

CALENDULA OFFICINALIS L. AMELIORATES LEVELS OF SOME BLOOD CONSTITUENTS AND BODY WEIGHT LOSS IN PASSIVE CIGARETTE SMOKE EXPOSURE OF RATS

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ABSTRACT

The aim of this study was to investigate the protective effect of *Calendula officinalis* L. (CO) on rats exposed to cigarette smoke (CS). For this purpose twenty one albino male Sprague Dawley rats were randomly divided into three groups as control, CS and CS+CO. The rats of the CS and CS+CO groups were subjected to CS during 1 h twice a day for 23 days. CO (100 mg/kg body weight) was given to the rats of the CS+CO group by oral gavage one hour before each administration period. Protective effect of CO was detected by comparing some serum biochemical parameters and mean body weight changes of groups. While aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine phosphokinase (CK) levels of CS group increased, amylase and lipase decreased significantly compared to control. AST, LDH and CK levels reversed to nearly their control group values by CO supplementation. There was a significant reduction in the HDL level of the CS group compared to control. But its level increased markedly in the CS+CO group. With regard to calcium, its amount escalated significantly in the CS and CS+CO groups compared with control. Administration of CO caused an amendment in the body weight loss. In conclusion, this study revealed that CO could relatively combat the hazardous effects of CS and thus may help to reduce development risk of some disorders related to CS.

KEYWORDS: Cigarette smoke, body weight, biochemical constituents, *Calendula officinalis* L.

1. INTRODUCTION

Cigarette smoke (CS) is a major risk factor for many diseases. The toxicity of CS is mainly due to nicotine, cadmium, benzopyrene, oxidants, and excitants of reactive oxygen species (ROS) that stimulate oxidative damage [1-5].

Assessment of organ specific enzymes in serum may play a key role in the diagnosis and monitoring of some disorders such as hepatic, renal and cardiovascular diseases [6]. Besides CS oxidizes lipids, disrupts cell membranes, changes lipoproteins, and impairs DNA [6-10]. Another harmful effect of CS is observed in body weight. Some investigations demonstrated an opposite relevance between smoking and body weight [11-15].

When investigating the effect of passive smoking in humans, the amount of dose is important in terms of accuracy of results [16]. For this reason, the results of controlled animal experiments related to this subject can offer us more clear information. For a more healthy life, some studies have been focused on protection of humans against risk factors by means of dietary meddling, chemopreventive drugs and conventional medicinal plants [6,17]. In this respect we decided to determine the protective influence of CO against passive CS exposure by evaluating body weight status and some biochemical parameters for the first time.

Calendula officinalis L. (Asteraceae) is an annual plant grows in Mediterranean region. It's known as pot marigold and has been widely used for reducing inflammation and antiseptic, wound-healing purposes [18,19]. Apart from that CO has been reported to possess a number of beneficial properties such as free radical scavenging and antioxidant activity [20,21].

The object of this investigation is to clarify the deleterious effects of CS on some biochemical parameters and body weight of albino rats and study out the positive act of CO flower extract in mitigation of these effects for the first time.

2. MATERIALS AND METHODS

2.1. Plant material and extraction

Calendula officinalis L. samples were obtained from herbal store in Sanliurfa, Turkey. The plant sample was authenticated by Esat Çetin, PhD. The extraction protocol was performed according to a modified method of Chandran

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and Kuttan [22]. Flowers of CO (400 g) were air-dried at room temperature and powdered coarsely. It was extracted with 350 ml ethyl alcohol at 25 °C for 5 days after maceration in the 225 ml distilled water. It was placed in a dark chamber at room temperature and shaken every day. Afterwards the blend filtered and stored in deep freeze for 24 h. The CO extract was concentrated under reduced pressure and lyophilized (Tel Star Cryodos) to get the dry residue. Finally extract was mixed with distilled water before administration. The concentration of CO dose (100 mg/kg body weight) used in this trial was adjusted according to the study reported by Chandran and Kuttan [22].

2.2. Experimental animals

Experiments were performed on 21 Sprague-Dawley male albino rats (250±20 g) that were housed in plastic cages (7 rats per cage) in a temperature-controlled room (22 °C) and food and water were given to the rats *ad libitum*. The 12-h light/dark cycle was from 7 am to 7 pm.

2.3. Experimental protocol

The animals were divided into three groups, each composed of seven animals. 1. Control group: inhaled fresh air and received 1 ml physiological saline (0.9 % NaCl) by intragastric administration during 23 days before sacrificing process. 2. CS group: exposed to CS during 1 h twice a day and received 1 ml physiological saline by intragastric administration for 23 days. 3. CS+CO group: exposed to CS during 1 h twice a day and received 1 ml CO (100 mg/kg bw) by intragastric administration during trial period. The body weight (bw) of the rats was measured and noted down at the initiation and end of the trial. All animals received humane care in compliance with the European Community Guidelines on the care and use of laboratory animals.

Rats were exposed to CS in a 44x44x100 glass box which was used for passive smoke exposure. A rubber tube, with one tip connected to blower, was placed into the box. Two lit cigarettes were mounted to the tip of tube in box and they were pumped by the blower during 60 min twice a day. There was a hole for air evacuation in the opposite wall of glass box. Moreover, fresh air was circulated regularly into the box from a further gap. Commercial filter-tipped cigarettes were used in current study. Food was not given to rats during the inhalation in box.

2.4. Analysis of Biochemical Parameters

At the end of the treatments, rats were anesthetized with ketamine (100 mg/kg ip) [23]. Blood samples were taken from these animals into tubes by injectors from the heart under light anesthesia. Afterwards rats were euthanized by CO₂ inhalation. The serum samples were obtained by centrifuging blood samples at 3000 rpm for 15 min at 4 °C. Biochemical parameters including aspartate aminotransferase (AST) (EC 2.6.1.1), alanin aminotransferase (ALT) (EC 2.6.1.2), lactate dehydrogenase (LDH) (EC 1.1.1.27), lipase (EC 3.1.1.3), creatine phosphokinase (CK, CK-MB) (EC 2.7.3.2), alkaline phosphatase (ALP) (EC 3.1.3.1), gamma glutamyl transferase (GGT) (EC 2.3.2.2), amylase (AMY) (EC 3.2.1.1), urea, creatinine, uric acid, total protein, albumin, triglyceride, cholesterol, high density lipoprotein (HDL), total bilirubin, direct bilirubin, glucose, calcium (Ca), rheumatoid factor-II (RF-II), C-reactive protein (CRP), iron, unsaturated iron binding capacity (UIBC), antistreptolysin O (ASO) were analyzed by an autoanalyser (Roche, Cobas Integra 400 plus) using the kits.

2.5. Statistical analysis

The statistical analyses were realized using one-way analysis of variance-ANOVA test. Minitab 13 for windows package program was used. The level of statistical significance was set at $p < 0.05$. Results were presented as means ± standard deviation (SD) (n=7).

3. RESULTS

The detrimental effect of passive CS exposure and protective influence of CO supplementation against this exposure were observed via recording the changes of body weight and measuring biochemical constituents in the serum samples obtained from control and treated rats.

3.1. Effect on serum enzymes

As shown in Table 1, while AST, LDH and CK levels of CS group increased, amylase and lipase decreased significantly compared to control. Lipase had a significant decline even after CO supplementation. AST, LDH and CK levels reversed to nearly their control group values by CO supplementation.

TABLE 1 - Protective effect of *Calendula officinalis* L. on serum enzymes of cigarette smoke exposed rats (Mean ± SD).

Parameters	Control	CS	CS+CO
AST U/L	142.33±18.24	172.40±16.76 ^a	152.20±6.69 ^b
ALT U/L	57.67±14.29	59.20±11.12	58.20±6.30
ALP U/L	203.00±25.16	210.00±20.00	209.20±20.09
LDH U/L	1141.67±79.30	1486.80±91.93 ^a	1309.00±88.91 ^{a,b}
GGT U/L	1.03±0.23	1.06±0.25	1.20±0.27
CK U/L	601.00±69.43	774.40±94.07 ^a	614.60±61.16 ^b
CK-MB U/L	493.00±151.97	517.20±90.99	437.40±121.28
AMYLASE U/L	1849.83±129.03	1675.00±78.17 ^a	1833.00±167.25
LIPASE U/L	4.75±0.36	4.12±0.52 ^a	4.26±0.342 ^a

Values are given as the means±SD. ^aControl versus treated $p < 0.05$ level. ^bCS versus treated $p < 0.05$ level.

TABLE 2 - Protective effect of *Calendula officinalis* L. on serum renal function markers and lipid profile of cigarette smoke exposed rats (Mean $\pm$ SD).

Parameters	Control	CS	CS+CO
Urea mg/dl	47.50 $\pm$ 4.04	46.00 $\pm$ 6.20	45.00 $\pm$ 6.36
Creatinine mg/dl	0.32 $\pm$ 0.05	0.34 $\pm$ 0.03	0.34 $\pm$ 0.03
Uric acid mg/dl	1.55 $\pm$ 0.26	1.66 $\pm$ 0.28	1.60 $\pm$ 0.33
T. protein mg/dl	7.58 $\pm$ 0.78	7.70 $\pm$ 0.51	7.60 $\pm$ 0.49
Albumin g/dl	3.63 $\pm$ 0.16	3.64 $\pm$ 0.35	3.54 $\pm$ 0.42
Triglyceride mg/dl	88.83 $\pm$ 9.91	93.20 $\pm$ 15.96	85.20 $\pm$ 10.43
Cholesterol mg/dl	65.67 $\pm$ 9.31	70.60 $\pm$ 4.88	66.00 $\pm$ 11.29
HDL mg/dl	21.50 $\pm$ 2.17	16.00 $\pm$ 3.46 ^a	20.60 $\pm$ 2.41 ^b

Values are given as the means $\pm$ SD. ^aControl versus treated $p < 0.05$ level. ^bCS versus treated $p < 0.05$ level.

TABLE 3 - Protective effect of *Calendula officinalis* L. on some biochemical parameters of cigarette smoke exposed rats (Mean $\pm$ SD).

Parameters	Control	CS	CS+CO
T. bilirubin mg/dl	0.055 $\pm$ 0.012	0.056 $\pm$ 0.011	0.056 $\pm$ 0.009
D. bilirubin mg/dl	0.032 $\pm$ 0.008	0.026 $\pm$ 0.005	0.032 $\pm$ 0.004
Glucose mg/dl	188.83 $\pm$ 15.12	180.00 $\pm$ 11.62	185.80 $\pm$ 17.54
Ca (mg/dl)	10.37 $\pm$ 1.54	12.10 $\pm$ 0.70 ^a	12.32 $\pm$ 0.76 ^a
RF-II (IU/ml)	10.58 $\pm$ 2.01	12.00 $\pm$ 1.90	10.84 $\pm$ 1.59
CRP (mg/l)	0.14 $\pm$ 0.02	0.15 $\pm$ 0.03	0.14 $\pm$ 0.02
Iron (μ g/dl)	159.33 $\pm$ 18.36	163.40 $\pm$ 9.07	157.00 $\pm$ 10.56
UIBC (μ g/dl)	516.50 $\pm$ 78.48	562.20 $\pm$ 134.22	559.60 $\pm$ 106.94
ASO (IU/ml)	9.00 $\pm$ 1.10	10.50 $\pm$ 3.04	10.60 $\pm$ 1.95

Values are given as the means $\pm$ SD. ^aControl versus treated $p < 0.05$ level

3.2. Effect on serum renal function markers and lipid profile

There was no significant difference in the levels of urea, creatinine, uric acid, total protein, albumin, triglyceride and cholesterol between control and treatment groups. While passive CS exposure led to significant decrease of HDL in comparison with control, its level was markedly increased in CS + CO group (Table 2).

3.3. Effect on other biochemical parameters

Ca amount increased markedly both in CS and CS+CO groups compared with control (Table 3).

3.4. Effect on body weight

While mean body weight of control group rats increased significantly in control group at the end of the experiment compared with the beginning, it decreased in CS group and did not change in CS+CO group (Figure 1).

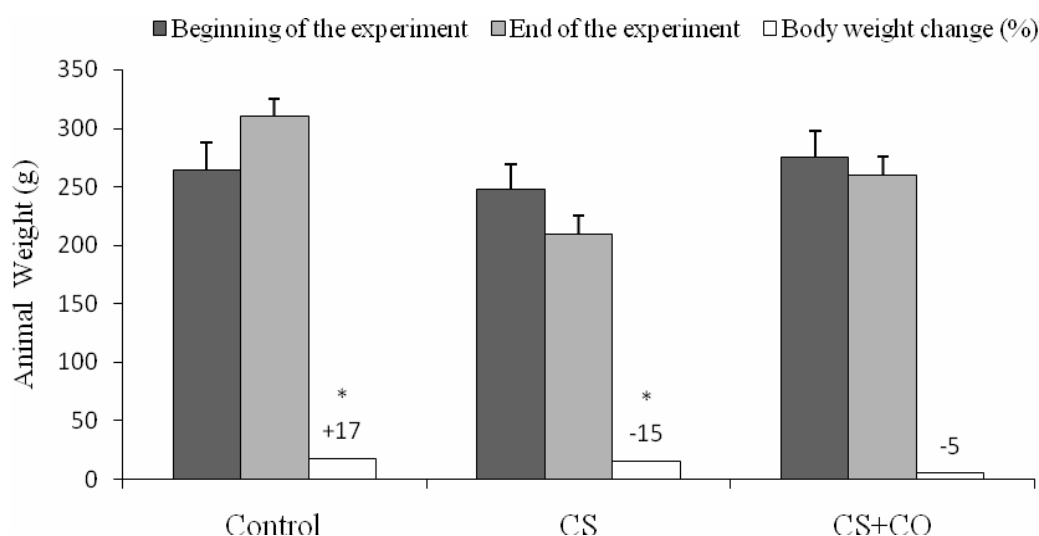


FIGURE 1 - The changes of body weight in groups (arithmetic mean $\pm$ SD; standard deviation). Values are given as the means $\pm$ SD. * "Beginning of the experiment" versus "end of the experiment" in control and CS groups $p < 0.05$ level.

4. DISCUSSION AND CONCLUSION

In the current study, we determined the alterations in the level of serum enzymes, renal function markers, lipid profile and also other biochemical parameters after passive CS exposure. In addition the mean body weight changes of control and treated groups were established. To the best of our knowledge ameliorative effect of CO against CS exposure by means of evaluating biochemical parameters and body weight alterations has been assessed in current study for the first time.

Enzymes as macromolecules leak from the injured tissues into the body fluids. Thus measurement of these molecules in serum has been found to be precious in the evaluation of related tissue damage. Hereof, serum enzymes are used as confidential markers in the identification of organ specific diseases [24]. In our study, while activities of AST, LDH and CK escalated, amylase and lipase activities inhibited markedly with CS exposure. Lipase had a significant decline even after CO supplementation. Activities of AST, LDH and CK significantly dropped in CS+CO group compared to CS group. Escalation of hepatic marker enzymes AST [25-28] and LDH [28] might be due to liver damage by CS exposure. Increased activity of CK may be associated with deterioration of cardiac [29, 30] and other muscle tissues in rats exposed to CS. Lipoprotein lipase activity which is responsible for triglyceride hydrolysis might have been diminished by the presence of carbonmonoxide in CS [10, 31]. In this trial CO might have regulated the activities of foregoing serum enzymes by reinforcing the cell membranes of tissues that may suffer from CS. Meanwhile Dontenwill et al. [32] reported that long-term exposure to CS did not impress the activities of serum enzymes.

With regard to lipid profile, while the cardio-protective HDL significantly reduced in CS group compared to control, it markedly increased in CS+CO group. There was no significant difference between groups in terms of triglyceride and cholesterol. Low level of HDL in CS group obtained from this study is compatible with data of some previous investigations suggesting that CS could oxidize lipids, change lipoproteins [6-10] and thus diminish HDL level. CO may have supplied relative protection against these detrimental effects of CS owing to its antioxidant features [20,21].

As shown in Table 3, Ca amount elevated crucially both in CS and CS+CO groups comparing to control. CS might have induced production and release of parathyroid hormone that is responsible for increase of serum calcium level [33,34].

While mean body weight of rats increased significantly in control group at the end of the experiment compared with the beginning, it decreased in CS group and did not change in CS+CO group. Accordingly it can be said that there is negative correlation between passive CS exposure and body weight. These results are in accordance with some previous studies [11-15]. Body weight

of rats in CS group might have reduced as a result of depletion in food intake due to CS exposure [35,36]. In our study supplementation of CO caused an amendment in the body weight loss.

In conclusion, assessment of some biochemical constituents and body weight alterations possess key role to detect physiological changes and general health status of rats exposed to CS. Our results suggest that CO could relatively combat the hazardous effects of CS and thus may help to reduce development risk of some disorders related to passive cigarette smoke.

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