



## 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.

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## 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<sub>2</sub>O<sub>2</sub>, 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 pilled 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 2<sup>nd</sup> 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 3<sup>rd</sup> 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 (4<sup>th</sup> group).

Finally, the remaining two groups (5<sup>th</sup> & 6<sup>th</sup>) 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 “2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup>” 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 fluorescence 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.

### 2.3.7. Estimation of lung hydroxyproline content

According to Woessner [24]; the content of hydroxyproline in tissue homogenates was measured quantitatively by a competitive inhibition enzyme immunoassay technique.

### 2.3.8. Determination of NO level

The level of NO was determined in pulmonary tissue according to Green et al. [25]; where, nitrous acid diazotize sulfanilamide was formed in an acid medium and in the presence of nitrite. Then nitrous acid diazotize sulfanilamide is coupled with N-(1-naphthyl) ethylene diamine forming Azo dye (a bright reddish-purple color) and it can be measured at 540 nm.

### 2.3.9. Determination of CAT activity

The pulmonary CAT activity was determined by the method of Bock et al. [26], where; CAT decomposes  $H_2O_2$  which can be followed directly by a decrease in the absorbance at 240 nm. The difference in the absorbance per unit time is a measure of the CAT activity.

### 2.4. Statistical analysis

The obtained data were presented as means  $\pm$  standard error. The statistical comparisons among the groups were carried out by using one-way ANOVA (Duncan's test); (SPSS version17.0).  $P \leq 0.05$  was considered as significant for all statistical analysis in this study.

## 3. Results

As shown in Fig. 1, 5-HT content in brainstem recorded a non-significant change as a result of daily oral administration each of amiodarone (30 mg/kg), carob aqueous extract (600 mg/kg) as well as exposure to waterpipe smoke (10 mg of tobacco) after 2 weeks. Similarly, the pre-treatment group showed a non-significant increase in 5-HT content as compared to control group. Moreover, a significant increase was observed in post-treatment group in 5-HT content of brainstem versus control group after 2 weeks. Likewise, a non-significant change was found in both pre- and post-treatment groups versus waterpipe smoke group.

After 4 weeks; 5-HT content in brainstem decreased significantly at  $P \leq 0.05$  as a result of daily amiodarone gavage and daily exposure to waterpipe smoke recording  $-23.44\%$  and  $-22.51\%$  as a percentage change respectively. In the same manner, a significant decrease was noticed in both pre- and post-treatment groups ( $-21.60\%$  and  $-23.20\%$ , respectively) as compared to control value. However, versus waterpipe smoke group; a significant decrease was cleared ( $-12.61\%$ ) in post-treatment group.

The daily amiodarone gavage for 6 weeks induced a significant reduction in 5-HT content in brainstem with a percentage change  $-16.00\%$ . Likewise, daily exposure to waterpipe smoke induced a significant reduction ( $-19.00\%$ ) versus control group. Carob extract administration before waterpipe smoke exposure caused a non-significant increase in 5-HT content versus control value, however as compared to waterpipe smoke group; 5-HT content showed a significant increase ( $P \leq 0.05$ ) recording  $34.61\%$  as a percentage change. Meanwhile, rats exposed to waterpipe smoke before gavage of carob aqueous extract influenced a significant increase in 5-HT content of brainstem after 6 weeks recording  $24.50\%$  and  $53.74\%$  as a percentage change as compared to control and waterpipe smoke groups respectively.

On the other hand, the daily oral administration of amiodarone caused a significant increase at  $P \leq 0.05$  in 5-HT content in brainstem on 8<sup>th</sup> week with a percentage change  $27.00\%$  versus control group (Fig. 1). Moreover, a non-significant change was observed in 5-HT content after 8 weeks of daily waterpipe exposure, carob extract administration and pre-/post-treatment group.

In cerebral cortex; 5-HT content recorded a significant increase ( $14.40\%$ ) after 2 weeks at  $P \leq 0.05$  as a result of daily oral administration of amiodarone (30 mg/kg). However, after 6 weeks 5-HT content cleared a significant decrease recording  $-22.50\%$  as a percentage change as compared to control group (Fig. 2). 5-HT content showed an increase in cerebral cortex in rats exposed to waterpipe smoke; it was non-significant on 2<sup>nd</sup> week while it was a significant on 4<sup>th</sup> week ( $14.60\%$ ). On contrary; the daily exposure to waterpipe smoke decreased 5-HT content significantly on 6<sup>th</sup> and 8<sup>th</sup> weeks with a percentage change ( $-36.70\%$ ) and ( $-25.10\%$ ) respectively versus control group.

Moreover, a significant reduction in 5-HT content was started on 4<sup>th</sup> week till 8<sup>th</sup> week in cerebral cortex as a result of daily oral administration of aqueous carob extract (600 mg/kg). The maximum reduction was found after 8 weeks. The pre-treatment with carob extract influenced a significant decrease in 5-HT content ( $P \leq 0.05$ ) on 8<sup>th</sup> week versus control group. However, in a comparison to waterpipe smoke group; 5-HT content decreased significantly on 4<sup>th</sup> week. On the other hand; the carob extract pre-treatment caused a significant increase in 5-HT content on 6<sup>th</sup> week. However, the post-treatment of carob extract induced a significant decrease in 5-HT content started from 6<sup>th</sup> week as compared to control group. Meanwhile, a significant decrease was recorded on 4<sup>th</sup> week ( $-14.51\%$ ) in 5-HT as compared to waterpipe smoke group as shown in Fig. 2.

Amiodarone administration (30 mg/kg) induced a significant decrease in DA content in brainstem after 2 weeks recording  $-24.90\%$  at  $p \leq 0.05$  as cleared in Fig. 3. On the other hand, amiodarone increased the content of DA in brainstem; it was

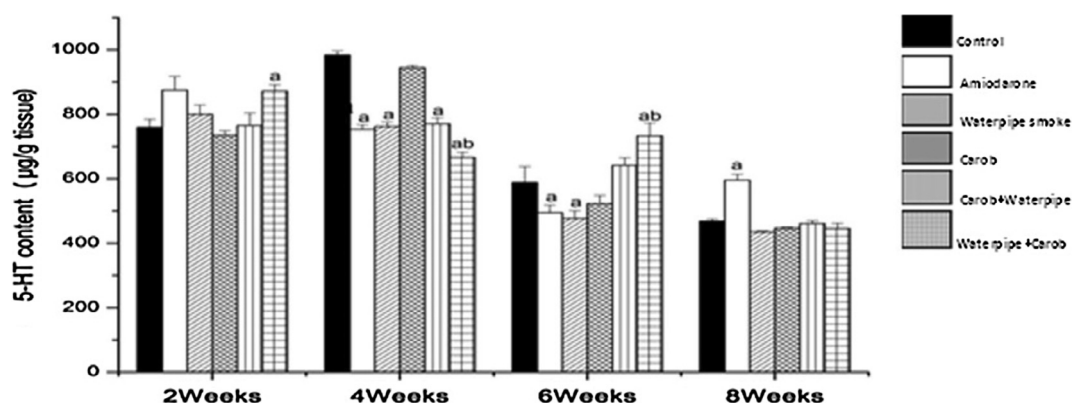
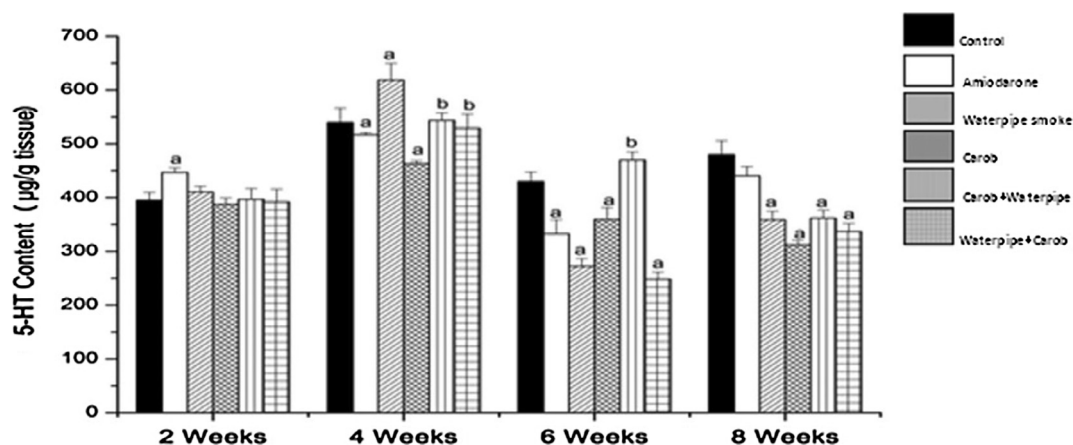


Fig. 1. Effect of daily exposure to waterpipe smoke on 5-HT content in brainstem of adult male albino rat pre- and post-treated with carob extract (600 mg/kg) for 8 weeks. Significant change at  $p \leq 0.05$  comparing to control group, (b) significant change at  $p \leq 0.05$  comparing to waterpipe smoking group.



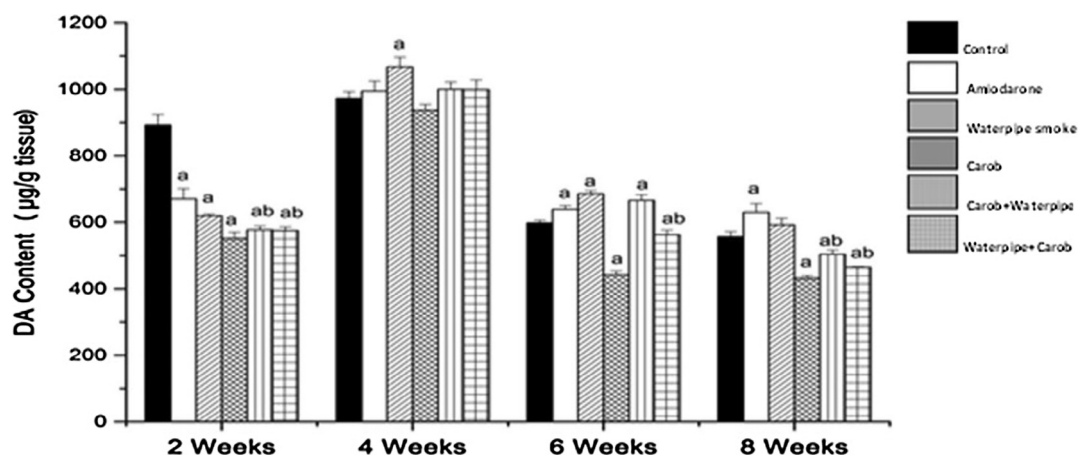
**Fig. 2.** Effect of daily exposure to waterpipe smoke on 5-HT content in cerebral cortex of adult male albino rat pre- and post-treated with carob extract (600 mg/kg) for 8 weeks. Significant change at  $p \leq 0.05$  comparing to control group, (b) significant change at  $p \leq 0.05$  comparing to waterpipe smoking group.

non-significant after 4 weeks; while, it was a significant on 6<sup>th</sup> and 8<sup>th</sup> weeks as compared to its corresponding control. However, there was a significant decrease in DA content in brainstem on 2<sup>nd</sup> week as a result of waterpipe smoke exposure. On contrary, there was a significant increase in DA content on 4<sup>th</sup> and 6<sup>th</sup> week versus corresponding control group. Moreover, the daily gavage of carob aqueous extract decreased the content of DA in brainstem significantly on 2<sup>nd</sup>, 6<sup>th</sup> and 8<sup>th</sup> week. The maximum reduction was found on 2<sup>nd</sup> week recording –38.20% as a percentage change. The pre-treatment of carob aqueous extract revealed a significant decrease in DA content on 2<sup>nd</sup> week, a significant increase on 6<sup>th</sup> week (11.33%); while the pre-treatment decreased DA content significantly on 8<sup>th</sup> week as compared to corresponding control. In a comparison to waterpipe smoke group; there was a significant decrease on 2<sup>nd</sup> and 8<sup>th</sup> week in DA content of brainstem.

In post-treatment group; DA content recorded a significant decrease on 2<sup>nd</sup>, 6<sup>th</sup> and 8<sup>th</sup> week versus corresponding control value. The maximum reduction was observed on 2<sup>nd</sup> week (–35.50%). Similarly, the rats were gavaged carob aqueous extract after the exposure to waterpipe smoke influenced a significant decrease in DA content in brainstem on 2<sup>nd</sup>, 6<sup>th</sup> and 8<sup>th</sup> week versus corresponding waterpipe smoke group. The maximum reduction was found on 8<sup>th</sup> week with a percentage change –21.60%.

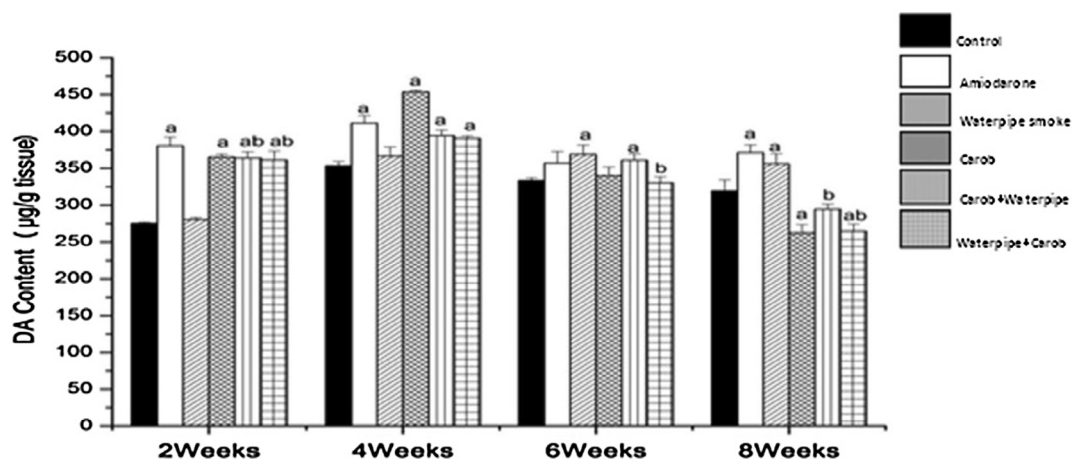
DA content in cerebral cortex revealed a significant increase on 2<sup>nd</sup>, 4<sup>th</sup> and 8<sup>th</sup> weeks as a result of daily administration of 30 mg/kg of amiodarone (Fig. 4). Meanwhile, rats exposed to waterpipe smoke showed a significant increase in DA content of cerebral cortex on 6<sup>th</sup> and 8<sup>th</sup> weeks recording 10.57% and 11.62% as a percentage change versus corresponding control value. Moreover, the daily oral administration of aqueous carob extract caused a significant increase in the content of DA in cerebral cortex on 2<sup>nd</sup> and 4<sup>th</sup> weeks. On contrary, a significant decrease in DA content was observed on 8<sup>th</sup> week as a result of carob extract (600 mg/kg) gavage.

A significant increase in DA content of cerebral cortex was started from 2<sup>nd</sup> till 6<sup>th</sup> week of the carob pre-treatment group; as compared to corresponding control group. The maximum increase was found on 2<sup>nd</sup> week with a percentage change (32.14%). In a comparison to waterpipe smoke group; carob extract pre-treatment induced a significant increase in cerebral cortex DA content on 2<sup>nd</sup> week; while it induced a significant decrease ( $p \leq 0.05$ ) in DA content on 8<sup>th</sup> week (–19.41%). The daily treatment of carob extract after rats being exposed to waterpipe smoke (post-treatment group) resulted in a significant increase after 2 and 4 weeks with percentage change 31.18% and 10.50% respectively, while a significant decrease was observed on 8 week



**Fig. 3.** Effect of daily exposure to waterpipe smoke on DA content in brainstem of adult male albino rat pre- and post-treated with carob extract (600 mg/kg) for 8 weeks. Significant change at  $p \leq 0.05$  comparing to control group, (b) significant change at  $p \leq 0.05$  comparing to waterpipe smoking group.





**Fig. 4.** Effect of daily exposure to waterpipe smoke on DA content in cerebral cortex of adult male albino rat pre- and post-treated with carob extract (600 mg/kg) for 8 weeks. Significant change at  $p \leq 0.05$  comparing to control group, (b) significant change at  $p \leq 0.05$  comparing to waterpipe smoking group.

(–17.14%) as compared to corresponding control group. On the other hand, when compared with waterpipe smoke group, DA content was significantly increased on 2 week (28.80%), on contrary; DA content was significantly decreased after 6 and 8 weeks (–10.40% and –25.77%) respectively (Fig. 4).

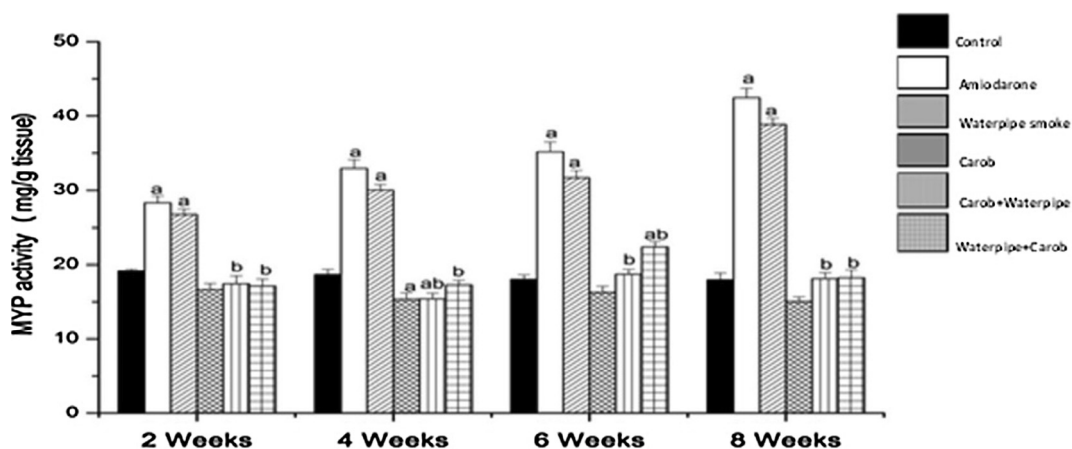
The daily oral administration of “30 mg/kg” of amiodarone caused a gradual and a significant increase in MPO activity in lung tissue all over the experimental period as compared to corresponding control value (Fig. 5). The maximum increase was observed at the end of treatment recording 136.50% as a percentage change. In the same manner, MPO activity revealed a gradual and a significant increase as a result of waterpipe smoke exposure (10 mg) all over experimental time. The rats were daily gavaged 600 mg/kg of carob aqueous extract showed a reduction in the activity of MPO all over the experimental time; it was a significant on 4<sup>th</sup> week with a percentage change (–18.00%). The rats were administered carob aqueous extract before their exposure to waterpipe smoke showed a significant decrease in the activity of MPO on 4<sup>th</sup> week (–17.48%) versus control group. Meanwhile, a significant decrease was noticed in pulmonary MPO activity all the experimental time interval as compared to waterpipe smoke group. The maximum reduction was recorded on 8<sup>th</sup> week.

Moreover, the treatment of carob extract after the waterpipe smoke exposure induced a significant increase on 6<sup>th</sup> week versus control value. While; in a comparison to waterpipe smoke the

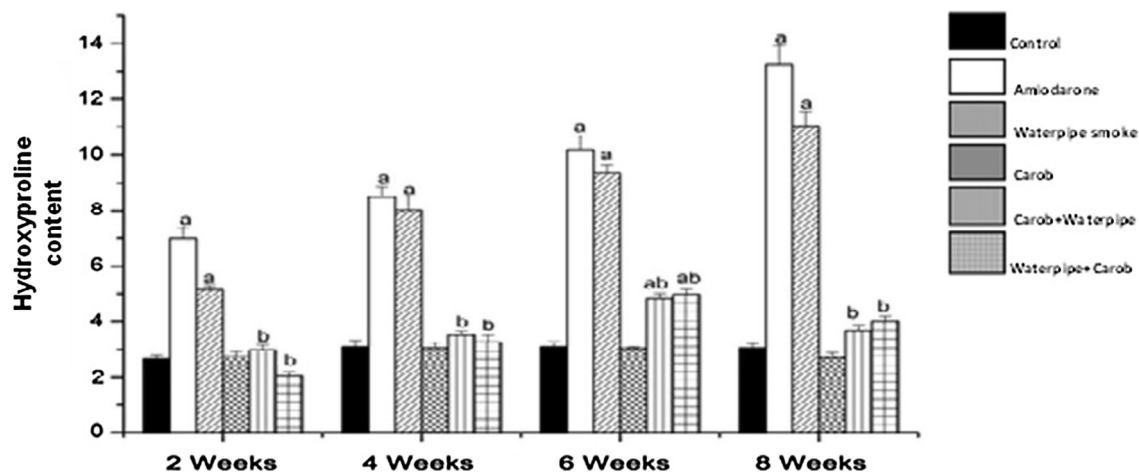
carob extract treatment induced a significant reduction in pulmonary MPO activity all over the time interval of the experiment. The maximum reduction was found on 8<sup>th</sup> week with a percentage change –53.16%.

As shown in Fig. 6; the daily administration of 30 mg/kg of amiodarone induced a significant elevation in pulmonary hydroxyproline content all over time intervals of the experiment with a percentage change 164.78%, 173.50%, 229.00% and 333.66% on 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> weeks respectively. Likewise, a gradual and a significant elevation was obtained in hydroxyproline content at  $p \leq 0.05$  as a result of exposure to waterpipe smoke all over experimental time as compared to control group. The maximum elevation was recorded on 8<sup>th</sup> week (260.79%). On the other hand, the daily administration of carob extract caused a non-significant change in hydroxyproline content all time intervals of the experiment. Moreover, in both pre- and post-treatment groups hydroxyproline content showed a significant increase on 6<sup>th</sup> week (55.50% and 60.64%) respectively versus control group. While; a significant decrease was observed in hydroxyproline content started from 2<sup>nd</sup> till 8<sup>th</sup> week versus waterpipe smoke group.

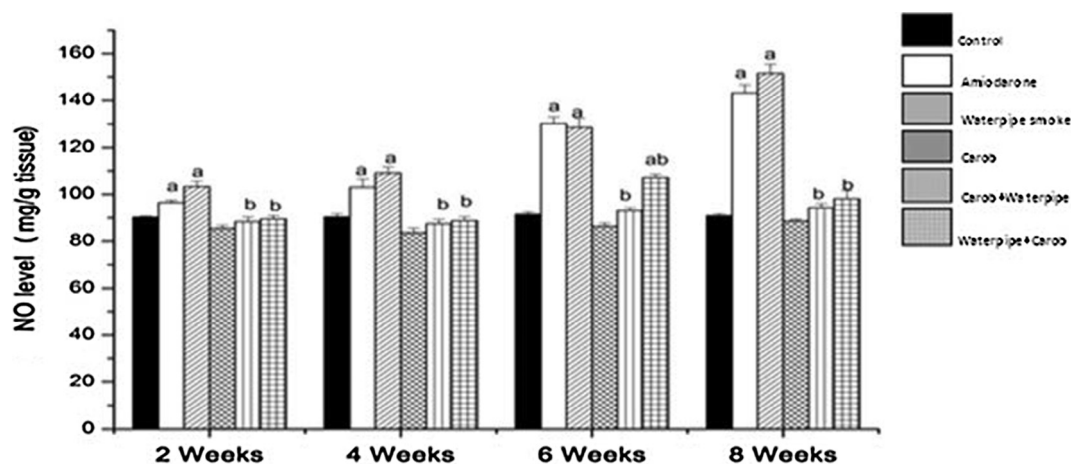
In Fig. 7; daily oral gavage of amiodarone/or daily exposure to waterpipe smoke induced a gradual and a significant increase in pulmonary NO level started from 2<sup>nd</sup> till 8<sup>th</sup> week as compared to corresponding control value. A non-significant change was found in NO level resulting from daily oral administration of 600 mg/kg



**Fig. 5.** Effect of daily exposure to waterpipe smoke on myeloperoxidase (MPO) activity in lung tissue of adult male albino rat pre- and post-treated with carob extract (600 mg/kg) for 8 weeks. (a) Significant change at  $p \leq 0.05$  comparing to control group, (b) significant change at  $p \leq 0.05$  comparing to waterpipe smoking group.



**Fig. 6.** Effect of daily exposure to waterpipe smoke on Hydroxyproline content in lung tissue of adult male albino rat pre- and post-treated with carob extract (600 mg/kg) for 8 week. Significant change at  $p \leq 0.05$  comparing to control group, (b) significant change at  $p \leq 0.05$  comparing to waterpipe smoking group.



**Fig. 7.** Effect of daily exposure to waterpipe smoke on NO level in lung tissue of adult male albino rat pre and post-treated with carob extract (600 mg/kg) for 8 weeks. Significant change at  $p \leq 0.05$  comparing to control group, (b) significant change at  $p \leq 0.05$  comparing to waterpipe smoking group.

carob aqueous extract before waterpipe smoke exposure (pre-treatment); versus corresponding control group. While, a significant decrease was recorded in NO level allover experimental time intervals as compared to waterpipe smoke values in pre-treatment group. The maximum reduction was observed on 8<sup>th</sup> week with a percentage change  $-37.72\%$ . Moreover, the post-treatment caused a non-significant change in NO level on 2<sup>nd</sup>, 4<sup>th</sup> and 8<sup>th</sup> week, meanwhile, a significant increase was obtained on 6<sup>th</sup> week ( $18.60\%$ ) as compared to control group. Oppositely; the post-treatment induced a significant decrease in NO level allover the experimental time interval as compared to corresponding waterpipe smoke group.

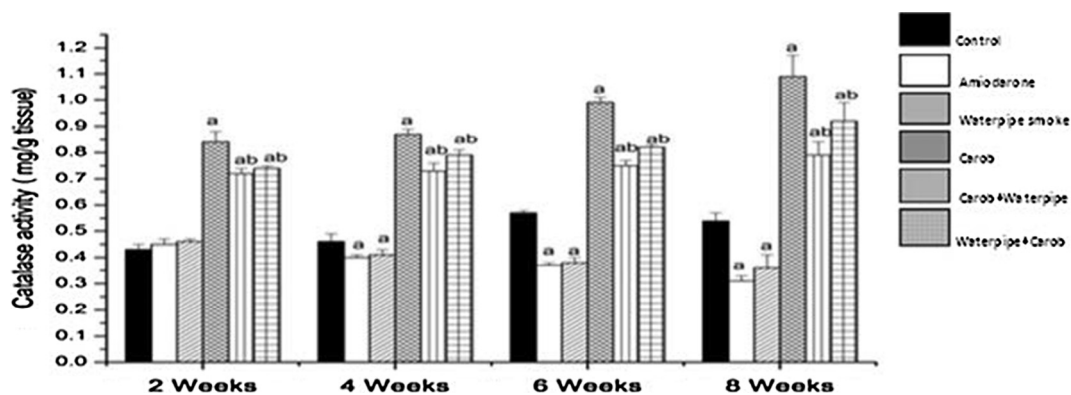
CAT activity in lung tissue was shown in Fig. 8; the daily gavage of amiodarone to rats induced a gradual and a significant decrease in CAT activity started from 4<sup>th</sup> till 8<sup>th</sup> week as compared to corresponding control value. Moreover, the daily exposure to 10 mg of waterpipe smoke for (15 min) caused a gradual and a significant decrease in CAT activity started on 4<sup>th</sup> and 6<sup>th</sup> week with a percentage change  $-10.86\%$  and  $-33.33\%$  respectively. However, the continuous exposure to waterpipe smoke recorded a constant and a significant reduction at  $p \leq 0.05$  in pulmonary CAT activity on 8<sup>th</sup> week ( $-33.33\%$ ) versus control group. On contrary, pulmonary CAT activity was increased significantly in rats were orally administered the aqueous extract of carob for 8 weeks; where the maxi-

imum increment was found on 8<sup>th</sup> week with a percentage change  $101.85\%$  as compared to control group. The pre-treatment with carob extract increased CAT activity in lung significantly allover time intervals as compared to control and waterpipe smoke groups. In the same line; post-treatment resulted in a significant increase in CAT activity at  $p \leq 0.05$  at all experimental time intervals versus both control and waterpipe smoke groups.

#### 4. Discussion

The oral administration of amiodarone caused a significant increase in 5-HT content after 8 weeks in brainstem and 2 weeks in cerebral cortex while, it caused a significant decrease in its content after 4 and 6 weeks in brainstem and 6 weeks in cerebral cortex. Also it caused a significant increase in DA content in brainstem and cerebral cortex at most of time intervals.

Wyss et al. [27] reported that amiodarone pass into the brain and it has an anticonvulsant and hypnotic effects. Moreover, amiodarone prolonged the sleeping time and behaved as central nervous system depressant drug in pentobarbital-induced sleeping in rat model [28]. Many reports attributed the anticonvulsant activity of amiodarone to its activity as a multiple ion-channel blocker drug which inhibit  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  inward and  $\text{K}^+$  outward currents [28,29]. It well known that when action potentials depolarize



**Fig. 8.** Effect of daily exposure to waterpipe smoke on catalase activity in lung tissue of adult male albino rat pre and post-treated with carob extract (600 mg/kg) for 8 weeks. (a) Significant change at  $p \leq 0.05$  comparing to control group, (b) significant change at  $p \leq 0.05$  comparing to waterpipe smoking group.

the plasma membrane of the axon terminal, voltage-gated  $\text{Ca}^{2+}$  channels is open. This permits  $\text{Ca}^{2+}$  to diffuse down its concentration gradient into the cytoplasm, where it stimulates the release of neurotransmitters which stored in synaptic vesicles by exocytosis [30]. Because of the amiodarone is a calcium ion-channel blocker [29]; so the neurotransmitters release is inhibited and as the result their content is increased in brain which may explain the increment in neurotransmitters content in some time intervals in the present study.

In laboratory animals, intravenous nicotine increases the sensitivity of brain reward systems and imprints an indelible memory of its effects; an action that appears unique to nicotine among drugs of abuse. This may partially explain the rapid return to former levels of smoking that frequently follows a relapse after a prolonged period of smoking abstinence [31]. Waterpipe smokers can be exposed to sufficient doses of nicotine which rapidly distribute to the brain and changes its structure and function so; it results in physical mood-altering effects by acting as a stimulant and a relaxant as well [32]. Moreover, nicotine is a psychomotor stimulant that binds to acetylcholine receptors and has short- and long-term effects. In the short-term, it can produce nausea or dizziness in new users and mild euphoria in experienced users. The mood effects are directly attributable to nicotine-mediated neurotransmitter release such as DA and 5-HT [32,33].

From the present results, it is clear that DA content is decreased after 2 weeks in brainstem of rats exposed to waterpipe smoke. The primary target for nicotine in the central nervous system is the  $\alpha 4\beta 2$  nicotinic acetylcholine receptor, which when activated by nicotine binding, results in the release of DA in the brain's "reward center" and provides the positive reinforcement observed with cigarette smoking [34].

However, the present study revealed that 5-HT content significantly decreased after 4 and 6 weeks in brainstem and 6 and 8 weeks in cerebral cortex after exposure to waterpipe smoke. It is clear that when nicotine binds to its receptors, which are ligand-gated ion channels, these channels are open in allowing the entry of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  ions which facilitate the release of neurotransmitters [32] and as results the contents of DA and 5-HT were decreased as shown in the present results.

The waterpipe smokers had increased cough and biologic abnormalities in several anatomic components in the lung compared to nonsmokers [35]. The cough reflex can be initiated by a wide variety of stimuli [36]. The vagal fibers for cough enter the brainstem (cough center) and relay in the nucleus of the solitary tract with connections to second-order neurons [37,38]. Afferent impulses travel to the dorsal medulla where the reflex is subject to cortical control where cerebral cortex has a role in influencing

cough. The brain then sends signals back to the lungs and respiratory muscles [39,40]. Neurotransmitters which involved in the central cough complex are tachykinins, glutamate, g-aminobutyric acid, N-methyl-D-aspartate, 5-HT and DA [37,38,41]. Increased release of substance P is involved in the development of cough and inflammation of the airway, and its production is regulated by dopaminergic neurons in the brainstem.

Previous study indicated that L-dopa (a precursor of DA) strengthens the coughing reflex [42]. So, the increment in DA content in the present study after exposure to waterpipe smoke after 4, 6 weeks in brainstem and after 6, 8 weeks in cerebral cortex may be due to increase its synthesis from L-dopa. In addition, Long-term use of nicotine can produce tolerance and dependence [43,44]. Neuroadaptation of the mesolimbic system in smokers occur. So, long term use of nicotine can produce tolerance and dependence [44], which may explain why there was a non-significant change in DA content in brainstem at the end of experiment in the present study.

From present results, it is clear that the oral administration of aqueous extract of carob induced a significant decrease in DA content in brainstem at almost time intervals while, in cerebral cortex, it caused a significant decrease after 8 weeks and a significant increase after 2 and 4 weeks. It also caused a significant decrease in 5-HT content at almost time intervals in cerebral cortex.

Previous studies indicated that carob has some medicinal uses where it is used in cough syrup [45,46] which may be due to decrease in DA synthesis via decrease in L- dopa to inhibit cough reflex as a result its content is decreased. Agrawal et al. [47] demonstrated that carob exhibited significant antidepressant effect in both tail suspension test and forced swim test. It also inhibits haloperidol-induced catalepsy and potentiates apomorphine-induced sniffing in rats. Grabowska et al. [48] indicated that apomorphine has been found to elevate 5-hydroxyindoleacetic acid content in rat and mouse brain. These findings were confirmed by the study of Scheel-Krieger and Hasselager [49] which indicated that the turnover of brain 5-HT is increased under the influence of apomorphine [48,50,51]. So, the decrement in 5-HT content after oral administration of carob extract in the present study may be due to increase in its turnover or inhibit its reuptake.

The most serious side effect of amiodarone is pulmonary toxicity [52]. So, it is used in this study as a model for pulmonary toxicity [53,54]. The present results revealed that the oral administration of amiodarone and the exposure to waterpipe smoke caused an increase in lung MPO activity. It is known that MPO was suggested as an early marker of systemic inflammation in smokers [55]. It is one of the most abundant proteins in neutrophils [56]. It stored in granules and released when neutrophils

are stimulated, it catalyzes the oxidation of chloride and other halide ions in the presence of hydrogen peroxide [57,58] to generate hypochlorous acid and other highly reactive products that mediate efficient antimicrobial action [59,60].

Previous studies revealed that amiodarone is metabolized to an aryl radical that may give rise to other reactive oxygen species (ROS) [61,62] which cause endothelial injury leading to oedema, thrombosis and inflammation contributing to morbidity and mortality in acute lung injury [63]. The activation of macrophages and release of inflammatory and cytotoxic mediators drive amiodarone-induced lung fibrosis [64]. *In vitro* studies have shown that alveolar leucocytes and macrophages from cigarette smokers spontaneously increase the release of oxidants amounts such as  $O_2$  and hydrogen peroxide ( $H_2O_2$ ) compared with non-smokers [65]. Neutrophils sequestered in the pulmonary circulation following cigarette smoke inhalation in the rabbit [66]. Also, in hamsters; the cigarette smoke inhalation activates neutrophils, increasing their adhesion to the endothelium of both arterioles and venules which is mediated by superoxide anion [67]. Thus, by several mechanisms involving oxidants, cigarette smoke causes neutrophil sequestration in the pulmonary microcirculation [68].

Also, Anderson et al. [69] demonstrated that passive cigarette smoking has been associated with increasing peripheral blood leucocytes counts and enhancement the release of ROS which directly damage the surrounding tissues and participate in inflammatory disorders [70]. In addition to the ROS production, the cytoplasmic granules of the neutrophils discharge hydrolytic, proteolytic enzymes and MPO [71]. So, the activation of neutrophils as a result of inflammation occurred by administration of amiodarone or exposure to waterpipe smoke may cause the increment in the activity of lung MPO as shown in the present study.

Moreover, the present results demonstrated that both amiodarone administration and exposure to waterpipe smoke increased pulmonary hydroxyproline content. These results are consistent with the previous study which demonstrated that amiodarone elevated hydroxyproline content in rats [64]. Oxidative stress, in particular lipid peroxidation, induces collagen synthesis [72]. Collagen constitutes measured by hydroxyproline, might serve as an index of lung tissue damage in the rat exposed to the various irritants present in waterpipe smoke for a period of time [73]. So, the increment in hydroxyproline content in the present results may be due to its mobilization for collagen formation by the fibroblasts which surrounding the injured lung tissues.

In the present study; NO level was significantly increased after amiodarone administration and the exposure to waterpipe smoke. Verifying that both amiodarone and waterpipe smoke induce markedly excessive ROS production that lead to serious oxidative damage. Punithavathi et al. [64] reported that amiodarone increased cellular oxidant production in treated rats. Moreover, amiodarone impaired lipid metabolism in lungs which damaged the endothelium and increased ROS production [62].

Nicotine, a major toxic component of cigarette smoke, is a well-established procarcinogen. In addition; it disrupts the mitochondrial respiratory chain leading to an increase in generation of superoxide anion and  $H_2O_2$  [74]. This may lead to oxidative damage of macromolecules including lipid, DNA and RNA [75]. Moreover; the heavy metals such as lead and cadmium which present in smoke are also capable of inducing lipid peroxidation and hence provokes damage of organs [76].

However, NO which is present in the gas phase of cigarette smoke, reacts with tyrosine to form nitrotyrosine products of proteins in plasma *in vitro* [77], these products can interfere with cell signaling pathways involving tyrosine phosphorylation and implicated in many lung diseases [78]. The increase in NO level is indicative of oxidant related tissue injury by formation of highly reactive nitrogen intermediates. NO reacts with superoxide and generates a

highly reactive metabolite, peroxynitrite, which is presumed to be largely responsible for most of the adverse effects of excessive generation of NO [79]. So, the increment of NO level in the present study may be due to increase in lipid peroxidation as a result of increasing the production of ROS and the presence of heavy metals and NO in waterpipe smoke.

The potential damage that can be caused by free radicals is normally minimized by a combination of biological antioxidant systems including enzymatic and non-enzymatic reactions [80]. An imbalance between oxidants and antioxidants has been proposed in the pathogenesis of chronic obstructive pulmonary disease [81].

CAT acts as preventive antioxidant [82]; its primary role is to scavenge  $H_2O_2$  that has been generated by free radicals and convert it to water [83]. The present results showed that the administration of amiodarone and the exposure to waterpipe smoke caused a significant decrease in CAT activity these results are consistent with the previous study of Ozbakis-Dengiz and Bakirci [28]; which demonstrated that administration of amiodarone decreased the activity of CAT in male mice and rats. Also, Al-Shammari et al. [52] postulated that the decrement in CAT activity in amiodarone administered group resulting from induction of oxidative stress.

In the experiment described by Baskaran et al. [84] showed that the limited time exposure of rats to tobacco smoke resulted in the induction of CAT and super oxide dismutase as adaptation to the oxidative insult. In the same line, Luchese et al. [85] demonstrated that the reduction of CAT activity may be related to excess  $H_2O_2$  production. Also, Mahapatra et al. [86] attributed the decreased the antioxidant enzymes activity to increase in their utilization to scavenge the free radicals generated from cigarette smoke. So, these may explain the decrement in CAT activity in rats treated with amiodarone and exposed to waterpipe smoke in the present study which may be due to its utilization to scavenge generated free radicals.

In the present study; the oral administration of aqueous extract of carob pod caused a significant increase in CAT activity while, it caused a non-significant change in the other studied parameter. Pre- and post-treatment with carob aqueous extract protected and ameliorated the variations in the levels of different investigated parameters in rats exposed to waterpipe smoke. These results are agreement with the study of Gulay et al. [15] which demonstrated that carob bean extract is non-toxic and have no significant adverse effects with its traditional use in male rabbits.

Previous studies indicated that carob possess antioxidant activity, where; Kumazawa et al. [87] reported that carob pod crude polyphenol had high antioxidant activity which acting as scavengers of singlet oxygen and free radicals [13]. It was reported that the carob powder is considered as a rich source of Fe, Ca, Na, K, P and S [88] and the trace element Cu, Zn and Se which act as cofactors of antioxidant enzymes to protect the body from oxygen free radicals that are produced during oxidative stress [89].

Rtibi et al. [71] reported that the aqueous extract of carob pod inhibited the MPO activity in a concentration dependent manner by its ability to reduce the production of hypochlorous acid from  $H_2O_2$  that could attenuate the inflammatory reactions. Also, it inhibits ROS, superoxide anion and  $H_2O_2$  production in a concentration dependent manner. Sebai et al. [13] showed that subacute (7 days) treatment with aqueous extract carob pod is able to alleviate lipid peroxidation in brain and heart. Consequently, it is involved in protection against several diseases such as cardiovascular, neuronal and others [89].

So, the pre- and post-treatment with carob aqueous extract protect and ameliorate the variations in the levels of different parameters in rats exposed to waterpipe smoke which may be due to its high levels of dietary fiber and polyphenol compounds. Besides the antioxidant activity the polyphenols, which mainly prevent or delay the oxidative damage, also the presence of trace element enhance the activity of antioxidant enzymes.



## 5. Conclusion

It is clear that the exposure to waterpipe smoke (passive smoking) caused serious side effects on brain and lung nearly like amiodarone which is used as a model for lung toxicity in rats. The pre-and post-treatment with carob aqueous extract ameliorated these effects due to its antioxidant effects. Moreover, the awareness about the usage or exposure to waterpipe smoke for all ages must be increased by Ministry of Health, the media and Schools. Also, the Government must be put hard rules for smoking in open areas. Carob is being acclaimed as an ingredient with a marked nutritional value due to its antidepressant, anti-inflammatory effects and its high levels of dietary fiber and phenol compounds as well as inhibit caught reflex which carob exert a preventative role against many diseases. So, carob powder should be increasing as an ingredient in the healthy food industry such as biscuits, bread and cakes.

## Conflict of interest

We declare that we have no conflict of interest with anyone.

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