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Effect of green tea and nicotine on haematological and biochemical parameters and histopathology of bone marrow and spleen on albino rats

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Abstract: We investigate the effects of green tea extract (GTE) on the attenuation of nicotine hematotoxicity, oxidative stress, inflammation and spleen and bone marrow structural lesions. Rats were treated by injecting nicotine (1,5mg/kg b.w. for 7 weeks) intraperitoneally and thereby supplementing GTE 2% orally to them. Haematological profiles, inflammation markers, neutrophil/lymphocyte (NLR), platelet/lymphocyte (PLR) and mean platelet volume (MPV/Plat) ratios- and erythrocyte sedimentation rate (ESR) were evaluated. Splenic levels of malondialdehyde (MDA), nitric oxide (NO), advanced protein oxidation products (AOPP) and catalase activity were measured. Femur bone and spleen were subjected to histological study. Nicotine-induced haematological abnormalities, a rise in the NLR and MPV/Plat ratios and ESR values with a drop in the PLR values compared to other experimental groups and leads to a significant increase in MDA, NO and AOPP levels-with a decrease in catalase activity compared to control groups. The bone marrow and spleen of nicotine exposed rats showed severe degenerative changes. GTE supplementation attenuates hematotoxicity, induce a decrease in the inflammation markers values, improved the levels of MDA, NO, AOPP and catalase activity and attenuate the adverse histological effects. GTE rich on polyphenols and flavonoids revealed by the *in vitro* study protects against the hazardous effects of nicotine.

Keywords: Nicotine, GTE, inflammation, oxidative stress, bone marrow, spleen.

INTRODUCTION

Cigarette smoking is still considered to be a serious health problem. Smoke habits are associated with large numbers of pathologies such as pulmonary and cardiovascular diseases and cancer (Ding *et al*., 2019). Nicotine, the principal alkaloid in tobacco, is a highly addictive drug, responsible for continuing use of tobacco products (Tutka *et al*, 2005). Several deleterious effects of nicotine both *in vivo* and *in vitro* are related to oxidative stress and inflammation (Yue *et al*., 2022).

Oxidative tissues injuries are the result of unbalance between the oxidant production and antioxidant cell defence system activity/ availability in favour of oxidants. The increase of pro-oxidant status led to lipid peroxidation, protein and DNA oxidation and cellular damage (Helmut *et al*., 2017). In addition, nicotine has been found to disturb the inflammatory response and therefore to damage healthy tissue. Nicotine causes alterations in cellular immunity. Chronic exposure to nicotine alters a wide range of immunological parameters in animal models (Paviaa and Plummer *et al*., 2020). Several previous studies on experimental animals have reported the adverse effects of nicotine on various tissues, including the heart, kidney, lung, liver and spleen (Haroun *et al*., 2017, Yue *et al*., 2022).

The hazardous effects of nicotine on the spleen and redbone have not been fully studied in comparison with other tissues. The spleen is essential to remove the old and damaged blood cells from circulation, metabolize drugs and regulate the response of the immune system to these toxic substances. The bone marrow contains hematopoietic stem cells that give rise to progenitors of different blood cell lineages. Bone marrow and spleen are sensitive targets to cytotoxic drugs (Ferjani *et al*., 2016). Much attention has been paid to the protective effects of natural antioxidants against drug-induced toxicity, mostly if free radicals are involved (Sinem *et al*., 2022)

Tea is a popular beverage, rich in polyphenol compounds, including catechins with potent anti-inflammatory, anti-oxidant and anti-apoptotic effects. The main catechins in green tea are (-)-epigallocatechin, (-)epicatechin gallate, (-)-epicatechin and (-)-epigallocatechin gallate. A positive correlation between the consumption of green tea (*camellia sinensis*) and beneficial antioxidant, anti-inflammatory and anti-carcinogenic effects have been suggested by several studies. The deleterious effects of oxidative stress are counteracted by natural antioxidants defence mechanisms that involve enzyme and non-enzyme detoxification systems (Sarkar *et al*., 2022). Green tea is attracted undergoing clinical trials due to its

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chemo-protector and anti-oxidant properties (Shakeela Begum *et al*., 2017).This present study aims to investigate the ameliorative role of green tea on nicotine-induced hematotoxicity, oxidative stress, inflammation and structural lesions in bone marrow and spleen.

MATERIALS AND METHODS

Study area

This study was performed in Boumerdes University Animal Research Laboratory from March 15, 2017 to June 30, 2018.

Preparation of green tea extract

According to the method described by Haroun *et al*.(2017) , a 2% instant green tea solution was obtained by soaking twenty grams of dry green tea leaves (*C. sinensis*, China, Lipton) in 1L of boiling water for 10 min, the preparation was then filtered . This green tea extract (GTE) was provided to rats as their sole source of drinking water. The collected aqueous extract was then lyophilized (Cryodos 80, −75°C, 5m^3/h) to obtain extract in yield of 0.15%. The extract was stored in sealed glass vials at ±4°C before be tested and analyzed .

Preparation of nicotine

Hydrogen tartrate salt of nicotine purchased from Sigma, N5260-25G was dissolved in 9‰ NaCl solution. The pH of the nicotine solution was adjusted to 7.4 by 0.2M NaOH.

Animals

Twenty-six male adults Wistar rats that weighed 90-120g were housed under conventional laboratory conditions, in stainless-steel cages with 12 h light/dark cycle, food and water were ad *labitum*, the room temperature was maintained at 24°C and the humidity kept constant at 60%. They were acclimatized under laboratory conditions, for one week before experiments, then weighed and randomly divided into four experimental groups. Group I was the control group (C; n=5).Group II was the control tea group (CT; n=5), rats given GTE (2%, w/v) orally. Group III, nicotine-treated rats, (N; n=8) were injected with a subcutaneous single dose of nicotine (1,5mg/kg b.w.). Group IV, nicotine-tea treated rats, (NT; n=8) received a subcutaneous single dose of nicotine (1,5mg/kg b.w.) and GTE (2% w/v) concomitantly. Then, after seven weeks, of treatment, the animals were made to fast for 12h, anaesthetized and then sacrificed by cervical decapitation. Blood samples were taken for haematological and plasma biochemical analyses. The spleens were isolated, washed with saline; part of the spleen was stored at -20°C for biochemical studies. The femur was also excised.

Haematological analyses were performed with a use of an automatic haematological analyser (Sysmex XT1800i (Germany) automatic. The number of white blood cells (WBC), red blood cells (RBC), granulocytes (GRA), platelet (P) haemoglobin concentration (Hb), hematocrit (Ht), Mean platelet volume (MPV) and platelet lymphocyte ratio (PLR), mean platelet volume platelet (MPV/Plat) ratio and Neutrophil lymphocyte ratio (NLR) are determined. Erythrocytes sedimentation rates (ESR) is determined as well, its consists of measuring in a graduated tube the sedimentation of red blood cells in plasma under the influence of gravity.

Methods

Chemical study

Total phenolic content

Total phenolic contents of the extract were determined using Folin-Ciocalteu reagent according to the method of Singleton *et al* (1998), using gallic acid as a standard and as modified by Djouahri *et al.* (2014). An aliquot (0.2mL) of extract solution containing 1000μg of GTE was mixed with 46 mL of distilled water and 1 mL of phenol reagent (Merck, 1090010100) in a volumetric flask. After spending 3min in the dark, 3mL of sodium carbonate solution (7.5%) was added. After shaking and spending an additional 2h in the dark, absorbance at 740 nm was measured in spectrophotometer (Shimadzu1800, Mulgrave, Victoria, Australia). The total phenolic content was evaluated from a standard calibration curve of gallic acid and results were expressed as microgram of gallic acid (GA) equivalents (E) per milligram of extract (μg GAE/mg).

Determination of total flavonoids

The total flavonoids were determined according to the Dowd method described by Djouahri *et al*. (2014). A diluted solution of GTE (4mL) was mixed with 4mL of aluminium trichloride solution (2% in methanol).The absorbance was measured at 415nm after 15 min. The total flavonoids content was evaluated from standard curve of quercetin and the results are expressed as µg QE/mg.

Antioxidant activity: Scavenging effect on DPPH radical

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out as described by Brand-Williams *et al.* (1995) . The GTE was dissolved in methanol. A sample of 25µL of each standard range of increasing concentration of GTA (100,200,400,600,800 and 1000 µg/mL) was added to the DPPH methanol solution (60µM, 975µL). The absorbance at 517nm was measured in UV spectrophotometer (Jaso, V-530) after 30 min of incubation at 25°C. The reference molecules that have been used are ascorbic acid and α-tocopherol. The radical scavenging activity was then evaluated from the equation: % of radical scavenging activity = [($Abs_{control}$-$Abso_{(extra)sample}$)/ Abscontrol]x 100, where $Abs_{control}$ is the absorption of the blank sample and Abs_{sample} is the absorbance of the tested extract.

Biochemical assays

Estimation of malondialdehyde (MDA)

The assay for lipid peroxidation in spleen homogenates was done according to the Heath and Packer method (1968). The MDA levels were measured by evaluating the reaction between MDA and thiobarbituric acid (TBA). About 1 mg of rat spleen was cut into small pieces into lyse buffer and the homogenates were centrifuged at 10000x g for 20 min at 4°C. Six hundred µl of TriChloroAcetic acid (TCA) 20% were mixed with Two hundred forty µL of the supernatants to discard proteins and 500 µL of TBA was added to the supernatant. The mixture was heated at 90°C for 15 min, cooled back on the ice for 5 min and centrifuged at 10000xg for 10 min at 4°C. The absorbance was read at 530 nm. The amount of MDA was expressed as mM of MDA formed per ml.

Catalase activity

The catalase activity was measured by the Claiborne method (1985). The principle is based on the disappearance of hydrogen peroxide in the presence of the enzyme source at 25°C. The reaction mixture (1mL) contained 500μL of phosphate buffer (KH_2PO_4, pH=7 and 0.1 M), 487,5μL of freshly prepared H_2O_2 (0.091 M) and 12,5µl of spleen homogenate. The reaction started by adding H_2O_2 and its decomposition was monitored at 560 nm. The absorbance was recorded two times *t*0 at the start of reaction and after two min.

Determination of Nitrogen Monoxide (NO).

The determination of the formation of NO is estimated indirectly by evaluating the levels of nitrates and nitrites which are the result of the oxidative degradation of NO. The supernatants were deproteinized by centrifugation at 10,000g for 10 minutes at -20°C. The supernatant will be used to evaluate the nitrate and nitrite levels. Nitrite are evaluated only by the Griess reaction. Before quantifying, nitrates should be reduced to nitrites. NO level measured represents the sum of nitrites and nitrates. The conversion of nitrate into nitrite requires reduction by cadmium and regeneration by CuSO4 solution in 5 Mm-NaOH buffer, in contact for 5 min. The nitrite content of all deproteinized and regenerated samples is quantified after addition of Griess reagent [0.1% N-(1 naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 5% phosphoric acid]. The absorbance was read at 543 nm.

Assay of plasma advanced protein oxidation products

The advanced protein oxidation product (AOPP) levels was evaluated by spectrophotometry by the modified Witko-Sarsat method**.** Samples were prepared according to the following way: 200μL samples and then 100μL of 1.16M potassium iodide added to each tube, followed by 200μL acetic acid two minute later. The absorbance of the reaction mixture was immediately estimated at 340 nm against a blank containing 200μL of phosphate buffer saline, 100μL of potassium iodide and 200μL of acetic acid. AOPP levels are expressed as micromoles/L of chloramine-T equivalents.

Protein quantification

Protein content was measured by the method of Bradford *et al*. (1976) using bovine serum albumin as the standard.

Histological study

Bone and portion of spleen tissue were fixed in 10% formalin at room temperature for 2h. Bone was decalcified in a decalcifying liquid which contains 10ml chloroform, 4ml HCL, 3ml glacial acetic acid, 10ml distilled water and 73ml absolute ethanol. The time required for decalcification was 4 weeks and the solution was changed every day. Spleen and bone were dehydrated in graded series of ethanol and embedded in paraffin. The sections were cut at 3 µm thick and stained with Masson's trichrome to be examined with microscope and photomicrographs were taken**.**

STATISTICAL ANALYSIS

Data were expressed as mean±SDS. Statistical analysis was performed with statistica version 6 by one-way ANOVA and by Tukey test. Value of $p<0.05$ was considered statistically significant.

RESULTS

Chemical study

Total phenolics and flavonoids content are given in table 1, GTE showed a high amount of phenolic and flavonoid compounds with values of 196.81 mg GAG/g and 19.75mg QE/g respectively.

Antioxidant activity

The principal antioxidant activity is based on the electrons' ability to neutralize free radicals. In this study, the antioxidant activity was evaluated by using scavenging DPPH free radical assay. The results shown in table 1 revealed that the GTE extract showed potent antiradical activity with an IC50 value of 1.83 ± 0.05µg/mL.

MDA levels

Table 2 indicates that compared to the control group and control-tea groups, the levels of splenic MDA increased significantly in nicotine-treated rats ($p<0.0001$), compared to the other experimental groups. However, MDA levels decreased significantly in rats treated with nicotine and GTE compared to the nicotine group ($p<0.0001$).

Catalase activity

Table 2 shows that the activity of the catalase, an antioxidant enzyme, significantly decreased in the nicotine-submitted group when compared to the control tea group ($p<0.0001$).

Table 1: Total phenolic and flavonoid contents and antioxidant activity of GTE

	Total phenolic content (mg GAE/g)	Total flavonoids (mg QE/g)	DPPH (IC_{50}) (µg/mL)
GTE	196.809 ± 0.5	19.735 ± 0.063	1.83 ± 0.05

Splenic oxidative stress markers

Table 2: Changes of splenic redox states in different experimental groups

Parameters	Control	Control-tea	Nicotine	Nicotine-tea
NO (µmol)	^{a}2.44±0.20	^{b}1.79±0.36$^{***a,\#c,\#d}$	^{c}3.81±0.21$^{\#a,\#b,\#d}$	^{d} 2.96±0.05$^{***a,\#b,\#c}$
MDA (mmol/ml)	^{a}0.12±0.038	^{b}0.076± 0.023	^{c}0.81± 0,11$^{\#a,\#b}$	^{d} 0.27±0.05$^{**a,***b,\#c}$
Catalase (UI min^{-1} mg^{-1} of protein)	^{a}0.35±0.033	^{b}0.67±0.08$^{\#a,\#c,\#d}$	^{c} 0.16±0.03$^{\#a,\#b,\#d}$	^{d}0.29±0.017$^{\#b,\#c}$
AOPP (µmole/L)	^{a}0.27±0.026	^{b}0.19±0.027^{***c,***d}	^{c}0.49±0.12^{***a,b,**d}	^{d}0.34±0.055^{***b,**c}

Values are the mean ± S.D. (a), (b), (c) , (d) correspond respectively to the groups control, control tea, nicotine and nicotine tea.^{*b,*d}p≤ 0.05 compared to control tea and nicotine tea; ^{**a,**c,**d} p<0.01 compared to control, nicotine and nicotine tea; ^{***a,} ^{***b,} ^{***c,} p < 0.001 compared to control, control tea, nicotine; $^{\#a,\#b,\#c,\#d}$p < 0.0001 compared to control, control tea, nicotine and nicotine tea.

Table 3: Haematological parameters in different groups

Parameters	Control	Control-tea	Nicotine	Nicotine-tea
HB(g/dl)	^{a}9.2±0,16	^{b}8.85±0.23	^{c}7.99±0.82^{**a}	7.86±0.54^{**a}
RBC **(**M/µl)	^{a}5.7±0.11	^{b}5.1±0.49^{*c}	^{c}4.20±0.47 ^{**a,*b,*d}	^{d}4.9±0.64^{*c}
WBC **(**K/µl)	^{a}6.26±0.17	^{b}7.32±0.29	8.11±0.74^{***a}	7.26±0.99
HT(%)	^{a}27.74±0.57	^{b}28.36±1.23^{***c}	^{c}20.43±1.38 ^{***a,b,d}	27.28±0.94^{***c}
Lym (K/µl)l	^{a}4.6±0.27	^{b}4.38±0.77	^{c}5.93±0.70^{*a}	^{d}5.61±1.17
PLa(K/µl)	^{a}737±34.43	^{b}749 ± 35.07^{***c}	^{c}411±65.97^{***a,b,d}	^{d}687.28±60.1^{***c}
Neut **(**K/µl)	^{a}3.61±0.14	^{b}3.9±0.47	^{c}5.59±0.18^{***a,b}	^{d}4.23±0.31^{**a,b,c}
MPV	^{a}7.06± 0.38	^{b}6.72±0.28^{**c,**d}	^{c}6.06±0.26^{***a,**b}	6±0.25^{***a,**b}

Values are the mean ± S.D. (a), (b), (c) , (d) correspond respectively to the groups control, control tea, nicotine and nicotine tea.^{*c}p ≤ 0.05 compared to nicotine; ^{**a} p<0.01 compared to control; ^{**b,} ^{**c,**d} p<0.01 compared to control tea, nicotine and nicotine tea; ^{***a,} ^{***b,} ^{***c,} ^{***d}p <0.001 compared to control, control tea, nicotine and nicotine tea

Table 4: Inflammation markers in different groups

Parameters	Control	Control-tea	Nicotine	Nicotine-tea
PLR	^{a} 160.79±13.56	^{b}147.28±9.90 ^{***c}	^{c}69.91±17.22^{***a,b,d}	^{d}127±27.32^{*a,***c}
NLR	^{a}0.71±0.055	^{b}0,71±0.14^{***c}	^{c}0.95±0.089^{***a,b,d}	^{d} 0.81±0.13^{*a,***c}
MPV/PLa	^{a} 0.0093±0.00026	^{b}0.009±0.00058^{**c}	^{c}0.014±0.0026^{**a,**b,***d}	^{d}0.0087±0.00071^{***c}
ESR1 (mm in^{1st}H)	^{a}1.8±0.44	^{b}1.79±0.45$^{\#c}$	^{c}4.88±1.95$^{\#a,\#b,\#d}$	^{d}1.75±0.88$^{\#c}$
ESR2 (mm in 2ndH)	^{a}3.4±1.14	^{b}3±0.71^{***c}	^{c}9.38±2.72^{***a,***b,***d}	^{d}3±1.3^{***c}

Values are the mean ± S.D. (a), (b), (c) , (d) correspond respectively to the groups control, control tea, nicotine and nicotine tea.^{*a}p ≤ 0,05 compared to control; ^{**b} p < 0.01 compared to control tea; ^{***a,} ^{***b,} ^{***c,} ^{***d}p<0.001 compared to control, control tea, nicotine and nicotine tea $^{\#b,\#c,\#d}$p < 0.0001 compared to control tea, nicotine and nicotine tea

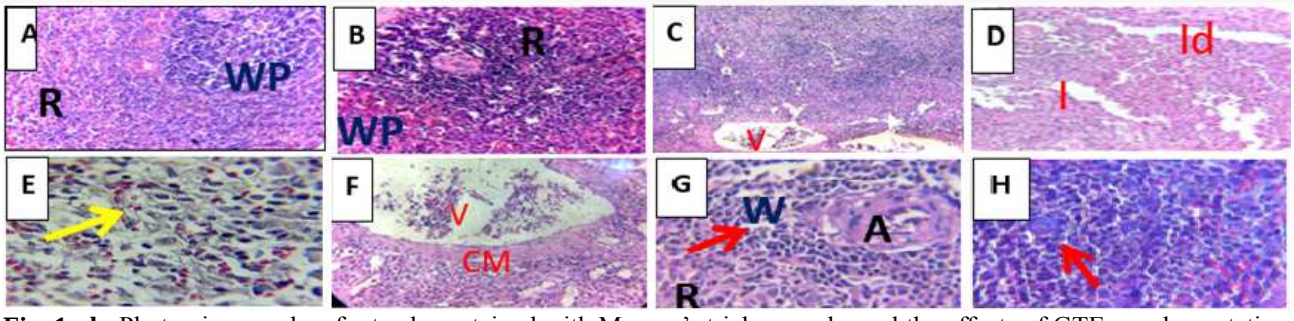

Fig. 1a-h: Photomicrographs of rat spleen stained with Masson's trichrome showed the effects of GTE supplementation after nicotine treatment**).**

(A) control groups (10x) normal structure with visible delineation of spleen pulps, the red pulp (RP) and white pulp (WP). (B) control tea group (200x) (2% w/v, oral for 2 months) presents no anomally. (C,4x), (D, 10x), (E, 100x), (F, 10x), the nicotine-treated group showed an undefined boundary between pulps, interstitial oedema (Id), accumulation of conjunctive material (CM) around large vessels (V) stuffed with cells, splenocytes exhibiting the classical morphology characteristics of apoptosis or necrosis: a reduction in nuclear size, chromatine condensation and nucleolus cap appearance (yellow arrow). (G,100x , (H,x 100) rats treated with nicotine (1,5mg/kg, i.p. for 7 weeks) and GTE (2% w/v oral for 7 weeks) showing the formation of lymph nodule around arteriole (A) with less oedematous space in the spleen (red arrow).

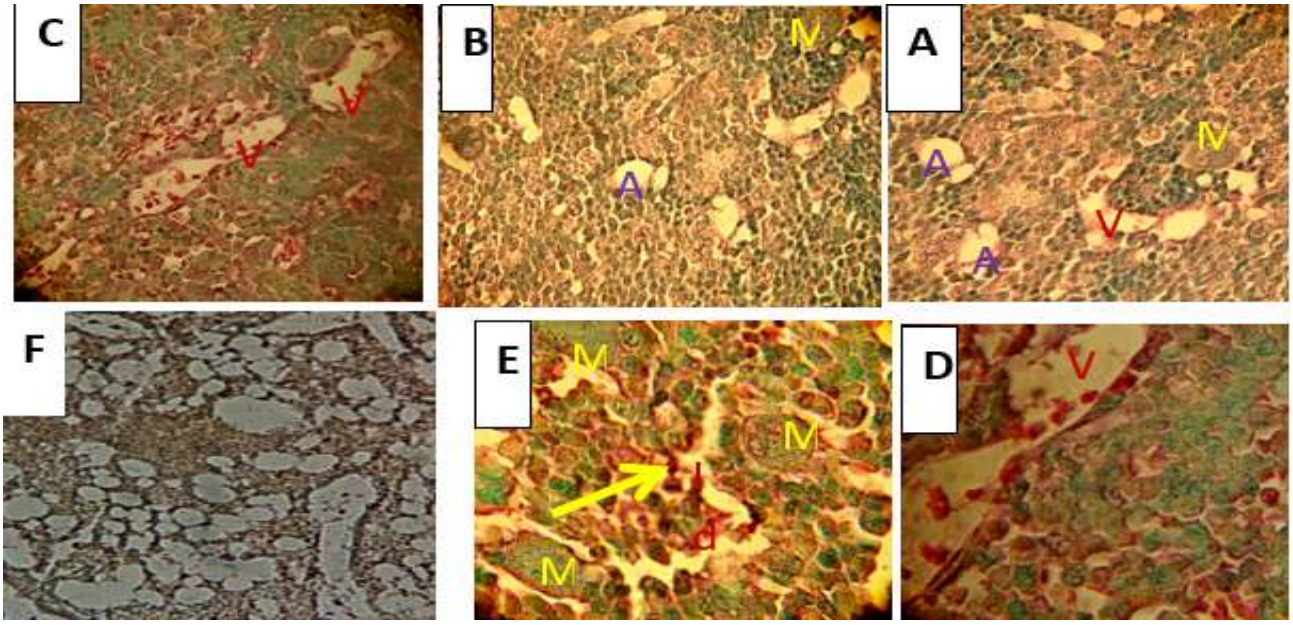

Fig. 2a-f: Photomicrograph of bone marrow rats stained with Masson's trichrome
(A,100x) control rat showing normal bone structure is composed of hematopoietic cells (a megakaryocyte (M) is visible and adipose (A) and blood vessel (V); (B)section from control tea, showing healthy marrow (C,100x) (D ,200x), (E,200x) bone sections from nicotine-treated animals (1mg/kg, i.p. for 2 months) showing the presence of the disorganized and irregular hematopoietic cells and interstitial edema (Id). The hematopoietic cells exhibiting the classical morphology characteristics of apoptosis or necrosis: a reduction in nuclear size, chromatin condensation and nucleolus cap appearance (yellow arrow); section (F,100x) from rats treated with nicotine (1,5mg/kg, *i.p.* for 7 weeks*)* and GTE (2% w/v oral for 7 weeks) a significant structural amelioration is observed.

A significant increase of the catalase activity in the control-tea group was observed compared to the control group ($p<0.0001$). The administration of GTE to the nicotine-treated group increased significantly ($p<0.0001$) the catalase activity compared to the nicotine group.

NO levels

The estimation of the oxidative and inflammatory marker, NO, as shown in table 2 revealed a significant increase in NO levels ($p<0.0001$) in nicotine-treated rats when compared with control and control tea. A significant decrease of splenic NO levels ($p<0.001$) was observed in control tea, compared to control. The GTE co-treatment in nicotine-treated rats induced a significant decrease ($p< 0.0001$) in NO levels compared to nicotine-treated rats.

Advanced protein oxidation products levels

The table 2 shows that the evaluation of advanced protein oxidation product (AOPP) levels revealed a significant increase in a nicotine-submitted group compared to control and control tea groups ($p<0.001$). The treatment of rats submitted to nicotine with GTE reduced significantly AOPP levels ($p<0.01$), when compared to nicotine-treated rats.

Hematological parameters

The haematological profiles of all animals were analyzed as shown in table 3. The results revealed a significant increase of neutrophil counts in samples of the nicotine-submitted group, compared to control and control-tea groups ($p<0.001$). Lymphocyte count showed a significant increase in nicotine-treated rats ($p\leq0.05$) compared to control. However, the RBC count significantly decreased in nicotine-treated rats, compared to control and control-tea groups ($p <0.01$; $p\leq0.05$ respectively). Likewise, this decrease also affects hematocrit, mean platelet volume (MPV) and the platelet count ($p<0.001$). Supplementation with GTE attenuated abnormalities in hematological parameters compared to nicotine-treated rats ($p<0.001$; $p<0.01$; $p\leq0.05$).

Inflammation markers

Table 4 shows that NLR and MPV/PLat ratios were significantly higher in nicotine-treated rats compared to control and control tea ($p<0.001$;$p<0.01$), but the PLR value was significantly lower compared to control and control tea ($p<0.001$). Likewise, the values of ESR in 1st hour and the 2nd hour were significantly higher in a nicotine-submitted group compared to control and control tea ($p<0.0001$; $p<0.001$ respectively). The treatment of rats submitted to nicotine with GTE reduced significantly NLR and MPV/PLat ratios compared to nicotine treated group ($p<0.001$), this decline also affects the ESR1 and ESR2 ($p<0$, 0001, $p<0,001$). However, PLR values increased significantly in the nicotine tea group compared to the nicotine group ($p<0.001$).

Histological results

Histological changes in spleen structure

Spleen sections of control and control-tea rats present normal structures. The white and red pulps are well-delineated as shown in fig.1A-B. The spleenic structures of the nicotine-treated group appeared less organized than that of the controls. It's difficult to identify the white pulps regions. Interstitial edema and large vessels surrounded with conjunctive material are observed through the spleen sections as shown in fig.1C-F). Splenocytes exhibited the classical morphology characteristics of apoptosis or necrosis: a reduction in nuclear size, chromatin condensation and the nucleolus cap appearance. Fig.1g-H show that the GTE co-treatment in nicotine-treated rats markedly reduced the degree of tissue injuries. Effective spleen lymph nodule proliferation and regenerative changes were observed.

Footnote: (A) control groups (10x) normal structure with visible delineation of spleen pulps, the red pulp (RP) and white pulp (WP). (B) control tea group (200x) (2% w/v, oral for 2 months) presents no anomaly. (C,4x), (D, 10x), (E, 100x), (F, 10 x), the nicotine-treated group showed an undefined boundary between pulps, interstitial edema (Id), accumulation of conjunctive material (CM) around large vessels (V) stuffed with cells, splenocytes exhibiting the classical morphology characteristics of apoptosis or necrosis: a reduction in nuclear size, chromatin condensation and nucleolus cap appearance (yellow arrow). (G,100x , (H,100x) rats treated with nicotine (1,5mg/kg, i.p. for 7 weeks) and GTE (2% w/v oral for 7 weeks) showing the formation of lymph nodule around arteriole (A) with less edematous space in the spleen (red arrow).

Histological changes in bone marrow structure

The histological photomicrographs of the bone marrow of control and control-tea are shown in fig 2-A-B), normal tissues structures, the bone reflects a good state of hematopoiesis, blood cells, megakaryocytes, blood vessel and adipocytes are visible. Bone marrow sections of nicotine treated group are shown in fig 2C-E revealed the presence of the disorganized and irregular hematopoietic cells and interstitial edema. The GTE co-treatment in nicotine-treated rats markedly reduced the degree of tissue lesions as shown in fig. 2F.

DISCUSSION

Total phenolic and flavonoid contents and antioxidant activity of GTE

In our *in vitro* studies, total phenolic and flavonoid contents were determined according to their importance as antioxidant compounds. That chemopreventive propriety of green tea has long been associated with total phenol content. The free radical scavenging activity of GTE was assessed using DDPH. The value of IC_{50} was used in the *in vitro* study. This potential antioxidant activity could be explained by the presence of the phenolic compounds related to different mechanisms, such as free radical scavenging, hydrogen donation, singlet oxygen quenching and metal ion-chelation and acting as substrates for radicals such as superoxide and hydroxyl (Kim *et al*., 2014, Sarkar *et al*., 2022).

Splenic oxidative stress markers

The present study was carried out to investigate the effects of GTE on the adverse effects of nicotine in the spleen and bone marrow of male adult's rats. Increased level of splenic NO and MDA of the nicotine-treated group compared to the other experimental group was observed in the present study. NO is an important intercellular mediator that reacts with superoxide anion to generate peroxynitrite, a potent oxidant that interacts with numerous biological molecules, thereby damaging them. MDA is a stable product of free-radical species that attack lipid components, leading to lipid peroxidation. From our data, splenic MDA levels were significantly higher in nicotine-treated groups than in controls. The evaluation of splenic protein oxidation levels shows an increase in AOPP levels in nicotine-treated rats compared to the others groups. Ahmed *et al*. (2014) have reported that nicotine induces a significant elevation in MDA and NO in spleen tissue. Green tea polyphenol acts as free radicals scavengers that decrease lipid peroxidation and protein oxidation (Haroun *et al*., 2017). The spleen catalase assay indicated that nicotine administration induced a significant decrease in the catalase activity after 7 weeks of nicotine treatment compared to the other groups. However, the excessive oxidation of proteins may lead to structural and functional alterations of many enzyme proteins. Supplementation with GTE enhanced the activity of splenic scavenging enzymes to some extent. Nicotine altered the cellular redox state through the generation of oxidant free radicals and the reduction of the antioxidant cell defense systems (Yue *et al*., 2022). GTE potentiate endogenous antioxidant enzyme activities. This result is similar to that obtained by Haroun *et al*. (2017).

Haematological parameters

Smoking is well-known as hematotoxicants; it is associated with various hematological abnormalities, such as leukocytosis, erythrocytosis and increased hemoglobin concentration and hematocrit (Shakeela Begum *et al*, 2017). Nicotine also alters the mechanisms that regulate blood cell formation. In our results, the administration of nicotine led to a significant fall in RBCs count and hematocrit. The reduction in hemoglobin level can be due to a suppressive effect of nicotine on erythropoiesis or the result of hemolysis, which was caused by excessive destruction of erythrocytes. Cigarette smoke, cotinine and nicotine altered erythrocyte membrane stability through the oxidation of proteins membrane-SH groups which is

greater than other tissues this is what leads to hemolysis (Burgara-Estrella *et al*, 2020). Nicotine had striking effects on the white blood cell count and platelets. This was probably caused by an intensive mobilization of cells from the bone marrow compartment. Hematological studies revealed that GTE co-treatment could enhance the rate of recovery from hematopoietic depression, especially the RBCs and platelet count restoration. The improvement in most hematological parameters after GTE administration might be related to the strong antioxidant effect of green tea polyphenols on hematopoietic cells that is vulnerable to oxidants *in vivo* (Shakeela Begum *et al*, 2017).

Inflammation markers

The NLR, PLR and MPV/PLat have been used as an indicator of inflammation and have been considered as prognostic markers of systemic diseases. In this study, it may be concluded that NLR and MPV/Plt ratios increase after nicotine administration. Gumus *et al* (2020) have shown that these ratios increase in smokers according to the number of cigarettes smoked and smoking duration. It is well-known that it is nicotine-induced oxidative stress that activates acute systemic inflammation (Zhang *et al.*, 2022) Moreover, inflammatory cells such as neutrophils and lymphocytes produce reactive oxygen species, which increases the number of harmful substances.

From this study, it may be concluded that nicotine increases ESR. This confirms the existence of inflammatory changes. This change has also been recognized by Gitte and Taklikar (2018) in male adult smokers. The administration of GTE in nicotine-treated groups reversed-this abnormal alteration of hematological parameters and inflammation markers. The immunostimmulation and immunosuppression of green tea both need to be tackled to regulate Immunological functioning. Immunomodulatory properties of GTE are caused by the synergistic effects of phytoconstituents, such as phenolic and flavonoids (Azambuja *et al*., 2021, Samynathan *et al*., 2021).

Histological changes in spleen and bone marrow structure

The histological examination of spleen and bone marrow sections revealed that nicotine-treated groups exhibited severe tissues injuries leading to congestion, edema, loss of splenocytes and hematopoietic cells. Improvement in the morphology of the spleen and bone marrow is observed in the nicotine-tea group. Bone disorders of cigarette smoke compounds have been observed in animal experiments and *in vitro* assays (Kaastrup and Grønbæk, 2021). *In vivo* exposure to cigarette smoke and *in vitro* treatment of long-term bone marrow cultures with nicotine result in inhibition of hematopoiesis. Nicotine treatment delayed the onset of hematopoietic foci and reduced their size (Cool *et al*., 2022). Cotinine, a metabolite of nicotine reduced the growth of human hematopoietic progenitor cells to a concentration range equivalent to its serum levels in smokers. Green tea catechin, on the other hand, can stimulate the proliferation of hematopoietic progenitor cells in mice (Shimura *et al.*, 2019). Diniz *et al*. (2013) reported the same morphological changes in the spleens of newborn mice exposed to cigarette smoke during pregnancy. Ahmed *et al*. (2014) found that in splenic tissues, nicotine significantly decreases the protein levels and mARN expression of P53, a transcription factor that promotes cell cycle progression or apoptosis and increases the protein levels of Bcl2. According to the same authors, the changes in spleen antioxidant activities following nicotine administration could be the result of altered gene expression. In this study, GTE provides mitigation against nicotine lesions.

The results obtained from the current study revealed that the supplementation with GTE conferred some protection against oxidative damages, hematotoxicity, inflammation and bone marrow and spleen lesions induced by nicotine. Further investigations are essential to elucidate the precise mechanism of GTE protection against nicotine.

CONCLUSION

Cigarette smoking is important risk factor in the induction and progression of diseases, nicotine is the potent addictive drogue in tobacco smoke. Nicotine induces oxidative stress, inflammation and organs lesions. Green tea antioxidants are more potent than those found in many fruits and vegetables. The beneficial health effects of tea has attracted attention of researchers**.** Supplementation with tea beverage on may be protect against nicotine toxicity. Our study purpose to examine if green tea extract may provide mitigation against nicotine injury**.** This study confirm that green tea attenuate haematotoxicity, oxidative stress, inflammation and spleen end bone marrow structural lesions induced by nicotine. This work will help scientists to elucidate new mechanisms of green tea protection against nicotine, it should be deepened, since tea is the second drink consumed in the world and humans are exposed to passive and active smoking.

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