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CONSERVATIVE TREATMENT OF MELATONIN ON THE OVARY AND SPINAL CORD OF THIOACETAMIDE-INTOXICATED ALBINO RATS

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ABSTRACT

The hormone melatonin (MT) is known to regulate ovarian function and neuro-protective action. 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 ovary and spinal cord. The present study aims to investigate the role of melatonin on ovary and spinal cord of rats intoxicated with thioacetamide (TAA). 30 female albino rats aging 3 and 12 months were arranged in three groups 5 rats for each. (1) Control group, (2) intoxicated group received a single high dose 300mg/kg/body weight of TAA and (3) treated group, administered the same dose of TAA with MT (5mg/kg/ body weight) daily injected orally for four weeks. Histological investigation of TAA intoxication revealed a highest grade of pathological alterations manifested in the ovary by decreased in the number of the different types of follicles. On the other hand, the number of atretic follicles was increased. Degeneration of the granulose cells. and ovarian stroma were noted.

Remarkable histological changes included changes in bodies of neurons, neuroglia cells, central canal cells and axons of the white matter were observed in the spinal cord. Cytomegaly and karyomegaly were noticed in spinal cord of young rats. Obvious necrosis of the intoxicated group neurons and degenerating changes were more pronounced in spinal cord of adult rats.

Combined treatment with TAA and MT exhibited improvements of ovary and spinal cord structure in intoxicated rats to a limited degree and not completely resolute the adverse histopathological alterations.

Finally, this study recommended using melatonin as a conservative treatment in case of toxication with thioacetamide.

INTRODUCTION

The mammalian pineal hormone melatonin is a very potent and efficient endogenous free radical scavenger. It reacts with the highly toxic radical and provides one side protection against oxidative damage. Melatonin acts as a primary nonenzymatic antioxidative uterus Abdel wahab,1997; Alonso et al.,

2006). In particular, many studies have focused on the antioxidant activity of melatonin (Reiter *et al.,* 1994; Susa *et al.,* 1997; Hilgier *et al.,* 1999 and Sanz *et al.*, 1999).

Melatonin participates in many physiological functions, including the control of seasonal reproduction and the immune system (Reiter, 1998). Melatonin may modulate intraovarian regulation of steroid-genesis as granulose cells have melatonin receptors; it stimulates progesterone synthesis by granulose-lutein cells in vitro (Webley and Luck, 1986). Voordouw *et al.* (1991) reported that since melatonin can suppress lutenizing hormone and follicle stimulating hormone, it can be used as a contraceptive using a high dose of melatonin.

Itoh *et al.* (1999) reported that the presence of melatonin and its precursors, serotonin and N- acetyl serotonin, was demonstrated in extracts of human ovary. In addition, activities of two melatonin- synthesizing enzymes were also found in human ovary homogenates and were similar to those reported for the pineal glands of humans and other mammals. These findings strongly suggest that the human ovary, like the pineal gland, may synthesize melatonin from serotonin by the sequential action of synthesizing enzymes.

Melatonin receptors have been demonstrated in various regions of human brain, in the gut, in the ovaries and blood vessels (Yie *et al.,* 1995). Neural melatonin receptors are likely to regulate circadian rhythm. While non neural receptors probably regulate reproductive function, cardiovascular function and body temperature (Brzezinski, 1997). Thioacetamide (TAA) reduced GSH content and catalase activity in the liver and brain, melatonin significantly, decreased the intensity of the changes produced by the administration of TAA. The results support the antioxidative and neuro-hepato-protective action of melatonin (Tunez *et al.*, 2005a). Tunez *et al.* (2005b) determined anti oxidative enzymes, lipid preoxidation product, glutathione (GSH) content in cerebral and hepatic homogenates. The results showed that TAA induced significant enhancement of lipid peroxidation products levels as well as in ammonia value, decrease in the antioxidant enzymes activities and GSH in both liver and brain. Melatonin, vitamin E and L- carnitine, although melatonin more significantly, decreased the intensity of the changes produced by the administration of TAA and show protective effect against oxidative stress and hepatic damage presented in fulminant hepatic failure.

Because of the lack of sufficient reports on the histological effects of TAA on the ovary and spinal cord. The present investigation was designed to study the effects of TAA on the ovary and spinal cord of rats and the role of melatonin on these tissues intoxicated with thioacetamide (TAA).

MATERIAL AND METHODS

Animals:

Thirty female Wistar rats aged three months weighing (110-125g) and one year weighing (450-500g) were obtained from 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 singe high dose of thioacetamide (TAA) (300mg/kg/body weight) freshly dissolved in 0.9 NaCl. was intraperitoneal (ip) injected in rats. Melatonin, (TLC) was purchased from Sigma Company, USA. A daily dose of orally injected (5mg/kg/ body weight) as recommended by Laurido *et al.* (2002) was given to rats for 4 weeks.

Design:

The animals were arranged into two groups each of 15rats according to their age as follows:

Group1: Animals aging 3 month (young rats)

Group 2: Animals aging 12 months (adult rats)

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

Group1: control group.

Group2: Thioacetamide treated group.

Group3: combined treatments of thioacetamide and melatonin.

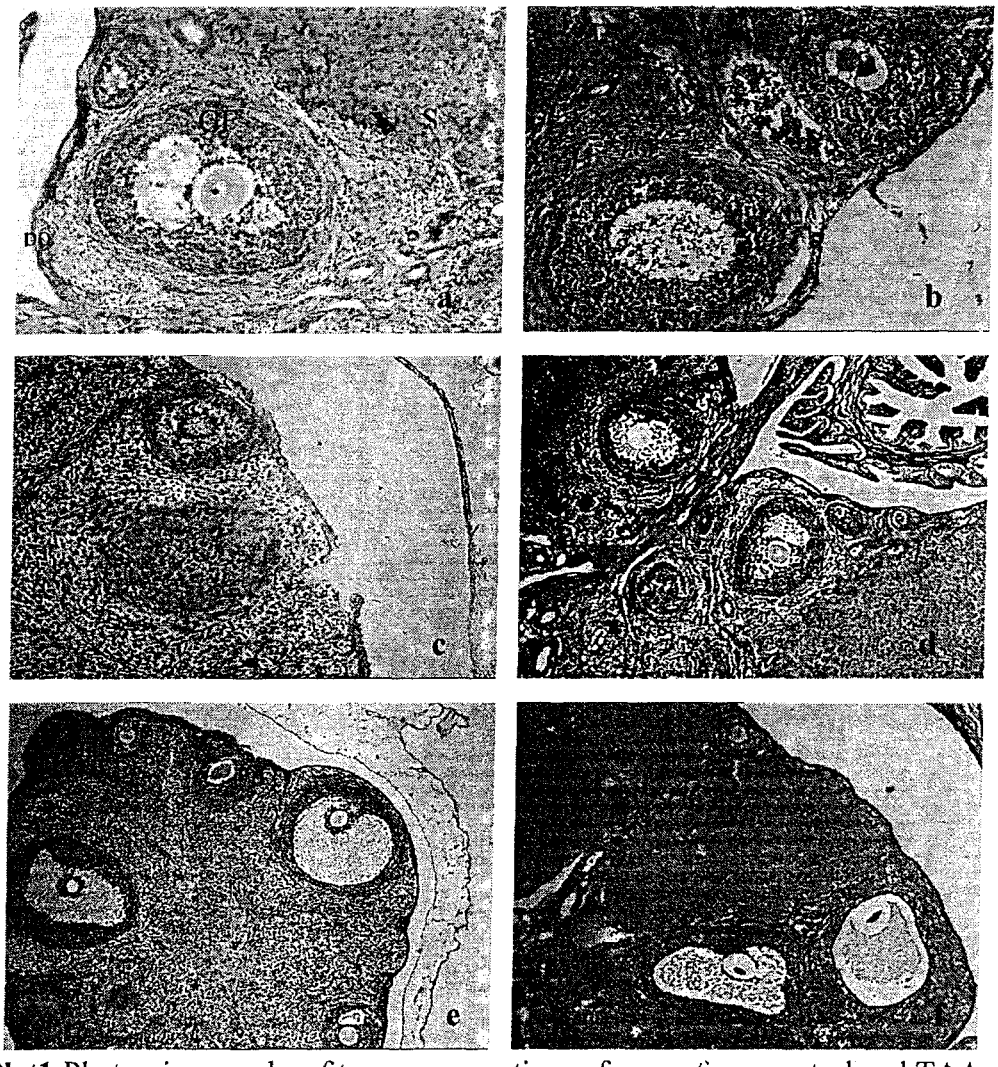
At the end of treatment, the animals of control and treated groups were sacrificed and the ovary and spinal cord were separated. Small pieces were fixed in 10% formol saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in molten paraplast at 56-60°c and cut at 5µm on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

RESULTS

Ovary:

The ovarian volume was increased with increase of the rat's age. The ovaries of control animals appeared oval in shape being covered by a thin layer of a simple epithelium called the germinal epithelium resting on a layer of collagenous connective tissue called the tunica albuginea. Under the germinal epithelium the stroma, consists of undifferentiated connective tissue elements, the primary interstitial tissue which differentiate from the secondary interstitial tissue with glandular appearance. Ovary is consists of a medullary region and a cortical region. Ovarian follicles "primary, secondary, Graafian and atretic", corpora lutea are embedded in the stroma of the cortex. (Plate.1.a).

The ovary of intoxicated rats with TAA displayed apparently smaller size than that of controls. The ovarian surface showed much degree of invaginations in the germinal epithelium along its surface. At the same time, the cells of this layer was disordered in arrangement and degenerated in both young and adult rats (Plate1.b&c). In the ovaries of young rats, some of the oocyte of primary follicles was degenerated and the other once appeared with eccentric nucleus. Also, some of the secondary follicles noticed with degenerated oocytes, the granulosa cells were degenerated, disarranged, became loose and separated from each other leaving a big space between them (Plate1.d). Abnormal Graafian follicles were noticed. The ovum was eliminated from its place into outside of the follicle within the theca follicle. While, the zona pellucida and cumulus oophorus were completely degenerated (Plate1.e)..



Plat1: Photomicrographs of transverse sections of ovary from control and TAA treated rats:

- **a.** *control group, showing different stages of oogenisis, germinal epithelium (GE), primary oocyte (po), secondary oocyte (so), Graafian follicle (GF) and stroma (S). (X250)*
- **b.** young TAA treated rats, note invagination and degeneration of germinal epithelium (X250).
- **c.** adult TAA treated rats. Obvious invagination of germinal epithelium. (X250).
- **d.** young TAA treated rats. Abnormal secondary oocyte (so) with degenerated granulose and degenerated stromal cells (S). (X250).
- **e** young TAA treated rats, illustrating abnormal Graafian follicle (X250).
- **f.** adult TAA treated rats, showing abnormal Graafian follicle the ovum appeared shrinked in size and the zona pellucida was disappeared (X250).

In adult intoxicated rats, abnormal follicles were observed. The primary follicles were completely destructed. The ovum of Graafian follicles appeared shrinked in size and the zona pellucida was disappeared. The granulose layer lost its normal shape and appeared with shrinking cells and pyknotic nuclei (Plate.1.f). Numerous atretic follicles were noticed in the stroma of these specimens (Plate.2.a). Corpora lutea occupied a large area of these ovaries (Plate.2.b).

In the young intoxicated rats, the interstitial tissue occupied a large area of the ovarian stroma. A large vacuoles detected in the stroma. The ovarian stroma showed congested blood vessels and the secondary interstitial cells appeared with many pyknotic nuclei (Plate.2.c). The secondary interstitial tissue of adult rats consisted of several clusters of highly luteinized cells interrupted with small darkly-stained intervening fibroblasts as well as blood capillaries which were distended and congested with blood. Such congested blood indicates clear phenomena of hemorrhage. The interstitial tissue was markedly large, vacuolated and degenerated (Plate.2.d).

The combined treatment of TAA and melatonin on the histology of rat ovaries exhibited marked improvement of ovary picture from that of non-treated with MT, but still lacked the normal integrity of control groups. Degenerated follicles as well as hyperplasia of cells in secondary interstitial tissue still existed. The stroma exhibited less dense blood congestion (Plate.2.e&f).

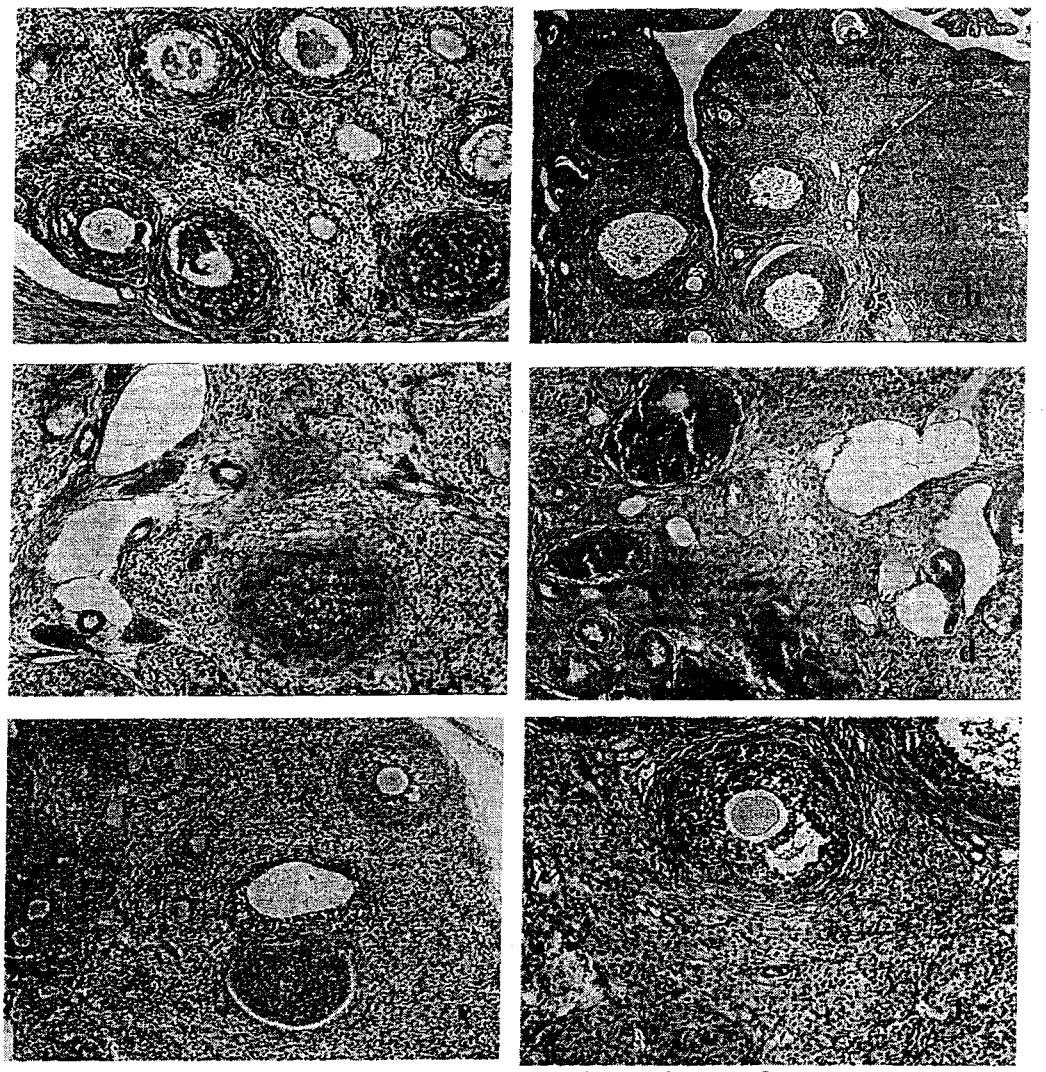


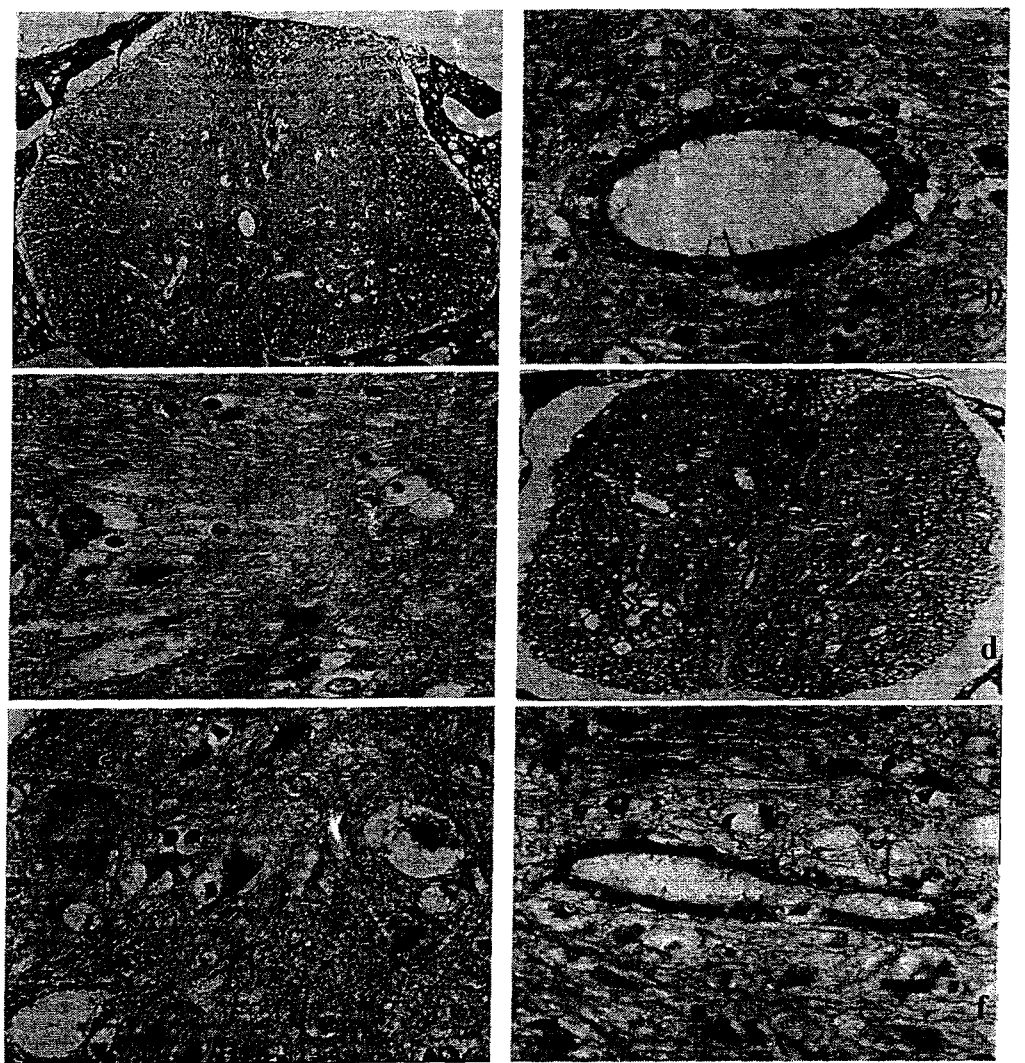
Plate2: Photomicrographs of transverse sections of ovary from TAA and TAA+MT treated rats:

- **a.** adult TAA treated rats, showing large number of atretic follicles (X250).
- **b.** adult TAA treated rats, showing large number of corpora lutea (X250).
- **c.** young treated TAA rats, large vacuoles detected in the interstitial tissue with congested blood vessels (X250)..
- **d.** adult TAA treated rats, illustrating a large area of the ovarian stroma, vacuolated and degenerated with remarkable congested blood vessels (X250).
- **e.** combined MT & TAA treated rats in young animals, displaying limited degree of healing of damaged tissue (X250).
- **f.** combined MT & TAA treated rats in adult animals, note less degree of healing (X250).

Spinal cord:

The spinal cord of control rats was differentiated into two distinct zones, a central zone (gray matter) that surrounds the central canal and peripheral zone (white matter). Two dorsal or posterior horns and two ventral or anterior horns extend from the gray matter (Plate.3.a). The cell bodies of neurons were scattered throughout the gray matter (Plate.3.b). The white matter contained a vast number of axons which originate from cell bodies of brain or spinal cord together with spinal ganglia. The gray matter contained also the neuroglia cells (Plate.3.c).

The spinal cord of intoxicated rats, exhibited obvious and severe histological changes in the spinal cord of the young and adult rats. The changes pronounced on the central nervous system include changes in the bodies of the neurons, neuroglia cells, central canal cells and axons of the white matter. The changes observed in the spinal cord of young intoxicated rats, were vacuolations in the gray matter, degeneration in the cells of central canal as well as degeneration of axons in the white matter (Plate.3.d&e). The central canal became narrower than that of control (Plate.3.e). Enlargement of the cytoplasm and in the nuclei of neurons were observed. Many necrotic cells showed a degree of lysis (Plate.3.f&g).

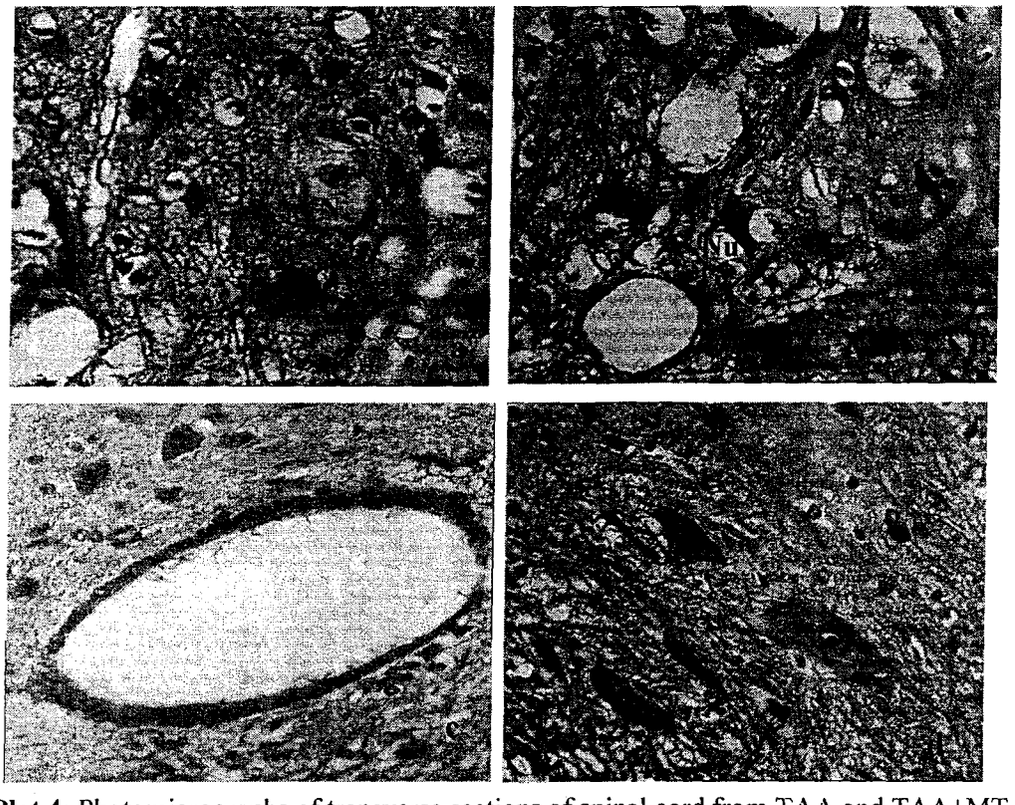


Plat 3: Photomicrographs of transverse sections of spinal cord from control and TAA treated rats:

- **a.** control group, showing the gray matter, scattered neurons and the white matter contained a vast number of axons (X250).
- **b & c.** high magnification of the above micrograph showing the central canal with normal cells. Neurons (Nu) and neuroglia cells (arrow) (X400).
- **d.** young treated TAA rats, showing vacuolations in the gray matter, degeneration of axons in white matter and the central canal become narrower (X250).
- **e. & f.** high magnification of the above micrograph showing central canal with enlargement and degenerated cells, vacuolations both in nucleus and in cytoplasm of the neurons (X400).

More cytomegaly and karyomegaly were noted in the spinal cord cells of adult intoxicated rats. Obvious vesiculation of the nuclei and vacuolations of the cytoplasm of neurons and of neuroglia cells were noted i.e necrosis (Plate.4.a&b).

After the combined treatment of TAA and MT the previous changes of the spinal cord of young and adult intoxicated rats were minimized. The spinal cord figures showed moderate degree of neurons neuroglia cells degeneration, moderate necrosis and moderate increase in the cellularity. The cells of central canal appeared almost normal and less vacuolations of the gray matter was seen (Plate.4.c&d).



Plat 4: Photomicrographs of transverse sections of spinal cord from TAA and TAA+MT treated rats:

- **a & b.** adult TAA treated rats, illustrating enlargement of neurons as well as neuroglia cells. Note necrotic neurons (Nu) with vacuolated cytoplasm (X400).
- **c.** combined MT & TAA treated rats in young animals showing the central canal with normal cells appearance (X400).

d. combined MT & TAA treated rats in adult animals. Note marked improvement of neurons and neuroglia cells (X400).

DISCUSSION

Thioacetamide (TAA), which was originally used as a fungicide, is a hepatotoxin, commonly used to induce liver cirrhosis in rats and other species (Dashti et al 1997). Clawson et al.(1997) reported that rats treated with low dose of the hepatocarcinogen TAA showed microscopic foci of hepatic injury. However, TAA has also been reported to cause chemically induced cell death via both apoptosis and necrosis (Witzmann et al.,1996).

Results of the present study indicated that TAA treatment caused destruction of the ovarian follicles. An obvious change in the ovarian follicular number, the number of all types of the follicles was reduced. On the other hand, the number of atretic follicles exhibited a remarkable increase. This observation indicate that the high number of these follicles is due to the high scales of degeneration of small follicles (Riad *et al.*, 1995a). In addition the corpora lutea occupied a very large area of the ovarian stroma. Similar observation was reported by Riad *et al.*(1995b) who attributed this changes to the fact that after hormonal administration and within few hours a quite large number of the growing follicles displayed follicular maturation and ovulation. It is suggested that TAA may cause disturbance in gonadotrophic hormones.

Blood congested was remarkable in the ovarian tissue of TAA treated young and adult rats; this impairment may attribute to the impact of TAA. Similar blood congestion was observed in liver of animals treated with TAA (Dashi *et al.*,1997, A-Rawi ,2007).

A number of necrotic cells in TAA treated rats in both ovarian and spinal cord tissues and increase of the nuclear size were observed in the present study. These results correspond to data reported by many workers (Clawson *et al.*, 1997; Ramaiah *et al.*, 1998; Sanz *et al.*, 1998 a & b; Torres *et al.*, 1998; Al-Rawi 2006; 2007). Bulera *et al.* (1998) reported that TAA has classically been used as a model to study hepatic necrosis; however, recent studies have shown that TAA can also induced apoptosis. Torres *et al.* (1998) studied cell damage caused by TAA in liver tissue leading to a decrease in cytoplasmic area together with increased nuclear and nucleolar size. Clawson *et al.* (1997) attributed the enlargement of nuclear size after TAA intoxication to direct selective nuclear oxidative damage.

The obtained data have also revealed that histopathological changes in both ovarian and spinal cord tissues were increased in adult TAA treated rats than in the young one. Al-Rawi (2007) found that adult rats treated with TAA showed more hepatic injury than that in newly weaned and old rats. Similar results were

reported by Hilgier *et al.*, 1999 and Sanz *et al.*, 1999 during their studies of age related changes on liver rats induced injury with TAA. Sanz *et al* (1998a) considered these differences as indication to the lower necrogenic response against the same dose of thioacetamide in young rats and may be due to the lower rate of thioacetamide biotransformation and to the earlier onset of cell division. According, the growing liver from young rats presents advantages against the necrogenic aggression of thioacetamide, first, because the diminished activity of its specific microsomal detoxification system, and second because the earlier increase in the proliferative response prevents the progression of injury permitting an earlier restoration of liver function. 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)

From the present findings, melatonin treatment of rats intoxicated with TAA revealed marked improvement of histological pictures in ovaries and spinal cords .Clemens *et al.* (2001) using immunoblot analysis and an anti-melatonin receptor antibody on both in vivo and in vitro mode of follicular development demonstrated that iodomelatonin binds especially to rat ovarian granulose cells membranes with high affinity. Because melatonin has been reported to alter the steriodogenic responses of ovarian tissue to gonadotropins. Berria and Mead (1990) found that the uterus like other than tissues was able to accumulate melatonin. Zhoa et al. (1998a&b) reported that melatonin may regulate the endometrial vascular permeability and decidualization. Sandyk *et al.* (1992) observed that deficient pineal melatonin functions in early pregnancy may be causally related to the development of spontaneous abortions. Abou-El-Naga (2002) reported that in ovarictomized rats and received Aluminium-intoxication, melatonin improved the histological alterations of uterine structure.

Many investigators studied the relationship between neurotoxicity induced by thioacetamide and protective effects of melatonin. They concluded that TAA induced oxidative stress with extensive tissue damage; melatonin prevented the oxidative stress- related changes and tissue damage and fibrosis associated with TAA toxicity (Bruck *et al.*, 2004; Karabay *et al.*, 2005; Tunez *et al.*, 2005a & b; Cruz *et al.*, 2005; Al-Rawi 2006; 2007).

Finally, it could be 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.

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