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Research article

Protective effect of Resveratrol against nephrotoxicity of nicotine in male rat: Antioxidant and histopathological approaches

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Key words: Nicotine, Resveratrol, Kidney, Oxidative stress, Histopathology.

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Abstract

Background and Objective: The deleterious health effects of cigarette smoking are remaining the most important convertible risk factor for improving overall mortality. In addition to being a risk factor for kidney disease, there is strong evidence that demonstrating a role for cigarette smoking in the progression of chronic kidney disease (CKD). Nicotine can freely cross the biological membranes of body organs. Resveratrol is a compound which is a member of plant compounds called polyphenols which possess a free radical scavenger. The study aimed to evaluate the influence of Resveratrol (Res) in rat with Nicotine (Nic) induced nephrotoxicity, by evaluating the biochemical and histopathological changes. **Methodology:** The experiment was carried out on four groups of male rats as follows; The first group served as a control group, 2nd group was treated with Nic (2.5 mg kg^{-1}) dissolved in physiological saline, 3rd group was received Res (20 mg Kg^{-1}), 4th group was treated with combination of Nic (2.5 mg Kg^{-1}) and Res (20 mg Kg^{-1}). **Results:** The results indicated that Nic increased Uric acid, Urea, Creatinine, lipid peroxidation, TOS level and decrease the antioxidant enzymes as well as decrease the thiol and G6PD levels. A serious congestion, correlated with kidney was observed in Nic treatment and the role of Res in improving all the oxidative damage and the histopathological changes in kidney. In the light of the present results, it is evident that Res was able to reduce the nephrotoxic effect of Nic in male rats.

Introduction

The use of tobacco products is the leading preventable cause of death in the world today, resulting in nearly six million deaths annually and assisting in the manifestation of many other diseases [1]. In addition, the economic damages caused by tobacco smoking in the US alone over the past four decades could exceed $7 trillion USD. Existing therapeutic treatments have proven to be more and more ineffective at assisting those with the desire to quit [2] and, if current trends continue, more than 1 billion people may die from smoking related diseases in the 21st century [1].

Nicotine is the main addictive compound found in tobacco products [3]. It has been shown to induce rewarding behavior through stimulation of brain mesolimbic dopamine neurons [4], prompting addiction and directly instigating the withdrawal symptoms experienced by those trying to quit [5].

Resveratrol is a polyphenolic phytoalexin that naturally occurs in many plants, including grapevines and berries, and exhibits pharmacologic health benefits including antioxidants [6], antimutagenic [7], anti-inflammatory [8], anticancer [9] and cardioprotective properties [10].

Resveratrol is a natural polyphenol that has been found in several plants, such as grapevines, cranberries and peanuts. This polyphenol exerts many physiological effects in mammals, including anti-inflammatory, anti-oxidant activities [11]. The antioxidant activities of reservatrol have been widely studied and there are apparently several mechanisms of activity.

Resveratrol decreases the intracellular and extracellular production of reactive oxygen and nitrogen species [12]. Resveratrol elicits inhibitory effects at all stages of the inflammatory response: it modifies the activity of inflammatory cells by inhibiting production of reactive oxygen and nitrogen species; it regulates enzyme expression, and it decreases the activity of several enzymes involved in the synthesis of proinflammatory

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mediators [13]. This polyphenol has effects on cell signaling pathways involved in inflammation.
It was imperative to investigate the effects of nicotine administration and these interactions with resveratrol supplementation.

Materials and methods

Chemicals
All reagents were of the highest purity available. Nicotine hydrogen bitartrate was obtained from Sigma Chemical Company, St.- Louis, Missouri, USA. All other chemicals and reagents used were of analytical grade. Other chemicals for measurements were purchased from Sigma Chemical Company, St. Louis, MO, USA, trans-Resveratrol (>98% purity) was obtained from Cayman Chemical Company (Ann Arbor, MI, USA).

Animals
Male albino Wistar rats, of 8 weeks of age and weighing about 150–180 g, were obtained from the king Fahad center for scientific purposes - King abdulaziz university, Jeddah , Saudi Arabia. Animals were housed into a polypropylene cage and provided with food and water ad libitum. Before beginning the experiment, all animals were acclimated for 2 weeks under well-controlled conditions of temperature (22- 25ºC) and a 12/12 h light-dark cycle. Animals were fed standard pellet diet.

Routes of administration and Experimental design
The dosages of the two chemicals were determined according to Kalpana and Menon [14] and Oktem *et al.* [15]. Rats were randomly distributed into four groups (n=10). And received their respective intraperitoneally (i.p) treatment for 4 weeks for sub-chronic study as follows:1stgroup served as non-treated control group which received physiological saline (NaCl 9%) (1 ml kg^{-1}), 2nd group treated with Nic (2.5 mg kg -1) dissolved in physiological saline, 3rd group treated with Res in a dose of (20 mg Kg-1).Finally , 4th group was treated with Nic plus Res at the same doses.

Blood collection
Blood samples of the fasted rats were collected from the medial retro-orbital venous plexus immediately with capillary tubes (Micro Hematocrit Capillaries, Mucaps, Jiangsu, China (Mainland) under ether anesthesia Boussarie [16]. Then, the blood was centrifuged at 1000 xg for 15 min and serum was collected for different biochemical analyses.

Tissue preparation
A kidney tissue (about 0.25 g) was used for the analysis of oxidative stress parameters.
Prior to dissection, tissue was perfused with a 50 mM (sodium phosphate buffer saline (100 mM Na_2HPO_4 /NaH_2PO_4) (pH 7.4) and 0.1 m ethylenediamine tetraacetic acid (EDTA) to remove any red blood cells and clots. Then, tissues were homogenized in 5 mL cold buffer per gram tissue and centrifuged at 2700 xg for 1hr. The resulting supernatant was transferred into Eppendorf tubes and preserved in a deep freeze until used for various oxidative assays.

Total antioxidant status assay (TAS)
Total antioxidant status (TAS) was measured in kidney using a commercially available kit from Rel Assay Diagnostics (Gaziantep, Turkey) Erel [17]. The method was based on the reduction of colored 2,20-azino-bis(3-ethylbenzotiazoline-6-sulfonic acid) (ABTS) radical to a colorless reduced form by antioxidants present in the sample. Absorbance was measured spectrophotometrically at a wavelength of 660 nm. Data were expressed as mmol Trolox equivalent (eq.) per liter (mmol Trolox eq./L).

Total oxidant status assay (TOS)
Total antioxidant status (TOS) was measured in kidney using a commercially available kit from Rel Assay Diagnostics Erel [18]. The method was based on the principle that the oxidants in the sample oxidized ferrous ions, previously bounded to a chelator, to ferric ions. The color intensity was measured spectrophotometrically at a wavelength of 530 nm and the results were expressed as l mol H_2O_2 eq./L.

Determination of oxidative and antioxidant parameter
Lipid peroxidation was determined by the procedure of Esterbauer and Cheeseman [19]. Tissue supernatant thiobarbituric acid-reactive substances (TBARS) were measured at 532 nm by using 2-thiobarbituric acid (2,6-dihydroxypyrimidine-2-thiol; TBA)MDA concentrations were determined using 1,1,3,3-tetraethoxypropane as standard and expressed as µmol/g tissue.
Superoxide dismutase (SOD) activity was measured according to the method described by Marklund and Marklund [20] by assaying the auto oxidation of pyrogallol at 440 nm for 3 min. One unit of SOD activity was calculated as the amount of protein that caused 50% pyrogallol autooxidation inhibition. A blank without homogenate was used as a control for non-enzymatic oxidation of pyrogallol in Tris–EDTA buffer (50 Mm Tris, 10 mM EDTA, pH 8.2). The SOD activity is expressed as U/mg protein.
Catalase (CAT) activity was measured, determined according to the method described by Aebi [21] by assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25˚C. Before determination of the CAT activity, samples

were diluted 1:9 with 1% (v/v) Triton X-100. CAT activity is expressed as mmol/mg protein.

Glutathione peroxidase (GPx) activity was determined by the assay described by Hafeman *et al.* [22]. The peroxide substrate (ROOH), GRx and NADPH are included in the reaction mixture. The formation of the oxidized form of glutathione (GSSG) that is catalyzed by GPx is coupled to the recycling of GSSG back to the reduced form of glutathione (GSH) using GRx. NADPH is oxidized to $NADP^+$. The change in A 340 due to NADPH oxidation is monitored and is indicative of GPx activity. The activity of GPx was expressed in terms of mmol GSH consumed/min/g wet weight tissue

Total antioxidant capacity was determined using ferric reducing antioxidant power assay according to the method of Prieto *et al.* [23]. FRAP reagent (300 mM acetate buffer, pH 3.6, 10 mM 2,4,6-Tri (Z-pyridy)-S (riazine, 99 %) (TPTZ) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the ratio of 10:1:1 was prepared. 1.5 mL FRAP reagent was added to 50 µL of homogenated kidney tissues and incubated at 37 ºC for exactly 5 min. The change in absorbance was measured at 593 nm due to the formation of a blue colored FeII-tripyridyltriazine complex from the colorless oxidized Fe^{III} form by the action of electron donating antioxidants. The absorbance of the sample was read against reagent blank (1.5 mL FRAP reagent and 50µL distilled water) at 593 nm.

Xanthine oxidase (XO) activity was assayed spectrophotometrically by the reaction of enzyme with xanthine, as a substrate, and the absorbance was measured at 650 nm, according to the method described in Kamel and Hamed, [24]. In two test tubes, 15 µl phosphate buffer (0.1 M, 7.4 pH) and 30 µl water (control) or substrate (test; xanthine, 38 mM, dissolved in distilled water by adding alkali and gentle heating) was added to 0.5 ml homogenates (Kidney) and incubated for 40 min at 37 °C. Then, 0.1 ml of this solution was mixed with 0.1 ml Na-tungstate 40%, 0.5 ml water and 0.1 ml 2N H_2SO4 complete to 1 ml and incubated for 1 h at 37°C then centrifuged at 3000 rpm for 10 min. 0.15 ml of supernatant was mixed with 0.75 ml water, 0.3 ml Foline reagent and 1.5 ml saturated Na-carbonate solution. The absorbance was measured at 650 nm after 10 min. XO activity was determined using the following equations: Concentration of xanthine in control or test = A (sample) /A (standard) × concentration of standard × 48. Xanthine oxidase activity (U/g tissue) = concentration of test concentration of control/0.284.

Determination of glucose-6 phosphate dehydrogenase (G6PD)

Tissue glucose-6-phosphate dehydrogenase (G6PD) activities were measured in kidney homogenates using the methods described by Deutsch Aebi [21]. The results were expressed as U/g protein.

Determination of non enzymatic antioxidant (Thiol level)

Total thiols level was determined according to Hu [25]. An aliquot of kidney homogenate (50 mL) was mixed with 1 mL of Tris base (0.25 M) – EDTA (20 mM) buffer, pH 8.2, and the absorbance at 412 nm was determined. To this was added 20 mL of 10 mM DTNB. After 15 min at ambient temperature, the absorbance was measured again with DTNB blank. Results are expressed in [mM].

Histological evaluation

For histological examination, a portion of the kidney was fixed in 10% neutral buffered formalin embedded in paraffin, sectioned and stained with Haematoxylin and Eosin as described by Gabe [26]. Then semi-thin sections (0.5-1 microns) were prepared by using LKB ultra microtome. The sections were stained with toluidine blue, examined with a light microscope and photographed.

Results

As shown in table 1 uric acid, creatinine and urea were significantly increased ($p< 0.05$) in Nic treated group alone. Treating the rats with the Res afforded non-significant changes in uric acid, urea and creatinine levels as compared to normal control group. All kidney function parameters had depressed significantly ($p < 0.01$) in the kidney tissues of Nic/Res treated group as compared to Nic treated group by 49, 53.75, and 44.34% in uric acid, creatinine and urea respectively.

Table 1. Effect of Nicotine and/or Resveratrol on some kidney functions in male rats

Nic: Nicotine; Res: Resveratrol, Values are expressed as means SE; n = 10 in each treatment group.

Significant differences are arranged alphabetically from higher to lower.

Parameters	Uric acid (mg)	Creatinine (mg)	Urea (mg)
Control group	22.09±1.25^{cd}	0.48±0.98^{d}	18.01±3.65^{c}
Nic group	68.04±1.02^{a}	2.66±1.65^{a}	44.29±2.52^{a}
Res group	21.11±2.35^{d}	0.59±2.24^{cd}	17.84±2.14^{c}
Nic +Res group	34.70±1.98^{b}	1.23±1.02^{b}	24.65±2.69^{b}

All the parameters of antioxidant enzymes (SOD, CAT, and GPx) were decreased when the male rats were exposed to the dose of Nic (Table 2). Administration of Res to the Nic-treated group with dose (2.5 mg Kg-1) had restored SOD, GPx, and CAT significantly ($p < 0.05$) as compared to Nic treated group alone. Treating the male rats with the Nic decreased the activity of SOD that has been increased again when Nic group has been treated with Res for 4 weeks. The activity of CAT had depressed significantly ($p < 0.01$) in the renal tissues of Nic treated group as compared to control group as shown in table 2.

The same observation has been noticed in the GPx activity that decreased by Nic treated group and increased by the treatment of the rat with Res after Nic.

To investigate the oxidative stress in renal tissues, Table 2 represents the levels of MDA (index of lipid peroxidation) in normal and treated rats. It was found that MDA levels in Nic treated rat were increased in renal tissues by approximately 57.40 -fold as compared to control after 4 weeks of treatment with Nic. The values of TAC were 94 %, 73.80%, 93.84% and 83.20 % for control, Nic, Res and Nic and Res respectively table 2.

Table 2 showed that XO activity was increased significantly in Nic treated group by 11.17-fold as compared to control group. Meanwhile, combination of Res with Nic afforded a significant decrease in XO activity as compared to Nic treated group and elicited a significant increase as compared to normal control group.

Data presented in (Table.3) showed that treatment of the male rat with Nic caused a significant decrease in the level of TAS. It decreased by1.07 fold as compared to control group. However, the concurrent administration of Res to the rat treated with Nic significantly ($p < 0.001$) increased the level of TOS as compared to its related group. But the levels were restored to near to normal values in group treated with Nic and Res respectively in both parameters (TAS and TOS) levels.

Thiol and G6PD levels were decreased by 3.08 -folds in Nic treated rat (Table. 4). Co-administration of Res with Nic practically prevented the changes in G6PD levels. Thiol level was decreased by 29.82% in Nic treated group as compared to control group and the thiol value was increased in Res treated group and Nic/Res treated group as compared to Nic treated group.

Table 2. Effect of Nicotine and/ or Resveratrol on some antioxidant /oxidative stress parameters in kidney of male rat

Parameters	SOD (U g^{-1})	CAT (U g^{-1})	GPx (U g^{-1})	MDA (U g^{-1})	TCA %	XO (U g^{-1})
Control group	15.04±1.25^{ab}	15.80±0.98^{b}	32.52±3.65^{a}	2.21±0.25^{d}	94±2.36^{a}	4.35±0.68^{d}
Nic group	5.27±1.02^{d}	6.27±1.65^{d}	16.52±2.52^{c}	59.61±5.35^{a}	73.80±2.14^{c}	15.52±1.25^{a}
Res group	13.36±2.35^{b}	17.06±2.24^{ab}	32.50±2.14^{a}	4.58±1.25^{cd}	93.84±1.69^{a}	5.03±0.69^{cd}
Nic +Res group	9.60±1.98^{c}	10.83±1.02^{c}	26.45±2.69^{b}	27.70±3.25^{b}	83.20±1.65^{b}	9.50±1.25^{b}

Table 3. Effect of Nicotine and/ or Resveratrol on TAS and TOS levels in kidney of male rat

Parameters	TAS (mmol Trolox eq./L)	TOS (µmol H_2O_2 eq./L)
Control group	1.62±0.98^{ab}	0.67±0.05^{cd}
Nic group	0.548±0.78^{d}	2.13±0.52^{a}
Res group	1.57±0.78^{b}	0.524±0.02^{d}
Nic +Res group	1.09±0.88^{c}	1.30±0.38^{b}

Nic: Nicotine; Res: Resveratrol; SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde; XO: xanthine oxidase; NO: nitrite; TAC: total antioxidant capacity. Values are expressed as means SE; n = 10 in each treatment group.

Significant differences are arranged alphabetically from higher to lower

Table 4. Effect of Nicotine and/ or Resveratrol on Thiol (SH) (mmole/ml) and G6PD (U/g) levels in kidney of male rat

Parameters	Thiol (mmole/ml)	G6PD (U/g)
Control group	0.57±0.05^{a}	4.19±0.14^{a}
Nic group	0.40±0.09^{c}	1.11±0.01^{c}
Res group	0.60±0.04^{a}	4.15±0.23^{a}
Nic +Res group	0.48±0.03^{b}	3.43±0.18^{b}

Nic: Nicotine; Res: Resveratrol; Values are expressed as means SE; n = 10 in each treatment group.

Significant differences are arranged alphabetically from higher to lower.

Histological finding

The histopathological changes in kidney after 4 weeks of Nic or/and Res treatment are presented in Figure 1 (A-D). All control rat and Res group had normal renal tissues with normal renal tubules formed of normal glomeruli surrounded by normal proximal and distal renal tubules (Figure 1A-1C). The renal tissues in Nic group had showing dilated congested vascular space and aggregated of inflammatory cells between the renal tubules (Figure 1B). Evident histological alterations in Nic + Res treated group already appeared 4 weeks after the treatment, showing mild condensed glomerulus and mild aggregates of inflammatory cells (Figure 1D). These modifications were more evident to the oxidative stress that has been taken place in renal tissues of Nic treated group.

Table 5 showed the recording of different histopathological findings in different slides obtained from the four different groups and the variation from absence of change ----+, change that was observed in almost groups +++-, change that is often observed in all animals ++- till the change that is often found ++++.

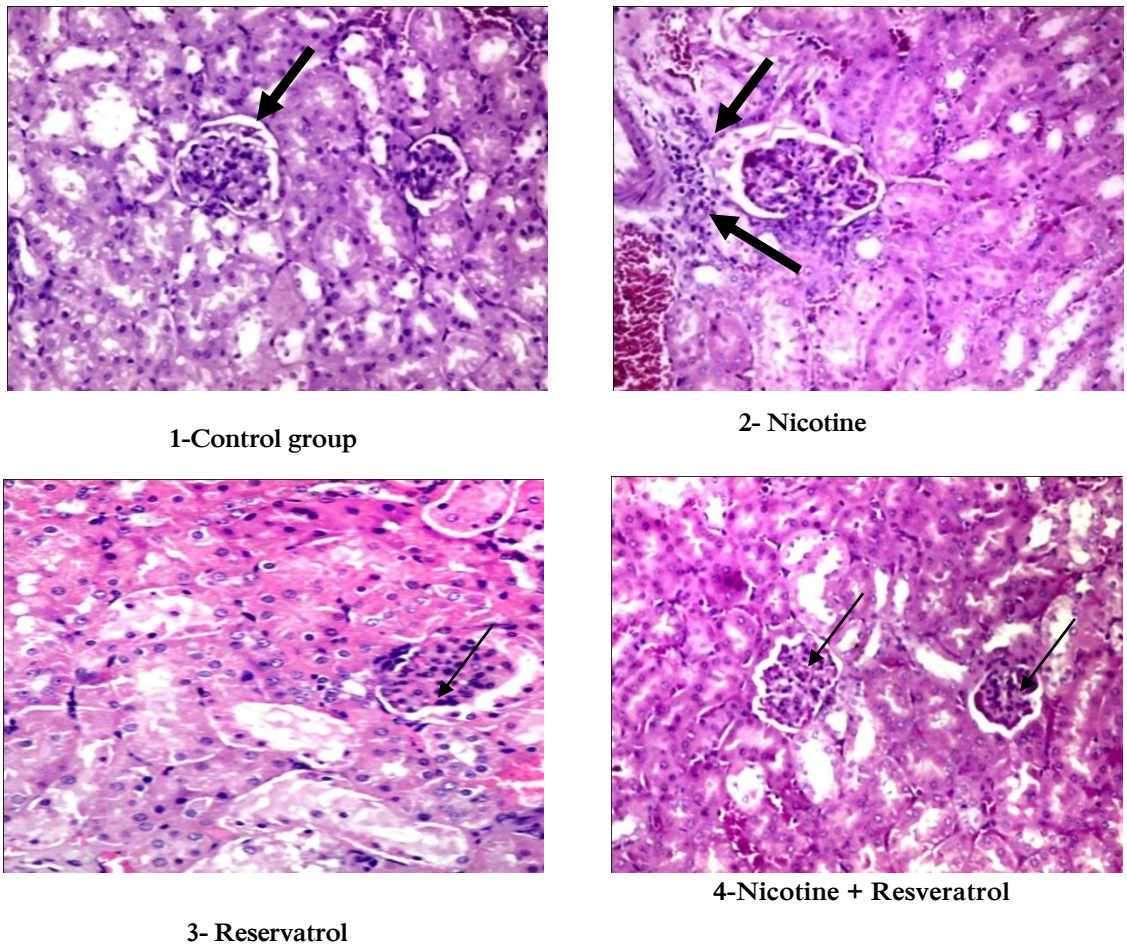

Figure 1. Effect of Resveratrol and Nicotine on renal tissues. (A) Control group: Histological section from control with normal renal tissues formed of normal glomeruli (Black arrow) surrounded by normal proximal and distal renal tubules (**) (H&E x 400); **(B) Nicotine group:** Photomicrograph of renal tissues exposed to Nic showing dilated congested space (*) and aggregated of inflammatory cells (Black arrow) between the renal tubules (H&E x 400). **(C) Resveratrol group:** Photomicrograph of normal renal tissues formed of normal glomeruli (Black arrow) surrounded by normal proximal and distal renal tubules (H&E x 400). **(D) Nicotine + Resveratrol group:** Photomicrograph of renal tissues treated with Nic + Res showing mild condensed glomerulus (Black arrow) and mild aggregates of inflammatory cells (**) (H&E x 400).

Table 5. Histopathological findings in kidney of rats treated with Nicotine and Resveratrol

Findings	Groups			
	Control	Nicotine	Resveratrol	Nicotine + Resveratrol
Normal renal tissues	++++	------	++++	+++-
Normal glomeruli	++++	+---	++++	+++-
Normal proximal and distal renal tubules	++++	+---	++++	+++-
Dilated congested vascular space	------	++++	------	+++–
Aggregated of inflammatory cells	------	++++	------	+++–
Mild condensed glomerulus	------	++++	------	++--

Symbol	Meaning
------	Absence of the change in the animals of the studied group.
++++	A change which was often found in all the studied animals of a group.
+++-	A change which was observed in almost all the studied animals of a group.
++–	A change not so often observed in all animals of a group.
—+	A change which was rare within a group

Discussion

Cigarette smoking causes stimulation in the sympathetic system, and an increase in blood pressure and heart rate leading to an increase of plasma concentrations of norepinephrine and epinephrine; these effects are intermediated by nicotine, and are seen in cigarettes with nicotine [27].

Administration of nicotine leads to significant increment in blood pressure accompanied by a decrease in glomerular filtration rate (GFR) [28]. It has been proposed that the response of the kidney can be impaired due to the increased blood pressure may in smokers, leading to an increase in GFR and possibly also increased intraglomerular pressure, compared to non-smokers who have the normal reno-protective decrease in GFR and renal plasma flow in the same scenario [29].

There is a vast evidence supporting the deleterious effects of cigarette smoking on renal function, with a significant impact on health care outcomes, as well as health care economics. The last decade has seen several significant studies to strongly suggest the role of cigarette smoking in progression of renal dysfunction. The effects of smoking start at an early age, and have long lasting effects. The increasing prevalence of chronic kidney disease calls for the progression of CKD and these previous findings support the obtained results in the current study as Nic induced a deleterious effects on kidney function parameters and oxidative stress as compared to control group and Res combined group with Nic which prove the beneficial effects of Res in reducing the side effects of Nic especially on kidney functions.

This is particularly important as patients with chronic kidney disease are also at risk for significant cardiovascular disease, and smoking offer a combined cardiovascular as well as renal dangerous [30]. Further studies are underway to investigate the mechanisms of nicotine induced renal injury, as well as possible ways to prevent this renal dysfunction.

The doses of Nic that was used induced significant oxidation status and histological modifications in the kidney. The increased LPO and TOS concentration, while decreasing TAS level observed in this study resulted from oxidative damage to the renal tissues after Nic-treatment. It is evident that Nic caused prooxidative activity.

Nicotine, once absorbed, is mainly metabolized by the liver to a number of major and minor metabolites [31, 32]. It has been considered highly susceptible organs to free radical generation and toxic effect of nicotine. In fact, numerous experimental and clinical evidences have been shown that nicotine through smoking induces an inflammatory response in the lung [33], promote pathogenesis of obstructive pulmonary diseases [34] and thus the new concept in the present study is the effect of nicotine on renal tissues from the oxidation aspect and histopathological approach through measuring also thiol and G6PD levels which are new concepts in the present finding and confirming the occurance of cell damage in Nic treated group which is diminished by administration of Res and thus proving its antioxidant activity.

In accordance with the present study findings, numerous investigations have revealed that nicotine treatment might augment toxicity and oxidative damage in rat tissues [35]. In reality, it has been demonstrated that nicotine as individual source for ROS production [36, 37] cause oxidative damage to important components of the cellular machinery [35]. In this respect and for the increasing rate of co-use and co-abuse of smoking cigarette [38, 39].

Resveratrol is a compound obtained from the roots of the Polygonum cuspidatum plant used in traditional Eastern medicine for the treatment of fungal diseases, skin inflammation, and cardiovascular and liver diseases [40]. Resveratrol is a phenolic phytoalexin [41] that occurs naturally in various foods, including grapes, plums, cranberries, and peanuts.

Its antioxidant effects have been demonstrated in the kidneys [42]. Res is a free radical scavenger that increases the activity of several antioxidant enzymes [43] and the present finding revealed its antioxidant activity against spleen toxicity induced by nicotine in male rats and this appear greatly and consequently through increasing the level of antioxidant enzymes and decreasing the final product of lipid peroxidation which reflect the antioxidant ability of Res against toxicity of Nic.

The present findings concides with obtained by Leonard *et al.,*[43] as Res is a free radical scavenger that increases the activity of several antioxidant enzymes. Resveratrol (3,5,40-trihydroxystibene) possesses antioxidant activity, due to its polyphenolic structure. It exists as trans-and cis-isomers. The trans-isomer is the naturally occurring form produced by plants and it is converted to the cis-isomer under exposure to light.

It was imperative to investigate the effects of nicotine administration and these interactions with resveratrol supplementation.

The obtained results came in accordance with that obtained by Mosbah *et al.*, [44] who reported that Nicotine is an active substance present in tobacco that causes oxidative stress and tissues damages leading to several diseases. Natural antioxidants that prevent the progression and severity of nicotine toxicity may have a significant health impact. They used green tea extract against nicotine toxicity and they proved that green tea extract played a protective role against nicotine-induced oxidative stress.

The present work also showed that the changes in LPO were accompanied by a concomitant decrease in the activities of antioxidant enzymes, namely SOD, CAT and GPx after 4 weeks of Nic treatment in renal tissues. SOD, CAT, and GPx constitute a mutually supportive team of

enzymes which provide a defense against the intermediates of dioxygen.

SOD constitutes an important link in the biological defense mechanism through dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 and $O_2.-$, which are deleterious to polyunsaturated fatty acids and proteins [45].

Nic administration to male rats induces free radical generation and hence the first line defense comes to the rescue as shown by the significant decrease in SOD in Nic-animals. Moreover, the reduced in CAT or GPx activity to degrade H_2O_2, more H_2O_2 could be converted to toxic hydroxyl radicals that may contribute to oxidative stress due to Nic as shown before in methanol intoxication with antioxidant enzymes [46].

A decline in the activities of these enzymes could be due to their inactivation by excess ROS production. The antioxidant enzymes CAT and GPx protect SOD against inactivation by H_2O_2. Reciprocally, SOD protects CAT and GPx against superoxide anion. The role of H_2O_2 in ROS generation has been observed in other studies [47].

In the present study, the activity of GPx decreased as the effect of Nic. A reduction in GPx activity results in increased H_2O_2 levels and hence severe cellular damage is observed in renal tissue.

Free radicals initially increase due to induction of Nic as the levels of NO and LPO increased as well as the activity of XO. LPO is associated with a wide variety of toxic effects, including decreased membrane fluidity and function, impaired functions of the mitochondria and Golgi apparatus and inhibition of enzymes.

The results of the present study go parallel with the results obtained by Salahshoor *et al.* [48] who indicated the effect of nicotine on vital organs and decreasing its antioxidant activity.

In accordance with the obtained results, Zhou *et al.,* [49] reported that nicotine stimulated apoptosis and it was observed that Nic are responsible for apoptosis related cell death.

The present results are in parallel with the previous results obtained by Sener *et al.,* [50] Chronic administration of nicotine has been described to be nephrotoxic in the rat. Indeed, nicotine accumulates preferentially in the kidney, where it creates an imbalance between the release of lipid peroxidation products and the endogenous antioxidant activity.

The current data are in accordance with Claude *et al.,* [51] who reported that Nic induced tubular damage score and histology of the kidney showed degeneration of kidney glomerulus.

Histological studies showed deleterious effects of nicotine on kidney. Urea showed a significant increase Nicotine treated group. These results indicate that nicotine exposure increase the risk of damage that occurs in the kidney with increasing the period of exposure to nicotine [52] which is in accordance with the current finding.

The current results are incomplete agreement with Ghada *et al.,* [53] who demonstrated that histological examination of nicotine-treated group rat kidneys, revealed marked tissue damage. The proximal and distal convoluted tubules showed partial destruction of the brush border of the proximal convoluted tubules and desquamated cells were observed inside their lumens. Apparent thickening of the basement membrane of the distal convoluted tubules was also found. Small pyknotic nuclei and collapsed necrotic glomerulus were present. There were irregular shapes of the nuclei of some tubular cells with dilation of glomerulus which is as the same of the obtained results.

Conclusion

Resveratrol had found to exert ameliorative effect on antioxidant parameters of the renal tissues. The present study indicates that a decrease in the antioxidant status is one of the main factors contributing to Nicotine toxicity to the kidney. The observed significant increase in the LPO and oxidative stress markers as well as XO activities in the renal tissues of Nic-exposed animals, suggests that the tissues are subjected to increased oxidative stress. Reversible oxidative/antioxidant and histological modifications were observed when Res used after Nic treatment. Res treatment found to remove the continuously generated free radicals, to prevent the endogenous antioxidant enzymes decrease and act to prevent oxidative cell damage induced by Nic.

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