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PROTECTIVE EFFECTS OF MELATONIN AND CAPTOPRIL AGAINST CARDIAC OXIDATIVE STRESS AND DYSFUNCTION INDUCED BY NICOTINE IN RATS

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Long-term exposure to nicotine accelerates the development and the progression of cardiovascular disease in smokers through cardiac damage. This research aims to investigate the ameliorative effects of melatonin and captopril in rats exposed to nicotine and report the effect of nicotine withdrawal. Sixty male rats were divided into 6 groups. A control group received saline, nicotine group received nicotine (1 mg/kg). The four other groups were administered melatonin (10 mg/kg), captopril (100 mg/kg), or a combination of melatonin and captopril, and a last group that received nicotine followed by 28 days of nicotine withdrawal. In all groups, biochemical parameters of cardiac toxicity in addition to evaluation of caspase-3 and TNF-α in cardiac tissue by immunohistochemistry were estimated. Administration of nicotine induced significant oxidative damaging effects as reflected by significant lowering of SOD, CAT and GPx levels and significant elevation of cardiac NO and MDA levels compared to control group. Furthermore, cardiac damage and collagen deposition were reported by histological and immunohistochemical evaluation. Treatment with captopril and melatonin reduced nicotine-induced biochemical disturbances and histological damage. Treatment with melatonin is superior to captopril on several parameters and nicotine withdrawal recorded the best ameliorating effect.

***Key words**: Captopril; melatonin; nicotine; cardiac toxicity and withdrawal*

INTRODUCTION

Smoking increases risk of developing many diseases including heart diseases. World health organization (2023) stated that smoking causes more than eight million deaths each year, of which about 1.2 millions are due to passive smoking[1]. Nicotine is the main component of cigarette smoke and long term exposure to it increases the possibilities of occurrence of cardiac diseases due to its cardiac damage[2]. Nicotine induces oxidative stress which elevates lipid peroxidation and reactive oxygen species[3]. Oxidative stress enhances mitochondrial respiration and causes cardiac dysfunction by impairing ATP production and necrosis[4]. In addition, nicotine stimulates the sympathetic nervous system to further increase heart rate, causing vasoconstriction and increasing peripheral resistance and blood pressure. This was followed by hypertrophy of the left ventricle and cardiac dysfunction due to hypertension[5]. Previous researches reported that prolonged administration of nicotine can cause cardiac dysfunction in rat models[6,7].

Melatonin is the main secretory hormone of the pineal gland in all mammals, it influences various biological processes such as circadian rhythm, cardiovascular, neuroendocrine, immune function and hormone regulation[8]. Kaneko et al. (2000) reported useful effects of melatonin on hypoxic/reoxygenated heart morphology and

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physiology. They used isolated rat hearts in their study and reported that melatonin significantly reduced the duration of ventricular arrhythmia compared to control hearts[9]. Administration of melatonin scavenges oxygen free radicals and reactive oxygen species and also enhances antioxidant enzymes; superoxide dismutase, glutathione reductase and glutathione peroxidase[10]. Melatonin protects DNA from oxidative damage induced by oxygen free radicals, especially highly toxic hydroxyl radical[11]. Poly (ADP- ribose) synthetase enzyme (core enzyme of the cell) can induce DNA damage by inducing very high energy expenditure within the cell, leading to necrotic cell death. Melatonin can inhibit the activity of these enzymes and protects organs from damage[12].

Angiotensin converting enzyme inhibitors such as captopril are widely used to treat cardiovascular diseases such as hypertension, myocardial infarction, atherosclerosis and heart failure[13]. Angiotensin II, a main component of the renin angiotensin system, induces tissue oxidative stress, pro-inflammatory cytokine production and apoptosis[14,15]. Captopril reduces plasma and tissue levels of angiotensin II. In addition, it is a potent thiol-containing antioxidant with anti-inflammatory properties[16]. Furthermore, the cardioprotective role of captopril is associated with reduction of reactive oxygen species in tissue ischemia and up-regulation of hypoxia-inducible factor 1α and stromal cell-derived factor 1 chemokines[17,18].

The current study was conducted to evaluate efficacy of captopril and melatonin in separate and their combined administration against nicotine-induced cardiac toxicity in male Wistar rats and reported the effect of nicotine withdrawal.

MATERIALS AND METHODS

Chemicals and drugs

Nicotine, Captopril and melatonin were obtained from Sigma-Aldrich Chemical Company. England. Superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPx) and malondialdehyde (MDA) kits were purchased from Elabscience Company. Houston, USA. Nitric oxide (NO), myeloperoxidase enzyme, and cardiac troponin 1 were obtained from Cusabio Company. Houston, USA. Ultravision detection system, Citrate buffer, and Mayer's hematoxylin were purchased from Labvision Thermo Scientific, California, USA. TNF-α and caspase 3 antibodies were obtained from Abcam, Massachusetts, USA.

Animals

The study was conducted on 60 adult male Wistar rats weighing 200-250g. Rats were obtained from the animal house of Sohag University Faculty of Medicine and housed at room temperature (24 ± 2 °C). Animals were fed a commercial pellet diet and housed under a normal light/dark cycle. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Sohag University, Egypt (approval number: Sohag-5-5-2022-01).

Experimental groups

Sixty male rats were randomly divided into six groups of ten rats each. All rats were treated for 28 days as follows:

- **Group I (control):** Rats received NaCl (0.9%) solution i.p. daily.
- **Group II (Nicotine):** Rats received nicotine (1 mg/kg/day. i.p)[19].
- **Group III (Melatonin):** Rats received melatonin (10 mg/kg/day. i.p)[20], followed by nicotine (1 mg/kg/day. i.p).
- **Group IV (Captopril):** Rats received captopril (100 mg/kg/day. i.p)[21], followed by nicotine (1 mg/kg/day. i.p).
- **Group V (Melatonin and captopril):** Rats received melatonin (10 mg/kg/day. i.p) and captopril (100 mg/kg/day. i.p), followed by nicotine (1 mg/kg/day. i.p).
- **Group VI (Withdrawal):** Rats received nicotine (1 mg/kg/day. i.p) daily for 28 days, then its administration was stopped and no treatment was given for this group for further 28 days before their scarification.

- Nicotine toxicity induction: Cardiotoxicity was induced in all rats of study groups (five groups) except normal control group by i.p injection of nicotine (1mg/kg) daily half an

hour after administration of the treated drugs; melatonin, captopril and combined melatonin and captopril. After 28 days of treatment, all rats were sacrificed except withdrawal group survived an additional 28 days without treatment.

Samples preparation

At the end of treatment, all rats were anesthetized with diethyl ether. A midline incision was made in the chest and heart was rapidly separated from surrounding tissue. Tissue heart samples were fixed with 10% buffered p-formaldehyde and embedded in paraffin for histopathological and immunohistochemical studies. Other cardiac tissues were homogenized in phosphate buffered saline after washing with ice-cold saline and then centrifuged; the supernatant was aliquoted and stored at -80°C for biochemical analysis.

Biochemical analysis

All the following biochemical parameters were measured in cardiac tissues by using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

- **Oxidative stress and antioxidant markers:** SOD, CAT, GPx, MDA and NO levels were estimated in cardiac tissues. SOD, CAT and GPx results were described as U/g tissue. NO results were presented in µmol/g tissue. MDA values were recorded as nmol/g tissue.
- **Myeloperoxidase activity:** Myeloperoxidase, a marker of inflammation and cardiac injury in cardiac tissues was analyzed and its results were expressed as U/g tissue.
- **Cardiac troponin I level:** Troponin I, a marker of cardiac injury was determined and its findings were indicated in ng/ml.

Histological studies

Tissue samples of the cardiac muscle of different groups were fixed in 10% formaldehyde solution for 24 hours. Tissues were dehydrated using graded alcohol, cleared in xylene and embedded in paraffin blocks. Five micrometer thick sections of paraffin blocks were obtained and subjected to the following techniques:

- **Histological examination**

hematoxylin and eosin (H&E) and masson's trichrome stains were used for determining distribution of collagen fibers [22. All sections were examined using Olympus microscope (CX40) and representative micro-photographs were obtained.

- **Immunohistochemical staining**

Tissue sections of the cardiac muscles were dipped in citrate buffer (AP9003) at pH 6.8 for antigen retrieval, washed properly with phosphate buffer saline, then incubated at room temperature for 30 minutes with either polyclonal anti-caspase 3 antibodies; ab13847[23] or anti-tumor necrosis factor alpha (TNF-α) antibodies[24]. Tissue sections were then treated with secondary antibody and the color was developed by avidin-biotin peroxidase and DAB protocol. Immuno-stained sections were examined using Olympus microscope (CX40) and the relative intensity of staining for both caspase 3 and TNF-α molecules was qualitatively measured.

Data analysis and statistics

Data were assayed by using SPSS software (Statistical Package for the Social Sciences, version 22, SPSS Inc, Chicago, IL, USA). Data were described as mean ± standard deviation (SD). Differences between two continuous variables were assessed by Mann-Whitney U test and differences between more than two groups were assessed by Kruskal-Wallis test. Differences between data were considered significant if P- value < 0.05.

RESULTS AND DISCUSSION

Results

Effect of melatonin, captopril and their combination on oxidative stress and antioxidant markers

Levels of SOD, CAT, GPx, MDA and NO in the different studied groups are summarized in table 1 and 2. Administration of nicotine for 28 days induced significant decrease in cardiac SOD, CAT and GPx levels compared to normal control group ($P < 0.001$) and significant increase in cardiac MDA and NO levels compared to normal control group ($P < 0.001$).

Comparing to nicotine group; treatment with melatonin and combination of melatonin and captopril elevated cardiac SOD, CAT and GPx levels significantly ($P < 0.001$) with significant reduction of cardiac MDA and NO

levels (P < 0.001). Administration of captopril separately induced significant increase in cardiac SOD and GPx levels and significant decrease in cardiac MDA level (P < 0.001), while captopril administration induced insignificant change in cardiac CAT and NO levels (0.22 & 0.06, respectively). Nicotine withdrawal for 28 days resulted in a significant decrease of cardiac MDA and NO levels (P < 0.001) and significant increase in cardiac SOD, GPx and CAT levels (P < 0.001).

The ameliorative effect of melatonin, captopril, their combination and withdrawal of nicotine have been compared. Melatonin administration induced significant improvement of cardiac SOD, CAT, GPx, MDA and NO levels (P < 0.05) when compared to captopril group. Combined administration of melatonin and captopril induced significant improvement of cardiac SOD, CAT, GPx, MDA and NO levels when compared to separate administration of captopril and significant improvement in cardiac GPx, MDA and NO levels when compared to separate administration of melatonin. Nicotine withdrawal induced significant improvement in cardiac SOD, CAT, GPx, MDA and NO levels when compared to separate or combined administration of melatonin and captopril (**Table 1 and 2**).

Table 1: Effect of melatonin & captopril and their combination on SOD, CAT and GPx levels in rats treated with nicotine.

Groups	SOD U/g tissue	CAT U/g tissue	GPx U/g tissue
I (control)	52.93±1.68	59.93± 1.74	0.79± 0.04
II (Nicotine)	27.22±1.08[a]	34.56±3.09[a]	0.27± 0.04[a]
III (Nicotine/Melatonin)	41.45± 1.62[b,d]	40.09±1.62[b,d]	0.54± 0.02[b,d]
IV (Nicotine/Captopril)	34.89± 1.20[b]	35.51±1.78	0.45± 0.15[b]
V (Nicotine/Melatonin/Captopril)	43.31± 1.36[bd]	42.98±1.36[b,d]	0.60± 0.06[b,c,d]
VI (Withdrawal)	49.78± 0.78 [b,c,d,e]	57.06±2.21[b,c,d,e]	0.68± 0.07[b,c,d,e]

The data was analysed using Mann-Whitney U and Kruskal-Wallis tests (SPSS, 22). The values represent mean ± SD (n=10 per group). [a], indicates significant difference from control group, [b] from nicotine group, [c] from melatonin group, [d] from captopril group and [e] from combined group**.** SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase. For all statistical comparisons, a P-value < 0.05 was considered statistically significant.

Table 2: Effect of melatonin & captopril and their combination on NO and MDA levels in rats treated with nicotine**.**

Groups	NO µmol/g tissue	MDA nmol/g tissue
I (control)	15.01± 0.75	18.09±1.36
II (Nicotine)	37.71±2.73[a]	41.35±1.64[a]
III (Nicotine/Melatonin)	29.83±2.50[b,d]	28.90± 2.23[b,d]
IV (Nicotine/Captopril)	35.76± 1.10	34.25±2.11[b]
V (Nicotine/Melatonin/Captopril)	23.13±2.27[b,c,d]	24.08± 2.40[b,c,d]
VI (Withdrawal)	17.50±1.20 [b,c,d,e]	19.57± 1.95 [b,c,d,e]

The data was analysed using Mann-Whitney U and Kruskal-Wallis tests (SPSS, 22). The values represent mean ± SD (n=10 per group). [a], indicates significant difference from control group, [b] from nicotine group, [c] from melatonin group, [d] from captopril group and [e] from combined group**.** NO: Nitric oxide; MDA: malondialdehyde. For all statistical comparisons, a P-value < 0.05 was considered statistically significant.

Effects of melatonin, captopril and their combination on cardiac Myeloperoxidase Activities

Fig. 1, demonstrates cardiac tissue levels of myeloperoxidase in different studied groups. Nicotine administration for 28 days induced a significant increase in cardiac myeloperoxidase levels compared to the control group ($P < 0.001$). Comparing to nicotine group; cardiac myeloperoxidase level was reduced significantly in melatonin and combined group ($P < 0.05$) with no significant difference in captopril group (0.07).Withdrawal of nicotine for 28 days resulted in a significant decrease of cardiac myeloperoxidase ($P < 0.001$).

Melatonin administration induced insignificant change in cardiac myeloperoxidase level when compared to captopril group. Combined administration of melatonin and captopril and nicotine withdrawal induced a significant reduction of cardiac myeloperoxidase level as compared to separate treatment with either melatonin or captopril ($P < 0.05$) (**Fig. 1**).

Effects of melatonin, captopril and their combination on cardiac Troponin I Levels

Administration of nicotine for 28 days induced a significant increase in cardiac troponin 1 level compared to normal control group ($P < 0.001$). When compared to nicotine group; treatment with melatonin, captopril and their combination reduced cardiac troponin 1 levels significantly ($P < 0.05$). Withdrawal of nicotine for 28 days resulted in a significant decrease in cardiac troponin 1 level ($P < 0.001$). Combined administration of melatonin and captopril and nicotine withdrawal decreased cardiac troponin 1 level significantly when compared to separate administration of either melatonin or captopril ($P < 0.05$) (**Fig. 2**).

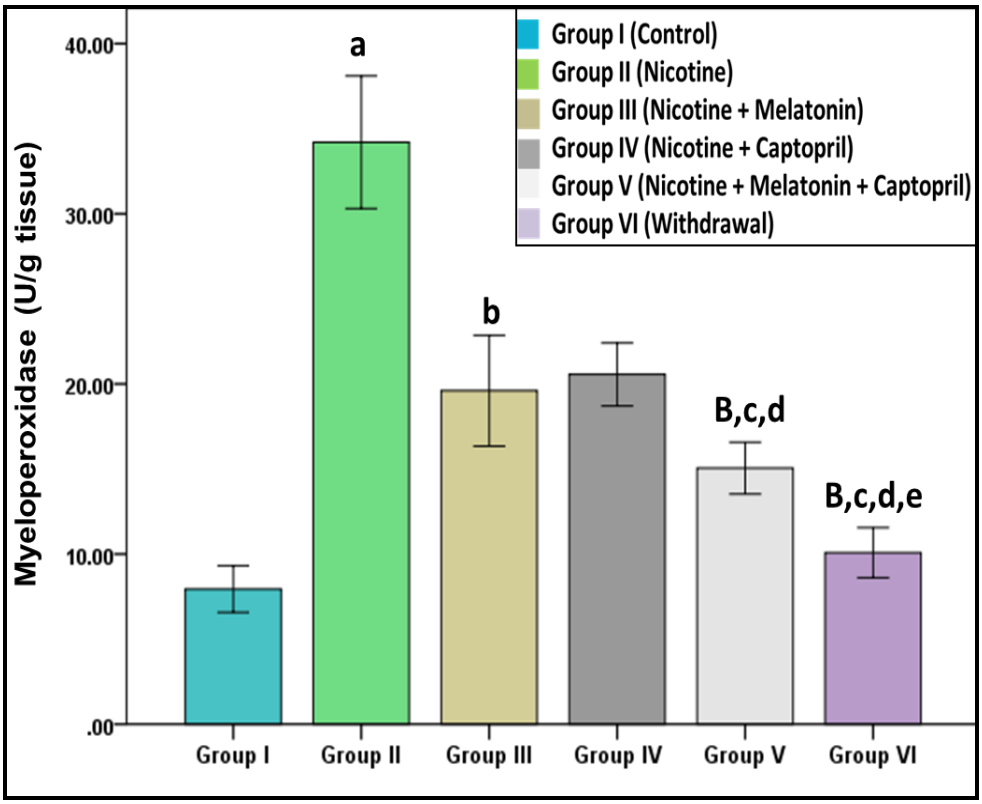

Fig.1: Myeloperoxidase levels in all groups. The data was analysed using Mann-Whitney U and Kruskal-Wallis tests (SPSS, 22). The values represent mean ± SD (n=10 per group). [a,] indicates significant difference from control group, [b] from nicotine group, [c] from melatonin group, [d] from captopril group and [e] from combined group. For all statistical comparisons, a P-value < 0.05 was considered statistically significant.

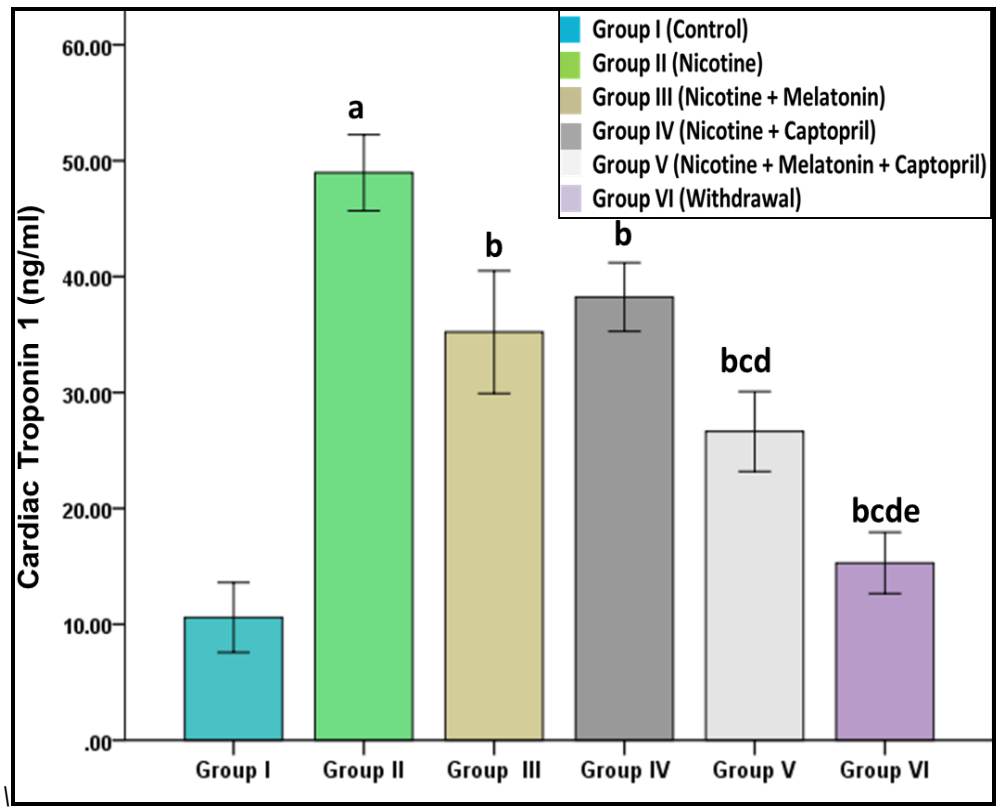

Fig. 2: Cardiac troponin 1 levels in all groups. The data was analysed using Mann-Whitney U and Kruskal-Wallis tests (SPSS, 22). The values represent mean ± SD (n=10 per group). [a,] indicates significant difference from control group, [b] from nicotine group, [c] from melatonin group, [d] from captopril group and [e] from combined group**.** For all statistical comparisons, a P-value < 0.05 was considered statistically significant.

Light microscopic examination (Hematoxylin and Eosin and Masson's trichrome)

In control rats; cardiac muscle has uniform arrangement of muscle bundles with uniform nuclei and identified cardiac striations. There is no evidence of necrosis, interstitial tissue hemorrhage or inflammatory reaction in normal control group (**Fig. 3A**).

On the other hand; cardiac muscle of nicotine treated rats showed histological indicators of myocardial injury (**Fig. 3B-3D**) in terms of granular cytoplasm of cardiomyocytes with loss of cardiac muscle striation. Focal cytoplasmic vacuolation was seen. There are patches of interstitial hemorrhage and focal infiltration by inflammatory cells mainly lymphocytes and neutrophils. Multiple congested capillaries were detected.

Treatment of melatonin and captopril in isolated or combined forms reduced the damaging effect of nicotine in cardiac muscle with stronger augmenting effect of combined melatonin and captopril to ameliorate the cardiotoxic effect of nicotine on heart muscle of rats. The histological changes in these rats include mild degenerative changes with patchy cytoplasmic vacuolation and retained cardiac muscle striations. Mild vascular congestion and minimal focal inflammation were observed (**Fig. 3E- 3G**). There is no evidence of necrosis, interstitial tissue hemorrhage or inflammatory reaction in nicotine withdrawal group. (**Fig. 3H**).

Fibrosis of cardiac muscle after nicotine treatment was evaluated by Masson`s trichrome stain (**Fig. 4**). Collagen deposition is obviously increased in nicotine treated rats compared to normal rats. Deposited collagen is reduced in melatonin treated rats, captopril treated rats and melatonin/captopril treated rats with stronger effect when combination was used. Rats after nicotine withdrawal showed small residual collagen fibers.

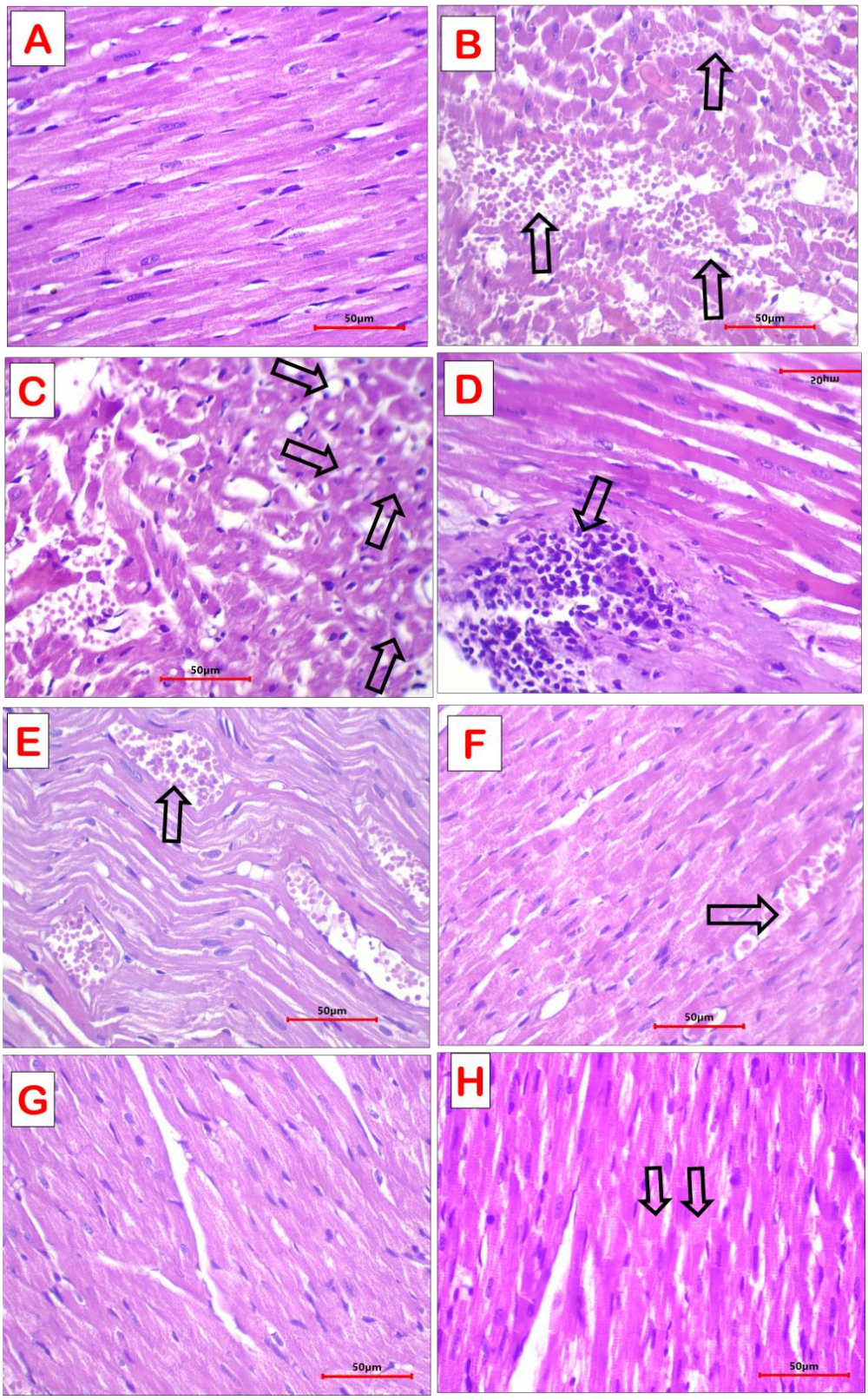

Fig. 3: Histological sections of cardiac muscle: Sections of normal control rats showed normal cardiac muscle bundles (**A**). Nicotine treated rats (**B, C and D**) showed interstitial tissue hemorrhage (**B**), cytoplasmic vacuolation (**C**) and inflammatory reactions (**D**). Melatonin (**E**), captopril (**F**) and melatonin/captopril (**G**) treated rats showed preserved muscle bundles with residual interstitial tissue hemorrhage. Nicotine withdrawal group with retained cardiac striation (**H**). H&E stain; magnification is x400 for all.

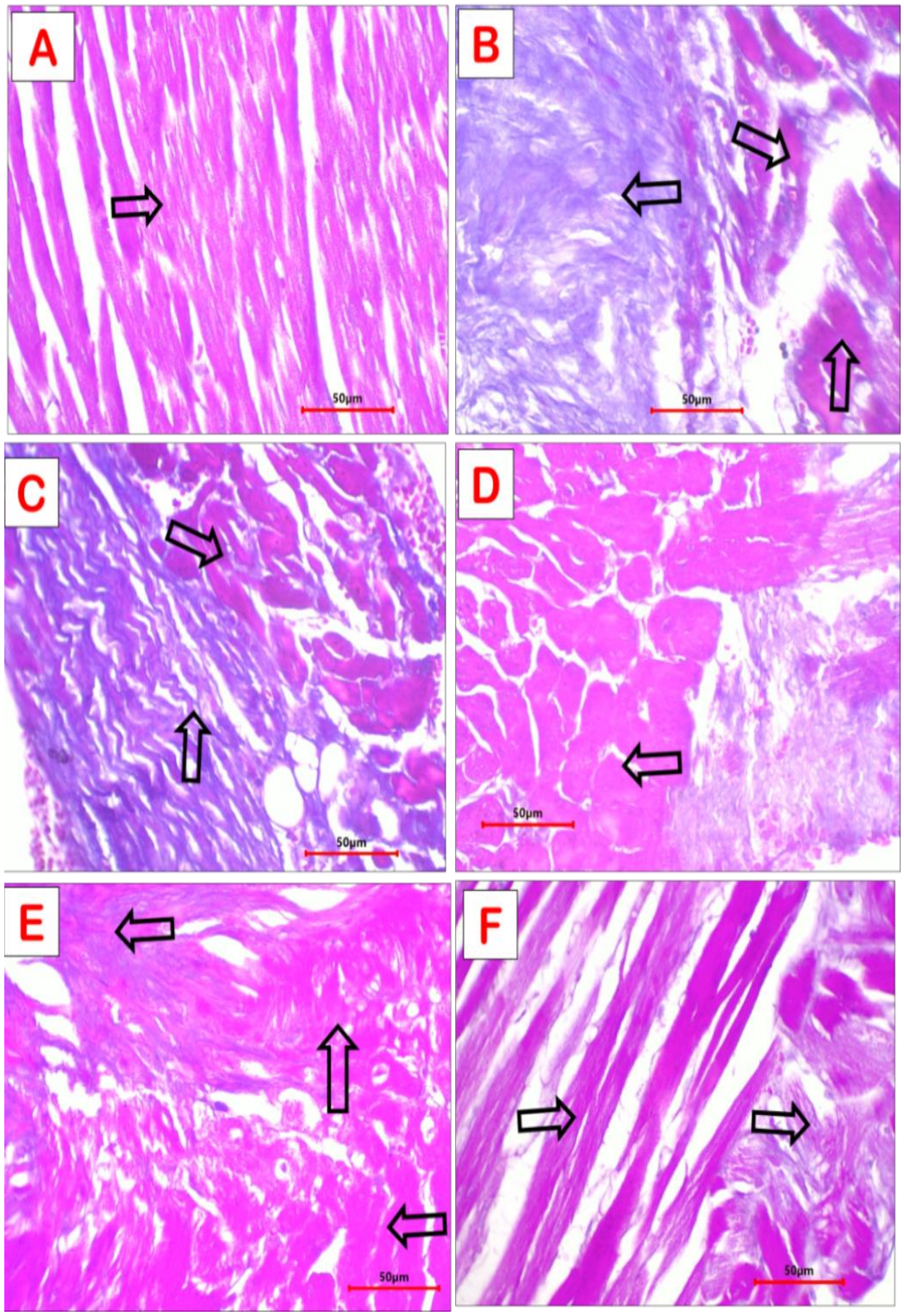

Fig. 4: Histological changes of cardiac muscle stained by Masson`s trichrome stain. Sections of normal rats showed eosinophilic color of cardiac muscle bundles with no collagen (**A)**. Sections of nicotine treated rats showed wide areas of blue stained collagen with residual eosinophilic stained cardiac muscle (**B**). Sections of melatonin treated rats (**C)**, captopril treated rats (**D**) and melatonin/captopril treated rats (**E**) showed less collagenous tissue and identified muscle bundles. Rats after nicotine withdrawal showed small residual collagen fibers (**F**). Magnification is x400 for all.

Immunohistochemical examination

Immune stained sections of caspase-3; an apoptosis related molecule showed strong expression in nicotine treated rats. There is weak to moderate expression of capsase-3 in melatonin and captopril treated rats. Capsase-3 expression was obviously weak and patchy in melatonin/captopril treated. It is very faint in rats after nicotine withdrawal (**Fig. 5**).

Expression of TNF-α; an inflammatory cytokine that has diverse signaling events leading to necrosis or apoptosis was evaluated in different studied groups (**Fig. 5**). TNF-α is strongly positive in nicotine treated rats with moderate expression in melatonin and captopril treated rats. Expression of TNF-α is weak positive and looks patchy in melatonin/captopril. It was weak after nicotine withdrawal.

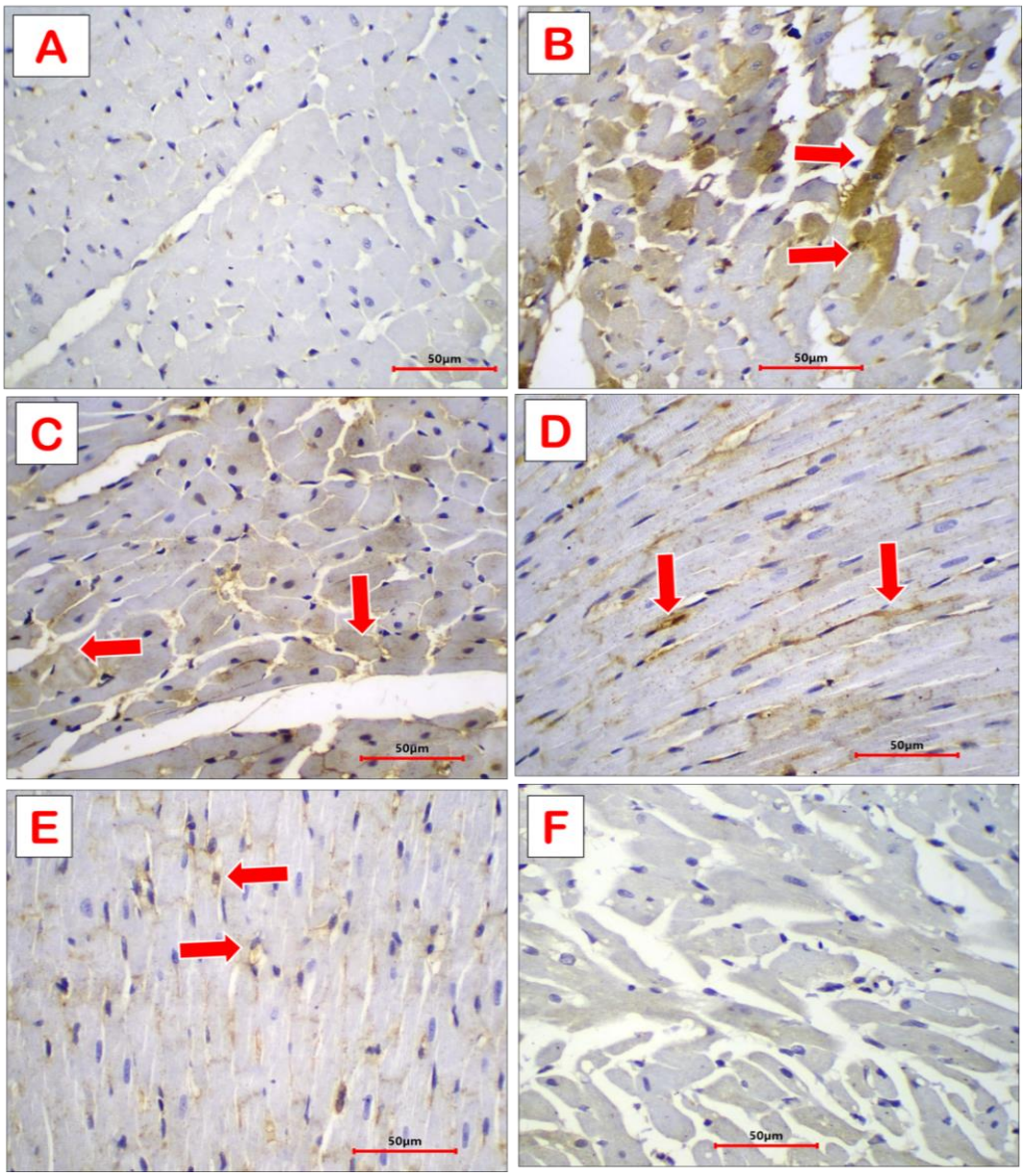

Fig. 5: Expression of caspase 3 in cardiac muscle of different study groups: The expression was negative in normal control rats (**A**). There is strong caspase-3 expression in nicotine treated rats (**B**). Caspase-3 expression was weak to moderate in melatonin (**C**) and captopril (**D**) treated rats and it was weak in melatonin/captopril treated rats (**E**). Caspase-3 expression is very faint in rats after nicotine withdrawal (**F**). Magnification is x400 for all.

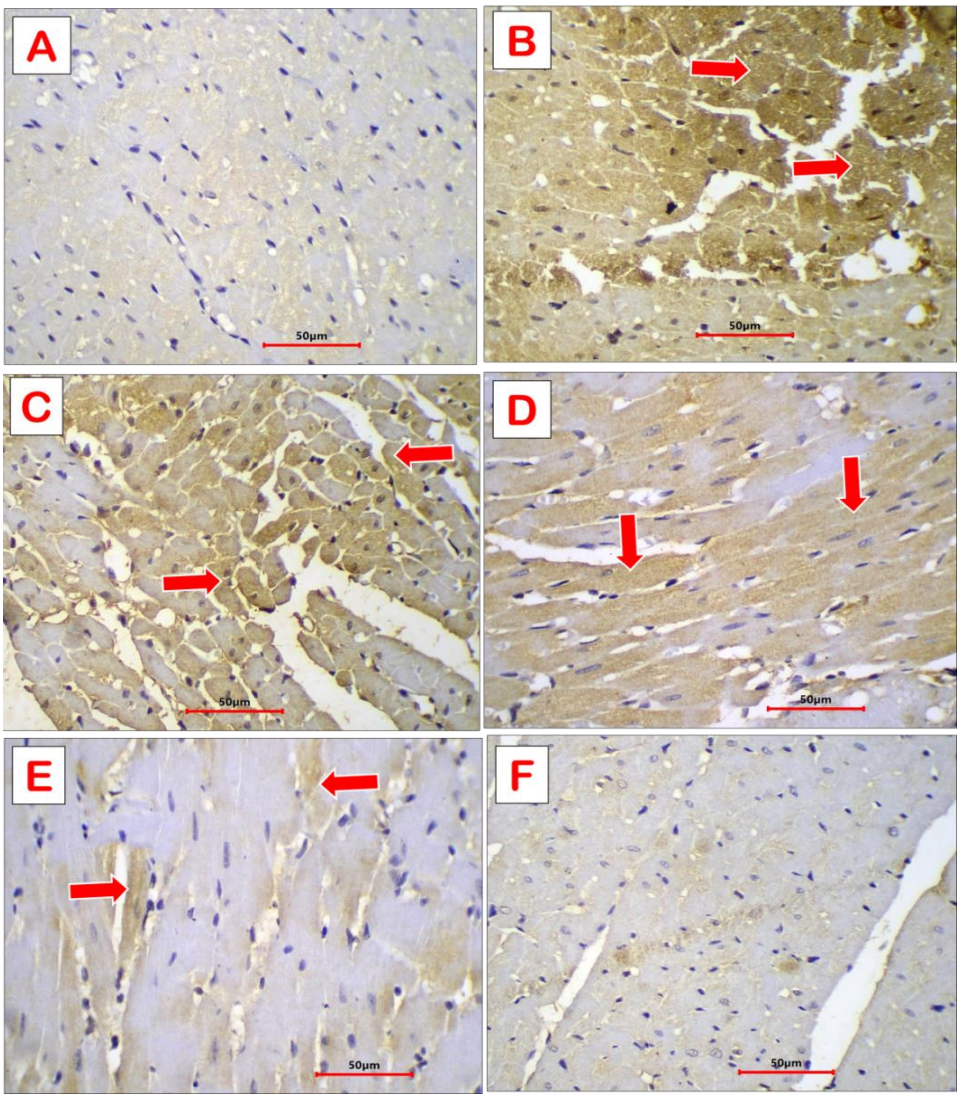

Fig. 6: Expression of TNF -α in cardiac muscle of different study groups: The expression was negative in normal control rats (**A)**. There is strong expression of TNFα in nicotine treated rats (**B**) with moderate expression in melatonin (**C**) and captopril (**D**) treated rats. TNFα showed weak and rather patchy expression in rats treated with melatonin/captopril combination (**E**). The expression was weak after nicotine withdrawal (**F**). Magnification is x400 for all.

Discussion

Nicotine is the main component of tobacco plant and is responsible for development of heart disease in smokers[2]. The current study was conducted to assess the cardiotoxic effect and possible protective effect of melatonin, captopril and their combination. Nicotine induced marked oxidative damage by significant reduction of SOD, GPx and CAT levels and significant increase in cardiac NO and MDA levels as compared to normal control group. These results were in line with results of Baykan and his colleagues[25] who reported lowering of SOD and GPx levels and elevation of NO and MDA levels after nicotine administration, they evaluated protective effects of melatonin on heart of neonatal rats born to mothers, which were treated with nicotine. Furthermore, nicotine administration induced oxidative stress that induced imbalance of peroxide/antioxidant levels within blood cells, plasma and tissues both *in vitro* and *in vivo*[26].

Previous research has reported decreased serum levels of SOD, which catalyzes decomposition of superoxide radicals into molecular oxygen and hydrogen peroxide. The observed decrease in serum SOD concentration

leads to a decrease in *de novo* synthesis of antioxidant enzymes[3]. SOD and GPx play effective roles in detoxifying oxygen free radicals[27]. Florek et al. indicated marked reduction of SOD and GPx activities in different tissues of pregnant and non pregnant female rats after exposure to cigarette smoking for 21 days[28].

The present study also reported that administration of nicotine increases cardiac NO level. This finding is consistent with Swami study[29] who reported that administration of nicotine induced oxidative stress with elevation of NO level among workers in tobacco factories. Lipid peroxidation of unsaturated fatty acids is often used as a marker of oxidative stress and damage. In the current study, nicotine-treated rats showed increased MDA levels compared to control group. High MDA levels may be a result of reduced antioxidant production in tissues of nicotine group, so, reactive oxygen species production is increased[30].

According to toxicity of nicotine on antioxidant parameters, we hypothesized that melatonin may have a protective effect on the deteriorated cardiac function by nicotine. In this study, melatonin induced significant rise in SOD, GPx and CAT levels and significant reduction of cardiac NO and MDA levels when compared to nicotine group. These data were in accordance with previous study which tested protective effect of melatonin on cardiotoxic effect of nicotine in rats[25]. The ability of melatonin to protect against oxidative damage has been studied both *in vivo*[31] and *in vitro*[32]. Melatonin has been demonstrated to have antioxidant properties because it directly neutralizes free radicals and related toxins[33]. It increases antioxidant enzymes activity such as SOD, GPx and CAT[34].

The study presented here showed that treatment with captopril before nicotine administration significantly increased SOD and GPx levels and significantly decreased cardiac MDA levels compared to the nicotine group. Previous study has used a similar protocol that examined effects of nicotine in heart of experimental animals and demonstrated that captopril inhihibts endothelial dysfunction induced by nicotine both *in vitro* and *in vivo* by its antioxidant effect[35]. Furthermore, it should be noted that captopril scavenges free radicals due to its sulfhydryl group and it has a great antioxidant capacity[36]. Addittionally, previous study reported ability of captopril to decrease serum lipid peroxide concentrations in diabetic patients[37].

Nicotine-treated rats showed an increase in cardiac troponin-1 compared to control rats. This result was in harmony with Nadruz study that examined effects of smoking on cardiac biomarkers, including troponin 1[38]. Association between tobacco smoking and elevation of cardiac troponin 1 may be due to myocardial damage, hypoxaemia and oxidative stress induced by smoking[39]. Administration of melatonin and captopril separately and in combination induced significant reduction of cardiac troponin 1 when compared to control group.

Myeloperoxidase is mainly present in primary neutrophils granules and its main function is to kill microorganisms, however, under certain conditions, over production causes tissue damage[40]. Nicotine stimulates production of reactive oxygen species from neutrophils and liberates oxidation promoting enzymes such as myeloperoxidase[41]. Our results showed that serum myeloperoxidase activity increases in nicotine-treated rats compared to control rats. Administration of melatonin alone and in combination with captopril significantly reduced cardiac myeloperoxidase activity. Interestingly, Galijasevic et al (2008) reported that melatonin is a potent inhibitor of myeloperoxidase activity; they demonstrated ability of melatonin to reduce catalytic activity of myeloperoxidase in several ways, including shifting myeloperoxidase activity from peroxidation to catalase-like activity and converting myeloperoxidase to an inactive form[42].

Administration of nicotine in our study showed histological indicators of myocardial injury in terms of granular cytoplasm of cardiomyocytes with loss of cardiac muscle striation and focal cytoplasmic vacuolation. There were patches of interstitial hemorrhage and focal infiltration by inflammatory cells with multiple congested capillaries. These results were constant with results of Baykan et al[25] who demonstrated marked cardiomyopathy which was associated with swelling, disorganization, interstitial necrosis and edema in cardiac tissues of all nicotine-treated rats.

Treatment with melatonin and captopril in isolated or combined forms reduced damaging effect of nicotine in cardiac muscle with stronger augmenting effect of their combination. Effect of melatonin on cardiac toxicity in our study was in agreement with previous study which demonstrated protective effect of melatonin on cardiac toxicity induced by doxorubicin[43]. Previous study demonstrated effect of captopril on heart of diabetic rats. Administration of captopril reduced myocardial injury and collagen fibers deposition within 4 and 8 weeks compared with diabetic rats[44].

Collagen deposition is obviously increased in nicotine-treated rats compared to normal rats. Deposited collagen is reduced in rats treated with melatonin and captopril, while their combination showed stronger effect. These findings were in accordance with Abdulwahab and colleagues who demonstrated reduction of collagen deposition in cardiac muscles of rats after treatment with melatonin[45]. Additionally, Attia and colleagues (2018) reported ability of captopril in diabetic rats to ameliorate collagen expression and reduce cardiac fibrosis[44].

In our study, a significant rise in immune-expression of caspase 3 and TNF-α was detected in nicotine-treated group. Overproduction, as caspase-3 and TNF-α is involved in tissue injury[46]. More interestingly, melatonin and captopril remarkably suppressed activation of TNF-α and caspase-3 induced by nicotine, suggesting protective effect of melatonin and captopril on nicotine induced inflammation and apoptosis through supressing TNF-α and caspase-3 activity. Our results matched with previous study that reported protective role of melatonin in cardiac apoptosis and inflammation caused by cisplatin in rats by reduction of caspase-3 and TNF-α activity[47]. Furthermore, our findings agreed with Saglam et al (2014) who indicated that captopril reduced TNF-α and caspase 3 in cardiopulmonary injuries induced by burn[48].

In withdrawal group, cardiac levels of SOD, CAT, GPx, NO, MDA and histological findings showed comparable results with the normal control group indicating that cardiotoxic effect of nicotine could be prevented by nicotine stoppage. Previous research reported relation between smoking and cardiovascular disease and hence, the importance of smoking cessation, they proved improvement of cardiac cells and markers from damage induced by continuous smoking[49].

To conclude, treatment with captopril and melatonin induced significant protective effects against cardiac toxicity of nicotine in rats. Melatonin administration has a better effect than captopril in some parameters. Beneficial effects of these drugs were their antioxidant, anti-inflammatory, antifibrotic and antiapoptotic abilities. Therefore, melatonin and captopril could be beneficial in protection and treatment of cardiac toxicity induced by nicotine. Smoking cessation would be the best choice to avoid nicotine cardiac dysfunction.

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التأثير الوقائي للميلاتونين و الكابتوبريل ضد الإجهاد التأكسدي القلبي والخلل الوظيفي الناتج عن النيكوتين في الفئران

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يؤدي التعرض طويل الأمد للنيكوتين إلى تدهور أمراض القلب والأوعية الدموية لدى المدخنين من خلال خلل وظائف القلب. يهدف هذا البحث إلى دراسة التأثيرات الوقائية للميلاتونين والكابتوبريل في الفئران المعرضة للنيكوتين ورصد تأثير انسحاب النيكوتين. تم تقسيم عدد ستين من الفئران الذكورالبالغة إلى ٦ مجموعات. تلقت المجموعة الضابطة محلول ملحي، وتلقت المجموعة الايجابية نيكوتين (١ مجم / كجم). تم إعطاء المجموعات الأربع الأخرى الميلاتونين (١٠ مجم / كجم)، كابتوبريل (١٠٠ مجم / كجم)، أو مزيج من الميلاتونين والكابتوبريل، والمجموعة الأخيرة التي تلقت النيكوتين تم ايقاف تعاطي النيكوتين لمدة ٢٨ يوما أخرى. في جميع المجموعات، تم تقدير التحليلات البيوكيميائية لتسمم القلب بالإضافة إلى تقييم caspase 3 وTNF-α في أنسجة القلب. تسبب النيكوتين في حدوث آثار مؤكسدة ضارة كبيرة كما يظهر ذلك في الانخفاض الكبير في مستويات SOD وCAT وGPx والارتفاع الكبير في مستويات NO وMDA في القلب مقارنة بالمجموعة الضابطة. علاوة على ذلك، تم حدوث تلف للقلب وترسيب المادة الليفية (كولاجين) من خلال التقييم النسيجي. أدى العلاج بالكابتوبريل والميلاتونين إلى تقليل الاضطرابات البيوكيميائية الناتجة عن النيكوتين والأضرار النسيجية. العلاج بالميلاتونين تفوق على الكابتوبريل في العديد من العوامل وتأثير انسحاب النيكوتين سجل أفضل تأثير.