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Full Length Article

Carob extract attenuates brain and lung injury in rats exposed to waterpipe smoke

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ABSTRACT

Waterpipe smoking is one of the most popular methods of tobacco consumption world-wide which induces many health problems. In the present study; we evaluated the toxic effects of waterpipe smoke by using amiodarone as a model for lung toxicity in adult male albino rats. Also, the protective and therapeutic effects of carob aqueous extract on these toxicity which produced by daily exposure to waterpipe smoke for 8 weeks. The amiodarone gavage significantly increased serotonin content in brainstem and cerebral cortex after 8 and 2 weeks respectively and dopamine content at most of time intervals. Moreover, waterpipe smoke exposure induced a significant decrease in dopamine content after 2 weeks in brainstem and in serotonin content after 4, 6 weeks in brainstem and 6, 8 weeks in cerebral cortex. While, increment in dopamine content after 4, 6 weeks in brainstem and 6, 8 weeks in cerebral cortex may be due to increase its synthesis which strengthens the coughing reflex. Amiodarone gavage and waterpipe smoke exposure induced a significant increase in myeloperoxidase activity, hydroxyproline content and nitric oxide level in lung; while, catalase activity was decreased significantly. Consequently, waterpipe smoke exposure for long time caused serious harmful effects on brain and lung nearly like amiodarone. Carob extract pre-and post-treatment has the ability to protect and ameliorate these effects due to its antioxidant and anti-coughing effects. So, further studies are necessary to elucidate the mechanism of action of the effective components in the extract.

1. Introduction

Waterpipe smoking is among the most popular methods of tobacco consumption world-wide especially among youth [1,2]; which cause more than 5 million deaths each year [3]. Tobacco is smoked in cigarette, cigar and waterpipe (shisha or narghile). Many people consider the waterpipe is less harmful than cigarette smoke [4]. In addition, the occasional waterpipe tobacco smoking may lead to tissue inflammation.

Tobacco smoke induces cancer, inflammation, oxidative stress in lung and other organs [5,6]. Waterpipe smokers have increased levels of carbon monoxide, so concentrations of carboxyhemoglobin are elevated and lead to tissue hypoxia [7]. However, Virués-Ortega et al. [8] reported that hypoxia affects cognitive functions and cause abnormal motor function.

Chronic tobacco smoking is associated with cognitive flexibility and intellectual abilities. In addition, it affects mood, learning and/or memory processing speed and working memory. Moreover, chronic smoking induces allover brain atrophy, abnormal decline in reasoning, structural and biochemical abnormalities in anterior frontal regions. Generally, it is associated with an increased risk for various forms of neurodegenerative diseases [9].

Amiodarone is an antiarrhythmic agent and is an iodine-containing drug which accumulates in lungs and in other several organs. In addition, amiodarone induces pulmonary toxicity [10]. The carob tree (*Ceratonia siliqua* L.) has been widely cultivated in Mediterranean regions. The carob pods have a prospect role in human healthy. Pods contain a large amount of tannins. Carob extracts have antioxidant and antimutagenic properties, antidiarrheal, cholesterol lowering activities and ameliorate the mice nephrotoxicity [11–14]. Carob is used in cough syrup due to its expectorant effect. However, traditional use of carob cures did not cause any toxicological effects in lung, brain and other organs in male rabbit [15].

According to Khabour et al. [16] the short-term and long-term health effects of waterpipe smoke still need to be investigated.

Abbreviations: 5-HT, serotonin; DA, dopamine; MPO, myeloperoxidase; NO, nitric oxide; CAT, catalase; H_2O_2, hydrogenperoxide; ROS, reactive oxygen species.

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Consequently, the object of the present study is to explore the waterpipe smoke toxicity in brains and lungs of rats using amiodarone as model of lung toxicity. Furthermore, to investigate the effect of carob aqueous extract pre-and post-treatment on rats exposed to waterpipe smoke.

2. Materials and methods

Adult male albino rats used in the present study were purchased from National Organization for Drug Control and Research. One hundred forty-four rats (120–150 g) were housed in clean transparent cages maintained in laboratory of physiology in Faculty of Science, Helwan University under normal environmental conditions of temperature, humidity and light. The standard pelled diet was allowed *ad libitum*. Before experimentation; the rats were kept for about one week to adapt the laboratory conditions and were approved by state authorities and followed Egyptian rules for animal protection.

2.1. Materials

2.1.1. Amiodarone

Amiodarone tablets were obtained from Sanofi-Aventis, Montpellier, France (Commercially found in the form of cordarone). Rats were received daily oral administration of 30 mg/kg b. wt. [17]. Amiodarone was used as a lung toxicity model in rats.

2.1.2. Waterpipe tobacco smoke

Tobacco (moassal) was obtained from Egyptian stores. In an isolated room; rats were transported in clean, isolated, transparent box to be daily exposed to waterpipe smoke (10 g) for 15 min [18]; thereafter, rats were returned to their room.

2.1.3. Carob (Ceratonia siliqua) pods aqueous extract preparation

Carob pods were obtained from Egyptian herbal markets, Cairo, Egypt. The pods were grinded and weighted. Thereafter, the rotary evaporator was used to prepare the aqueous extract according to Ayaz et al. [19]. Rats were received daily oral gavage of 600 mg/kg b. wt. [20].

2.2. Study design

Rats were divided into 6 groups (6 rats per group); 1st group was served as a control group, rats were orally received distilled water daily for 8 weeks. The 2nd group was considered to be "lung toxicity model"; rats were orally administered amiodarone tablets at a dose level of 30 mg/kg b. wt. daily for 8 weeks. The rats of the 3rd group were daily exposed to waterpipe smoke 10 mg (15 min) for 8 weeks. In addition, carob aqueous extract (600 mg/kg b. wt.) was daily administered to rats for 8 weeks (4th group).

Finally, the remaining two groups (5th & 6th) are protective and therapeutic groups respectively. In protective group; rats were daily gavaged aqueous extract of carob (600 mg/kg b. wt.) then 30 min after rats were exposed to waterpipe smoke (15 min) for 8 weeks. However, rats of therapeutic group were daily exposed to waterpipe smoke 10 mg (15 min) then 30 min afterwards rats were daily gavaged carob aqueous extract (600 mg/kg b. wt.) for 8 weeks.

2.3. Methods

For neuro- and biochemical investigations; rats were killed by sudden decapitation at different time intervals "2nd, 4th, 6th and 8th" weeks during the experiment.

2.3.1. Brain tissue preparation and neuro-investigations

Brains were rapidly excised from skulls, blotted with filter paper then dissection was performed on an ice cooled glass plate. Brains were divided into two hemispheres; each one was separated into brainstem and cerebral cortex according to Glowinski and Iversen [21]. The selected brain areas were weighed, wrapped in plastic films then in aluminum foil and quickly frozen in a refrigerator (−70 °C) till used for estimation of monoamines (serotonin "5-HT" and dopamine "DA") according to the method of Ciarlone [22].

2.3.2. Lung tissue preparation and biochemical investigations

Lungs were excised and only 0.40 g were weighed to be homogenized in ice-cold Tris-HCl buffer solution (pH 7.4) and centrifuged at 2000 r.p.m. for 10 min to separate the supernatant and quickly frozen in dry ice (−70 °C) till use for further determination for myeloperoxidase (MPO) activity, hydroxyproline content, nitric oxide (NO) level and catalase (CAT) activity.

2.3.3. Monoamines estimation

In acidified n-butanol; cerebral cortex and brainstem were homogenized to be centrifuged (2000 r.p.m.; 5 min); then the supernatant fluid (2.5 ml) were transferred to tubes containing 1.6 ml of 0.2 N acetic acid and n-heptane (5 ml). The tubes were placed in a vortex mixer for 30 sec. After centrifugation (1000 r.p.m. 5 min); the aqueous phase was separated and stored at −70 °C for 5-HT & DA estimations [22].

2.3.4. Estimation of 5-HT content

1. Three external standards for 5-HT were prepared in different concentrations in 0.2 N acetic acid and made up to a total volume of 0.3 ml.
2. To all 5-HT tubes 1.2 ml of OPT (4 mg/100 ml, 10 N HCl) was added and mixed well. The tubes placed in a boiling water bath for 10 min, all tubes were cooled by tap water and read the florescence in a fluorometer. Excitation and emission were 355 and 470 nm, respectively [22].

2.3.5. Estimation of DA content

1. The external standards were prepared for DA using three different concentrations in 0.1 N HCl and completed to a final volume of 1.6 ml and followed by adding 2.5 ml n-butanol and 5 ml n-heptane to the tubes. Thereafter; to be acted as samples to get the aqueous phase.
2. To all the tubes and reagent blank (1 ml of 0.1 N HCl), 0.2 ml of 0.1 M EDTA was added, the mixture was adjusted to a pH 6.5; then 0.1 ml of (0.1 N) iodine was added to oxidize the catecholamine.
3. Exactly after 2 min; the oxidation was stopped by the addition of 0.2 ml of alkaline sulfite and exactly 2 min. Later, the solution was adjusted to a pH 5.4 by the addition 0.2 ml of 5 N acetic acid.
4. To assay DA, read the florescence after heating in a boiling water bath for exactly 5 min. and cooling all tubes by using tap water. Excitation and emission were 320 and 372 nm, respectively [22].

2.3.6. Estimation of lung MPO activity

By a sandwich enzyme immunoassay for *in vitro* quantitative measurement of myeloperoxidase activity in tissue homogenate [23], at the end of the experiment the color change is measured spectrophotometrically at a wavelength of 450 nm ± 10 nm.