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Protective effects of *Mentha spicata* against nicotine-induced toxicity in liver and erythrocytes of wistar rats

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Abstract

The aim of this study was to investigate the protective effect of *Mentha spicata* supplementation against nicotine-induced oxidative damage in the liver and erythrocytes of wistar rats. Bioactive substances were determined by liquid chromatography-electrospray ionization-tandem mass spectrometry analysis. Animals were divided into four groups of six rats each: a normal control group, a nicotine-treated group (1 mg/kg), a group receiving *M. spicata* extract (100 mg/kg), and a group receiving both *M. spicata* extract (100 mg/kg) and nicotine (1 mg/kg). Many phenolic acids were identified in the *M. spicata* aqueous extract. After 2 months treatment, nicotine induced an increase in the level of white blood cells and a marked decrease in erythrocytes, hemoglobin and haematocrit. Aspartate transaminase, alanine transaminase, alkaline phosphatase and lactate dehydrogenase activities were also found to be higher in nicotine-treated group than those of the control one. Furthermore, nicotine-treated rats exhibited oxidative stress, as evidenced by a decrease in antioxidant enzymes activities and an increase in lipid peroxidation level in liver and erythrocytes. Interestingly, the oral administration of *M. spicata* extract by nicotine-treated rats alleviated such disturbances. *M. spicata* contained bioactive compounds that possess important antioxidant potential and protected liver and erythrocytes against nicotine-induced damage.

Keywords: *Mentha spicata*; phytochemistry analysis; tobacco; liver; erythrocytes; oxidative stress

Introduction

Tobacco smoking is one of the major public health problems in all countries around the world. The world health organization has estimated that over a billion people are users of tobacco (Natsume et al. 2003). There is growing evidence that cigarette smoking and tobacco chewing have a profound negative impact on general health associated with high risk of morbidity. Nicotine is the most abundant volatile alkaloid extracted from the dried leaves and stems of the *Nicotiana tabacum* and *Nicotiana rustica* and it features prominently among the over 4000 chemicals found in tobacco products (Rustemeier et al. 2002). In fact, nicotine is rapidly absorbed by the circulatory system and most of this compound is metabolized in the liver (Omar et al. 2015). It is an active compound on the nervous system, including the neuroendocrine axis, as it binds stereo-selectively to nicotinic cholinergic receptors in the autonomic ganglia, chromaffin cells of the adrenal medulla, neuromuscular junctions and brain (Tundulawessa et al. 2010). Furthermore, the deleterious toxic effects of nicotine are at least in part due to genotoxic, immunologic, as well as reproductive effects in both sexes. Consequently, it is generally regarded to be a primary risk factor in the development of cardiovascular and pulmonary diseases (Edwards-Jones et al. 2004; Polyzos et al. 2009).

The genus *Mentha* (Lamiaceae) comprises diverse species that contain bioactive substances such as phenolic compounds. Many of these compounds are famous for their antioxidant activity *via* preventing carcinogen bioactivation and increasing the detoxification of reactive oxygen species (ROS) (Yamaguchi et al. 2002). *Mentha spicata* L. as perennial aromatic plant is distributed across the Europe, Africa, Australia and North America. For centuries, traditional *M. spicata* has been used for gastrointestinal discomfort, stomach and chest pain, and suppression of indigestion symptoms (Samarth and Kumar 2003; Arumugam and Ramesh 2009). The mint plant is also known for its insecticidal, antimicrobial, antispasmodic, anti-inflammatory and antiplatelet properties (Papachristos and Stamopoulos

2002; Özgen et al. 2006; Tognolini et al. 2006). According to several studies, *M. spicata* is known for its important content of vitamins, phenolics, flavonoids and terpenoids compounds (She et al. 2010; Krzyzanowska et al. 2011).

The current study was designed to evaluate the hepatoprotective effect of *M. spicata* against nicotine-induced toxicity in liver and erythrocytes of wistar rats. The effect of the *M. spicata* aqueous extract (ME) on hematological parameters, liver toxicity indices, antioxidant enzyme activities and lipid peroxidation were investigated. Furthermore, the ME was analyzed by liquid chromatography-electrospray ionization-tandem mass spectrometry analysis (LC-ESI-MS) technique in order to identify the bioactive compounds frequently associated with the antioxidant activity.

Materials and methods

Plant material

The aerial parts of *Mentha spicata* L. were collected in March from Gafsa (Tunisia). The plant was identified and authenticated by a taxonomist and a voucher specimen is deposited at the Faculty of Sciences of Gafsa (Gafsa, Tunisia) under the number MS 0315.

Antioxidant activity and LC-ESI-MS analysis of M. spicata extract

M. spicata extract preparation

The air-dried plant material was extracted by maceration in water (10 g/100 ml) at ambient temperature for 24 h with continuous stirring. After that, the extract was filtrated with Whatmann Millipore filter paper and concentrated to dryness with a rotary evaporator at 40°C. Finally, the *M. spicata* aqueous extract (ME) was kept in the dark at 4°C until further analysis.

Total phenolics, flavonoids, tannins and antioxidant activity

Total phenolics content of ME was determined by the Folin–Ciocalteu method (Chen et al. 2007). Gallic acid monohydrate was used as standard for the calibration curve. Total

phenolics content was expressed as mg gallic acid equivalent (GAE)/g extract. Flavonoids content of the sample was determined as previously described (Djeridane et al. 2006) and rutin was used as standard. The results were expressed as mg rutin equivalent (RE)/g extract. Tannins content in *M. spicata* extract was also measured using the vanillin assay described by Julkunen-Tiitto (1985). Tannins content was expressed as mg catechin equivalent (CE)/g extract.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical-scavenging and superoxide anion radical-scavenging activities of the *M. spicata* extract were measured as previously described (Yen and Chen 1995; Yildirim et al. 2001). Results of DPPH• radical-scavenging and superoxide anion radical-scavenging activities were presented by IC₅₀ values, which was defined as the extract concentration needed to scavenge 50% of DPPH• and 50% of superoxide anion, respectively. Lower IC₅₀ values reflected better antioxidant activity.

Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS) analysis

The ME was dissolved in ethanol and the resulted solution (4 mg/ml) was filtered through a 0.45 µm membrane filter before injection into the HPLC system. LC-ESI-MS analysis was performed using a LCMS-2020 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionisation source (ESI) and operated in negative ionization mode. The mass spectrometer was coupled online with an ultra-fast liquid chromatography system consisted of a LC-20AD XR binary pump system, SIL-20AC XR autosampler, CTO-20AC column oven and DGU-20A 3R degasser (Shimadzu, Kyoto, Japan). An Aquasil C18 column (Thermo Electron, Dreieich, Germany) (150 mm × 3 mm, 3 µm) preceded by an Aquasil C18 guard column (10 mm × 3 mm, 3 µm, Thermo Electron) were applied for analysis. The mobile phase was composed of A (0.1% formic acid in H₂O, v/v) and B (0.1% formic acid in methanol, v/v) with a linear gradient elution: 0-45 min, 10-100%

B; 45-55 min, 100% B. Re-equilibration duration was 5 min between individual runs. The flow rate of the mobile phase was 0.4 ml/min, the column temperature was maintained at 40°C and the injection volume was 5 µl. Spectra were monitored in mode SIM (Selected Ion Monitoring) and processed using Shimadzu Lab Solutions LC-MS software. High-purity nitrogen was used as the nebulizer and auxiliary gas. The mass spectrometer was operated in negative ion mode with a capillary voltage of -3.5 V, a nebulizing gas flow of 1.5 l/min, a dry gas flow rate of 12 l/min, a DL (dissolving line) temperature of 250°C, a block source temperature of 400°C, a voltage detector of 1.2 V and the full scan spectra from 50 to 2000 Da.

Animals and treatments

A total of 24 Wistar male rats (3 months old), weighing approximately 140 g, were obtained from the Central Pharmacy of Tunisia (SIPHAT, Tunis, Tunisia). The animals were maintained for a two-week adaptation period under standard environmental conditions of temperature (22±2°C), relative humidity (50±4%) and a constant photoperiod (12 h light/dark cycle). Animals had *ad libitum* access to pellet diet (SICO, Sfax, Tunisia) and water. The handling of the animals was approved by the Medical Ethics Committee for the Care and Use of Laboratory Animals of the Pasteur Institute of Tunis (approval number: FST/LNFP/Pro 152012) and carried out according to the European convention for the protection of living animals used in scientific investigations (Council of European Communities 1986).

After the adaptation period the animals were divided into four groups, of six animals each, and were subjected to the following treatments during 2 months:

- (i) group 1: untreated rats that were considered as control animals and referred to as “C”;
- (ii) group 2: animals that were injected intraperitoneally with nicotine in aqueous solution (1 mg/kg body/day) and referred to as “NT”;

(iii) group 3: animals receiving *M. spicata* extract (100 mg/kg body mass/day) administered by gastric gavage and referred to as “ME”;

(iv) group 4: animals receiving *Mentha spicata* extract (100 mg/kg body mass/day) administered by gastric gavage and then injected intraperitoneally with nicotine in aqueous solution (1 mg/kg body/day) and referred to as “NI+ME”.

At the end of all treatments, the rats were sacrificed by decapitation and their trunk bloods were collected in plastic tubes. Ethylenediaminetetraacetic acid (EDTA) was added to blood samples used for haematological parameters determination. Heparin was added to the blood samples used for biochemical assays and then the serum samples were recovered by centrifugation ($3000 \times g$, 15 min, 4°C) before being stored at -80°C until use.

Preparation of erythrocytes

The sediment containing erythrocytes were twice suspended in phosphate buffer saline (0.9% NaCl in 0.01 M phosphate buffer pH 7.4) and centrifuged at $3000 \times g$ for 15 min and at 4°C . The hemolysats were then aliquoted and stored at -80°C before their use for antioxidant enzyme activities and the determination of thiobarbituric acid reactive substances (TBARS) content.

Preparation of liver extracts

Samples of 1 g liver tissues were homogenized in 2 ml tris-buffered saline, pH 7.4 using an Ultra-Turrax grinder. Then, the homogenates were centrifuged at $9000 \times g$ for 15 min and at 4°C and the supernatants were recovered and stored at -80°C until use.

Haematological variables

Haematological parameters such as erythrocytes, white blood cells, haematocrit and, haemoglobin were measured using a Coulter automated cell counter MAX-M (Beckman Coulter Inc., Fullerton, CA, USA).

Biochemical assays

The analyses of the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities were performed using commercial kits (Spinreact, Girona, Spain) on an automatic biochemical analyzer (Vitalab Flexor E, Spankeren, Netherlands) in the biochemical laboratory of Regional Gafsa Hospital (Gafsa, Tunisia).

Lipid peroxidation in the liver was measured by the quantification TBARS content (mmol/mg protein) as previously described (Buege and Aust 1978). The catalase (CAT) activity was measured as previously described by Aebi (1984) and expressed as $\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg}$ protein. The superoxide dismutase (SOD) activity was measured by the method of Beyer and Fridovich (1987) and expressed as U/mg protein. The glutathione peroxidase (GPX) activity was measured by the method described by Flohe and Gunzler (1984) and expressed as $\mu\text{mol GSH}/\text{min}/\text{mg}$ protein. The protein concentration was determined as previously described by the method of Bradford (1976) using bovine serum albumin ($E_{1\text{cm}}^{1\%} = 6.7$) as standard.

Histological analyses

The liver samples from rats of different treatments were fixed in 10% formalin solution for 24 h, embedded in paraffin and then sections of 5 μm thickness were stained with hematoxylin-eosin. The slides were photographed with an Olympus U-TU1X-2 camera linked to an Olympus CX41 microscope (Olympus, Tokyo, Japan).

Statistical analysis

All data were presented as means \pm standard deviation (SD). Determination of all parameters was performed from six animals per group. Significant differences between treatment effects were determined using one-way ANOVA, followed by Tukey's HSD Post-Hoc tests for multiple comparisons with statistical significance of $p \leq 0.05$.

Results

Phytochemistry analysis and antioxidant activity of M. spicata

The amounts of total phenolics, flavonoids and tannins contents in *M. spicata* aqueous extract (ME) were determined (Table 1). The ME contained relatively high level of total phenolics (256 mg GAE/g extract), which correlate with its appreciable antioxidant potential in 2,2-diphenyl-1-picrylhydrazyl DPPH• radical-scavenging (IC_{50} : 0.58 mg/ml) and superoxide anion radical-scavenging (IC_{50} : 0.87 mg/ml) assays (Table 1). The results of the phytochemical profile of the ME were presented in Table 2. In fact, high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS) analysis of ME resulted in the identification of 10 phenolic compounds that were divided into 8 phenolic acids and 2 flavonoids (Table 2). The compounds identification was carried out by comparing retention times and mass spectra with those of the authentic standards. Phenolic acids constituted the largest group accounting for 97.40% of the total identified compounds, among which the salvianolic acid (1498.77 μ g/g extract) and quinic acid (1066.92 μ g/g extract) were found to be the major compounds.

M. spicata effect on the hematological parameters

Nicotine caused a significant decrease ($p \leq 0.05$) in the levels of erythrocytes, haemoglobin and haematocrit, while a significant increase ($p \leq 0.01$) in white blood cells as compared to the control rats was observed. Interestingly, in (NI+ME) group these parameters were restored to normal levels as compared to nicotine-treated group. Furthermore, no significant differences ($p \geq 0.05$) were observed between (NI+ME)-treated group and control one (Table 3).

M. spicata effect on hepatic dysfunction parameters

Nicotine treatment caused significant increase ($p \leq 0.01$) in ALT, AST, ALP and LDH activities as compared to control rats (Table 4), suggesting hepatocellular damage as a result

of nicotine toxicity. Interestingly, ME administration showed an important protective action in nicotine-treated rats by reducing the hepatic toxicity. Indeed, (NI+ME) treatment significantly decreased ($p \leq 0.01$) ALT and ALP activities as compared to nicotine-treated group and the obtained values were similar to those of the control rats (Table 3).

Evaluation of the antioxidant enzymes activities and lipid peroxidation in liver and erythrocytes

The effect of nicotine and ME administration on lipid peroxidation (Fig. 1) and antioxidant enzymes (Tables 5 and 6) in liver and erythrocytes was investigated. The obtained results showed that the antioxidant enzyme activities such as CAT, SOD and GPX significantly decreased ($p \leq 0.01$) in the liver and erythrocytes of nicotine-treated rats when compared to the normal rats, which proved the development of severe oxidative stress status. Interestingly, ME administration to nicotine-treated rats significantly increased ($p \leq 0.01$) the antioxidant enzymes activities, which indicated an antioxidant status improvement. For example, as compared to the nicotine-treated animals, the SOD activity raised by 2.2 and 2.68 folds in the liver and erythrocytes, respectively. A similar trend was also observed for CAT and GPX activities (Tables 5 and 6).

Fig. 1 also presents the TBARS content in the liver and erythrocytes, which gave an idea about the polyunsaturated fatty acids peroxidation level. As it can be clearly observed, the ME administration to nicotine-treated rats significantly, and the values were comparable to the normal rats in control group. These results suggested the positive effect of ME on improving the rat's antioxidant status by activating its enzymatic antioxidants, and in turn, reducing the lipid peroxidation reactions in vital tissues.

Liver histological examination

The findings relative to ME protective effect on nicotine-treated rats obtained through biochemical assays were further confirmed by liver histological analysis (Fig. 2). Liver of the

normal rats had regular histological structure with a characteristic pattern of hexagonal lobules (Fig. 2A). Accordingly, no histological alterations were observed in the liver of ME-treated rats (Fig. 2C). In contrast, livers of nicotine-treated rats presented slight histopathological injuries such as sinusoidal dilatation and congested central veins (Fig. 2B). Interestingly, the livers of (NI+ME)-treated group showed prominent recovery in the form of hepatic histoarchitecture (Fig. 2D).

Discussion

Phenolic compounds are mainly responsible for the antioxidant properties and several studies were devoted to find natural antioxidants in cheap plant materials. The phytochemical analysis revealed that *M. spicata* aqueous extract was rich in phenolic acids and exhibited an interesting scavenging activity. A survey of the literature shows that most of the identified compounds in ME had potent antioxidant potential. In fact, the IC₅₀ values relative to the DPPH• radical-scavenging activities of compounds 2, 3, 4, 5, 6 and 7 were 0.71, 0.89, 2.43, 0.50, 3.34 and 1.7 µg/ml, respectively (Yokozawa et al. 1998; Chiang et al. 2004; Mishra et al. 2012; Zhao et al. 2013; Kakkar and Bais 2014) (Table 2). It's well known that dietary antioxidants from plants contribute to the defense system against oxidative stress. As a result, they protect cells against oxidative damage and therefore may prevent chronic diseases (Ferrari and Torres 2003). Thus, *M. spicata* consumption would provide antioxidant potential and consequently health benefits.

The decrease in antioxidant enzyme activities (SOD, CAT and GPX) as well as the increase in the lipid peroxidation reactions in the liver and erythrocytes indicated an oxidative stress status in nicotine-treated animals. SOD is responsible for the fast conversion of superoxide radicals to H₂O₂ and is considered as the first enzyme involved in the detoxifying process, while CAT eliminates H₂O₂ by its conversion into H₂O and O₂. The GPX is also considered to be a powerful ROS scavenger by its broader substrate specifications and strong

affinity for H₂O₂ (Pincemail et al. 2002). Reduction in SOD activity in nicotine-exposed animals could be attributed to an enhanced superoxide production during nicotine metabolism (Maurya et al. 2005). The decrease of CAT activity may be a consequence of hydrogen peroxide accumulation (Mertens et al. 1991). The GPX activity reduction in nicotine-treated rats as observed in this study indicated damage in the analyzed tissues (Rikans et al. 1991).

Previous studies reported that nicotine compounds during their cell metabolism generated ROS such as superoxide anion, hydroxyl radical, H₂O₂ and nitric oxide leading to an oxidative stress status (Maurya et al. 2005; Hritcu et al. 2017). An excessive level of lipid peroxidation, expressed by thiobarbituric acid reactive substances (TBARS) accumulation, is now recognized to be a critically important phenomenon resulting from the oxidative stress (Hritcu et al. 2017).

To reduce nicotine toxicity and to protect against nicotine-induced oxidative stress, antioxidant enzymes are produced, since they are considered to be the first line of cellular defense against oxidative damage. In the present study, the significant decrease ($p \leq 0.05$) in the antioxidant enzyme activities (SOD, CAT and GPx) proved the failure of antioxidant defense system to overcome the influx of ROS generated by nicotine-treatment. Thus, the antioxidant enzymes inhibition in nicotine-treated rats could promote lipid peroxidation, altered gene expression and lead to cell death (Halliwell 2006). Interestingly, ME treatment reduced oxidative damage (Fig. 1) and increased antioxidant enzymes levels in liver and erythrocytes tissues (Tables 5 and 6) that indicated that *M. spicata* might attenuate the antioxidant stress status and prevent nicotine-induced lipid peroxidation.

As compared to the control, nicotine treatment caused a significant decrease ($p \leq 0.05$) in the haemoglobin and erythrocytes levels, suggesting haematological disturbance. This status might come in part from the effects of free radicals generated by the nicotine on erythrocytes. In fact, several studies suggested that erythrocytes are particularly vulnerable to

oxidative stress since they are exposed to high concentrations of oxygen and thus can easily be oxidized (Karafakıoglu et al. 2008). Furthermore, nicotine treatment induced a significant increase ($p \leq 0.01$) in white blood cells count that might be indicative of immune system activation and reflected the incidence of tissue edema and inflammation (Maiti et al. 2015). Obtained results were in line with previous reports, which demonstrated that nicotine exposure altered erythropoiesis in rats (WHO 2008). Supplementation of *M. spicata* extract in the diet of nicotine-treated rats restored the erythropoiesis mechanism and enhanced erythropoietine production. In fact, ME protective effect could be explained by its antioxidant potential and also its anti-inflammatory property reported in several previous studies (Farkhani et al. 2007; Arumugam et al. 2008).

Liver is a major site for metabolism of exogenous chemicals, resulting in the formation of metabolites which may be more or less toxic than the parent compound. In the present study, administration of nicotine at 1 mg/kg corporal mass for 2 months induced a significant increase ($p \leq 0.01$) in AST, ALT, ALP and LDH activities, suggesting hepatocellular damage. These findings were in agreement with those reported earlier by others (Karafakıoglu et al. 2009; Azzalini et al. 2010; Ateyya et al. 2016). The observed disorders in nicotine-treated rats may be explained by the oxidative stress damage caused by the nicotine. It was also suggested that hepatic damage may cause this abnormal rise in liver enzymes levels (Asante et al. 2016). Moreover, the biochemical parameters were correlated with the liver histological study that showed some injuries (Fig. 2B), which agree with the previous findings (Chen et al. 2016). Given with nicotine, *M. spicata* extract decreased significantly ($p \leq 0.01$) the liver enzymes levels and provided protection on hepatic histoarchitecture, indicating a potential hepatoprotective effect of this plant. Previous studies reported that nutraceutical benefits of *M. spicata* were attributed to its phenolic compounds (Murad et al. 2016). Many other studies, reported the protective action of some medicinal plants against

liver damage and some authors explained this protective effect by the antioxidant capacity of these plants (Maurya et al. 2005).

Conclusions

It is evident that nicotine induced oxidative stress, which contributes to the activation of various downstream signaling cascade causing structural and functional damages in different tissues. The present study interestingly revealed that *M. spicata* during nicotine treatment provided important protection to the altered hematological parameters, biochemical markers, antioxidant enzymes and limited the extent of liver histopathological injuries. Taking into consideration the demonstrated protective potential, these results encourage further exploration of *M. spicata* in preventing diseases arising from oxidative damage.

Conflicts of interest

The authors declare no conflicts of interest.

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Draft

Table 1. Total phenolics, flavonoids and tannins contents, and antioxidant activity of *M. spicata* aqueous extract.

Parameters	
Total phenolics (mg gallic acid equivalents/g extract)	256.0±2.12
Flavonoids (mg rutin equivalents/g extract)	30.20±1.23
Tannins (mg catechin equivalents/g extract)	9.30±0.45
DPPH• scavenging activity (IC ₅₀ , mg/ml)	0.58±2.03
Superoxide anion scavenging activity (IC ₅₀ , mg/ml)	0.87±1.13

Draft

Table 2. LC-ESI-MS analysis of *M. spicata* aqueous extract and literature review of their DPPH• radical-scavenging presented as IC₅₀ values.

No ^a	Compounds ^b	Molecular formula	Molecular mass	[M-H] ⁻ m/z	RT (min)	Content (µg/g extract)	IC ₅₀ (µg/ml)	References
1	Quinic acid	C ₇ H ₁₂ O ₆	192	191	2.607	1066.92	> 191	Izuta et al. 2009
2	Gallic acid	C ₇ H ₆ O ₅	170	169	3.710	48.28	0.71	Mishra et al. 2012
3	Protocatechuic acid	C ₇ H ₆ O ₄	154	153	5.145	42.79	0.89	Kakkar and Bais 2014
4	Caffeic acid	C ₉ H ₈ O ₄	180	179	7.868	122.39	2.43	Yokozawa et al. 1998
5	Syringic acid	C ₉ H ₁₀ O ₅	198	197	7.737	257.57	0.50	Zhao et al. 2013
6	<i>trans</i> -Ferulic acid	C ₁₀ H ₁₀ O ₄	194	193	10.013	503.48	3.34	Mishra et al. 2012
7	3,4-di- <i>O</i> -Caffeoylquinic acid	C ₂₅ H ₂₄ O ₁₂	516	515	12.659	32.23	1.70	Chiang et al. 2004
8	Salvianolic acid	C ₃₆ H ₃₀ O ₁₆	718	717	12.785	1498.77	94	Zhao et al. 2008
9	Naringenin	C ₁₅ H ₁₂ O ₅	272	271	16.501	18.81	282	Khanduja and Bhardwaj 2003
10	Acacetin	C ₁₆ H ₁₂ O ₅	284	283	31.788	76.45	> 142	Yokozawa et al. 1998

^aThe numbering refers to elution order of compounds from an Aquasil C18 column. ^bIdentification was confirmed using 31 authentic commercial standards.

Table 3. Hematological parameters.

	C	NI	ME	NI+ME
Erythrocytes ($\times 10^6/\mu\text{l}$)	9.86 \pm 0.21	8.32 \pm 0.36*	9.89 \pm 0.55 ⁺	9.12 \pm 0.32 ⁺
White blood cells ($\times 10^3/\mu\text{l}$)	9.23 \pm 1.32	12.02 \pm 0.62**	10.33 \pm 1.36 ⁺⁺	10.36 \pm 0.23 ⁺⁺
Haemoglobin (g/dl)	16.23 \pm 0.23	14.18 \pm 0.53**	17.53 \pm 1.23 ⁺⁺	16.12 \pm 0.53 ⁺⁺
Haematocrit (%)	46.23 \pm 0.59	44.23 \pm 0.23**	47.56 \pm 1.04 ⁺⁺	46.01 \pm 0.13 ⁺⁺

Data represented mean \pm SD ($n = 6$ for each group). C indicated control animals. NI indicated animals injected intraperitoneally with nicotine. ME indicated animals receiving *Mentha spicata* aqueous extract. NI+ME indicated animals receiving nicotine and *Mentha spicata* aqueous extract. (* $p \leq 0.05$, ** $p \leq 0.01$) indicated significant differences as compared to control (C) group. (⁺ $p \leq 0.05$, ⁺⁺ $p \leq 0.01$) indicated significant differences as compared to nicotine-treated (NI) group.

Draft

Table 4. Liver toxicity indices.

	C	NI	ME	NI+ME
AST (U/l)	120.71±5.36	264.72±1.53**	128.0±1.20 ⁺⁺	189.32±5.23 ⁺⁺
ALT (U/l)	146.23±3.02	249.32±5.36**	126.3±2.28 ⁺⁺	125.20±4.32 ⁺⁺
ALP (U/l)	120.02±2.13	184.30±3.20**	103.03±6.32 ⁺⁺	117.0±9.03 ⁺⁺
LDH (U/l)	620.0±3.12	865±3.02**	530.0±3.75 ⁺⁺	759.01±5.36 ⁺⁺

Data represented mean±SD ($n = 6$ for each group). C indicated control animals. NI indicated animals injected intraperitoneally with nicotine. ME indicated animals receiving *Mentha spicata* extract. NI+ME indicated animals receiving nicotine and *Mentha spicata* extract. (** $p \leq 0.01$) indicated significant differences as compared to control (C) group. (⁺⁺ $p \leq 0.01$) indicated significant differences as compared to nicotine-treated (NI) group.

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Table 5. Antioxidant enzyme activities (CAT, SOD and GPX) in liver tissue.

	C	NI	ME	NI+ME
CAT ($\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg}$)	8.23 \pm 0.09	6.31 \pm 0.13**	8.54 \pm 0.35 ⁺⁺	7.89 \pm 0.31 ⁺⁺
SOD (U/mg)	0.91 \pm 0.07	0.45 \pm 0.06**	0.98 \pm 0.08 ⁺⁺	0.99 \pm 0.06 ⁺⁺
GPX ($\mu\text{mol GSH}/\text{min}/\text{mg}$)	0.19 \pm 0.008	0.16 \pm 0.005*	0.19 \pm 0.05 ⁺	0.18 \pm 0.03 ⁺

Data represented mean \pm SD ($n = 6$ for each group). C indicated control animals. NI indicated animals injected intraperitoneally with nicotine. ME indicated animals receiving *Mentha spicata* extract. NI+ME indicated animals receiving nicotine and *Mentha spicata* extract. (* $p \leq 0.05$, ** $p \leq 0.01$) indicated significant differences as compared to control (C) group. (⁺ $p \leq 0.05$, ⁺⁺ $p \leq 0.01$) indicated significant differences as compared to nicotine-treated (NI) group.

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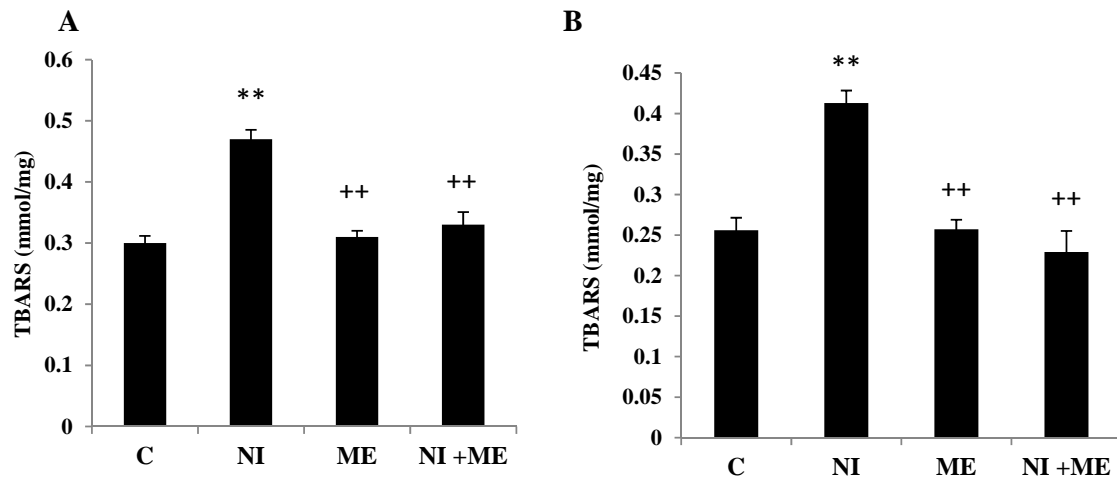
Table 6. Antioxidant enzyme activities (CAT, SOD and GPX) in erythrocytes.

	C	NI	ME	NI+ME
CAT ($\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg}$)	2.89 \pm 0.46	1.62 \pm 0.02*	2.01 \pm 0.03 ⁺	1.89 \pm 0.03 ⁺
SOD (U/mg)	1.37 \pm 0.05	0.41 \pm 0.05**	1.39 \pm 0.01 ⁺⁺	1.10 \pm 0.03 ⁺⁺
GPX ($\mu\text{mol GSH}/\text{min}/\text{mg}$)	0.18 \pm 0.003	0.14 \pm 0.003*	0.19 \pm 0.006 ⁺	0.17 \pm 0.003 ⁺

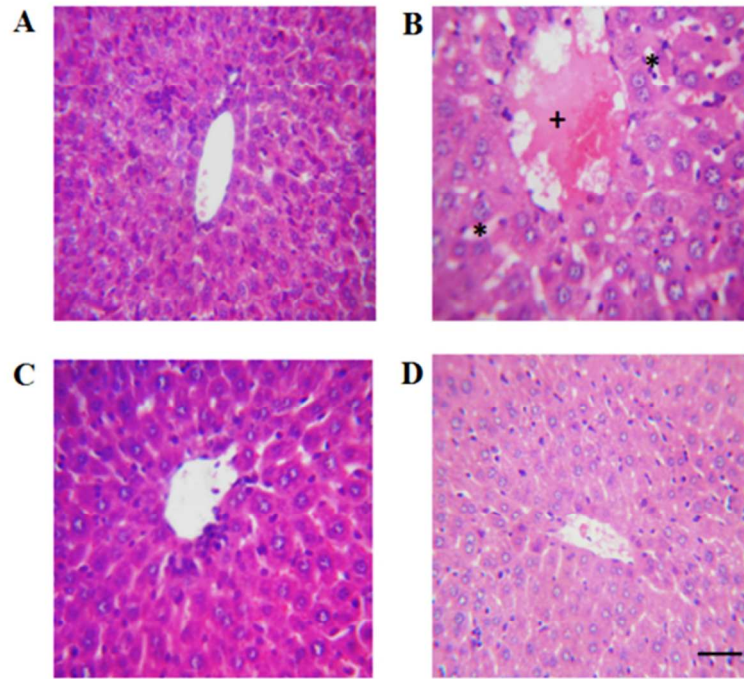
Data represented mean \pm SD ($n = 6$ for each group). C indicated control animals. NI indicated animals injected intraperitoneally with nicotine. ME indicated animals receiving *Mentha spicata* extract. NI+ME indicated animals receiving nicotine and *Mentha spicata* extract. (* $p \leq 0.05$, ** $p \leq 0.01$) indicated significant differences as compared to control (C) group. (⁺ $p \leq 0.05$, ⁺⁺ $p \leq 0.01$) indicated significant differences as compared to nicotine-treated (NI) group.

Draft

Figure 1



Draft



67x50mm (300 x 300 DPI)