P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com
Research Article
https://doi.org/10.47278/journal.ijvs/2020.022

Efficacy of Melatonin against Oxidative Stress, DNA Damage and Histopathological Changes Induced by Nicotine in Liver and Kidneys of Male Rats

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Article History: 20-103 Received: 18-April-20 Revised: 13-Aug-20 Accepted: 06-Sept-20

ABSTRACT

The present study was carried out to investigate and compare the effect of nicotine alone and in combination with melatonin on some oxidants and antioxidant parameters, histopathological changes and DNA integrity in the liver and kidneys of male rats. For this purpose 75 mature male rats weighing 120-140g were randomly divided into five groups; control group (1% ethanol in saline), nicotine group (rats administrated nicotine at a dose of 0.6mg/kg body weight; BW) and nicotine and melatonin groups (rats administrated the same dose of nicotine plus 1, 5 or 10mg/kg BW melatonin, respectively). Nicotine and melatonin were injected intraperitoneally daily for 21days. Fasting blood samples were collected from each rat one day after the end of last injection (at 22nd day) and sera were collected for determination of total antioxidant capacity (TAC). Five rats were sacrificed from each group; Liver and kidneys were collected for estimation of oxidative stress parameters (MDA, SOD and GSH), histopathological examination and for estimation of DNA damage. The results revealed that nicotine increased MDA, decreased TAC, SOD and GSH, induced histopathological changes and increased the percentage of DNA damage in the liver and kidneys Melatonin administration with nicotine counteracted the effect of nicotine on previous parameters. The effect of melatonin was dose dependent and the 10mg dose produced the highest protective effect. It is concluded that melatonin can ameliorate the harmful effect of nicotine on the liver and kidneys of male rats.

Key words: Nicotine, Melatonin, Antioxidants, DNA, Histopathology, Rats.

INTRODUCTION

Cigarette smoking was found to have harmful effect on the body. It causes severe diseases that threaten health of human being, reduces fertility in both males and females and induces hormonal disturbance (Gaur et al. 2010). Smoking also acts as a risk factor for many diseases such as pulmonary and cardiovascular diseases, insulin resistance and diabetes as well as osteoporosis and cancers (Tweed et al. 2012). Nicotine, the most important component of cigarette (Milad and Zahra 2019), was found to produce oxidative stress by generation of free radicals due to reduction of antioxidant enzymes activity and increasing lipid peroxidation (Sener et al. 2007). The free radicals induce oxidative DNA damage with mutations and alterations of chromosomal structures with loss of genetic information (De Marini 2004).

Melatonin, the pineal gland hormone, was found to be a strong antioxidant (Singh and Jadhav 2014). It can easily cross cell membranes and blood–brain barriers due to its lipophilic and hydrophilic nature (Reiter et al. 2000). It also has a protective role against development of cancer (Reiter et al. 2007), acts as a positive regulator of immune system (Szczepanik, 2008), plays a role in reproduction in different animal species (Revel et al. 2009) and plays a role in regulation of circadian rhythm and sleep promotion (Zisapel 2018). Thus the aim of the present study was to investigate the ameliorating effect of melatonin on the nicotine -induced oxidative stress and tissue damage in the liver and kidney of male rats.

MATERIALS AND METHODS

Animals

The present study was carried out at Physiology department, Faculty of Veterinary Medicine, Cairo University during the period from July to August 2018. The experimental protocols and procedures were approved by the institutional animal care and use committee of the Faculty of Veterinary Medicine Cairo University (vet CU/23012020104).

Cite This Article as: Ahmed HH, NES El-Toukhey, SS Abd El-Rahman and AKH Hendawy, 2021. Efficacy of melatonin against oxidative stress, DNA damage and histopathological changes induced by nicotine in liver and kidneys of male rats. International Journal of Veterinary Science 10(1): 31-36. https://doi.org/10.47278/journal.ijvs/2020.022

The study was carried on 75 mature male albino rats weighing 120-140 g obtained from the Animal House Colony of Abou Rawish, Giza, Egypt. The rats were fed on a standard granulated ration which was obtained from Cairo Company, Cairo, Egypt. The ration contained crude protein not less than 21%, crude fat not less than 4.43% and crude fibers not more than 2.94%. The rats were maintained in a special room, with natural ventilation with 12 hrs. Light/dark cycle. Water and feed were available ad libitum.

Chemicals

Melatonin

Melatonin was obtained from Memphis Company for Pharmacy and Chemistry Industry and used in three different concentrations (1, 5 and 10mg/kg BW). The required dose of melatonin was dissolved daily in a minimum volume of absolute ethanol and then diluted to the desired concentration with physiological saline to give final concentration of 1% ethanol (Hermoso et al. 2016). The purity and strength of melatonin was tested using HPLC it was 97%.

Nicotine

Nicotine hydrogen bitartarate used was obtained from Sigma Aldrich Company. Nicotine used at a dose of 0.6 mg/kg BW dissolved in saline (Sener et al. 2007).

Experimental design

The study was carried out for 21 successive days. Before starting all animals were subjected to acclimatization for 2 weeks. The rats were divided into 5 groups with 3 replicate cages having 5 rats in each as follow: the 1st group (control group) received daily I/P injection of 1% ethanol in saline. The 2nd group (nicotine group) received daily I/P injection of nicotine hydrogen bitartarate at a dose of 0.6mg/kg BW. The 3rd, 4th and 5th groups (nicotine and melatonin groups) received daily I/P injection of the same dose of nicotine followed 30 minutes later by I/P injection of melatonin at a dose of 1, 5 or 10mg/kg BW, respectively. All treatments were given 2 hrs. before sunset, when melatonin receptors were active (Mercan and Eren 2012).

Sampling

Blood samples

Fasting blood samples were collected from each rat at the end of the experiment (on the morning of the 22nd day) by orbital sinus puncture using heparinized capillary tubes under isoflurane anesthesia. Sera were collected and stored at -80˚C to be used for determination of total antioxidant capacity according to the method of Koracevic et al. (2001) using a commercial kit purchased from Biodiagnostic Company-Egypt.

Tissue samples

After collection of blood samples, 5 rats from each group were sacrificed by cervical dislocation and the following organs were collected and used as follow:

1) Tissue samples from liver and kidneys were collected and stored at -80°C to be used for estimation of GSH concentration, SOD activity MDA concentration according to the methods of Beutler et al. (1963), Nishikimi et al. (1972) and Ohkawa et al. (1979) respectively. All kits used in this part were purchased from Biodiagnostic Company, Egypt.
2) Tissue samples from liver and kidneys were obtained and stored at -80°c to be used for determination of DNA damage according to the method described by Perandones et al. (1993).
3) Tissue samples from liver and kidneys were collected and fixed in neutral buffered formalin 10% for histopathological examination according to methods developed by Bancroft and Gamble (2008), for preparation of paraffin blocks and staining tissue sections with H &E stain.

Statistical analysis

All Data were presented as Mean±SE. They were subjected to one-way analysis of variance test (ANOVA), followed by the Tukey-Kramer multiple comparison test using statistical analysis system program (Instat-3).

RESULTS

Effect of nicotine alone and in combination with melatonin on some oxidative stress parameters

Total antioxidants

The data represented in Fig. 1 revealed that serum total antioxidants capacity was significantly lower in the nicotine treated group than control group ($P<0.05$) and groups co-administrated with nicotine plus 5and 10mg melatonin ($P<0.01$ and $P<0.001$, respectively), while melatonin at doses of 5 and 10mg could completely counteract the nicotine-induced decrease in total antioxidants capacity and melatonin at a dose of mg could not counteract the nicotine -induced decrease in TAC.

Liver antioxidants parameters

The data shown in Fig. 2 revealed that; MDA concentration in the hepatic tissue was significantly higher in the nicotine treated group than control group ($P<0.001$) and groups co-administrated with nicotine plus1, 5 and 10mg melatonin ($P<0.05$ and $P<0.001$, respectively). There was no significant difference between the control group and group co- treated with 10mg melatonin, while MDA in groups co-treated with 1 and 5mg melatonin was significantly higher than control group ($P<0.001$). Meanwhile nicotine administrated group exhibited highly significant decrease in hepatic SOD activity than control group and groups co-administrated with nicotine plus 5 or 10mg melatonin ($P<0.01$ and $P<0.001$, respectively). There was no significant difference between the control group and group co-treated with 10mg melatonin, while SOD activity in groups co-administrated with nicotine plus 1 or 5mg melatonin was significantly lower than control group ($P<0.001$) and significantly higher than nicotine supplemented group ($P<0.05$ and $P<0.01$, respectively). At the same time nicotine administrated group exhibited a non-significant decrease in hepatic GSH concentration versus control group and group co-administrated with nicotine plus 1 mg melatonin. Group co-administrated with nicotine plus 5 or 10mg melatonin exhibited the highest concentration of GSH. It was significantly higher than all other groups ($P<0.001$).

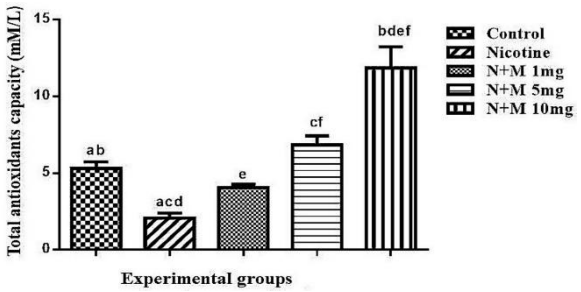

Fig. 1: Effect of nicotine alone and in combination with melatonin on serum total antioxidants capacity of male rats. Groups having the same letter are significantly different.

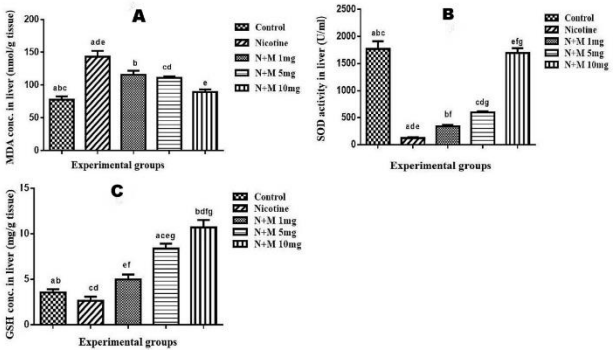

Fig. 2: Effect of nicotine alone and in combination with melatonin on: A) MDA concentration, B) superoxide dismutase activity and C) glutathione concentration in liver Groups having the same letter are significantly different.

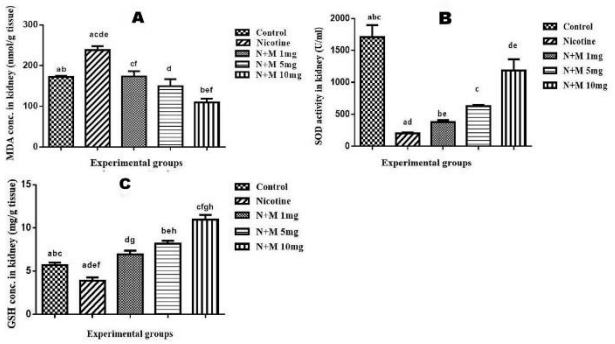

Fig. 3: Effect of nicotine alone and in combination with melatonin on: A) MDA concentration, B) Superoxide dismutase activity and C) glutathione concentration in kidney. Groups having the same letter are significantly different.

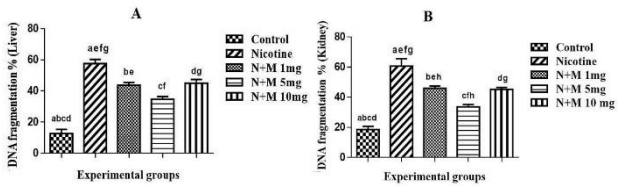

Fig. 4: Effect of nicotine alone and in combination with melatonin on DNA fragmentation % A) Liver and B) kidney Groups having the same letter are significantly different.

Kidneys antioxidants parameters

Data illustrated in Fig. 3 clarified that MDA concentration was significantly higher in the nicotine treated group than control group (P<0.01) and groups co-administrated with nicotine plus 1, 5 and 10mg melatonin

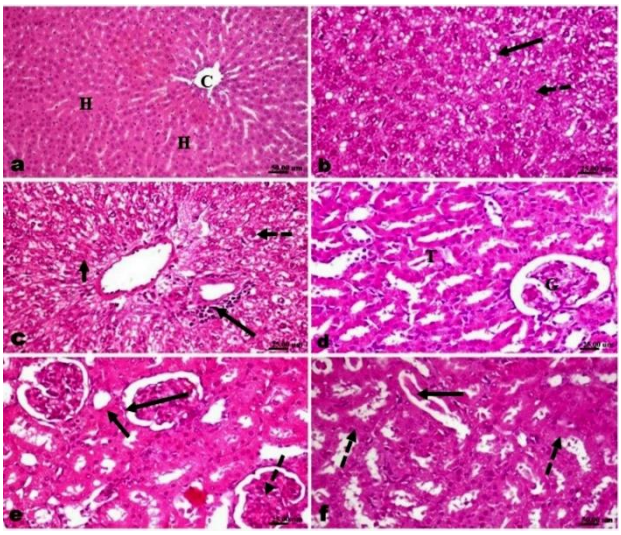

Fig. 5: H&E stained sections. (a) Liver of control rat showing normal histological structure of the central vein (C) and hepatic cells (H), (b and c) Liver of nicotine administrated rat showing (b) hepatocellular vacuolar degeneration (short arrow), many necrotic cells (dashed arrow), (c) mild inflammatory cells infiltration (arrow) in portal areas with the wide spread vacuolar degeneration (dashed arrow) and necrosis (short arrow) of the hepatic cells. (d) Kidney of control rat showing normal histological structure of the renal tubules (T) and renal glomeruli (G). (e and f) Kidney of nicotine administrated rat showing necrotic changes (short arrow) of the renal tubular epithelium, thickening of the parietal layer (long arrow) of the Bowman's; capsule with mesangial hyalinization (dashed arrow), (f) granular cast in the tubular lumens (arrow) with scattered necrotic tubular epithelial cells (dashed arrow).

(P<0.01, <0.001 and<0.001, respectively). There was no significant difference between the control group and groups co- treated with 1 and 5mg melatonin. Group co-administrated with nicotine plus 10mg melatonin exhibited the highest decrease in renal MDA concentration. It was significantly lower than control (P<0.01) and nicotine treated (P<0.001) group. On the other hand SOD activity was significantly lower in the nicotine administrated group than control group (P<0.001) and group co-administrated with nicotine plus 10mg melatonin (P<0.001), also SOD activity in the control group was significantly higher than that in the groups co-administrated with nicotine plus 1 or 5mg melatonin (P<0.001). There was non-significant difference between the control group and group co- treated with 10mg melatonin as well as between nicotine treated group and groups co-administrated with nicotine plus 1 or 5mg melatonin. GSH concentration was significantly lower in the nicotine treated group than control group (P<0.05) and groups co-administrated with nicotine plus 1, 5 and 10mg melatonin (P<0.001), GSH concentration was significantly higher in groups supplemented with nicotine plus 5 or 10mg melatonin than control group (P<0.01 and P<0.001, respectively).

Effect of nicotine alone and in combination with melatonin on DNA fragmentation % in liver and kidneys

The data shown in Fig. 4 revealed that nicotine significantly increased the percentage of DNA damage in the tissues of the liver and kidney (P<0.001) versus control.

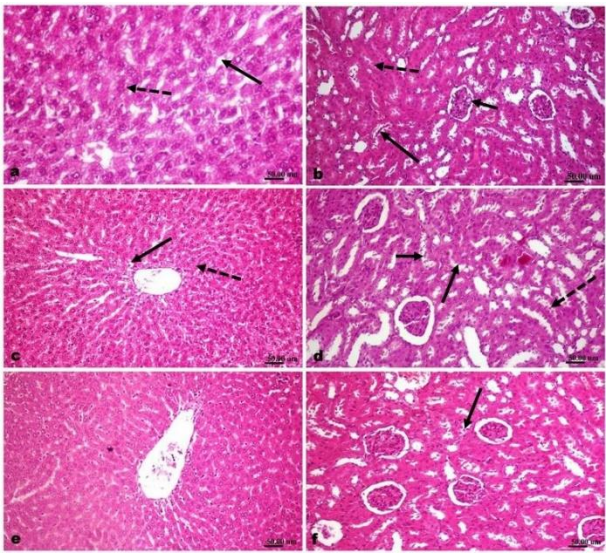

Fig. 6: H&E stained sections. (a and b) Liver and kidney of nicotine and 1mg melatonin co-administrated rat that treated with showing (a) sinusoidal dilatation (arrow), hepatocellular degeneration and many necrotic cells (dashed arrow), (b) granular cast (long arrow) in the lumen of most of the renal tubules, necrotic changes of the tubular epithelial lining (dashed arrow) and mesangial vacuolation (short arrow). (c and d) Liver and kidney sections of nicotine and 5mg melatonin co-administrated rat showing mild degree of hepatocellular vacuolar degeneration (arrow) and necrosis (dashed arrow) particularly in the centrilobular area, (d) moderate degree of degeneration, necrosis (long arrow) with few desquamation (dashed arrow) of the renal tubular epithelial linings and very few granular cast (short arrow). (e and f) Liver and kidney of nicotine and 10mg melatonin co-administrated rat showing near to normal appearance of the hepatic parenchymal cells with only scares degenerative changes of some cells and (f) mild necrotic changes of the renal tubular epithelium with few desquamated cells (arrow).

Melatonin at 1, 5 and 10mg significantly ameliorated the percentage of DNA damage in the liver and kidney ($P<0.01$, $P<0.001$ and $P<0.05$, respectively) versus nicotine treated groups. The 3 doses of melatonin could ameliorate the nicotine induced increase in the percentage of DNA damage in the liver and kidney to a certain extent; however, they could not bring the percentage of DNA damage to the control (Partial inhibition).

Effect of nicotine alone and in combination with melatonin on histopathological changes in the liver and kidneys of male rats

Microscopic examination of liver and kidneys of control rats revealed normal histological structure (Fig. 5a and 5d). Administration of nicotine induced vacuolar degeneration and necrosis of the hepatic cells as well as dilatation of the hepatic sinusoids and activation of Kupffer cells (Fig. 5b and 5c). kidneys showed degeneration and necrosis of the renal tubular epithelium, thickening of the parietal layer of the Bowman's capsule, hyalinization of the mesangial cells (Fig. 5e) and presence of granular cast in the renal tubular lumens (Fig. 5f). However, the co-administration of nicotine and 5 or 10mg melatonin induced a dose related ameliorating effect on the deleterious effect of nicotine. The 1mg dose of melatonin had no observable effect, while 10mg had the highest curative effect. Histopathological changes of the liver in group supplemented with nicotine plus 1 mg nicotine showed sinusoidal dilatation, hepatocellular granular and vacuolar degeneration as well as many necrotic cells (Fig. 6a). Meanwhile, kidneys' tissue of those rats showed granular cast in the lumen of most of the renal tubules, necrosis of the tubular epithelial linings and mesangial vacuolation (Fig. 6b).

Regarding the co-treated group with 5mg melatonin, livers of which revealed mild degree of vacuolar degeneration and necrosis of the hepatic cells particularly in the centrilobular area (Fig. 6c). Kidneys showed moderate degree of degeneration, necrosis with few desquamations of the renal tubular epithelial linings and very few granular casts (Fig. 6d).

In regard to the group co-treated with 10mg melatonin, the liver showed near to normal appearance of the hepatic parenchymal cells with only scares degenerative changes of some cells (Fig. 6e), while kidneys' tissue of those rats showed mild degeneration and necrosis of the renal tubular epithelium and few desquamated cells (Fig. 6f).

DISCUSSION

The results of the present study revealed that nicotine administration increased lipid peroxidation and decreased activity of antioxidant enzymes in all examined tissues. It significantly increased MDA concentration in liver and kidneys and significantly decreased TAC, SOD activity in the liver and kidneys and GSH concentration in kidneys as compared to control group. These results are in accordance with the findings of Muthukumaran et al. (2008) who reported that nicotine significantly decreased SOD activity, GSH concentration and increased MDA concentration in liver and kidney of rats. Moreover, the more recent study of Milad and Zahra (2019) also indicated that nicotine induced a significant decrease in SOD activity and GSH concentration and a significant increase in MDA concentration in liver and kidneys. They attributed the harmful effect of nicotine to the production of free radicals which interact with viable molecule in cell such as proteins, lipids, DNA and RNA causing oxidative stress in different organs leading to decrease or loss of their function.

Regarding the effect of adding of melatonin to nicotine, the results obtained during the present study revealed that all groups co-treated with nicotine plus melatonin exhibited a dose dependent decrease in MDA concentration in the liver and kidneys versus nicotine administrated group and the 10mg dose of melatonin induced the highest effect. It completely counteracted the nicotine induced increase in MDA concentration and returned it back as in the control group. Meanwhile, the 1 and 5mg doses of melatonin completely ameliorated the nicotine induced increase in MDA concentration in the kidneys, while the 5mg dose could not bring MDA concentration in the liver tissue back as in the control group and partially counteracted the nicotine induced increase in MDA concentration. Moreover, addition of the three doses of melatonin to nicotine in the present study produced a dose dependent increase in SOD activity in all examined tissues versus group treated with nicotine alone; however, this increase was only significant in the groups treated with 5 and 10mg melatonin and the 10mg dose of melatonin still produced the highest increase. It could bring the SOD activity back as in the control group.

Regarding GSH, the obtained data revealed that nicotine alone decreased GSH concentration in the liver and kidney than control group; however, this decrease was only significant in the kidney tissue. Addition of the 3 doses of melatonin induced a dose dependent increase in GSH concentration in all examined tissues. The 5 and 10mg doses produced the highest increase. It was significantly higher than that in the control and nicotine treated groups.

The results recorded in the present study are in agreement with the findings of Elbe et al. (2015) who reported that melatonin significantly increased SOD activity, GSH concentration and decreased MDA concentration in liver of diabetic rats. Also Karakilik et al. (2015) stated that melatonin significantly increased SOD activity, GSH concentration in liver and kidney of Adriamycin treated rats. This antioxidant effect of melatonin may be due to its ability to cross different physiological barriers easily as a result of its lipophilic and hydrophilic criteria (El-Sokkary et al. 2006), its free radical scavenging power As well as Its ability to deactivate other oxidant compounds (Park et al. 2013).

Concerning the integrity of DNA, the present results indicated that nicotine alone produced a significant increase in the percentage of DNA damage in the liver and kidney compared to control group and all groups treated with melatonin.

The three doses of melatonin significantly decrease the percentage of DNA damage in the liver and kidney versus group administrated with nicotine alone. Although the three doses of melatonin significantly decreased the nicotine induced increase in the percentage of DNA damage; however, they could not bring it down as in the control group and produced only partial amelioration of the nicotine induced increase in the percentage of DNA damage in the liver and kidney. In the same concern, Lee et al. (2018) reported that smoking causes DNA damage and reduces DNA repair machinery in mice. Concerning the effect melatonin on the nicotine induces increase in DNA damage; Galano et al. (2018) found that melatonin can protect DNA against oxidative damage through inhibition of metal caused DNA damage, activation of antioxidant enzymes, inhibition of pro-oxidative enzymes and enhancement of DNA repair machinery.

The result of the current study revealed that nicotine rats showed degeneration and necrosis of the hepatic cells and dilatation of the hepatic sinusoids. Adedayo et al. (2011) reported that administration of male rats with nicotine induced vacuolation in the liver parenchyma, necrosis of hepatocytes and degeneration of the cells lining the bile ducts, also Mercan and Eren (2012) found that administration of mice with nicotine induced mononuclear cell infiltration, sinusoidal dilatation and hydropic degeneration. These histopathological changes may be due to increasing metabolic activity of cells for removal of toxins from the body during the toxification process (Milad and Zahra, 2019).

Concerning the effect of melatonin on the nicotine induced liver histopathological changes, the obtained result indicated that melatonin at a dose of 10mg completely counteracted the nicotine induced hepatic damage and retuned it back to normal as in the control group. These results are matching with those of Mercan and Eren (2012) who found that melatonin at 10mg could protect liver of nicotine treated mice from histopathological changes and this may be due to its antioxidants effect, moreover, Moradpour et al. (2020) in his recent study recorded that melatonin improved liver damage and histopathological changes caused by mobile phone radiation in mice model.

Concerning the kidney, the obtained result indicated that nicotine administrated rats showed degeneration and necrosis of the tubular epithelium, thickening of the parietal layer of the Bowman's capsule, hyalinization of the mesangial cells and granular casts in lumens of renal tubules. Adedayo et al. (2011) reported that administration of male rats with nicotine caused degenerative changes in the proximal convoluted tubules with shrinkage and hemorrhage of renal glomeruli. In the same regard Mahmoud and Amer (2014) found that nicotine induced acute renal Injuries together with dilatation and destruction of the cells lining renal tubules of male rats.

Regarding the effect of melatonin on kidney histopathological changes, the result indicated that melatonin at a dose of 10mg ameliorated the nicotine induced renal damage and return it back to the control. These results confirm the finding of Tavakoli et al. (2014) who reported that melatonin administration restored renal function and structure due to its antioxidant effect.

Conclusion

The results of the present study indicated that melatonin can ameliorate the disturbance in oxidant and antioxidant parameters, DNA damage and histopathological changes induced by nicotine and the 10mg dose of melatonin produced the highest effect in most cases.

Author's contribution

All authors contributed to the reagents/materials/ analysis tools, collected the material, analyzed the data and wrote and revised the manuscript.

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