally for four weeks. Histological investigation of TAA intoxication revealed a highest grade of pathological alterations manifested by changes of width and length of most villi in ileum of all intoxicated groups. The epithelial cells lining the top of the villi were denuded in ileum sections of newly weaned and young intoxicated animals, while they appeared degenerated with exposed lamina propria in ileum sections of adult group. There was also an increase in the number of goblet cells in most villi and in crypts of Lieberkuhn of intestinal cells. Mitotic figures were observed obviously in the crypts of onset ileum cells of intoxicated animals. Lymphocytic infiltration in lamina propria and submucosa were more obvious in the intestinal cells of young intoxicated groups. However, congestion and dilatation of blood vessels were more pronounced in ileum sections of adult intestinal groups. The combined treatment with TAA and MT inhibited intestinal tissue damage. The recovery towards normal is more noticeable in ileum sections in newly weaned rats, delayed in those of young and low in the adult rats. The results for the present work indicated that melatonin could prevent cell death and intestinal dysfunction after TAA intoxication.

Keywords: *ileum, thioacetamide, melatonin, histopathology, rats.*

المستخلص: تناول هذا البحث دراسة تأثير هرمون الميلاتونين على أنسجة أمعاء الجرذان بعد معاملتها بمركب ثيوأسيتاميد السام، حيث ان هرمون الميلاتونين له تأثير فعال على إزالة الشوارد الحرة. من المعروف ان مركب ثيوأسيتاميد والمستخدم عادة كمضاد للفطريات، له تأثير سمي على الكبد محدثاً تليفاً كبدياً في الجرذان وأنواع أخرى من الحيوانات. افتقرت معظم الدراسات على توضيح تأثير مركب ثيوأسيتاميد على خلايا الأمعاء ونشاطها، لذلك كان من الضروري دراسة التأثير الضار لهذا المركب على تلك الخلايا وعلاقتها بعمر الحيوان وإيضاح مدى التأثير الوقائي لهرمون الميلاتونين عند استخدامه. أجريت هذه الدراسة على أنسجة أمعاء الجرذان في أعمار مختلفة من الجرذان بعد معاملتها بمركب ثيوأسيتاميد. استخدم عدد 54 من ذكور الجرذان البيضاء في أعمار شهر وثلاثة أشهر واثنا عشر شهرا تم تقسيمهم إلى ثلاث مجموعات، مجموعة

ضابطة ومجموعة أعطيت جرعة عالية من مركب ثيوأسيتاميد TAA (300 ملجم/كجم/وزن الجسم) ومجموعة ثالثة أعطيت نفس الجرعة من TAA مع جرعة يومية من هرمون الميلاتونين MT (5 ملجم/كجم/وزن الجسم) لمدة اربعة اسابيع. تم دراسة التغيرات المرضية الواضحة في نسيج اللفائفي بالمجهر الضوئي. في المجموعة المسممة بمركب ثيواسيتاميد، اظهرت الدراسة النسيجية اختلاف عرض وطول الزغب المتجاور في معظم خلايا الامعاء في مجموعات الحيوانات المستخدمة. بدت الخلايا الطلائية التي تغطي قمة الزغب متحللة وظهر النسيج الضام (الطبقة الاساسية) معرى وكان ذلك اكثر وضوحا في الخلايا الموجودة في مجموعة الحيوانات البالغة. ظهر زيادة في عدد الخلايا المخاطية في الزغب وجيوب ليبركهن في خلايا اللفائفي في جميع المجموعات. شوهدت الانقسامات الفتيلية في الجيوب في خلايا امعاء جميع المجموعات. ظهر ارتشاح للخلايا اللمفاوية بداخل الزغب في الصفيحة الاساسية وفي الطبقة تحت المخاطية مع ازدياد في حجمها بشكل ملحوظ في قطاعات امعاء المجموعة الثانية (الحيوانات غير البالغة). اظهر الفحص احتقان وتمدد في الاوعية الدموية وكان ذلك اكثر وضوحا في خلايا امعاء مجموعة الحيوانات البالغة. عند استخدام هرمون الميلاتونين لمعالجة الجرذان المسممة بمركب الاسيتاميد، اظهرت نتائج الفحص النسيجي تحسنا ملحوظا على المستوى النسيجي والخلوي وكان بمعدل اكبر في الجرذان في عمر شهر وبمعدل اقل في الجرذان ذات عمر ثلاثة اشهر وكان التحسن منخفض في الجرذان في عمر سنة. مما يدل على ان لهرمون الميلاتونين تاثير وقائي للانسجة والخلايا من التاثير السمي لمركب الثيواسيتاميد.

كلمات مدخلية : *امعاء دقيقة، ثيواستياميد، ميلاتونين، دراسة تغيير مرضي، جرذان.*

INTRODUCTION

Extensive works were carried out by many investigators to study the toxic effects of thioacetamide on different animals and tissues. The oral intake of TAA caused nodular liver cirrhosis in rats characterized by extensive fibrosis occupying most of the hepatic parenchyma. TAA caused cell damage and affected the ultrastructure of hepatocytes leading to a decrease in the cytoplasmic area together with increase in the nuclear and nucleolar sizes (Torres, *et al.* 1998; Bruck, *et al.* 2004; Al-Rawi, 2007a). Following a single low-dose administration, TAA produces apparently permanent alterations of hepatocyte nuclei, which encompass increased nuclear size (Brini, *et al.* 1993), DNA content (Clawson, *et al.* 1992), and enzymatic activities (Clawson, *et al.* 1997). TAA also induced toxic effects in the kidney (Kunduzova, *et al.* 2003; Tunez, *et al.* 2005; Al-Rawi, 2006), pancreas (Barker and Smuckler, 1972), ovary and spinal cord (Al-Rawi, 2007b). Moreover, TAA has also been reported to cause chemically-induced cell death via both apoptosis and necrosis (Witzmann, *et al.* 1996). Although the exact molecular mechanism by which TAA induces the toxic effects in different organs is not understood, it is known that TAA interferes with the ribosomal activity, thereby interfering with protein synthesis (Al-Bader, *et al.* 1998). Acute TAA administration has also been shown to stimulate DNA synthesis (Morley and Buyer, 1977).

Over the past decade, the potential of the pineal hormone melatonin as a therapeutic agent in a variety of diseases has been recognized. Melatonin effects in sleep disorders, its possible use as an immunoregulatory agent and clinical results obtained in cancer immunotherapy (Tunez, *et al.* 2005). Several papers are devoted to the pharmacological and molecular characterization of melatonin receptors in a variety of cell types. Other contributions further investigated the immunoenhancing effect of melatonin, such as in viral encephalitis and bacterial infections, and considered possible therapeutic indications. Melatonin is also reported to exert important hematopoietic effects by stimulating the production of novel T helper cells opioid cytokines (El-Sokkary, 2000; Maestroni *et al.*, 2001; Ozacmak, *et al.* 2005).

Melatonin is a very potent and efficient endogenous free radical scavenger (Tan, *et al.* 1993). Bruck, *et al.* (2004) reported that melatonin seems to be more effective than other antioxidant in protecting against oxidative damage, thus it may provide protection against diseases that cause degenerative or proliferative changes by shielding macromolecules, particularly DNA, from such injuries.

Because of the lack of sufficient reports on histological effects of TAA on the intestine, the present investigation was designed to determine whether MT could prevent cell death and tissue injury after intoxicated with TAA.

MATERIAL AND METHODS

Animals

Forty five male Wistar rats aged one months weighing 85-100g, three months weighing 110-125g and one year weighing 450-500g, were obtained from the Animal House of King Abdel Aziz University and were housed in good aerated chambers. Excess food and water were allowed *ad libitum* for ten days before experimentation.

Drugs and chemicals

A single high dose of thioacetamide (TAA) (300 mg/ kg/ body weight) freshly dissolved in 0.9 NaCl was injected intraperitoneally (ip) in rats. Melatonin, (TLC) was purchased from Sigma Company, USA. A daily dose of 5mg/kg/ body weight-as recommended by Laurido *et al.* (2002) was given orally to rats for 4 weeks.

Design

The animals were divided into three groups each consists of 15 rats according to their age as follows:

Group 1: Animals aging 1 month (newly weaned rats).
Group 2: Animals aging 3 months (young rats).
Group 3: Animals aging 1 year (adult rats).

Each group was divided into three groups each of 5 rats as follows:

Group A: control group.
Group B: Thioacetamide treated group.
Group C: combined treatments of thioacetamide and melatonin.

At the end of treatment, the animals of control and treated groups were sacrificed and the ileum was separated. Small pieces were fixed in 10% formol saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in molten paraplast at 56-60°C and cut at 5 μm on rotary microtome. The paraffin sections were stained with haematoxylin and eosin for histological studies and examined under light Leitz microscope.

RESULTS

Histology of ileum of control animals

Histological examination of the transverse sections of ileum of control rats showed light microscopic description similar to that found in other mammals i.e., it is essentially formed of serosa, muscularis of longitudinal and circular fibers. The mucosal surface is thrown up into numerous finger-like folds called villi, while the mucosa between the bases of the villi is formed into crypts, called crypts of Lieberkuhn. The villi are lined by a simple columnar epithelium which is continuous with that of the crypts. The columnar cells are seen as brush border and many goblet cells are scattered among them. The connective tissue in the villi and in the submucosa is called lamina propria, it contains lymphatic vessels (lacteal) as well as blood vessels (Figures 1 and 2).

Effect of TAA treatment on the histology of ileum

Marked histopathological changes in ileum of rats of all TAA intoxicated groups were shown. In the newly weaned rats, a decrease in the length of some intestinal villi could be observed. The length of the neighboring villi is greatly different. The epithelial cells covering the tip of the villi were denuded and the lamina propria of the villi appeared nearly exposed .Some of the villi showed very narrow base (Figures 3 and 4). Mitotic figures were noticed in the crypts (Fig. 5). There was an increase in the number of goblet cells in the villi and the crypt (Figures 4 and 5).

In the young rats the differences in the length and width of the villi were more pronounced. The epithelial cells lining the top of the villi were degenerated and atrophied and the lamina propria appeared exposed (Figure 6). Lymphocyte infiltration in the lamina propria of the villi was noticeable (Figure 7). The most characteristic feature in the present study was the abnormal growth in the submucosa with lymphocytic infiltration (Figure 8). The smooth muscle fibres were not densely packed, with thinner layer of muscles in some parts (Figure 9). Goblet cells were increased in the villi and the crypt (Figure 9).

In the adult rats, one of the features of ileum injury is loss of the normal architecture of villi (Figure10). The epithelial cells of the villi showed degrees of degeneration and necrosis (Figure 11). Mitotic figures were noticed in the crypts (Figure 10). The smooth muscle fibers were loose; lymphocytes were noticed in-between muscle

fibres in some regions (Figure 12). The blood vessels and blood capillaries showed dilatation in the lamina propria and submucosa (Figure 13).

Effect of combined treatment of TAA and melatonin on the histology of ileum

The histological examination of ileum rat from TAA and MT treated groups exhibited some degree of regeneration. They showed less necrosis and less inflammation. The villi showed improvement in the shape and structure; this was more pronounced in ileum sections of newly weaned rats (compare Figure 14, 15, and 16). It has also been noticed that the smooth muscle fibres appeared irregular and they were densely packed (Figure 17).

Inhibition of mitotic activity was noticed in most ileum sections of rats treated with TAA and MT (Figure 18). Moreover, a decrease number of goblet cells were observed in between the columnar cells of ileum sections, when compared with ileum sections of rats treated with TAA only.

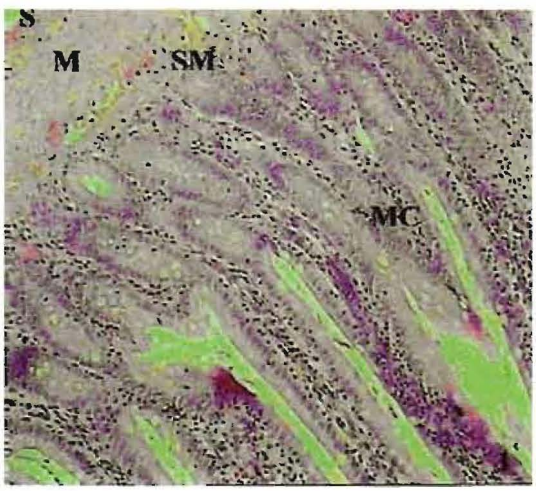

Fig. 1. Section in the ileum of control rat, serosa (S), muscularis (M), submucosa (SM) and mucosa (MC). (X250).

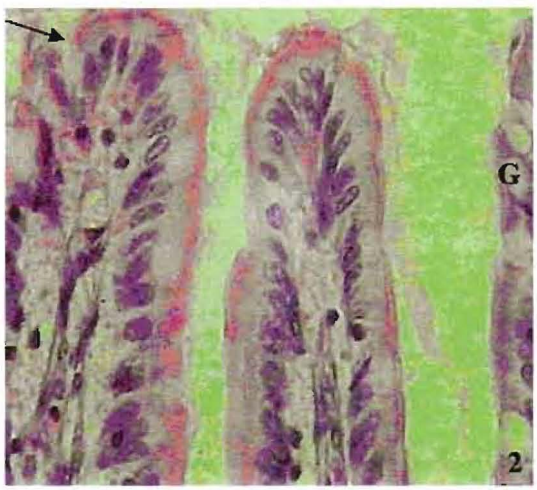

Fig. 2. Section in the ileum of control rat showing two villi. Notice exfoliated cells at the tip of the villi (arrow), few goblet cells (G). (X400).

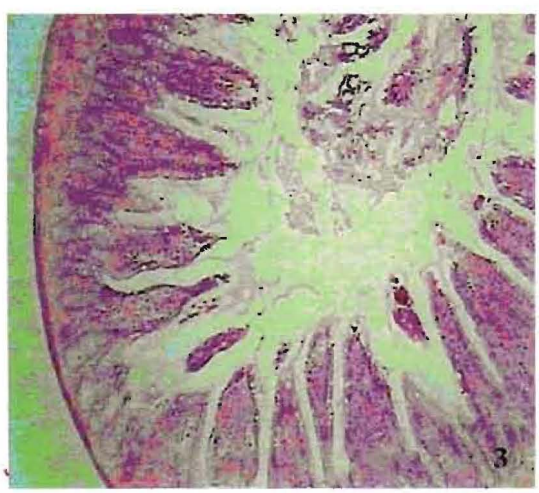

Fig. 3. Section in the ileum of 1 month old TAA treated rat, showing denuded epithelial cells at the tip of the villi. Some of the villi showed very narrow base (X150).

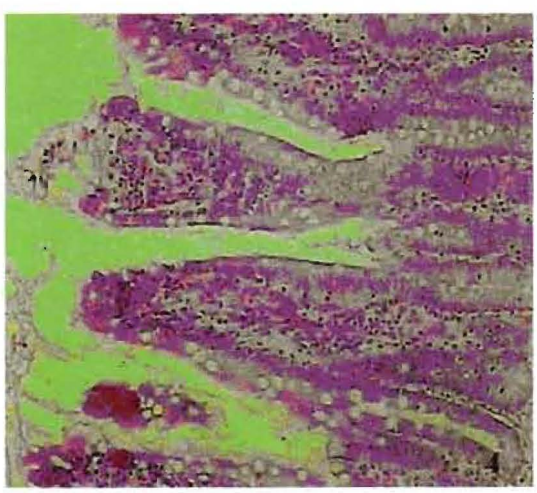

Fig. 4. Section in the ileum of 1 month old TAA treated rat, showing degenerated epithelial cells at the tip of the villi and the lamina propria of the villi appeared nearly exposed (X250).

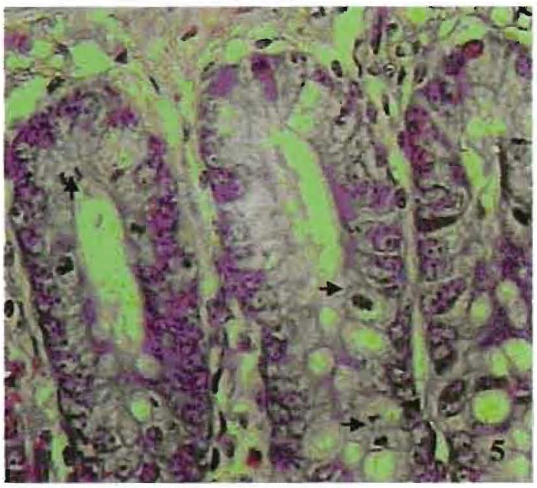

Fig. 5. Section in the ileum of 1 month old TAA treated rat. Notice mitotic figures in the crypts of Lieberkuhn (head arrow) (X400).

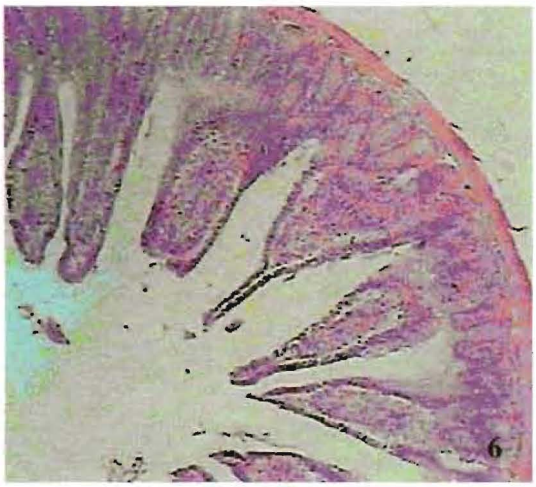

Fig. 6. Section in the ileum of 3 months old TAA treated rat, showing degenerated and atrophied villi and the lamina propria appeared exposed (X150).

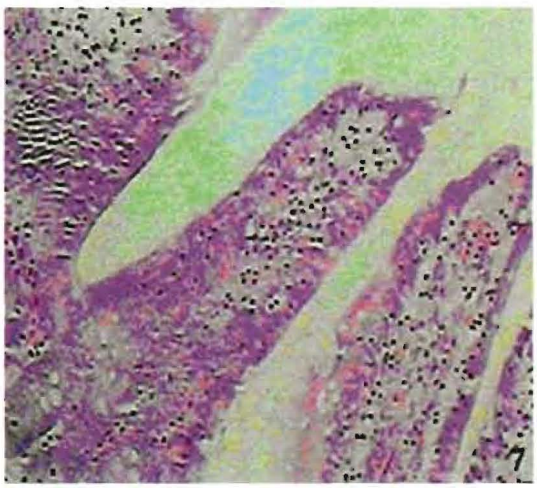

Fig. 7. Section in the ileum of 3 months old TAA treated rat, showing lyococytic infiltration in the lamina propria of villi (X250).

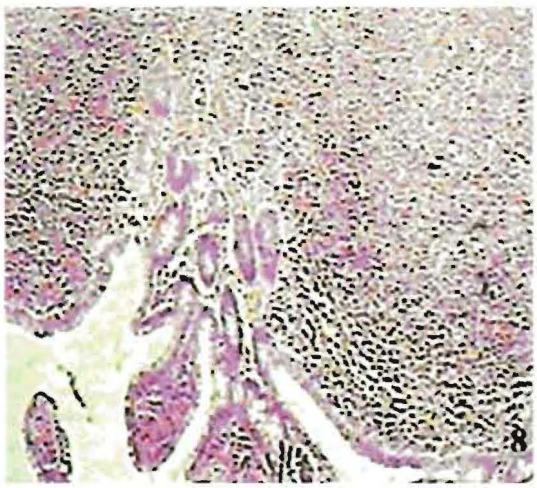

Fig. 8. Section in the ileum of 3 months old TAA treated rat, showing metaplasia in the submucosa with lymphocytic infiltration (X250).

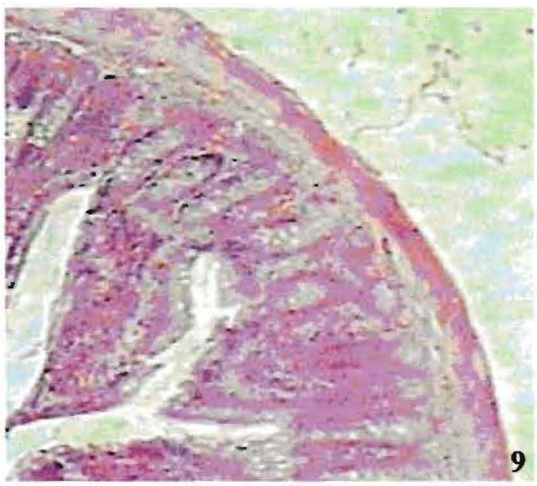

Fig. 9. Section in the ileum of 3 months old TAA treated rat, showing the smooth muscle fibers with thinner layer of muscles in some parts (X250).

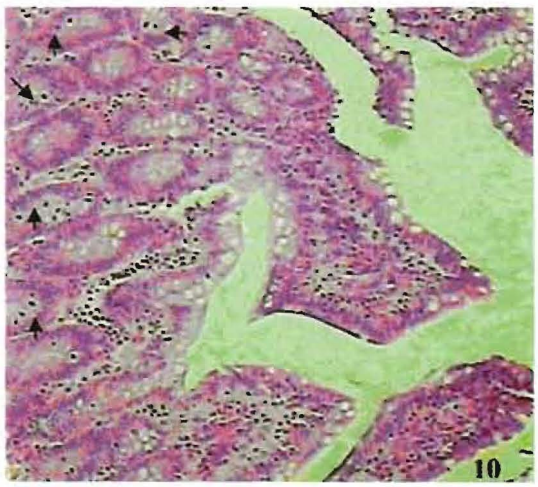

Fig. 10. Section in the ileum of 12 months old TAA treated rat. Showing loss of the normal architecture of villi. Notice mitotic figures in the crypts (head arrow) (X250).

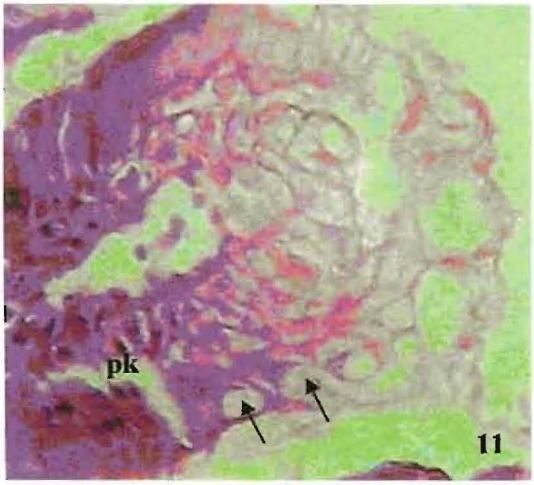

Fig. 11. Section in the ileum of 12 months old TAA treated rat, showing epithelial cells at the tip of the villi showed lysis cells (arrow) and pyknotic nuclei (pk) (X500).

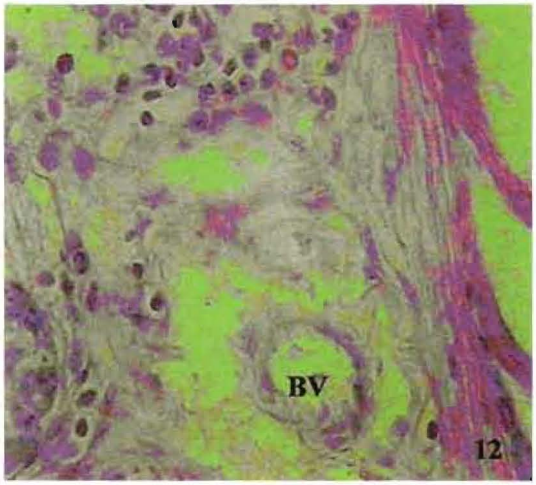

Fig. 12. Section in the ileum of 12 months old TAA treated rat, showing smooth muscle fibers were loose, dilated and degenerated blood vessel (BV) (X400).

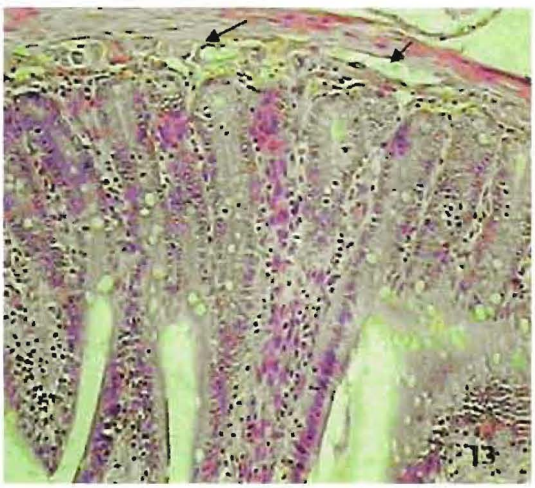

Fig. 13. Section in the ileum of 12 months old TAA treated rat, showing dilatation blood vessels and blood capillaries in submucosa (arrow) (X250).

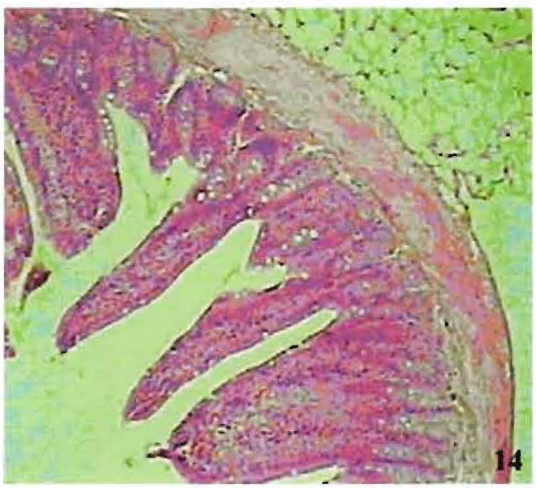

Fig. 14. Section in the ileum of 1 month old treated rat with TAA+MT, showed less inflammation. Most cells of the villi appeared normal (X400).

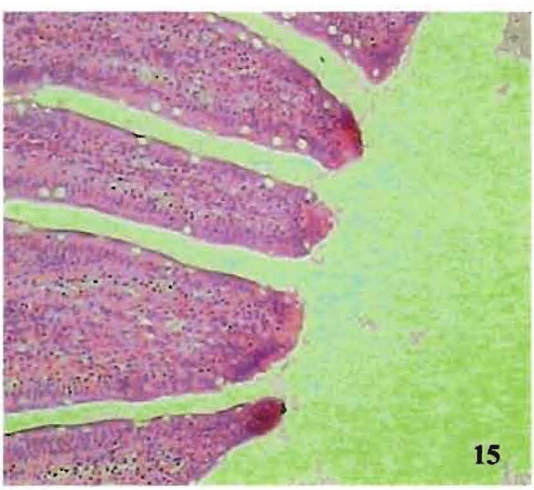

Fig. 15. Section in the ileum of 3 month old treated rat with TAA+MT, showed regeneration in villi, some of them were seen normal shape with less exfoliation in the tips (X250).

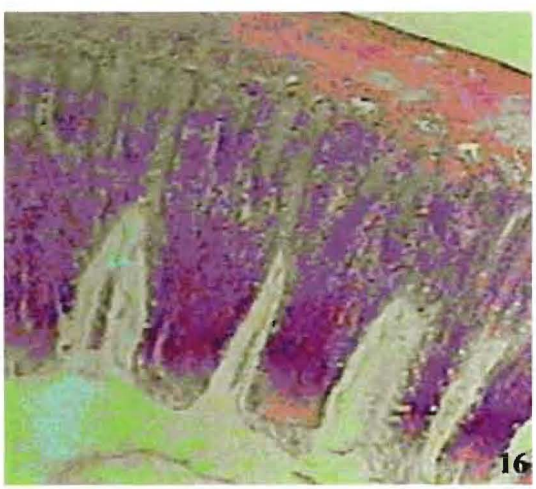

Fig. 16. Section in the ileum of 12 month old treated rat with TAA+MT, showing less improvement and less recovery in the villi (X250).

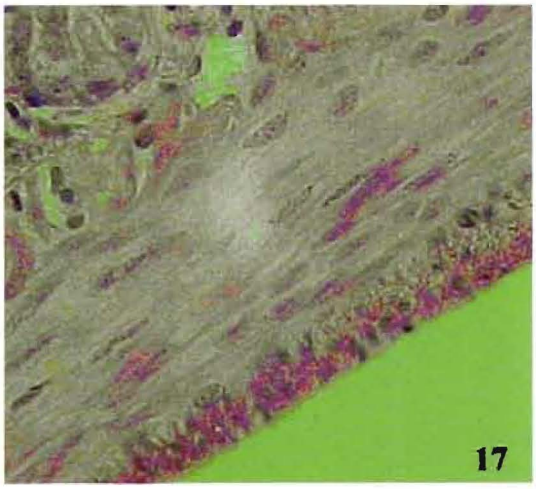

Fig. 17. Section in the ileum of 1 month old treated rat with TAA+MT, showing smooth muscle fibers were irregularly and densely packed (X400).

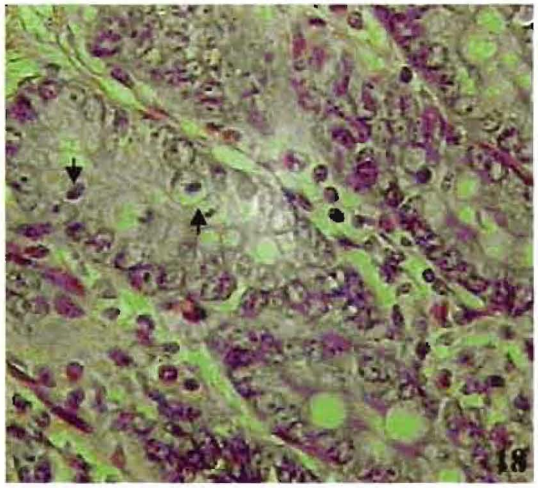

Fig. 18. Section in the ileum of 1 month old treated rat with TAA+MT, showing inhibition of mitotic activity (head arrow)(X400).

DISCUSSION

Although TAA-induced hepatic pathology is well characterized, only a few reports have focused on the role of administered TAA in the induction of biliary disease (dysplasia and/or cholangiocarcinoma "CCA") (Chun, *et al.* 2004). Praet and Roels (1984) reported a universal incidence of CCAs after prolonged (12 months) administration of TAA to male albino rats.

In the present study, changes in the width of ileum villi in TAA treated animals were compared to the control. Some of the villi showed narrow bases and atrophy at their tips. These results were obvious in adult treated group than others. These changes might be due to the effect of TAA on the disturbance of metabolic activity and protein synthesis (Abdel Wahab, 1997; Dashti, *et al.* 1997), leading to marked increase of the exfoliation rate of the epithelial cells at the tip of the villi.

Obvious lymphocytic infiltration in lamina propria was observed in the present study. Al-Rawi (2007a) reported large irregularly phagocytic cells (Kupffer cells) in liver cirrhosis after a single dose of TAA (300 mg/kg/body weight) in rats. There was a marked increase of inflammatory leukocytic infiltrations in kidney tissue after the same dose of TAA (Al-Rawi, 2006).

The present observation showed capillary dilatation in the connective tissue of the lamina propria and the submucosa. Al-Rawi (2006) reported that a single high dose of TAA caused impairment in the walls of renal arteries, blood vessels congestion with blood in the young and adult rats. Blood congested was also remarkable in the ovarian tissue of TAA treated young and adult rats; this impairment may attribute to the impact of TAA (Al-Rawi 2007b).

Results of the present study indicated that TAA treatment caused dissolution of nuclear material with necrotic nuclei. Similar results were reported by Abul, *et al.* (2006), on hepatic tissue of TAA-induced cirrhotic rats, caused a decrease in the antioxidant level indicate an increase in free radical level and increase in cellular damage. Chun, *et al.* (2004) demonstrated prominent intra-luminal necrosis ('comedonecrosis'), an indication of high cellular turnover. Increased number of goblet cells was observed in ileum of all treated rats with TAA. Similar observation were discussed by Chun, *et al.* (2004) who reported that biliary dysplasia was accompanied in many instances by metaplastic intestinal- type goblet cells, which has been described as a precursor lesion in the subset of intestinal- type CCA in man (Albores-Saavedra, *et al.* 1992).

The present observations showed that the severity of injury was higher in the ileum of adult rats than that in ileum of newly weaned and young rats. Similar results were reported by Hilgier, *et al.* (1999) and Sanz, *et al.* (1999) during their studies age related changes on liver rats induced injury with TAA. Sanz, *et al* (1998) reported that these

differences indicate that the lower necrogenic response against the same dose of thioacetamide in newly rats may be due to the lower rate of thioacetamide biotransformation and to the earlier onset of cell division. The expansion of metabolic capacity in adult rats results in higher generation of oxidants, developing an increased severity, and demanding a higher response against oxidative stress (Rikans, *et al.* 1993).

Data in the present study demonstrate that melatonin has beneficial effect on histopathological changes in ileum produced by thioacetamide administration. Similarly, Ozacmak, *et al.* (2005) reported that melatonin has been considered as an antioxidant that prevents injuries resulted from intestinal ischemia-reperfusion such as changes in motility and mucosal damage, but did not return them to the normal level.

This protective effect of melatonin may be due to: 1) Its ability to scavenger the free radical induced by thioacetamide; and 2) It also functions as an indirect antioxidant by stimulating m (RNA) levels and the activities of superoxide dismutase (Kotler, *et al.* 1998), glutathione peroxidase and glutathione reductase (Pablose, *et al.* 1998). These enzymes function to reduce OH generation by metabolizing as precursors to non-toxic products. Limson, *et al* (1998) reported that melatonin and its precursors have a high metal binding affinity.

Chwelatick, *et al.* (2006) suggested that melatonin co-treatment brought about a dose-dependent decrease in the renal, hepatic and intestinal cadmium concentrations and accumulation in mice. Al-Rawi (2007b) concluded that usage of melatonin as a conservative treatment in case of toxication with thioacetamide is of great importance which makes cross linkage with other cellular components and produce the dramatic effect in ovarian and spinal cord tissues in rats.

The obtained results emphasize the protective effects of melatonin, as melatonin co-treatment prevents intestinal cell death after TAA intoxication. These results may provide new therapeutic implications in the treatment of intestine disease characterized by necrotic cell death.

REFERENCES

Abdel Wahab, MH (1997) Protective effect of melatonin against carbon tetrachloride-induced hepatic damage. *J. Pharmacol. Sci.* **19**: 179-189.

Abul, H, Mathew, TC, Dashti, HM, and **Al-Bader, A** (2006) Level of superoxide dismutase, glutathione peroxidase and uric acid in thioacetamide- induced cirrhotic rats. *J. Gastroenterol. and Hepatol.* **21**(2): 358-366.

Al-Bader, AA, Abul H, Hussain, T, Al-Moosawi , M, Mathew, TC, and **Dashti, H** (1998) Selenium and liver cirrhosis. *Molecul. Cellul. Biochem.* **185**(1/2): 1-6.

Albores-Saavedra, J, Henson, DE, and **Sobin, LH** (1992) The WHO histological classification of tumors of the gallbladder and extrahepatic bile ducts. A commentary on the second edition. Cancer, **70**: 410– 414.

Al-Rawi, MM (2006) Melatonin protect from renal impairment with thioacetamide. *Alx. J. Vet. Med.* **24**(1): 231-241.

Al-Rawi, MM (2007a) Inhibitory effect of melatonin on histological changes in rat liver following thioacetamide intoxication. *Pakistan J. Biol. Sciences* **7**(1): 10-18.

Al-Rawi, MM (2007b) Conservative treatment of Melatonin on the ovary and spinal cord thioacetamide-intoxicated Albino rats. *Sci. J. Fac. Sci. Minufiya Univ.* **21** (1): 1- 15.

Baker, EA, and **Smuckler, EA** (1972) Altered microsome function during acute rhinaceramide poisoning. *Molec. Pharmacol.* **8**: 318-326.

Brini, A, Lee G, and **Kinet, J** (1993) Involvement of Alu sequences in the cell- specific regulation of transcription of the gamma chair of Fe and T cell receptors. *J. Biol. Chem.* **268**: 1355-1361.

Bruck, R, Aeed H, Avni, Y, Shirin, H, Matas, Z, and **Zozulya, G** (2004) Melatonin inhibits nuclear factor kappa B activation and oxidative stress and protects against thioacetamide induced liver damage in rats. *J. Hepatol.* **40**(1): 86-93.

Chun, NY, Anirban, M, Kam-Fai L, and **Miiin-Fu, C** (2004) Thioacetamide-induced intestinal-type cholangiocarcinoma in rat: an animal model recapitulating the multi-stage progression of human cholangiocarcinoma. *Oxford J.* **25** (4): 631-636

Chwelatick, E, Wlostowski, T, Alicija, K, and **Bonda, E** (2006) The effect of orally administered melatonin on tissue accumulation and toxicity of cadmium in mice. *J. Toxicol.* **19**(17): 259-265.

Clawson, G, Blankenship, L, Rhame, J, and **Wilkinson, D** (1992) Nuclear enlargement induced by hepatocarcinogens alters ploidy. *Cancer Res.* **52**: 1304-1308.

Clawson, GA, Benedict, CM, Kelley, MR, and **Weisz, J** (1997) Focal nuclear hepatocyte response to oxidative damage following law dose thioacetamide intoxication. *Carcinogenesis* **18**(8): 1663-1671.

Dashti, H, Mathew, TC, Jadaun, MM, and **Ashkanani, E** (1997) Zinc and liver cirrhosis: biochemical and histopathologic assessment. *Nutrition* **13**(3): 206-218.

El-Sokkary, GH (2000) Melatonin protects against oxidative stress induced by the kidney carcinogen KBrO3. *J. Neuroendocrin.* **21**: 461-468.

Hilgier, W, Zielinska, M, Borkowska, HD, Gadamski, R, Walski, M, Oja, SS, Saransaari, P, and **Albrechi, J** (1999) Changes in the extracellular profiles of neuroactive amino acids in the rat striatum at the asymptomatic stage of hepatic failure. *J. Neurosci.Res.* **56**(1): 76-84.

Kotler, M, Rodriquez, C, Sainz, RM, Antolin, I, and **Menen dez-Peiaez, A** (1998) Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. *J. Pineal Res.* **24**: 83-89.

Kunduzova, OR, Escourrou, G and **Seguelas, MH** (2003) Prevention of apoptotic and necrotic cell death, caspase-3 activation, and renal dysfunction by melatonin after ischemia/ reperfusion. *J. Faseb*, **17**: 872-874.

Laurido, C, Pelissie, T, Sotomoyano, R, Valladares, L, Flores, F, and **Hernandez, A** (2002) Effect of melatonin on rat spinal cord nociceptive transmission. *Neuroreport.* **13**(1): 89-91.

Limson, J, Nyokong, T, and **Daya, S** (1998) The interaction of melatonin and its precursors with aluminum cadmium, copper, iron, lead and zink an adsorptive voltammetric study. *J. Pineal Res.* **24** (1): 15-21.

Maestroni, GJ, Conti, A, and **Reiter, RJ** (2001) *Therapeutic potential of melatonin. 2 ed.* Karger Publishers. Locarno.

Morley, CG, and **Buyer, JL** (1977) Stimulation of hepatocellular proliferation by a serum factor from thioacetamide treated rats. *Biochem. Biophys. Acta.* **477**: 165-176.

Ozacmak, H, Sayan, H, Arslan, SO, Altaner, S, and **Gulhan, R** (2005) Protective effect of melatonin on contractile activity and oxidative injury induced by ischemia and reperfusion of rat ileum. *J. Life Sci.* **76**(14): 1575-1588.

Pablose, MI, Reiter, RJ, Ortiz, GG, Guerreto, JM, and **Agapito, MT** (1998) Rhythms of glutathione peroxidase and glutathione reductase in brain of chick and their inhibition by light. *Neurochem. Int.* **5** (1): 61-66.

Praet, MM, and **Roels, HJ** (1984) Histogenesis of cholangiomas and cholangiocarcinomas in thioacetamide fed rats. *Exp. Pathol.* **26** (1): 3-14.

Rikans, LE, Cai, Y, Kosante, SD, and **Venkatarman, PS** (1993) Redoxcyclin and hepatotxicity in aging male Fischer 344 rats. *Drug. Metab. Dispo.* **21**: 605-610.

Sanz, N, Diez-Fernandez, C, Alberto, M, Lourdes- Fernandez, S, and **Cascales, M** (1999) Age-related changes on parameters of experimentally-induced liver injury and regeneration. *Toxicol. Applied Pharmacol.* **154** (1): 40-49.

Sanz, N, Diez-Fernandez, C, and **Cascales, M** (1998) Aging delays the post-necrotic restoration of liver function. *Biofactors* **8**(1/2): 103-112.

Tan, DX, Chen, LD, Poeggeler, B, and **Reiter, RJ** (1993) Melatonin a potent endogenous hydroxylradical scavenger. *J. Endocrine.* **1** (1): 53-60.

Torres, MI, Fernandez, MI, Gil I, and **Rios, A** (1998) Dietary nucleotides have cytoprotective properties in rat liver damaged by thioacetamide. *Life Sci.* **62**(1): 13-22.

Tsujimoto, T, Kuriyama, S, Tominaga, K, *et al.* (2001) Augmented hepatocellular carcinoma progression and depressed Kupffer cell activity in rat cirrhotic livers. *Int. J. Oncol.* **18** (1): 41– 47.

Tunez, I, Munoz, MC, Villavicencio, MA, Medina, FJ, and **Prado, P** (2005) Hepato- and neurotoxicity induced by thioacetamide: Protective effect of melatonin and

dimethylsulfoxide. *J. Pharmacol. Res.* **52**(3): 223-228.

Witzmann, FA, Fultz, CD, Mangipudy, RS, and **Mehendale, HM** (1996) Two-dimensional electrophoretic analysis of compartment-specific hepatic protein charge modification induced by thioacetamide exposure in rats. *Fundam. Applied Toxicol.* **31**(1):124-132.

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