Protective effect of lobelia and hops mixture against cigarette inhalation toxicity in female rats

Amel A. Refaie[a], Yasser M. Diab[b]

[a]Environmental Toxicology Research Unit (ETRU), Department of Pesticide Chemistry, National Research Centre (NRC), [b]Poisonous and Drugs in Toxicology Centre, Cairo, Egypt

Correspondence to Amel A. Refaie, Assistant Professor, Department of Pesticide Chemistry, National Research Centre, 33 Bohouth Street, PO Box 12622 Dokki, Giza, 12611, Egypt
Tel: +20 109 548 3939; fax: (202)-33370931; e-mail: dramelrefaie@yahoo.com

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Background/Aim
Cigarette inhalation is dangerous for the health of both nonsmokers and smokers. It is the most important cause of death. The current study is important for studying the protective role of lobelia (*Lobelia inflata*) and hops (*Humulus lupulus*) against the toxic effects of smoking inhalation in adult female rats.
Materials and methods
This study was performed on 24 female rats divided into four groups (six each) including control group (G1), cigarettes inhalation group (G2), mixture of lobelia and hops inhalation group (G3), and mixture of herbal plants plus cigarettes inhalation group (G4). Female rats were exposed to inhalation of a mixture of lobelia and hops or cigarettes separately or mixed for 4 weeks with a concentration of 4.4 mg/kg for each dose. At the end of the experiment, serum samples and whole blood were used for biochemical and hematological investigation as well as histological examination on liver, kidney, lung, and cardiac tissue.
Results
The cigarette-treated rats showed significant increases ($P<0.05$) in levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, protein, urea, and creatinine, whereas a significant decrease ($P<0.05$) in albumin level was estimated. The exposure to the mixture of herbal plants only showed normal levels of liver and kidney biomarkers in comparison with the control group. However, the combination of cigarette plus mixture of herbal plants inhalations revealed normal levels of liver and kidney parameters after 4 weeks of inhalation. Histological studies showed morphological alterations in all vital organs of rats in cigarette inhalation-treated group, whereas normal histological examination in group of rats treated with cigarette plus mixture of herbal plants inhalations.
Conclusion
These findings concluded that exposure to cigarette inhalation in female rats activated harmful effects to the vital organs; this toxicity can be improved by exposure to mixtures of herbal plants from lobelia (*L. inflata*) and hops (*H. lupulus*).

Keywords:
cigarette inhalation, hepatotoxicity, herbal plants, liver, nephrotoxicity, rats

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Introduction

Smoking inhalation or tobacco roll is a product made from tobacco leaves, wrapped or stuffed in a paper wrapping in the form of a cylinder, usually 120 mm in length and 10 mm in diameter. Some cigar manufacturers allow its composition not only from tobacco leaves but even from plant stems, as they are crushed and cut. The cigarette is lit from one end and the other placed in the mouth. Cigars were unknown in Europe before Christopher Columbus discovered America when he saw Native Americans smoking. Cigarette components have been modified; it contains tobacco, which includes about 500 different compounds depending on the type of cigarette, including tar and oxidized carbon. Among the most dangerous substances, it contains nicotine, tar, and carbon monoxide [1].

Nicotine is the active substance in the cigarette. It speeds up breathing and heartbeat, raises blood pressure, activates the vomiting center, causes nausea in beginners, dampens the center of hunger, and reduces the smoker's appetite to eat, along with its nervous effects ranging from mild stimulation to sedation, a feeling of depression, and contraction according to the dose and smoker's condition. A tiny amount of pure nicotine, amounting to 40–60 mg is fatal. One cigarette contains 1 mg on average. The word nicotine came from the French ambassador, 'John Nicot,' who defended cigarettes and claimed that they are useful in treating some diseases. Carbon monoxide in the blood will result in a profound decrease in the capacity of blood to carry oxygen. Although the partial pressure of dissolved

oxygen in the blood may be normal, tissues may show severe hypoxia, because much of the hemoglobin will be irreversibly bound by carbon monoxide rather than oxygen. Carbon monoxide and nicotine also contribute to increase in arterial lipid deposition and cholesterol, as well as blood clotting. Tar (tobacco) is a viscous substance that is deposited in the alveoli, disrupting the exchange of gases in them. It also contains highly harmful substances, the most important of which are carcinogenic hydrocarbons [1]. Nicotine disperses generally into different organs, including liver, lung, kidney, brain, and spleen [2]. The time critical for nicotine accumulation in many organs of the body highly depends on the way and rate of dosing. Nicotine reaches the brain in 10–20 s [3–5]. The initiation of lipid peroxidation caused by increasing the free radicals, like hydroxyl radicals, superoxide, and other reactive oxygen species like H_2O_2, causes cellular injury. Lipid peroxidation levels increased in tissues (lung, liver, and heart) after intraperitoneal administration of nicotine (0.6 mg/kg body weight) to rats [6,7].

On the contrary, lobelia and hops are herbal plants cultivated in north Africa and Europe and classified under the major families of Campanulaceae and Cannabaceae, respectively. Herbal plants contain herbs as opposed to tobacco leaves as the crude material. Additionally, herbal plants were an identical formula to solve an enormous problem, because the smokers can use it without adverse effects found within the current cigarette, whereas smoking inhalation consists of a complicated blend of substance constituents, several of which are recognized to be located in trace concentrations. The numerous constituents are formed by warm combination responses and warm deterioration happening in the smoking procedure. There were 4994 affirmed segments in tobacco leaves and 5311 affirmed parts in cigarette smoking [8].

Lobeline is a pyridine alkaloid found in a variety of plants, particularly those in the genus lobelia, and has numerous systems of activity, going about as a VMAT2 ligand [9–11], which animates dopamine discharge to a moderate degree, when controlled only, in any case, lessens the dopamine discharge brought about by methamphetamine ligand [12,13]. It also inhibits the reuptake of dopamine and serotonin [10]. Reality, lobeline can reduce the effects of nicotine in the body, especially dopamine release. Dopamine is a chemical in the brain which plays a number of significant roles in the brain. It is also involved in opioid abuse, so researchers suggest that lobeline might be able to treat addiction with some promise, and goes as a blended agonist-antagonist at acetylcholine nicotinic receptors [14], which it ties at the subunit interfaces of the extracellular area [15]. It seems to be a P-glycoprotein inhibitor, according to a past study [16]. It has been hypothesized that P-glycoprotein inhibition reduces chemotherapeutic resistance in cancer [17]. There is recounted proof that proposes that lobelia (likewise called Indian tobacco) can help individuals to quit smoking. Lobeline, the dynamic fixing in the lobelia plant, is thought to work by authoritative action to a similar receptor locale in the cerebrum as nicotine, causing increasing levels of the neurotransmitter dopamine in the brain. Lobeline was once a public ingredient in over-the-counter products used to improve symptoms associated with nicotine withdrawal. Lobelia may also be active in assisting in clearing excess mucus from the respiratory tract, including the lungs, throat, and bronchial tubes that smokers often experience in quitting; however, more research is needed to determine if this is accurate [18].

Moreover, the pharmacological action of Humulus is essential because of pitch (lupulin) from the dried, female-blossoming parts (strobiles). This operation and to report their mode of action on the central nervous system (CNS). The key site of rapid synaptic inhibition in the central nervous system is gamma-aminobutyric acid type A (GABAA) receptors corrosive, the leading inhibitory synapse of the central nervous system. Its calming effects might be an aftereffect of three distinct classifications of constituents acting synergistically: alpha acids, beta acids, and basic oils [19,20]. Beta acids appear to show stimulant action, however, with less calming effects, most likely by influencing GABA neurotransmission movement. Different parts of Humulus basic oil were found to include the terpene hydrocarbons, myrcene, humulene, and caryophyllene, with the concentrations around 80–90% of all fundamental oil contents [21,22].

Many smokers who wish to quit smoking use nicotine-replacement treatment [23,24]. The herbal plants in the form of cigarette may be the solution for many smokers, because it does not include any carcinogenic components. Several components recognized in old smoking cigarettes were involved in a catalog of harmful components in the International Agency for Research on Cancer. Nine constituents of those carcinogenic components inside normal smoking cigarettes have an effect on the body of a human and classified as a carcinogen. The WHO identified 18 constituents, and Health Canada identified 44

constituents [25]. Generally, tobacco scientists observed 44 toxic components as Hoffmann list. Aids to stop smoking like patches of nicotine, gum of nicotine, cigarette of herbal plants, and electronic cigarettes are accustomed to support an individual to halt smoking. Scientists recommend that many smoking human use cigarette of herbs as an aid against smoking in several countries [26].

This study was performed to determine the possible protective effect of mixture from lobelia (*Lobelia inflate*) and hops (*H. lupulus*) against the toxic effect of cigarette inhalation in female rats.

Materials and methods

Animals

A total of 24 female albino rats, weighing 110±5 g (4 weeks), were used in this study. The animals were obtained from the animal house of the National Research Centre and Research in Dokki, Giza, Egypt. Rats were housed under standard environmental conditions of humidity and temperature allowed to acclimatize to the new environment for 1 week before starting the experiment. Animal rooms (23±2°C) were maintained on a 12 : 12 h light/dark photoperiod. Animals were provided with food with free access to a standard pellet diet and water *ad libitum*. All techniques were conducted according to the acceptance standards of animal care following NODCAR Guidelines [27].

Ethical consideration

All the experiments were done in compliance with the public health guide for the care and use of laboratory animals and followed the ethical consideration of the National Research Centre and Helsinki Declaration [28].

Regular cigarette

The cigarette was purchased from the Egyptian market in Giza, Egypt. The selective dose of LM cigarette (4.4 mg/kg) is based on Saha *et al.* [29] and Cosci *et al.* [30].

Herbal plants used

Leaves and flowers of lobelia and hops used are purchased from a local market in Cairo, Egypt, and were ground separately and then mixed by equal volume 1 : 1 before used.

Inhalation method

Cigarettes were from a commercial brand. They were burned in an appropriated apparatus, and smoke inhalation was pulled to a hermetically closed glass chambers (50×30×30 cm), with a controlled negative airflow ventilation (10 l/min), preserved constant by a vacuum pump. Cigarette-negative control groups were also exposed to the chamber but with environmental fresh air circulating. Animals were exposed to cigarette inhalation and herbal cigarette inhalation for 3 h per day for 4 weeks and adjusted weekly for body weight alterations.

Experimental design

The female rats were divided into four groups, with six rats each as follows:

Group 1 (G1) received water and served as control.
Group 2 (G2) received the cigarette inhalation at a dose of 4.4 mg/kg [29,30] for 4 weeks.
Group 3 (G3) received mixture of herbal plants from lobelia and hops for 4 weeks by inhalation at a dose of 4.4 mg/kg.
Group 4 (G4) received cigarette plus mixture of herbal plants inhalation as dose of 4.4 mg/kg for 4 weeks.

Samples preparation

At the end of the experimental period, rats were fasted overnight, and blood samples were collected and left to clot in clean dry tubes and centrifuged at 3000 rpm (600*g*) for 10 min at 4°C using Heraeus Labofuge 400 R (Kendro Laboratory Products GmbH, Germany) to get the sera. Sera were stored at −20°C for further biochemical analysis, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, bilirubin, albumin, creatinine, urea, and total protein content. Blood samples were collected from animals by heart puncture and placed into EDTA-containing tubes. The parameters analyzed included hemoglobin level, red blood cell count, and white blood cell count. The liver, kidney, heart, and lung organs were removed immediately after killing, washed in saline, and weighed. A small part of each was homogenized in 10% (w/v) ice-cold 100 mM phosphate buffer (pH 7.4) and centrifuged at 10 000 rpm, and then small pieces of liver, kidney, heart, and lung were cut and kept in 10% formalin solution for histological studies.

Biochemical analysis

Serum ALT and AST were measured according to the procedures of Reitman and Frankel [31], and ALP according to Young *et al.* [32], albumin by Lukicheva *et al.* [33], and serum bilirubin (serum total and direct bilirubin) was measured according to Rutkowski and Debaare [34], and total protein content was determined according to Gornall *et al.* [35]. All biomarkers were performed according to the details

given in the Biodiagnostic kit's instructions (Biodiagnostic Company, Dokki, Giza, Egypt). Serum creatinine was measured as described by Tietz *et al.* [36] and urea as described by Henry [37].

Hematological analysis

Red blood cells and white blood cells were counted by Britton [38] and Seivered [39] method using Multiscan Skyhigh microplate spectrophotometer. Hemoglobin measurements were carried out to Wintrobe [40] method using an automatic hematological assay analyzer, Advia 60 Hematology system (Bayer Diagnostics Europe Ltd, Ireland).

Histological study

At the end of the experimental period, rats were killed, as well as liver, kidney, heart, and lung were dissected and fixed in 10% neutral formalin, dehydrated in ascending grades of alcohol, and embedded in paraffin wax. Paraffin sections (5 μm thick) were stained for routine histological study using hematoxylin and eosin. Two slides were prepared for each rat; each slide contains two sections. Ten field areas for each section were selected and examined for histopathological changes (×160) under a light microscope. The liver, kidney, heart, and lung fields were scored as follows: normal appearance (–), minimal cellular disruption in less than 1% of field area (+), mild cellular disruption of 1–30% of field area (++), moderate cellular disruption of 31–60% of field area (+++), severe cell disruption of 61–90% of field area (++++), and very severe cellular disruption of 91–100% of field area (++++) [41].

Statistical analysis

Data were reported as mean±SE (stander error). Data were analyzed using one-way analysis of variance followed by Duncan's test. The level of significance between mean values was set at P value less than or equal to 0.05. All statistical analyses were performed using SPSS software (SPSS 18.0 for windows, SPSS Inc. 233 South Wacker Drive, 11th Floor Chicago, IL 60606-6412).

Results

In the current study, no mortality or signs of toxicity were observed in female rats exposed to cigarette inhalation for 4 weeks, but their body weight was reduced by 25.64%, whereas there was a significant increase in liver weight, accounting for 3.35%, compared with the control female rats. The present result exhibited a normal value in body weight (%) in the mixtures of lobelia and hops-treated group of rat, as shown in Table 1. Exposure to cigarette plus lobelia and hops mixtures inhalation caused improvement in body weight and decrease in liver weight in comparison with control group. The present result exhibited also insignificant changes in relative kidney weight in all groups.

Exposure to cigarette inhalation to female rats resulted in a significant increase ($P<0.05$) in serum ALT, AST, ALP and total bilirubin in addition a significant decrease ($P<0.05$) in serum albumin and total protein compared with the control group, whereas exposure to mixtures of lobelia and hops induced normal levels of liver parameters. Exposure to cigarette plus mixtures of lobelia and hops inhalation-treated groups caused recovery in all liver parameters compared with the control group (Table 2).

Table 1 Body, relative liver, and kidney weights of rats exposed to cigarette inhalation, and protective effect of lobelia and hops for 30 consecutive days

Treatments	Body weight (%)	Relative liver weight (%)	Relative kidney weight (%)
G1: control	32.37 ±0.33^{a}	2.75±0.04^{a}	0.77±0.005^{a}
G2: cigarettes	25.64 ±0.22^{b}	3.35±0.08^{b}	0.79±0.003^{a}
G3: mixture	32.37 ±0.21^{a}	2.86±0.01^{a}	0.76±0.003^{a}
G4: mixture +cigarette	30.29 ±0.39^{c}	2.89±0.02^{a}	0.75±0.003^{b}

All values are expressed as mean±SE. Values with different letters (a, b, c, d) within the same column are significant at P value less than 0.05.

Table 2 Liver parameters of female rats exposed to cigarette and mixtures of herbal plants inhalation from lobelia and hops for 4 weeks

Groups	Alanine aminotransferase (u/l)	Aspartate aminotransferase (u/l)	Alkaline phosphatase (u/l)	Total bilirubin (mg/l)	Albumin (g/dl)	Total protein (g/dl)
G1: control	45.49±0.35^{a}	38.55±0.53^{a}	38.98±0.16^{a}	1.98±0.001^{a}	3.25 ±0.05^{a}	6.84±0.04^{a}
G2: cigarettes	65.62±0.71^{c}	41.04±0.38^{b}	43.97±0.21^{c}	2.06±0.024^{b}	2.47 ±0.02^{b}	5.28±0.06^{b}
G3: mixture	45.47±0.40^{a}	38.82±0.40^{a}	38.95±0.33^{a}	1.97±0.002^{a}	3.23 ±0.03^{a}	6.78±0.08^{a}
G4: mixture +cigarette	52.88±0.43^{b}	38.06±0.59^{a}	41.1±0.31^{b}	1.99±0.001^{a}	3.20 ±0.01^{a}	6.82±0.06^{a}

All values are expressed as mean±SE. Values with different letters (a, b, c, d) within the same column are significant at P value less than 0.05.

The present results revealed that exposure to cigarette inhalation resulted in a significant increase ($P<0.05$) in creatinine and urea concentration compared with the normal control group. These increases accounted for 1.39 and 42.95 mg/dl. Exposure to mixtures of cigarette plus lobelia and hops inhalation induced improvement in the level of blood urea in comparison with the control group of rats (Table 3).

On the contrary, there was a significant increase ($P<0.05$) in hematological parameters such as red blood cell count, hemoglobin content, and white blood cell count in the cigarette inhalation-treated group when compared with the control group, whereas the exposure to mixture of lobelia and hops only caused a significant decrease ($P<0.05$) in their values, near to the control group. Exposure of cigarette plus mixture of lobelia and hops inhalation created recovery in the aforementioned selected parameters of hematology (Table 4).

The histopathological findings in the liver and kidney tissues of cigarette inhalation-treated female rats support the results of liver and kidney function biomarkers. In the present study, the severity score of damage in the liver, kidney, heart, and lung is presented in Table 5. The histopathological examination (Figs 1–4) showed focal hemorrhage associated with a diffuse degenerative change in the hepatocytes in the cigarette inhalation-treated group, whereas the kidney tissue of cigarette inhalation-treated rat showed nuclear pyknosis and degeneration in the epithelial cells in the kidney and lymphoid hyperplasia and dilatation in the blood vessels, associated with collapse and emphysema in the air alveoli. The mixtures of lobelia and hops inhalation-treated group or mixtures of lobelia and hops plus cigarette inhalation-treated group showed no histological change in heart, and lung tissue.

Table 3 Kidney parameters of female rats exposed to cigarette and mixtures of herbal plants inhalation from lobelia and hops for 4 weeks

Groups	Creatinine (mg/dl)	Urea (mg/dl)
G1: control	1.06±0.02[a]	37.24±0.03[a]
G2: cigarettes	1.39±0.03[b]	42.95±0.17[b]
G3: mixture	1.03±0.01[a]	37.24±0.03[a]
G4: mixture+cigarette	1.24±0.04[b]	38.11±0.31[a]

All values are expressed as mean±SE. Values with different letters (a, b, c, d) within the same column are significant at *P* value less than 0.05.

Discussion

Cigarette inhalation has long been known to be a major risk factor for cardiovascular disease, obstructive pulmonary diseases, and carcinogenesis in humans [42]. As of late, numerous experimental examinations have used to create models of cigarette inhalation presentation for the evaluation of poisonous effects and hindrance of cancer prevention agent/supportive of oxidant balance and its effect on health status [43]. Body and organ weights for the determination of toxicity are essential parameters in toxicological studies. In the current study, significant increases in liver weight was observed; these results are supported by El-Aziz *et al.* [44], who found increases in relative liver weight in cigarette inhalation-treated rats. The decrease in body weight is upheld by past investigations that expressed lower body weight among

Table 4 Hematological analysis of female rats exposed to cigarette and mixtures of herbal plants inhalation from lobelia and hops for 4 weeks

Groups	Red cell count ($\times10^6$/cmm)	White cell count ($\times10^3$/cmm)	Hemoglobin (g/dl)
G1: control	4.48±0.015[a]	15.26±0.55[a]	13.42±0.09[a]
G2: cigarette	7.28±0.037[c]	18.38±0.36[b]	14.44±0.09[c]
G3: mixture	4.38±0.003[a]	15.24±0.31[a]	13.41±0.08[b]
G4: mixture +cigarette	5.20±0.104[b]	15.76±0.34[a]	13.77±0.07[a]

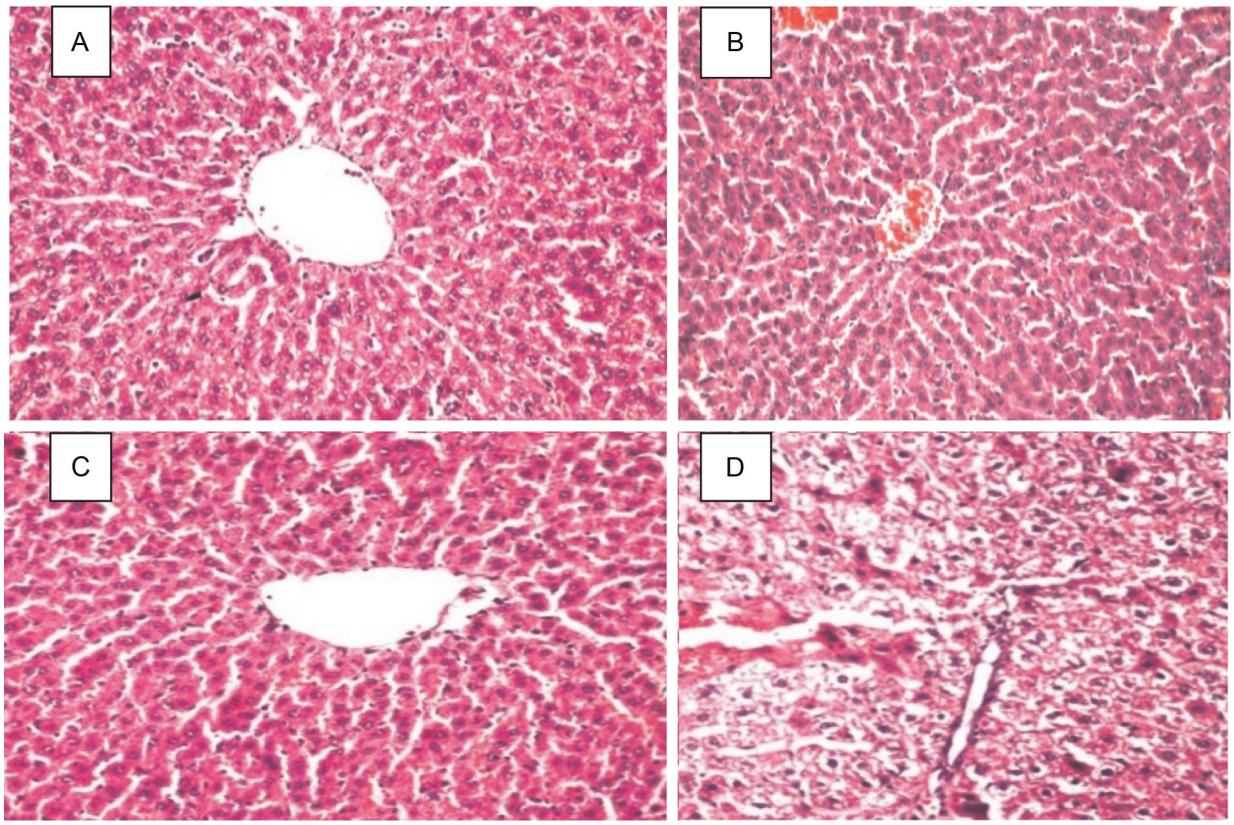
All values are expressed as mean±SE. Values with different letters (a, b, c, d) within the same column are significant at *P* value less than 0.05.

Table 5 Scoring severity of injury in the vital organs of female rats exposed to cigarette and mixtures of herbal plants inhalation from lobelia and hops for 4 weeks

Organs	Observation	G1	G2	G3	G4
Liver	Focal hemorrhage	–	–	+++	+
	Degenerative change	–	–	+++	–
	Congestion	–	–	–	–
	Kupffer cells proliferation	–	–	–	–
Kidney	Nuclear pyknosis and degeneration in tubular lining epithelial cells	–	–	+	–
Heart	Focal inflammatory cells infiltration in the myocardium	–	–	++	–
	Congestion in the myocardial blood vessels	–	–	–	–
Lung	Peribronchiolar lymphoid hyperplasia	–	–	++	–
	Collapse and emphysema in the air alveoli	–	–	++	–
	Congestion	–	–	–	–

–, normal; +, minimal; ++, mild; +++, moderate.

Figure 1

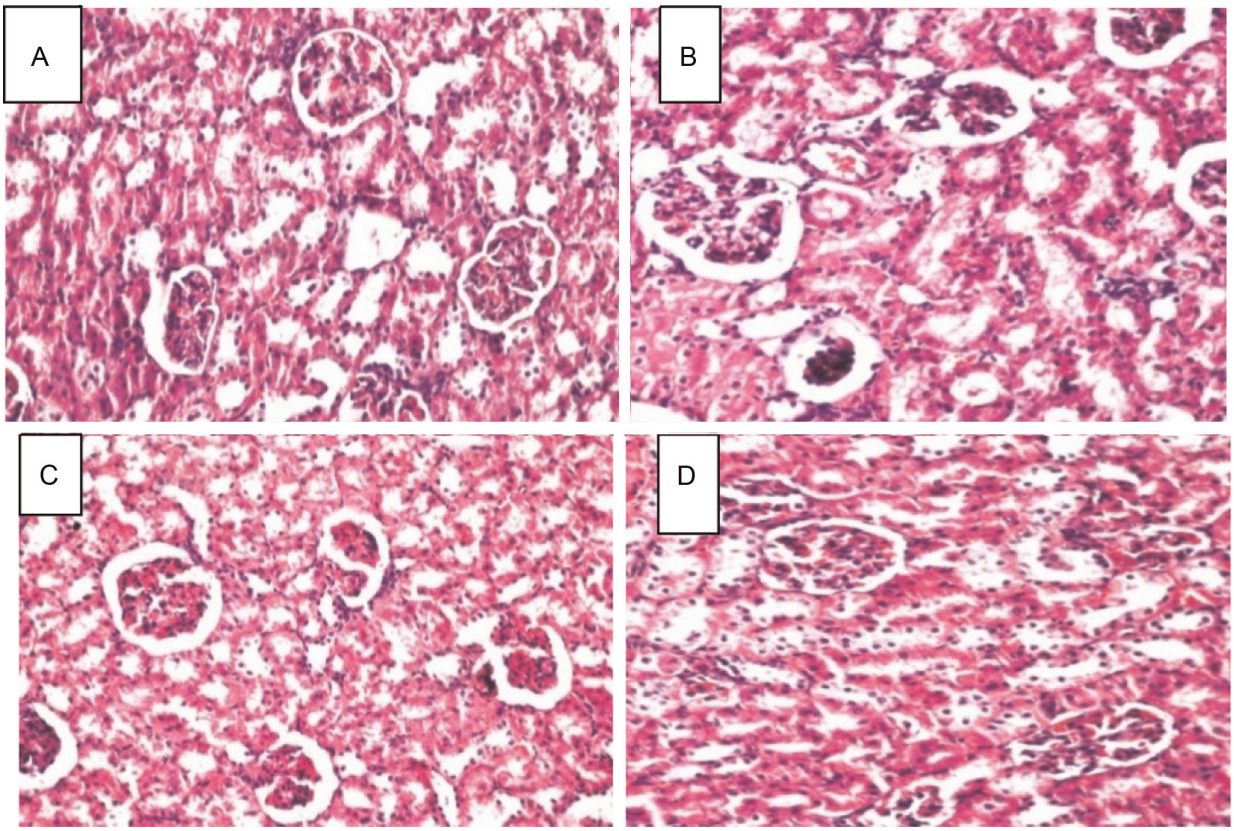


Photomicrograph of liver sections from rats of (A) G1, showing normal liver tissue. Normal hepatocytes and central vein hepatic sinusoids arefound. (C) G3 respectively, G2 (B) which were given cigarette inhalation, showing newly formed bile ducts with congestion in the portal vein and (D) G4, which was treated with mixtures of lobelia and hops plus cigarette inhalation showing focal hemorrhage in the hepatic parenchyma (hematoxylin and eosin, ×100).

smokers compared with the normal individuals [45,46].

In metabolism, the liver has a central function. It is also extremely susceptible to toxicity caused by chemicals. Chemical liver damage is a significant health concern that accounts for the majorly of acute liver failure [47]. The present study showed that exposure to cigarette inhalation to female rats resulted in a significant increase in serum ALT, AST, ALP and total bilirubin in addition a significant decrease in serum albumin and protein level. Some investigators claimed that ALT level increased by cigarette inhalation or nicotine administration [47], which was approved with our results. In addition, this increase may be indicative of initial cell injury occurring in advance of gross hepatic pathology. It causes hepatocyte damages and changes membrane integrity and as a result enzymes in hepatocytes leak out. Liver enzymes were effectively influenced by smoking, which is an indicator of liver cell injury owing to damage to the functional integrity of cell membranes. Subsequently, they are the dependable makers for liver function [48,49]. Serum ALP was used as good criteria for liver damage and hepatotoxicity. Furthermore, high levels show interstitial liver and lung fibrosis following their damage [50,51]. However, collecting data have shown that serum bilirubin has antioxidative, anti-inflammatory, and immunosuppressive functions in altered diseases [52]. There was a significant increase in the value of bilirubin in the cigarette inhalation-treated group when contrasted with the control group. Thus, it tends to be accepted that the excretory capacity of the liver in the treated rats is not antagonistically influenced as bilirubin level is a valuable record of the excretory capacity of the liver [53–55]. Albumin is one of the essential elements of the kidneys to eliminate creatinine, which is the waste result of muscle breakdown, from the circulation. Albumin is a protein created by the liver. Albumin helps to save fluid in the blood stream, so it does not outflow into other tissues. It also carries various substances throughout the body, including vitamins, hormones, and enzymes. Low albumin amounts can point to a problem with the liver or kidneys [56]. The reduced protein content in serum possibly will be owing to restraint genetic alterations and are congenital in the

Figure 2



Photomicrograph of kidney sections from rats of (A) G1, showing normal kidney tissue. Normal histological structure of the glomeruli and tubulesat the cortex. (C) G3, (B) G2, showing nuclear pyknosis and degeneration in the lining epithelial cells of some tubules at the cortex and (D) G4, which were treated with cigarette inhalation plus mixtures of lobelia and hops showing focal inflammatory cells infiltration in between the tubules at the cortex (hematoxylin and eosin, ×100).

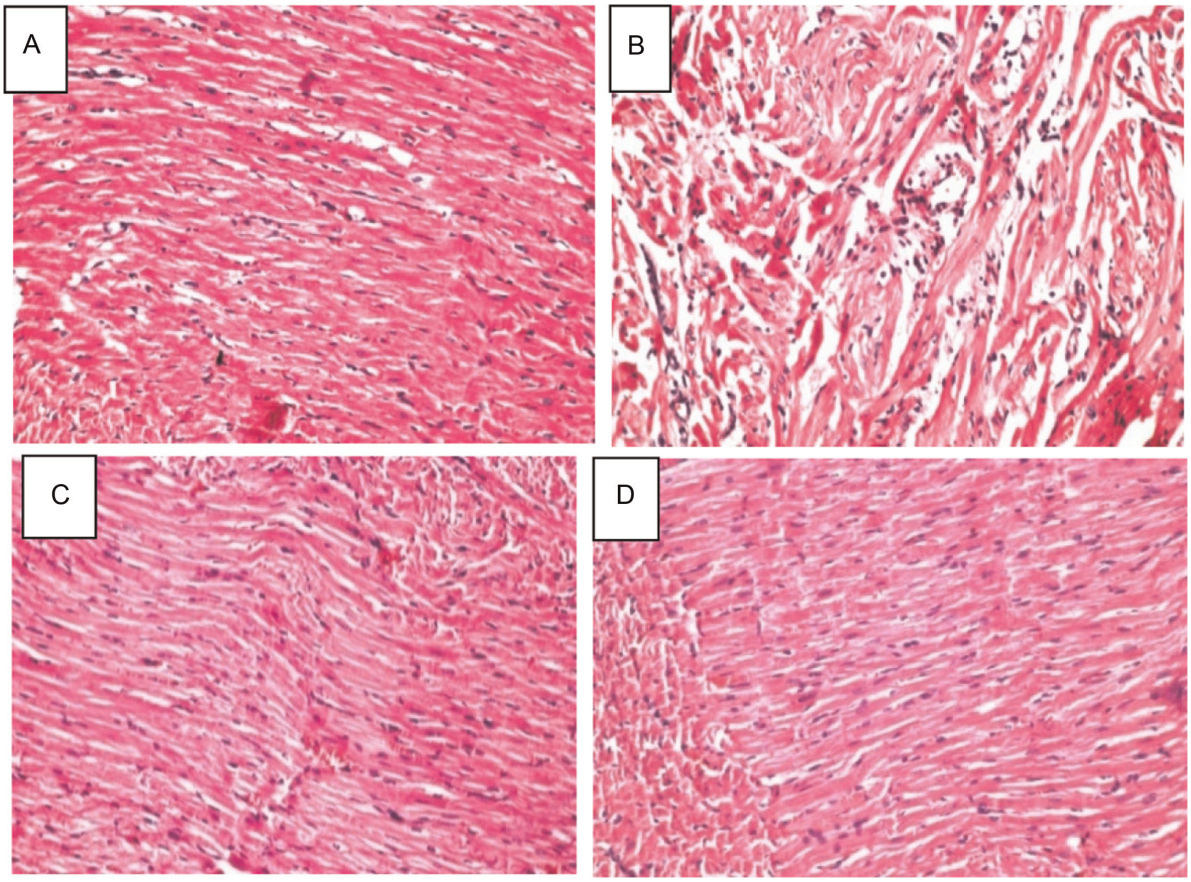
DNA instructions, usually causing the cell to create too little protein content [57].

The present study revealed that exposure to cigarette inhalation resulted in a significant increase in creatinine and urea concentration compared with the normal control group. Creatinine is commonly estimated as a list of glomerular capacity [58]. Urea is an adverse effect of the breakdown of protein. Practically 90% of urea produced is discharged through the kidney [59]. Recent data from the latest two decades have shown its vital antioxidant properties, which provide defense against improved oxidative stress [60].

Hemoglobin concentration values are directly connecting with red blood cell count. This is owing to the synergistic link involving these blood parameters in all vertebrates. This close correlation between hemoglobin and erythrocyte count concentration value was also stated for other vertebrates including man [61]. We showed a normalized level of red blood cell count, white blood cells, and hemoglobin after exposure to the mixtures of lobelia and hops. The expansion in total red blood cell count in cigarette inhalation-treated rats seems to reflect that overwhelming tobacco may also accelerate erythropoiesis. Lacking aspiratory work in smokeless tobacco shoppers may confer a need for energizing erythropoiesis for satisfying the oxygen stresses of the human body. Consequently, the rise in hemoglobin degrees following expansion in erythrocyte production is much anticipated [62]. In concomitant with the current results, Mukherjee and Chatterjee [63] declared increase in total red blood cells and hemoglobin levels. The current investigation is similar together with the earlier observations of Holy *et al.* [64], who found an increase in white blood cell count, which is indicative of leukocytosis.

Exposure to mixtures of lobelia and hops inhalation alone or in combination with cigarette caused significant improvement in body and liver weight, as well as in the levels of liver and kidney functions, red blood cells, white blood cell content, and hemoglobin concentration. Compounds in other types of lobelia, especially the alkaloid lobinaline found in *Lobelia cardinalis*, have been shown to act as antioxidants

Figure 3



Photomicrograph of heart sections from rats of (A) G1, showing normal heart tissue. Normal histological structure of the myocardial bundles with the nuclei is found. (C) G3 and (D) G4, which were given mixtures of lobelia and hops and cigarette plus mixtures of lobelia and hops inhalation, respectively, and (B) G2, treated with cigarette inhalation showing focal inflammatory cells infiltration in the myocardium (hematoxylin and eosin, ×100).

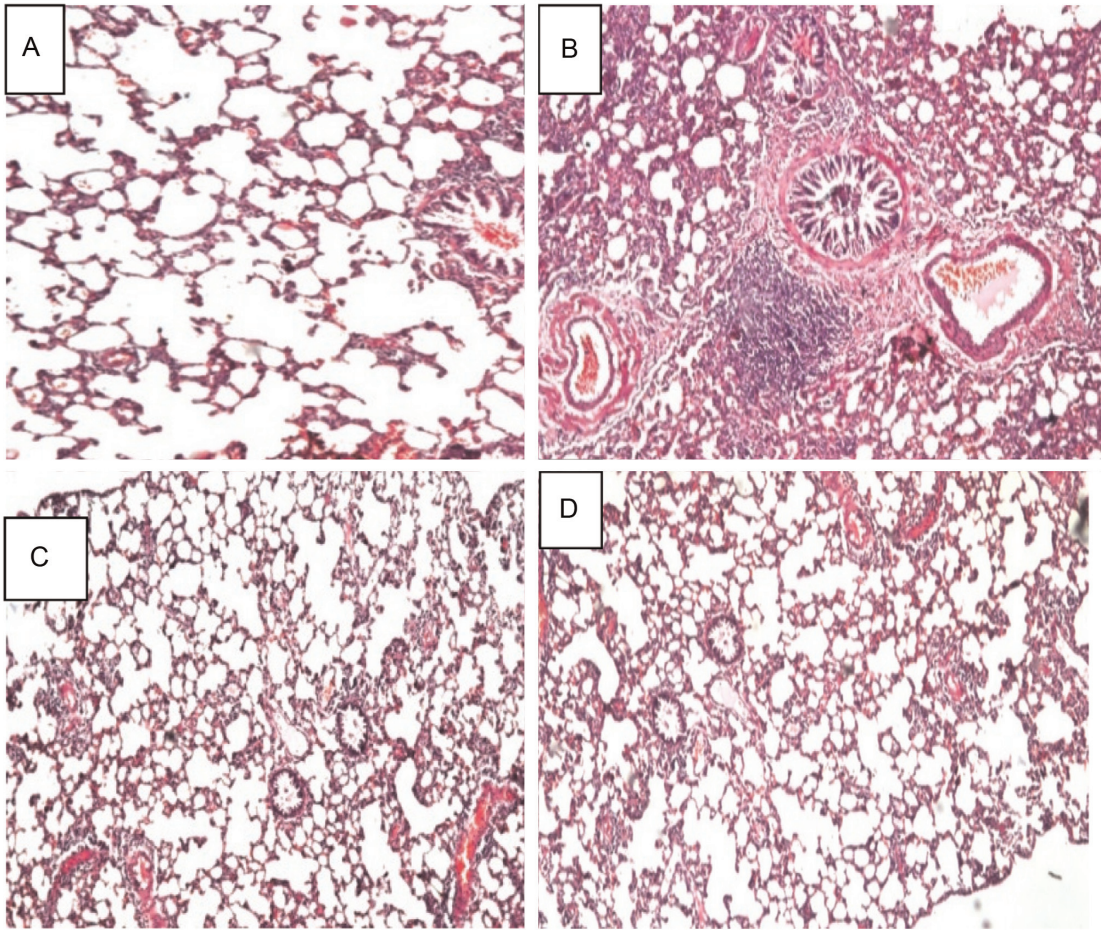
that fight free radicals. These are reactive molecules that can damage cells in your body and increase your risk for illnesses, such as cancer and heart disease, and the mechanism of action of the resin of hop consists of increasing the activity of the neurotransmitter GABA and inhibiting the central nervous system, and also, hops has been used as a medicinal plant for a long history owing to its richness in a variety of phenolic compounds [65].

Nicotine, the major component of cigarette inhalation, plays a vital role in the development of liver and lung problems, because nicotine is mostly metabolized in the liver and excreted by the kidney [66,67]. It initiates oxidative damage to the liver, lung, kidney, and heart; it is a potential oxidant that is capable of producing free radicals and reactive oxygen species. Nicotine creates free radicals that react with biomembranes causing oxidative damage of polyunsaturated fatty acids and forming cytotoxic aldehydes by lipid peroxidation [68]. The histopathological findings in liver and kidney in all groups of female rats support the results of biochemical parameters in the current study. However, our results showed histopathological alterations in liver, kidney, heart, and lung tissues in cigarette inhalation-treated group, whereas exposure of mixture of herbal plants only or in combination with cigarette inhalation to rats caused normalized in all organs tissue. Previous studies reported that oxidative stress-induced free radicals that caused damage in organs have been implicated in malignant transformation [69].

Conclusion

In conclusion, the present investigation showed that cigarette inhalation caused toxic effects on the liver, kidney, heart, and lung in female albino rats. However, the exposure to herbal plants mixture from lobelia (*Lobelia inflata*), and hops (*H. lupulus*) by inhalation alone or mixed with cigarette inhalation can ameliorate this toxic effect. These outcomes could be valuable for adding knowledge about the possible harmful effect of cigarette inhalation in humans and in suggesting that

Figure 4



Photomicrograph of lung sections from rats of (A) G1, showing normal lung tissue. The normal histological structure of the bronchiolar andsurrounding air alveoli and blood vessels are found. (C) G3 and (D) G4), respectively, given mixtures of lobelia and hops cigarette plus mixtures oflobelia and hops inhalation, respectively, and (B) G2, treated with cigarette inhalation, showing the lymphoid hyperplasia and dilatation in theblood vessels associated with collapse and emphysema in the air alveoli (hematoxylin and eosin, ×100).

the herbal plant mixture can be used in herbal cigarette form.

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Conflict of interest

There are no conflicts of interest.

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