

AMELIORATIVE EFFECT OF CAROB AQUEOUS EXTRACT ON WATER PIPE SMOKE INDUCED-TOXICITY IN ADULT MALE ALBINO RATS

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ABSTRACT

Objective: The aim of the present study was to investigate the daily effect of pre and post treatment of carob aqueous extract (600 mg/kg b. wt.); on rats exposed to were exposed to water pipe smoke (10 mg/kg; 15 minutes) for 2, 4, 6 and 8 w.

Methods: Rats were divided into 6 groups; 1st group was served as a control. Rats of the 2nd group were administered amiodarone (an antiarrhythmic medication used as a model for lung toxicity) at a dose level of 30 mg/kg. The rats of 3rd and 4th groups were exposed to water pipe smoke and treated with carob extract respectively. However, the two remaining groups (5th and 6th) were the pre-and post-treatment groups with carob extract respectively.

Results: Amiodarone and water pipe smoke induced a significant increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP); on the contrary, induced a significant decrease in serum total protein and albumin. In addition; serum creatinine and urea showed a significant increase; as well as; amiodarone and water pipe smoke increased the oxidative stress (malondialdehyde/glutathione; MDA/GSH) in lung tissue homogenate. While; carob extract gavage showed protective and curative effects in liver and kidney functions; in addition; it decreased MDA level and increased GSH level significantly.

Conclusion: Finally, we can conclude that the carob aqueous extract treatment revealed ameliorative effects on rats were exposed to water pipe smoke.

Keywords: Water pipe smoke, Amiodarone, Carob, Liver, Kidney, Lung, Rat

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INTRODUCTION

The water pipe (shisha, narghile) is a tobacco smoking device. It has long been used in the Middle East, Africa and Asia. Water pipe smoking appears to be more popular among younger people. Moreover, two or more smokers may share the same pipe [1, 2].

Water pipe smoke produces noxious chemicals: "tar", nicotine, carbon mono-oxide, nitric oxide and various carcinogens [1]. The most common belief among water pipe smoke users across all regions of the world is that water pipe smoke is less harmful and less addictive than cigarette smoking [3, 4].

Meanwhile, Cobb *et al.* [5] suggested that water pipe smoke is associated with cigarette smoking risks. So, water pipe smokers, like cigarette smokers, are at risk for nicotine/tobacco dependence, cardiovascular disease and respiratory tract hazards. Moreover, water pipe smoke induces free radicals accumulation [6].

In addition, smoking induces adverse effects on organs that have no direct contact with the smoke itself such as the liver. Heavy smoking yields toxins, which induce inflammation and increase the severity of hepatic lesions [7]. In the same manner; there is an association between smoking and respiratory tract, lung, stomach, liver, kidneys and urinary tract malignancies [8]. Amiodarone is a potent antiarrhythmic agent, associated with pulmonary fibrosis and hepatotoxicity [9]. Likewise, Gado and Aldahmash [10] reported that amiodarone-induced lung toxicity in rats and it is used as a model for lung toxicity [11].

During the past years; the use of natural products as alternative medicines gained an important among the public, especially medicinal herbs are used in treating some diseases and as protective agents against the toxic effect of some drugs and other toxic compounds [12].

Carob (*Ceratonia siliqua* L.) is a typical tree of the semiarid environments in the Mediterranean area [13]. It has a long history of the application as a source of health products [14].

The major chemical constituents of the carob are moisture, carbohydrates, proteins, fat, minerals, vitamins, polyphenols and dietary fiber [15]. Polyphenols have anti-oxidative properties which prevent the coronary heart disease and have vasorelaxation effects [16]. In addition, carob extract reduces the rate of hepatic synthesis of cholesterol [17].

Since there is no enough information about the effect of carob extract on the toxicity of water pipe smoke. So, the aim of the present study was to investigate the effect of pre-and post-treatment of carob aqueous extract on liver, kidney and lung functions in rats exposed to water pipe smoke by using amiodarone as a model for lung toxicity.

MATERIALS AND METHODS

Experimental animals

Adult male albino rats (*Rattus rattus*) weighing 120-150g, obtained from National Organization for Drug Control and Research. They were housed in polypropylene cages maintained in the laboratory of physiology in Faculty of Science, Helwan University under normal environmental conditions of temperature, humidity and light. They were allowed food on a standard pelleted diet (*ad libitum*). The rats were kept for about one week before experimentation to adapt the laboratory conditions.

Experimental design

a- Carob (*Ceratonia siliqua*) pods aqueous extract

Carob pods were obtained from Egyptian herbal markets, Cairo, Egypt. The pods were grinded and weighted. According to Ayaz *et al.* [18] the aqueous extract was prepared and evaporated by using rotary evaporator. Rats were received daily oral administration of 600 mg/kg body weight (b. wt.) [19].

b- 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. [20].

c- Water pipe tobacco smoke

Tobacco (moassal) was obtained from Egyptian stores. Animals were exposed to hookah smoke (10 gm) for 15 minutes [21]. Rats were placed in an isolated box during the experiment in an isolated room and after being exposed to water pipe smoke, the rats were returned to their room. To do this, a special apparatus was designed to have the ability to keep the rats for 15 minutes in a situation very similar to water pipe smoking by a human.

Animal grouping

Animals were divided into 6 groups (6 animals per group)

-**The 1st group:** Rats were received daily oral administration of distilled water for 8 w and served as control.

-**The 2nd group (lung toxicity model):** Rats were received daily oral administration of amiodarone tablets (30 mg/kg b. wt.) for 8 w.

-**The 3rd group:** Rats were daily exposed to water pipe smoke 10 mg/kg b. wt. (15 minutes) for 8 w.

-**The 4th group:** Rats were received daily oral intubation of carob aqueous extract (600 mg/kg b. wt.).

-**The 5th group (Protective group):** Rats were received daily oral gavage of carob extract (600 mg/kg b. wt.) then 30 minutes after rats were exposed to water pipe smoke (15 minutes)

-**The 6th group (Therapeutic group):** Rats were daily exposed to water pipe smoke (15 minutes) daily then 30 minutes after rats were received daily oral gavage of carob extract (600 mg/kg b. wt.).

The experimental duration extended to 8 w. The animals were killed by sudden decapitation at different time intervals 2, 4, 6 and 8 w during the experiment. The ethics of animal care and use was followed in this study.

Methods and techniques

Tissues sampling and biochemical assay

Rats were sacrificed by sudden decapitation on the 2nd, 4th, 6th and 8th weeks. Blood was collected, allowed to stand for 15 minutes, and then centrifuged at 3000 r. p. m. for 15 minutes to separate serum and quickly frozen in dry ice (-70 °C) till use for further determination of liver and kidney functions by using Biodiagnostic kits (Biodiagnostic, Dokki, Giza, Egypt).

Serum activities of ALT and AST were determined according to the method of Reitman and Frankel [22]. The activity of ALP was determined by using the method of King and King [23], serum albumin level by the method Kinsley and Frankel [24] and serum total protein level by Lowry *et al.* [25].

Moreover, serum creatinine and urea levels were determined according to Bartles *et al.* [26] and Fawcett and Scott [27] respectively. The color intensity was measured in a spectrophotometer.

Lung tissue (40 mg from each rat) was homogenised in ice-cold Tris-HCl buffer solution PH (7.4) and centrifuged at 2000 r. p. m. for 10 minutes to separate supernatant and quickly frozen in dry ice (-70 °C) till use for malondialdehyde (MDA), reduced glutathione (GSH) determinations.

Briefly; MDA level (the end product of lipid peroxidation) in the lung was determined as thiobarbituric acid reactive substance according to a modification of the method of Ohkawa *et al.* [28] and the sample absorbance was measured at 535 nm in a spectrophotometer.

Reduced GSH in homogenate was determined according to Ellman [29]. The procedure is based on the reduction of Ellman's reagent [5, 5'-dithiobis-(2-nitrobenzoic acid)] by SH groups to form 1 mole of 2-nitro-5-mercaptobenzoic acid per 1 mole of SH. The nitro-mercaptobenzoic acid anion has intense yellow color and can be determined spectrophotometrically at 412 nm.

Statistical analysis

The statistical comparisons among the groups were carried out by using one-way ANOVA (Duncan's test); (SPSS version17.0). P<0.05 was considered as significant for all statistical analysis in this study.

RESULTS

The data tabulated in table (1) showed that amiodarone daily oral administration (30 mg/kg) caused a significant increase in ALT activity in serum all over the experimental periods starting from the 2nd week till the 8th week of the administration recording 32.00%, 54.64%, 92.11% and 131.24% on 2nd, 4th, 6th and 8th week respectively as compared to control group. The maximal increase was recorded after 8 w.

Similarly, the daily exposure to water pipe smoke (10 mg/kg, b. wt.) for 15 minute induced a significant increase in serum ALT activity after 2, 4, 6 and 8 w with a percentage change 25.72%, 41.36%, 78.50% and 106.00% respectively as compared to control group. The maximal increase was recorded after 8 w. However; the daily oral administration of carob aqueous extract showed a non-significant change in the activity of ALT all over the weeks as compared to control group. The pre-treatment with carob extract (600 mg/kg) for 8 w resulting in a significant increase in serum ALT activity after 2, 4, 6 and 8 w recording 16.63%, 20.00%, 32.82% and 26.00% respectively versus their respective control values. In contrary, ALT activity showed a non-significant change in ALT activity after 2 w and significantly reduction after 4, 6 and 8 w (-15.11%, -25.60% and -38.78% respectively) as compared to water pipe smoke group.

Table 1: Effect of daily exposure to water pipe smoke on alanine aminotransferase (ALT) activity in serum of adult male albino rat pre- and post-treated with carob extract (600 mg/kg) for 8 w

Time Groups	2 W	4 W	6 W	8 W
Control	11.00±0.68	12.50±0.43	11.67±0.67	11.17±0.48
Amiodarone	14.52±0.52 (32.00% ^a)	19.33±0.71 (54.64% ^a)	22.42±0.80 (92.11% ^a)	25.83±0.60 (131.24% ^a)
Water pipe smoke	13.83±0.40 (25.72% ^a)	17.67±0.61 (41.36% ^a)	20.83±0.70 (78.50% ^a)	23.00±0.58 (106.00% ^a)
Carob	12.00±0.70 (9.00%)	11.33±0.49 (-9.36%)	10.33±0.33 (-11.50%)	10.07±0.32 (-9.85%)
Carob+water pipe smoke	12.83±0.48 (16.63% ^a) (-7.23%)	15.00±0.45 (20.00% ^a) (-15.11% ^b)	15.50±0.43 (32.82% ^a) (-25.60% ^b)	14.08±0.55 (26.00% ^a) (-38.78% ^b)
Water pipe smoke+ Carob	12.50±0.56 (13.63%) (-9.62%)	13.67±0.56 (9.36%) (-22.64% ^b)	13.00±0.93 (11.40%) (-37.60% ^b)	12.00±0.58 (7.43%) (-47.83% ^b)

• mean±SE. Statistical analysis performed by using Student's t-test (n=6), ^aSignificant change at p<0.05 comparing to control group, ^bSignificant change at p<0.05 comparing to water pipe smoking group, (%) percentage change.

A non-significant increase in serum ALT activity was observed all over experimental time intervals; as a result of daily carob extract

treatment after exposure to water pipe smoke for 8 w (post-treatment) as compared to control group. On the other hand, when

compared with water pipe smoke group; ALT activity was significantly decreased after 4, 6 and 8 w (-22.64%,-37.60% and-47.83% respectively).

As shown in table (2); daily amiodarone intubation (at a dose level 30 mg/kg b. wt.) indicated a gradual and significant increase (p<0.05) in serum AST activity all over time intervals with a percentage change 24.25%, 30.66%, 34.82% and 67.21% on the 2nd, 4th, 6th, 8th week respectively versus control group. The maximal increase was recorded after 8 w. Likewise, the daily water pipe smoke exposure (10 mg/kg, b. wt.) for 15 minutes increased serum AST activity significantly (18.32%, 21.40%, 26.40% and 56.00%) all over the administered period and the maximal increase was recorded after 8 w.

The daily oral gavage of carob aqueous extract induced a non-significant increase in serum AST activity all over experimental time

intervals. Moreover, a constant and gradual increment occurred in serum AST activity as a result of carob extract pre-treatment recording 13.60%, 17.34%, 15.20% and 19.70% as a percentage change on 2nd, 4th, 6th and 8th weeks respectively as compared to control values (table, 2).

On the contrary, a significant decrease was occurred in AST activity started from 6th week with a percentage change (-8.90% and-23.30%) on the 6th and 8th week respectively; versus water pipe smoke group.

In post-treatment group; a constant and significant increase in AST activity started from 4th week till 8th week as compared to control group. Meanwhile, in a comparison to water pipe smoke group a constant and significant reduction was found with a percentage change-9.50%,-9.51%,-13.33% and-27.00% on 2, 4, 6 and 8 w respectively.

Table 2: Effect of daily exposure to water pipe smoke on aspartate aminotransferase (AST) activity in serum of adult male albino rat pre- and post-treated with carob extract (600 mg/kg) for 8 w

Time Groups	2 W	4 W	6 W	8 W
Control	28.17±0.91	28.83±0.75	29.67±0.77	29.80±0.59
Amiodarone	35.00±0.73 (24.25% ^a)	37.67±1.20 (30.66% ^a)	40.00±0.58 (34.82% ^a)	49.83±1.25 (67.21% ^a)
Water pipe smoke	33.33±0.71 (18.32% ^a)	35.00±1.15 (21.40% ^a)	37.50±1.07 (26.40% ^a)	46.50±1.26 (56.00% ^a)
Carob	29.67±1.02 (5.33%)	30.17±0.60 (4.65%)	30.50±0.70 (2.80%)	31.20±0.55 (4.70%)
Carob+water pipe smoke	32.00±0.82 (13.60% ^a)	33.83±1.17 (17.34% ^a)	34.17±1.12 (15.20% ^a)	35.67±1.05 (19.70% ^a)
Water pipe smoke+Carob	30.17±0.79 (7.10%) (-9.50% ^b)	31.67±1.15 (9.85% ^a) (-9.51% ^b)	32.50±0.91 (9.54% ^a) (-13.33% ^b)	34.00±0.82 (14.10% ^a) (-27.00% ^b)

• mean±SE. Statistical analysis performed by using Student’s t-test (n=6), ^aSignificant change at p<0.05 comparing to control group, ^bSignificant change at p<0.05 comparing to water pipe smoking group, (%) percentage change.

The level of total protein in serum cleared a gradual, and significant reduction started from 4th week as a result of daily oral gavage of 30 mg/kg b. wt. amiodarone treatment and water pipe smoke exposure (10 mg/kg b. wt.) for 15 minutes as compared to control value. However, the maximal reduction was observed on 8th week recording-35.34% and-23.10% respectively as a percentage change (table, 3).

In carob aqueous extract group and the pre-treatment group, there was a non-significant change in total protein level throughout the

experimental period versus control value. While, a significant increase in total protein level started from 4th to 8th week in the pre-treatment group as compared to water pipe smoke group (7.86%, 18.20% and 24.08%).

In the same manner, carob extract gavage after water pipe smoke exposure indicated a significant increase in total protein level on 4th, 6th and 8th week (12.43%, 27.37% and 29.03%) respectively as compared to water pipe smoke group.

Table 3: Effect of daily exposure to water pipe smoke on total protein content in serum of adult male albino rat pre- and post-treated with carob extract (600 mg/kg) for 8 w

Time Groups	2 W	4 W	6 W	8 W
Control	7.5±0.29	8.00±0.13	7.90±0.21	8.15±0.20
Amiodarone	7.00±0.21 (-6.67%)	6.43±0.19 (-19.63% ^a)	6.00±0.20 (-24.05% ^a)	5.27±0.11 (-35.34% ^a)
Water pipe smoke	7.20±0.29 (-4.00%)	7.00±0.28 (-12.50% ^a)	6.32±0.20 (-20.00% ^a)	6.27±0.18 (-23.10% ^a)
Carob	7.75±0.12 (3.33%)	8.12±0.16 (1.5%)	8.10±0.12 (2.53%)	8.20±0.05 (0.61%)
Carob+water pipe smoke	7.76±0.18 (3.46%) (7.78%)	7.55±0.08 (-5.63%) (7.86% ^b)	7.47±0.17 (-5.44%) (18.20% ^b)	7.78±0.14 (-4.54%) (24.08% ^b)
Water pipe smoke+carob	7.25±0.18 (-3.33%) (0.70%)	7.87±0.12 (-1.63%) (12.43% ^b)	8.05±0.21 (2.00%) (27.37% ^b)	8.09±0.19 (-0.74%) (29.03% ^b)

• mean±SE. Statistical analysis performed by using Student’s t-test (n=6), ^aSignificant change at p<0.05 comparing to control group, ^bSignificant change at p<0.05 comparing to water pipe smoking group, % Percentage change.

There was a significant reduction in serum albumin level started from 6th and 8th week resulting from amiodarone treatment and exposure of

rats to water pipe smoke versus its control value (table, 4). Moreover, the daily oral intubation of 600 mg/kg b. wt. of carob aqueous

extract induced a significant increase in albumin level on 6th week (5.76%) and 8th week (11.52%) as compared to control group.

In table (4); a significant decrease in albumin level was occurred on 8th week (-10.10%) in the pre-treatment group as compared

to control group. Meanwhile, a significant increase was observed on 6th and 8th weeks with a percentage change 9.68% and 8.33% respectively as compared to water pipe smoke. In the same manner, a significant increase in albumin level started from 6th week in a post-treatment group versus water pipe smoke group.

Table 4: Effect of daily exposure to water pipe smoke on albumin content in serum of adult male albino rat pre-and post-treated with carob extract (600 mg/kg) for 8 w

Time Groups	2 W	4 W	6 W	8 W
Control	3.48±0.05	3.47±0.06	3.47±0.02	3.47±0.03
Amiodarone	3.37±0.08 (-3.16%)	3.28±0.06 (-5.48%)	3.07±0.03 (-11.52% ^a)	2.70±0.09 (-22.20% ^a)
Water pipe smoke	3.40±0.09 (-2.30%)	3.30±0.08 (-4.90%)	3.10±0.05 (-10.66% ^a)	2.88±0.08 (-17.00% ^a)
Carob	3.43±0.04 (-1.44%)	3.52±0.09 (1.44%)	3.67±0.09 (5.76% ^a)	3.87±0.15 (11.52% ^a)
Carob+water pipe smoke	3.40±0.05 (-2.30%)	3.42±0.03 (-1.44%)	3.40±0.06 (-2.00%)	3.12±0.10 (-10.10% ^a)
Water pipe smoke+Carob	3.42±0.04 (-1.72%)	3.47±0.04 (0%)	3.45±0.02 (-0.58%)	3.40±0.09 (-2.00%)
	(0.59%)	(5.51%)	(11.30% ^b)	(18.05% ^b)

• mean±SE. Statistical analysis performed by using Student's t-test (n=6), ^aSignificant change at p<0.05 comparing to control group, ^bSignificant change at p<0.05 comparing to water pipe smoking group, %percentage change.

A gradual and significant increase in serum ALP activity was found as a result of amiodarone treatment started from 2nd till 8th weeks recording 23.00%, 45.56%, 47.00% and 62.73% on 2nd, 4th, 6th and 8th week respectively as compared to control group (table, 5). Similarly, the exposure to water pipe smoke induced a significant increase in ALP activity throughout the experimental period; however, ALP activity showed a constant increase on 4th and 6th week (44.32% and 44.16%) respectively and the maximum increase was found on 8th week (55.00%).

In pre-treatment group; there was a significant increase in ALP activity on 4th, 6th and 8th week with a percentage change (18.00%,

23.00% and 30.31%) respectively as compared to control group. On the other hand, a significant decrease was noticed on 4th, 6th and 8th week with a percentage change -18.27%, -14.67% and -15.93% respectively versus water pipe smoke group.

The carob extract gavage after water pipe smoke exposure revealed a significant increase in ALP activity started from 4th week recording 16.00%, 17.78% and 27.34% on the 4th, 6th and 8th weeks respectively versus control value.

On the contrary, a significant decrease was cleared in ALP activity on the 4th, 6th and 8th weeks as compared to water pipe smoke group.

Table 5: Effect of daily exposure to water pipe smoke on alkaline phosphatase (ALP) activity in serum of adult male albino rat pre-and post-treated with carob extract (600 mg/kg) for 8 w

Time Groups	2 W	4 W	6 W	8 W
Control	150.83±5.69	146.67±3.80	152.83±2.87	151.17±3.52
Amiodarone	185.50±9.48 (23.00% ^a)	213.50±5.12 (45.56% ^a)	224.67±2.06 (47.00% ^a)	246.00±1.34 (62.73% ^a)
Water pipe smoke	180.17±5.19 (19.45% ^a)	211.67±7.24 (44.32% ^a)	220.33±3.53 (44.16% ^a)	234.33±4.75 (55.00% ^a)
Carob	159.50±2.05 (5.75%)	153.50±3.77 (4.66%)	148.65±3.59 (-2.74%)	144.68±1.89 (-4.30%)
Carob+water pipe smoke	168.67±6.93 (11.83%)	173.00±9.33 (18.00% ^a)	188.00±3.42 (23.00% ^a)	197.00±6.73 (30.31% ^a)
Water pipe smoke+carob	164.00±4.02 (8.73%)	170.17±4.87 (16.00% ^a)	180±3.17 (17.78% ^a)	192.50±5.44 (27.34% ^a)
	(-8.97%)	(-19.61% ^b)	(-18.30% ^b)	(-17.85% ^b)

• mean±SE. Statistical analysis performed by using Student's t-test (n=6), ^aSignificant change at p<0.05 comparing to control group, ^bSignificant change at p<0.05 comparing to water pipe smoking group, % percentage change.

As shown in table (6); the daily oral administration of amiodarone (30 mg/kg) caused a significant increase in serum creatinine level all over the experimental periods starting from the 2nd week of the treatment recording 45.71%, 108.33%, 131.58% and 148.65% at p<0.05 as a percentage change on 2nd, 4th, 6th and 8th week respectively as compared to control group. The maximal increase was recorded after 8 w. likewise; the daily exposure to water pipe smoke (10 mg/kg) for 15 minutes induced a significant increase in serum creatinine level all experimental time intervals versus control value. However; the maximum increment was noticed after 8 w.

The gavage of aqueous extract of carob showed a non-significant change in creatinine level throughout the experimental period. Moreover, in a pretreatment group a significant increase was found in creatinine level started from 4th to 8th week as compared to control group. While; in a comparison to water pipe smoke group a significant decrease was observed in serum creatinine level on 2nd, 4th, 6th and 8th week recording -18.75%, -24.29%, -25.64% and -14.77% as a percentage change respectively.

The maximum reduction was observed after 6th week.

The rats were gavaged carob extract after water pipe smoke exposure revealed a gradual and significant increment in creatinine level on 4th, 6th and 8th week versus the control group.

On the other hand, a gradual and significant decrease was recorded all over time intervals as compared to water pipe smoke group.

Table 6: Effect of daily exposure to water pipe smoke on creatinine content in serum of adult male albino rat pre-and post-treated with carob extract (600 mg/kg) for 8 w

Time Groups	2 W	4 W	6 W	8 W
Control	0.35±0.02	0.36±0.02	0.38±0.01	0.37±0.02
Amiodarone	0.51±0.04 (45.71% ^a)	0.75±0.04 (108.33% ^a)	0.88±0.06 (131.58% ^a)	0.92±0.05 (148.65% ^a)
Water pipe smoke	0.48±0.03 (37.14% ^a)	0.70±0.03 (94.44% ^a)	0.78±0.05 (105.26% ^a)	0.88±0.05 (137.84% ^a)
Carob	0.31±0.02 (-11.43%)	0.37±0.02 (2.78%)	0.39±0.01 (2.63%)	0.41±0.01 (10.81%)
Carob+wateripe smoke	0.39±0.02 (11.43%) (-18.75% ^b)	0.53±0.03 (47.22% ^a) (-24.29% ^b)	0.58±0.03 (52.63% ^a) (-25.64% ^b)	0.75±0.02 (102.70% ^a) (-14.77% ^b)
Water pipe smoke+ carob	0.37±0.02 (5.71%) (-22.91% ^b)	0.50±0.04 (38.90% ^a) (-28.57% ^b)	0.54±0.04 (42.00% ^a) (-30.77% ^b)	0.58±0.04 (56.76% ^a) (-34.09% ^b)

• mean±SE. Statistical analysis performed by using Student's t-test (n=6), ^aSignificant change at p<0.05 comparing to control group, ^bSignificant change at p<0.05 comparing to water pipe smoking group, % percentage change.

Urea level in serum showed a gradual and significant increment resulting from administration of 30 mg/kg b. wt. of amiodarone on 2nd, 4th, 6th and 8th week with a percentage change 20.86%, 77.23%, 83.00% and 104.25% respectively (table 7). The maximum increase occurred on 8th week. Similarly; rats were exposed to water pipe smoke showed a significant increase in serum urea level all overtime period, recording the maximum increase on 8th week with 97.48% as a percentage change. The rats were intoxicated by water pipe smoke after carob extract gavage showed a significant increase in urea level on 6th week (18.71%) and 8th week (42.00%) versus control value. On the contrary, a significant decrease was observed

on 4th week (-17.56%), 6th week (-23.56%) and 8th week (-28.12%) versus water pipe smoke group.

Moreover, in post-treatment group urea level showed a significant increase started from 4th till 8th week and the maximum increment was found on 8th week recording 52.12% as a percentage change as compared to control group.

Meanwhile; a significant decrease was noticed in serum urea level on 4th, 6th and 8th week with a percentage change -14.51%, -16.44% and -22.97% respectively; versus water pipe group".

Table 7: Effect of daily exposure to water pipe smoke on urea content in serum of adult male albino rat pre-and post-treated with carob extract (600 mg/kg) for 8 w

Time Groups	2 W	4 W	6 W	8 W
Control	25.17±1.01	35.83±1.25	39.17±1.55	39.33±1.23
Amiodarone	30.42±1.05 (20.86% ^a)	63.50±2.50 (77.23% ^a)	71.67±1.33 (83.00% ^a)	80.33±2.59 (104.25% ^a)
Water pipe smoke	28.67±0.99 (13.91% ^a)	49.33±1.31 (37.68% ^a)	60.83±2.25 (55.30% ^a)	77.67±1.50 (97.48% ^a)
Carob	26.50±0.52 (5.02%)	37.67±0.84 (5.14%)	43.33±1.22 (10.62%)	40.67±1.36 (3.41%)
Carob+water pipe smoke	27.25±0.93 (8.26%) (-4.95%)	40.67±1.36 (13.51%) (-17.56% ^b)	46.50±1.76 (18.71% ^a) (-23.56% ^b)	55.83±1.08 (42.00% ^a) (-28.12% ^b)
Water pipe smoke+ carob	27.42±0.49 (8.94%) (-4.36%)	42.17±1.85 (17.70% ^a) (-14.51% ^b)	50.83±2.51 (29.77% ^a) (-16.44% ^b)	59.83±1.25 (52.12% ^a) (-22.97% ^b)

• mean±SE. Statistical analysis performed by using Student's t-test (n=6), ^aSignificant change at p<0.05 comparing to control group, ^bSignificant change at p<0.05 comparing to water pipe smoking group, % percentage change.

In table (8); pulmonary MDA level showed a significant elevation (P<0.05) as a result of daily oral administration of amiodarone (30 mg/kg b.wt.) recording 101.0%, 118.0% and 184.0% as a percentage change on 2nd, 4th and 6th week, in addition, a sudden elevation in MDA level was found on 8th week (301.0%) as compared to control group.

In the same manner, the daily exposure to water pipe smoke (10 mg/kg b. wt.) induced a significant increment in pulmonary MDA level on 2nd, 4th and 6th week (82.0%, 96.73% and 174.0%) respectively, moreover; a sudden elevation was occurred in level of

pulmonary MDA on the end of the treatment recording 268.0% as a percentage change versus control value.

In pre-treatment group; the rats exposed to water pipe smoke after carob extract administration showed a significant increment on 4th week (29.50%), moreover, on 6th and 8th week there was a sudden and significant elevation in pulmonary MDA level recording 110.0% and 109.0% respectively versus control value. Oppositely, in a comparison to water pipe smoke group; there was a significant reduction in MDA level all over the experimental time period.

However, the carob extract gavage after water pipe smoke exposure (post-treatment) indicated a significant increase ($P<0.05$) in MDA level in lung tissue on 2nd and 4th week (32.87% and 43.75%) respectively; in addition; a sudden elevation was observed on 6th and

8th week recording 123.0% and 140.0% respectively versus its corresponding control. While, a constant and significant decrease was occurred in MDA level all time intervals versus water pipe smoke group.

Table 8: Effect of daily exposure to water pipe smoke on malondialdehyde (MDA) level in lung tissue of adult male albino rat pre-and post-treated with carob extract (600 mg/kg) for 8 w

Time Groups	2 W	4 W	6 W	8 W
Control	17.22±0.71	18.65±0.53	19.86±0.90	19.33±0.44
Amiodarone	34.64±0.65 (101.00% ^a)	40.64±0.98 (118.00% ^a)	56.49±1.00 (184.00% ^a)	77.60±1.43 (301.00% ^a)
Water pipe smoke	31.33±0.64 (82.00% ^a)	36.69±0.83 (96.73% ^a)	54.51±1.06 (174.00% ^a)	71.13±2.60 (268.00% ^a)
Carob	17.92±0.82 (4.06%)	18.10±0.95 (-2.95%)	18.38±0.71 (-7.45%)	19.10±0.71 (-1.20%)
Carob+water pipe smoke	18.33±0.57 (6.45%) (-41.50% ^b)	24.15±1.05 (29.50% ^a) (-34.18% ^b)	41.77±1.16 (110.00% ^a) (-23.37% ^b)	40.42±0.89 (109.00% ^a) (-43.17% ^b)
Water pipe smoke+ Carob	22.88±0.58 (32.87% ^a) (-26.97% ^b)	26.81±1.21 (43.75% ^a) (-26.93% ^b)	44.29±0.86 (123.00% ^a) (-18.75% ^b)	46.45±1.25 (140.00% ^a) (-34.70% ^b)

• mean±SE. Statistical analysis performed by using Student's t-test (n=6), ^aSignificant change at $p<0.05$ comparing to control group, ^bSignificant change at $p<0.05$ comparing to water pipe smoking group, % percentage change.

The daily oral gavage of amiodarone at a dose level of 30 mg/kg b. wt. decreased GSH content significantly in lung tissue on 4th, 6th and 8th week with a percentage change -23.40%, -41.00% and -48.31% respectively. Likewise, a significant decrease was recorded resulting from daily exposure to water pipe smoke (10 mg/kg b. wt.) for 15 minutes; the maximum reduction occurred on 8th week (-42.00%) as shown in the table (9).

Carob aqueous extract gavage (600 mg/kg b. wt.) indicated a significant increase in lung GSH content on 4th, 6th and 8th week with a percentage change 20.53%, 33.00% and 53.68% respectively.

There was a significant decrease in lung GSH level in pretreatment group started from 4th till 8th week versus control value. While, a non-significant increase was found in GSH content as compared to water pipe smoke group.

The carob extract administration after water pipe smoke exposure (post-treatment group) caused a non-significant change in pulmonary GSH content all over experimental time intervals as compared to control value. Meanwhile, a significant increase was observed in GSH content started from 4th till 8th week versus water pipe smoke group.

Table 9: Effect of daily exposure to water pipe smoke on glutathione (GSH) level in lung tissue of adult male albino rat pre-and post-treated with carob extract (600 mg/kg) for 8 w

Time Groups	2 W	4 W	6 W	8 W
Control	16.17±0.33	16.37±0.87	16.08±0.33	16.02±0.52
Amiodarone	15.77±0.34 (-2.47%)	12.54±0.51 (-23.40% ^a)	9.48±0.39 (-41.00% ^a)	8.28±0.30 (-48.31% ^a)
Water pipe smoke	15.81±0.29 (-2.22%)	13.66±0.67 (-16.60% ^a)	10.30±0.36 (-36.00% ^a)	9.29±0.41 (-42.00% ^a)
Carob	17.35±0.41 (7.30%)	19.73±0.32 (20.53% ^a)	21.39±0.54 (33.00% ^a)	24.62±0.59 (53.68% ^a)
Carob+water pipe smoke	16.53±0.81 (2.23%) (4.55%)	14.20±0.34 (-13.26% ^a) (3.95%)	11.08±0.35 (-31.00% ^a) (7.57%)	10.25±0.43 (-36.00% ^a) (10.37%)
Water pipe smoke+ carob	16.21±0.34 (0.25%) (2.53%)	15.71±0.51 (-4.00%) (15.00% ^b)	15.16±0.41 (-5.72%) (47.18% ^b)	15.09±0.14 (-5.80%) (62.63% ^b)

• mean±SE. Statistical analysis performed by using Student's t-test (n=6), ^aSignificant change at $p<0.05$ comparing to control group, ^bSignificant change at $p<0.05$ comparing to water pipe smoking group, % percentage change.

DISCUSSION

The daily exposure to water pipe smoke (15 minutes) and oral amiodarone (30 mg/kg) administration increased serum ALT, AST and ALP activities and decreased serum total protein and albumin levels significantly. Moreover, the carob pre-and post-treatment to rats were exposed to water pipe smoke returned the activities of ALT, AST and ALP as well as the levels of serum total protein and albumin to control values. Our results are in agreement with [30-33].

Babatin *et al.* [34] reported that low cumulative dose of amiodarone-induced acute hepatitis, hepatic failure. Water pipe smoking elevated plasma ALT, AST and ALP activities and it decreased total protein and albumin levels [32, 33].

The increased activity of ALP was produced by liver, kidney and bone which is a mainly marker of the liver turnover [35]. In the same manner, change in serum transaminase activity is an indicator of early hepatic inflammation and liver cell damage [36].

However, the reduction in plasma protein of smokers could be attributed to the damaging effect of harmful compounds from cigarette smoking on liver cells as confirmed by the increase in the activities of plasma ALT, AST and ALP [37].

Gulay *et al.* [31] demonstrated that traditional use of carob extract is non-toxic and have no significant adverse effects in rabbits, so serum ALT, AST and ALP activities were within the normal ranges [30].

In the present study the daily oral administration of amiodarone (30 mg/kg) and exposure to water pipe smoke (15 minute) induced an increase in serum creatinine and urea levels, while oral administration of carob before/after water pipe exposure decreased the elevated levels of creatinine and urea in serum significantly.

It was reported that the increased level of serum creatinine was associated with amiodarone therapy [38, 39]; which are in agreement with the present study. In addition, Kannan *et al.* [40] concluded that amiodarone-induced renal damage in rat.

Hallan and Orth [41] demonstrated that smoking-induced renal failure which increased urea reabsorption [41-43]. Smoking of 20 cigarettes per day results in inhalation of Cd, which is a cumulative nephrotoxicant [44]; which increased tubular proteinuria and induced glomerular dysfunction evidenced by an increased level of creatinine in plasma [45].

The daily oral administration of amiodarone and intoxication by water pipe smoking increased MDA level significantly in pulmonary tissue, on the contrary; level of GSH was reduced significantly. The pre-and post-treatment groups showed that carob indicated a significant decrease in MDA level and a significant increase in GSH level in lung tissue. Our results go hand in hand with that of [46, 47, 48, 6].

Amiodarone has been shown to generate free radicals that may be involved in the pathogenesis of its toxicity [48].

Wolfram *et al.* [49] reported that a significant increase in MDA, as a biomarker for oxidative stress and a significant decrease in vitamin C as a potent antioxidant in water pipe smokers were recorded. So, Ben Saad *et al.* [46] and Sharma *et al.* [47] emphasised that the regular water pipe smoke increased oxidative stress significantly which leads to an imbalance in the production/consumption level of reactive oxygen species. In the same manner, Al-Awaida *et al.* [6] deduced that the repeated daily smoking-induced a persistent, long-lasting oxidation injury. Some studies have shown the antioxidant activity of carob which is attributed to the presence of phenolic compounds [50, 51]; mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [52], so polyphenols enhances the prevention or delay the oxidative damage [53].

Nicotine, a major constituent of tobacco smoke, is mainly metabolised in the liver and induces lesions characterised by focal or confluent necrosis, and varying degrees of fibrosis [54].

CONCLUSION

From our results and previous studies, it could be concluded that the pre and post-treatment of carob aqueous extract ameliorated the impairments in liver, kidney and lung functions and decreased the oxidative stress that induced by exposure to water pipe smoke and amiodarone in rats. Collectively, carob extract is very promising for the effective phytomedicine; so further studies are required to investigate the bioactive fractions in the carob pods aqueous extract.

CONFLICTS OF INTERESTS

The authors report no conflicts of interest in this work.

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