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Original Article

Resveratrol Nanoemulsion; A Promising Inhibitor against Mitogen-Activated Protein Kinase - Dependent Inflammation and Ameliorates Nicotine induced-lung Toxicity in Rats

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ABSTRACT

Background: Nicotine, a major component of cigarette smoke, plays an important role in the development of cardiovascular disease and lung cancer in smokers. The aim of the present article was to investigate protective activity of resveratrol nanoemulsion (RENE) against lung toxicity induced by nicotine in adult rats as compared to basic resveratrol. **Materials and Methods:** RENE was prepared using bovine serum albumin method, then characterized for their particle size and zeta potential. Furthermore, Adult albino rats weighing around 150 ±10 g were used for the evaluation of lung protective activity of RENE (50 mg/k.b.w.) against nicotine-induced lung toxicity in rats. **Results:** The mean particle size of RENE was 49.5 ± 0.05 nm and zeta potential of +15.75 with the observed shapes of nanoparticle was spherical. The daily oral administration of the RENE at a concentration of 39.75 mg/kg body weight for 30 days to rats treated with nicotine (2.5 mg/kg.b.w.) resulted in a significant improve plasma cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol as well as serum tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and growth factor (TGF)-β1 in nicotine treated groups rats. On the other hand, oral administration of RENE elevated the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and total protein kinase-1 (Akt-1) as well as reduced the level malondialdehyde (MDA) in lung rats treated with nicotine. In addition, RENE reduced the expression of lung inducible nitric oxide synthase (iNOS) and mitogen-activated protein kinase (p38-MAPK) levels as compared to nicotine treated control group. Also, RENE and resveratrol almost normalized these effects in the histoarchitecture of the lung. **Conclusion:** The obtained biochemical, molecular biology and histological results of our study proved the lung protective activity of RENE against nicotine induced lung toxicity in rats.

Key words: Resveratrol nanoemulsion, Nicotine, Lung, Nanoparticles and p38-MAPK.

INTRODUCTION

Smoking is a major risk factor for cardiovascular morbidity and mortality.[1,2] 25% of middle-aged cardiovascular deaths are attributable to smoking.[3]

Nicotine is classed as an alkaloid (like morphine and cocaine) and meets the criteria of a highly addictive drug. 1.2-2.9 mg of nicotine, in each cigarette.[4] As an addictive drug, nicotine has 2 very potent effects: it is a stimulant and it is also a depressant.[5,6] Also, nicotine increases serum levels of HDL-C and LDL-C.[7,8] It has been recognized to result in oxidative stress by inducing the generation of reactive oxygen species (ROS).[9] These ROS in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation.[10] Many plant products have been shown to have significant antioxidant activity,[11] which may be an important property of medicinal plants associated with the treatment of several ill-fated diseases.[12]

Resveratrol is found in more than 70 species of plants, particularly in grapes, blueberries and peanuts[13] as well as red wine.[14] Not surprisingly, a phenolic compound such as resveratrol, an excellent scavenger of reactive oxygen species[15] and anticholestatic activity.[11] It is also speculated that resveratrol could be effective in controlling of hyperglycemia and dyslipidemia in diabetes.[16]

Nanotechnology is at the forefront of cancer research. This technology allows scientists to target cancer cells.[17] Nanoparticles can decrease side effects in patients by directly targeting the area of disease and eliminating the need to circulate through the body.[18,19] When encapsulating drugs into nanoparticles,

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researchers observed improved drug solubility, controlled drug release, enhanced bioavailability, increased stability and improved long-term storage (versus non-encapsulated drugs).[20] These attributes are promising and could be the traits needed to combat disease.[21] As an extension of my interested research program to evaluate the medicinal importance of resveratrol (11, 15 and 21) we report herein, a facile route to evaluate the therapeutic potential of resveratrol nanoemulsion on rat lung toxicity induced by nicotine.

MATERIALS AND METHODS

Materials

Resveratrol (97%.5%), Trypan Blue, Bovine serum albumin, Glutaraldehyde 50%, nicotine (98%), RPMI1640 (Roswell park memorial institute) medium with L-glutamine (Cambrex, Belgium) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used in this study were of the analytical grade.

Resveratrol nano-emulsion preparation

Resveratrol nano-emulsion was prepared according the method of Hussein *et al*.[21] 100 mg bovine serum albumin (BSA) is dissolved in 12 ml distilled water; dissolve the resveratrol 24 mg in 24 ml ethanol then add drop wise the ethanolic solution on the BSA solution under stirring (500 rpm); then add 3 ml 11% glutaraldehyde and leave on stirring overnight.

Resveratrol nano-emulsion characterization

The crystalline nature and grain size of RENE was carried out by X- ray diffraction pattern at 25-28°C with a D8 Advance X –ray diffractometer (Bruker – Germany) with a nickel (Ni) filtered using CuKα (λ= 1.54184 A0) radiations as an X-ray source. Infrared spectrum (IR) of sample is registered using Nicolet 6700. The thermal analysis was measured using Thermo gravimetric analysis. Morphology and size of RENE were examined by Scanning Electron Microscope and Field Emission Transmission Electron Microscopy at an accelerating voltage of 15Kv and 200 Kv.

Animals

Male albino rats weighing around 150±10 g were obtained from animal house of Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. They were housed in plastic cages with stainless steel covers at the National Cancer Institute Animal House. The animals were maintained at a temperature of 22±1°C and a humidity of 55–60% in a light-controlled room. The animals were kept for 1 week to acclimatize and provided with standard diet and water *ad libitum*.

Experimental setup

This experiment was carried out to examine the protective effect of RENE against nicotine-induced lung toxicity. This experiment was conducted in accordance with guidelines established by the Animal Care and Use Committee of October 6th University. Adult albino rats were divided into six groups with six animals in each. The treatment groups are described in Table (1).

Biochemical assays

Blood samples were withdrawn from the retro-orbital vein of each fasted animal. Blood was collected using sodium fluoride as anticoagulant, centrifuged and plasma was used freshly for estimation of triglyceride, total cholesterol and HDL-C were determined.[23-25] Plasma LDL-cholesterol level was calculated from Falholt *et al*.[26] formula.

Plasma tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6) and total Akt-1 levels were performed using a series of ELISA kits. The lung

Table 1: Description of treatment groups.

Group	Group name	Treatment description
I	Normal control A	3 mL of distilled water, orally for 30 days
II	Normal control B	3 mL of tween 80, 1%, orally for 30 days
III	Nicotine	Subcutaneous injection of 2.5 mg/kg b.w. nicotine[22] in tween 80, 1%, for 30 days
IV	RENE	Oral suspension of 39.75 mg/kg b.w. in tween 80, 1%.[21]
V	RENE + Nicotine	Oral suspension of 39.75 mg/kg b.w. RENE in tween 80, 1%. + subcutaneous injection of 2.5 mg/kg b.w. nicotine in tween 80, 1%, for 30 days in a single daily dose
VI	Basic resveratrol + Nicotine	Oral suspension of 50 mg/kg b.w. basic resveratrol in tween 80, 1%. (11) + subcutaneous injection of 2.5 mg/kg b.w. nicotine in tween 80, 1%, for 30 days in a single daily dose

superoxide dismutase assay kit utilizes a tetrazolium salt.[27] The glutathione peroxidase assay kit measures GPx activity indirectly by a coupled reaction with GR (28). Also, A thiobarbituric-acid-reactive substance assay was used to measure the lung lipid peroxidation products, malondialdehyde (MDA) equivalents.[29]

Western blot

Lung samples of three rats from each group were taken 3 min after the last administration. The samples were added with the lysis buffer on the ice for cracking for 1 hr and then centrifuged at 16,009.2× *g* to obtain the supernatant. The tissue protein concentration in the supernatant was determined by bicinchoninic acid (BCA) method and 10% SDS-PAGE gel electrophoresis was used to isolate β-actin, iNOS and p38 MAPK. The proteins were transferred onto PVDF membrane for 2 hr and the membrane was rinsed with Tris buffer saline Tween (TBST) for 5 min and then blocked with the blocking buffer for 1 hr. After the incubation at room temperature, the blocking buffer was discarded. The first antibodies of iNOS (1:1000), β-actin (1:1000) and p38 MAPK (1:1000) Santa Cruz Biotechnology, Inc, Calif, USA) were added onto the membrane, respectively, which was incubated at 4°C overnight and then washed with TBST five times, 5 min each time; the second antibody (1:2,000) were added onto the membrane, which was incubated for 2 hr and then washed five times with TBST, 5 min each time and finally, ECL color solution was added onto the membrane for its development.

Histological assessment

The lung was sliced and pieces were fixed in 10% buffered formaldehyde solution for histological study. Sections of 5 ml in thickness were prepared and then stained with hematoxylin and eosin for light microscopy analyses according to the method of Bancroft and Steven.[30]

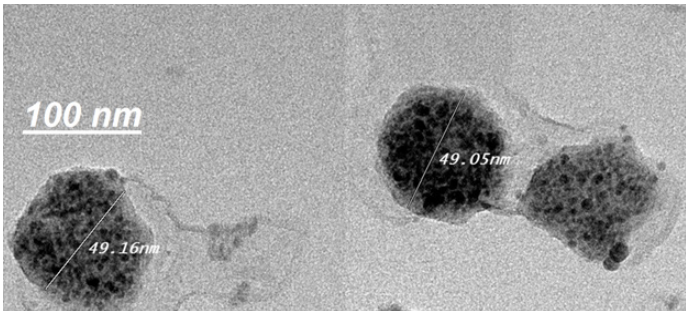
Statistical analysis

The results were expressed as mean ± SD for eight separate determinations. All the data were statistically evaluated with SPSS/18 Software. *P* values of < 0.01 were considered to indicate statistical significance.

RESULTS

IR spectrum infrared spectrogram of the basic resveratrol and resveratrol nanoemulsion shows a phenol hydroxyl groups absorption peak at 3252 and 3436cm^{-1}, respectively, as well as benzene ring absorption peaks at 2827, 2920 exists.

LST as well as TEM analysis shows that resveratrol nanoemulsion had size of around 49.5 ± 0.05 nm with negative zeta potential of +15.75.



Tables 2 show plasma lipid profile levels. Nicotine administration led to significant increase of biochemical marker levels for cholesterol, triglycerides and LDL-cholesterol while significantly decreasing HDL-cholesterol, respectively, as compared with the normal control group ($P < 0.05$), indicating acute lung injury. Treatment of animals with RENE and basic resveratrol 39.75 and 50 mg/kg.b.w., significantly reduced the level of cholesterol, triglycerides and LDL-cholesterol as well as significantly increased HDL- cholesterol, respectively, ($P < 0.05$), as compared with the nicotine treated group.

Table 3 revealed a significant elevation in plasma TNF-α and IL-6 as well as significant decrease in TGF-β1 levels ($p<0.05$) in the second group which represents nicotine (2.5 mg/kg) treated the group of rats compared with control group. The administration of RENE and basic resveratrol showed significantly decreased in TNF-α and IL-6 as well as significant increase in TGF-β1 levels relative to nicotine treated the group of rats after 30 days ($p< 0.05$).

Table 2: Effect of resveratrol nanoemulsion (RENE) and basic resveratrol on plasma lipid profile in rats treated with nicotine.

Groups	Treatment Description	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-Cholesterol (mmol/L)	LDL-Cholesterol (mmol/L)
I	Normal control A 3 mL of distilled water, orally	92.6 ± 3.17[a]	65.37 ± 2.97[a]	33.60 ± 2.57[a]	45.93 ± 4.86[a]
II	Normal control B 3 mL of tween 80, 1%, orally	93.44 ± 5.30[a]	60.40 ± 3.46[a]	31.88 ± 3.09[a]	49.48 ± 5.03[a]
III	RENE 39.75 mg/kg b.w. in tween 80, 1%, orally	90.54 ± 3.21[b]	63.08 ± 6.00[a]	30.65 ± 4.00[a]	47.27 ±4.21[a]
IV	Nicotine 2.5 mg/kg b.w. in tween 80, 1%, subcutaneous injection	136.76 ± 4.11[e]	97.50 ± 3.89[d]	19.80 ± 2.43[a]	97.46 ± 4.62[d]
V	RENE (39.75 mg/kg b.w.) + (Nicotine 2.5 mg/kg b.w.)	100.68 ± 6.17[c]	68.72 ± 7.25[b]	35.11 ± 3.90[a]	51.83 ±3.90[b]
VI	Basic resveratrol (50 mg/kg b.w.) + Nicotine (2.5 mg/kg b.w.)	110.37 ± 5.21[d]	85.20 ± 5.09[c]	26.49 ± 2.80[b]	66.84 ± 5.35[c]

Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at $P \leq 0.05$.

Table 3: Effect of resveratrol nanoemulsion (RENE) and basic resveratrol on levels of serum tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and growth factor (TGF)-β1 in rats treated with nicotine.

Groups	Treatment Description	TNF-α (pg/ml)	IL-10 (pg/mL)	TGF- β1 (pg/mL)
I	Normal control A 3 mL of distilled water, orally	15.64 ± 1.65[a]	12.65 ± 1.26[a]	63.54 ± 4.00[a]
II	Normal control B 3 mL of tween 80, 1%, orally	14.88 ± 2.03[a]	13.61 ± 1.54[a]	62.10 ± 4.35[a]
III	RENE 39.75 mg/kg b.w. in tween 80, 1%, orally	90.54 ± 3.21[b]	63.08 ± 6.00[a]	30.65 ± 4.00[a]
IV	Nicotine 2.5 mg/kg b.w. in tween 80, 1%, subcutaneous injection	58.70 ± 3.20[d]	26.54 ± 2.00[c]	197.60 ± 11.25[d]
V	RENE (39.75 mg/kg b.w.) + (Nicotine 2.5 mg/kg b.w.)	19.65 ± 2.05[b]	16.90 ± 1.56[b]	73.25 ± 5.42[b]
VI	Basic resveratrol (50 mg/kg b.w.) + Nicotine (2.5 mg/kg b.w.)	29.00 ± 2.17[c]	17.80 ± 2.09[b]	82.30 ± 4.70[c]

Data shown are mean ± standard deviation of number of observations within each treatment.

Data followed by the same letter are not significantly different at $P \leq 0.05$.

Table 4 show a significantly ($P < 0.05$) decreased activities of lung antioxidant enzymes SOD, GPx and total Akt-1 while significantly increasing lung MDA, were observed in the nicotine-treated rats as compared with the normal control group ($P<0.05$), indicating acute lung damage. RENE and basic resveratrol treatment significantly ($P < 0.05$) enhanced the lung enzymes activities SOD, GPx and total Akt-1 in rats and decrease MDA level, as compared to the nicotine-treated group.

Figure 1 displayed that nicotine (2,5 mg/ kg) promoted the iNOS protein expression in nicotine- treated the group of rats compared with control group. Administration of RENE and basic resveratrol treatment led to a statistically significant decrease of iNOS protein expression relative to nicotine treated group of rats ($p< 0.05$). Agarose gel electrophoresis images of iNOS and β-actin by RT-PCR support the present results Figure 2.

RT-PCR results (Figure 3) showed that significant increase in the expression levels of p38 MAPK in group of treated rats with nicotine (2.5 mg/ kg) when compared with normal control group of rats. Also, Administration of RENE and basic resveratrol treatment led to a statistically significant decrease of p38 MAPK protein expression relative to nicotine treated rats ($p< 0.05$). Agarose gel electrophoresis images of p38 MAPK and β-actin by RT-PCR support the present results Figure 2.

Histopathology examination

Histopathological examination of lung sections of the normal groups (I and II) as well as RENE treated group (III) demonstrated normal morphological features of lung parenchyma with apparent intact respiratory airways epithelium as well as alveolar walls (arrows) with intact vasculatures. Figure 4 (I-III)

Table 4: Effect of resveratrol nanoemulsion (RENE) and basic resveratrol on activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and total protein kinase-1 (Akt-1) as well as malondialdehyde (MDA) level in lung rats treated with nicotine.

Groups	Treatment Description	SOD (U/mg protein)	GPx (U/mg protein)	Total Akt-1 (ng/mg protein)	MDA (nmol/ mg protein)
I	Normal control A 3 mL of distilled water, orally	15.4 ±1.66[a]	11.7 ±0.65[a]	3.73 ±0.84[a]	1.86 ± 0.09[a]
II	Normal control B 3 mL of tween 80, 1%, orally	14.76 ±1.53[a]	10.8 ±0.48[a]	3.62 ±0.79[a]	1.83 ± 0.16[a]
III	RENE 39.75 mg/kg b.w. in tween 80, 1%, orally	90.54 ± 3.21[b]	63.08 ± 6.00[a]	30.65 ± 4.00[a]	47.27 ±4.21[a]
IV	Nicotine 2.5 mg/kg b.w. in tween 80, 1%, subcutaneous injection	8.25 ±0.94[b]	6.94 ±0.25[b]	1.90 ±0.44[c]	3.33 ± 0.14[b]
V	RENE (39.75 mg/kg b.w.) + (Nicotine 2.5 mg/ kg b.w.)	14.30 ±1.65[a]	9.64 ±0.79[a]	3.61 ±0.66[a]	1.88 ± 0.19[a]
VI	Basic resveratrol (50 mg/kg b.w.) + Nicotine (2.5 mg/ kg b.w.)	14.25 ±1.76[a]	9.00 ±0.65[a]	3.30 ±0.42[b]	1.90 ± 0.16[a]

Values are given as mean ± SD for groups of six animals each. Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at $P \leq 0.05$. SOD: one unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1min/mg protein; GPx: µg of GSH consumed/min mg protein;

On the other hand, in the lung of nicotine-treated control group (IV), histological examination showed marked diffuse hemorrhagic pneumonia with extravasation of blood into alveolar lumen and intrabronchiolar lumen (stars) accompanied with sever thickening of interalveiolar walls and peribronchiolar tissue with many inflammatory cells infiltrates (arrow). Figure 4 (IV)

Histopathological examination also showed good recovery of nicotine-induced lung toxicity (V) by RNE as compared with the nicotine-treated group and showed almost the same records as Groups I- III. Figure 4 (V)

Group (VI) all samples of nicotine treated rats recovery by treatment with basic resveratrol and showed almost intact morphological features of pulmonary tissue with minimal records of inflammatory cells infiltrates. Figure 4 (VI)

DISCUSSION

Smoking ranks among the top causes of cardiovascular disease, including coronary heart disease, ischemic stroke, peripheral artery disease and abdominal aortic aneurysm.[4] It is also associated with an increased risk of certain types of cancer and is a major cause of chronic obstructive pulmonary disease.[31]

In the present study, the levels of total cholesterol and triglycerides and low-density lipoprotein (LDL) by rats during experimental period were significantly increased in nicotine-treated rats when compared with

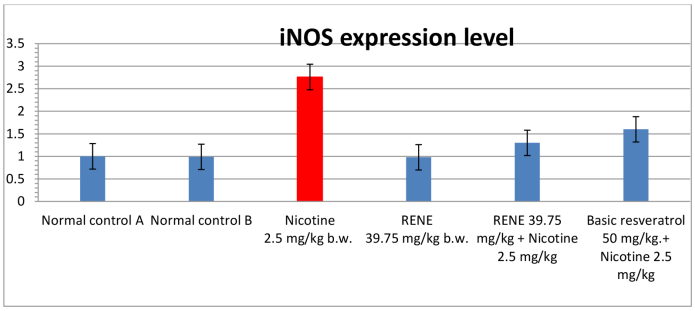


Figure 1: Effect of resveratrol nanoemulsion (RENE) and basic resveratrol on levels of lung inducible nitric oxide synthase (iNOS) in rats. Representative bar diagram of three independent experiments are presented.

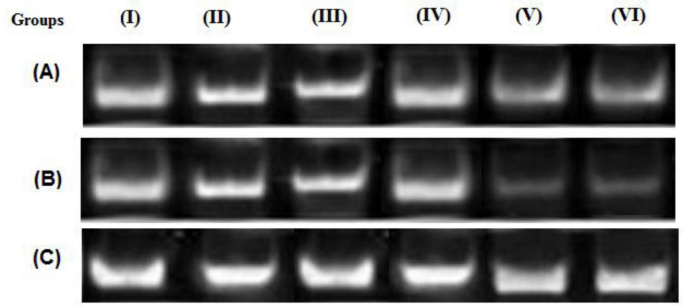


Figure 2: An agarose gel electrophoresis shows PCR products of lung inducible nitric oxide synthase (iNOS) (A) and p38 mitogen-activated protein kinase (p38-MAPK) (B) and beta actin (C) in different studied groups.

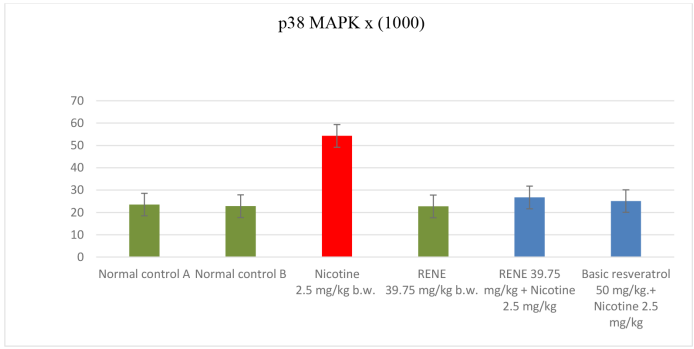


Figure 3: Effect of resveratrol nanoemulsion (RENE) and basic resveratrol on lung phosphospecific p38 mitogen-activated protein kinase (p38-MAPK) in rats. Representative bar diagram of three independent experiments are presented.

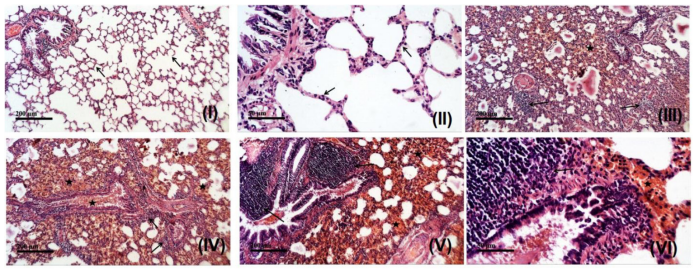


Figure 4 (I-VI): Histological examination of different pulmonary tissue samples.

normal control rats. On the other hand, nicotine lowers plasma levels of high-density lipoprotein (HDL), a powerful protective factor against the development of atherosclerosis.[32]

Also, oral administration of RENE and basic resveratrol at 39.75 and 50 mg/kg b.w., respectively, showed significant protection against nicotine induced increase in plasma cholesterol, triglycerides and LDL.

The deposited cholesterol esters in the tissue need hydrolysis to release free cholesterol. One of the hydrolysis factors is HDL, since HDL-cholesterol level was found to be decreased in atherogenic diet fed rats,[33] the insufficient HDL level may lead to free cholesterol in plasma, enhancing the pathogenesis. Our results showed that resveratrol nanoemulsion (RENE) and basic resveratrol enhanced HDL in treated rats. The most obvious effect of resveratrol on lipid profile was its action on *in vivo* LDL. Resveratrol nanoemulsion (RENE) and basic resveratrol reduced the LDL in nicotine treated rats. LDL promotes atherosclerosis both by providing lipids signals that initially activate macrophages and by stimulating foam cell formation.[33]

The present results show that RENE could inhibit serum TNF-α, IL-6 and TGF-β1 levels in the nicotine-treated group. Free radicals are involved in the regulation of cell proliferation and death, as well as gene expression such as TNF-α, IL-6, TGF-β1 and MDA.[11] Evidence indicates that free radicals, oxidative stress and lipid peroxidation are present in organs damage.[34] It has been shown that in chronic lung toxicity, the increased lung concentration of as TNF-α, IL-6, TGF-β1 and MDA and decrease activity of SOD, GPx and Akt-1 induces mitochondrial toxicity and free-radical generation.[35] TNF-α, TGF-β1 and interleukin-6 are the most extensively studied mitogenic and fibrogenic factors. RES is also able to inhibit proinflammatory cytokine expression.[36] Taken together, these results indicate that the antifibrotic effect of RENE is associated with the blockade of mitogenic and/or fibrogenic signaling. TNF-α was reported to induce NO formation.[37] RENE and basic resveratrol are a potent reactive oxygen species (ROS) scavenger[38] and normalized the oxidative stress biomarkers SOD, GPx, Akt-1 and MDA, resulting in reduced oxidative stress, which contributes to suppression of lung inflammation by nicotine. In the present study, the significant decrease in lung SOD, GPx and Akt-1 activity were detected after nicotine administration.

In the present study, lung toxicity by nicotine, iNOS begins to express and generate a large number of NO and endogenous NO is massively released into regional damaged lung tissue, which directly react or interact with other factors, indirectly involved in scar formation and evolution process by adjusting firoblasts, endothelial cells and other functions.[39]

There are more major findings in the resveratrol study. First, the present results shown that TNF-α-induced increased monocyte adhesiveness to Human Coronary Artery Endothelial Cells (HCAECs) is NF-κB dependent and it can be inhibited by resveratrol.[40] IL-10 and iNOS protein expression also elicited endothelial activation and this effect also could be attenuated by resveratrol. It is significant that resveratrol also attenuated H_2O_2-induced monocyte adhesion to HCAECs in a similar concentration range. The second important finding is that TNF-α -induced NF-κB activation in HCAECs is inhibited by treatment with resveratrol.[41] The present study was in confirmed with other studies[42] suggested that resveratrol was effective against iNOS protein expression, IL-10 and TGF-β1-induced NF-κB activation in intact blood vessels.

In lung cells, TNF-α, IL-6, TGF-β1 can induce P38MAPK activation and increase its activity, thereby inducing cardiac endothelial cell death and stimulating neutrophil function, leading to the increase of TNF-α, IL-6, TGF-β1 -α and the accumulation of neutrophils in lung tissue and damage it's cells.[43] In our study, we found that baicalin significantly down-regulated P38MAPK protein expression in rat with nicotine induced lung toxicity group. Usta *et al*. concluded that treatment with resveratrol suppresses migration, invasion and metastasis through p38MAPK signaling pathway in human cardiac tissue.[43]

Indeed, there was remarkable reduction in fibrosis extent and a decrease of stellate infiltration in rats treated with RENE groups compared to the control nicotine treated group. Histological studies confirmed the lung protective effect of RES. Since the proliferation of lung is an early event in toxicity-related changes, the attenuation of lung injury and fibrosis in rats by RENE might be associated with alleviation of inflammatory reaction. Prophylactic effect of RENE against nicotine-induced lung toxicity has not been reported earlier to my knowledge and this study is perhaps the first observation of its kind.

CONCLUSION

The present study showed that RENE has powerful lung protective activity against nicotine-induced lung toxicity, via normalize the levels oxidative stress biomarkers and gene expression of inflammatory mediators.

Ethics approval and consent to participate

Ethical approval for the data collection was granted by the Research Ethics Committee at the Faculty of Applied Medical Sciences, October 6 University, Egypt (No. 20191102). No Humans were used studies that are base of this research, *in-vivo* study using rats only.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ABBREVIATIONS

RENE: Resveratrol nanoemulsion; **HDL-C:** HDL-cholesterol; **LDL-C:** LDL-cholesterol; **TNF-α:** Tumor necrosis factor alpha; **IL-6:** interleukin 6; **TGF-β1:** Growth factor, **SOD:** Superoxide dismutase; **GPx:** Glutathione peroxidase; **Akt-1:** total protein kinase-1; **iNOS:** Inducible nitric oxide synthase; **p38-MAPK:** mitogen-activated protein kinase.

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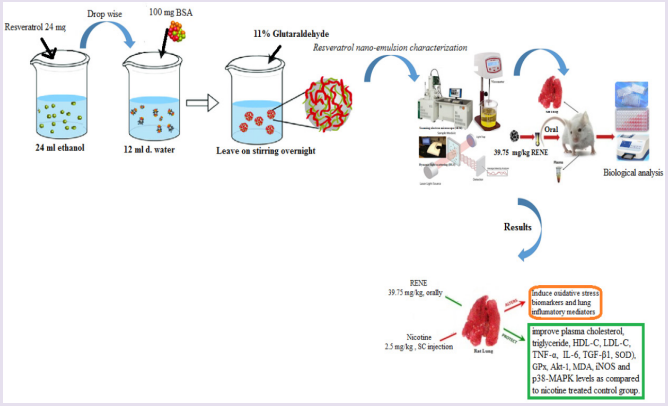
GRAPHICAL ABSTRACT



SUMMARY

In the present study, RENE was prepared, characterized and evaluated for its lung protective activity against nicotine-induced lung toxicity using the following biochemical molecular parameters; cholesterol, triglyceride, HDL-C, LDL-C,TNF-α,IL-6, TGF-β1, SOD, GPx, Akt-1, iNOS and p38-MAPK. The obtained biochemical, molecular biology and histological results of our study proved the lung protective activity of RENE against nicotine induced lung toxicity in rats.

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