Original article

Black cumin may be a potential drug for development of carbontetrachloride-induced lung damage in rats

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Summary. The study examines whether Black cumin (*Nigella sativa* L.) plays a protective role against the damage in the lung by administering carbontetrachloride (CCl_4) to rats. 28 male Wistar albino (n=28, 8 weeks old) rats were used in the study. The rats divided into 4 groups according to their live weights. The groups were: (i) Negative Control (NC): Normal water consuming group to which no CCl_4 and *Black cumin* (BC) is administered; (ii) Positive Control (PC): Normal water consuming group to which no CCl_4 is administered but BC is administered; (iii) CCl_4 Group: Normal water consuming and group to which CCl_4 is administered (1.5 ml/kg live weight, ip); (iv) CCl_4 + BC group: CCl_4 and BC administered group (1.5 ml/kg live weight, ip). Tissue apoptotic index was examined via TUNEL method. MDA (malondialdehyde) determination in lung tissue was made using spectrophotometer. As a results, MDA amount decreased in the CCl_4 + BC group (6,33 ± 1,54 nmol/g) in comparison to CCl_4 group (8,66 ± 1,58 nmol/g) whereas it was observed in the CCl_4+BC group (15,35 ± 0,21%) that the apoptotic index (TUNEL results) decreases in comparison with the CCl4 group (27,48 ± 0,28%) thus approaching normal values. DNA damage ratio decreased in the CCl_4 + BC group in comparison to CCl_4 group. These results show that BC plant protects the lung against oxidative damage.

Key words: Apoptosis, DNA damage, lung, new drug, TUNEL

Introduction

Carbon tetrachloride (CCl_4) is one of the effective models to induce damage in tissues during studies carried out on experimental animals and it is a poisonous chemical that induces oxidative stress and causes damage in the tissues. It has been put forth in various studies that CCl_4 causes free radical formation in many tissues such as lungs, heart, kidney, brain, liver as well as in the blood (1, 2). CCl_4 is a highly stable dense fluid with quick volatility property that is transparent and non-flammable. This substance can be found in nature while it can also be generated as a result of many chemical reactions (3). Mammal lungs are located in the thoracic cavity with its spongy structure and moist epithelium. Lungs of many mammals are made up of many alveoles (air sacks) where gas exchange takes place. Thus, respiration surface is increased and occupies a smaller space in the body. There are two lungs, the left and the right; the right lung is made up of three lobes, whereas the left lung is made up of two lobes. Left lung is smaller than the right lung since the heart is located underneath it. The number of alveoles in each lung is about 300 million and each have a diameter of about 0.25 mm. Hence, the surface area of the lungs is about 70-100 m^2 (4,5). Some plants have been used in the treatment of many diseases for centuries. Parallel to the developments in the science of biology, the chemical content and protective effects

of many plants are known and they have been classified under the title of medicinal plants and have been started to be used for treatment purposes. *Black cumin* (BC) is one of these plants and its therapeutic effects have been used since the ancient times. When recent studies are taken into account, it is thought that BC also has significant protective effects on the lungs. BC is the seed of plants of the *Nigella sativa* species in the Ranunculaceae family that is formed in a capsule and has been used as a medicinal plant in many regions of the world since 2000 years. Recent studies put forth its many therapeutic effects such as antibacterial, antidiabetic, bronchodilator, antitumoral, antiasthmatic effects (6-8). Thymoquinone is the active substance of BC and is found in the volatile oil of the seed. (9). The importance of plants such as BC that protect the lungs can be understood more easily when one considers the negativities that damages in the lungs which is a vital part of human body can cause in people's lives (2, 10). Apoptosis is a physiological process that is also known as programmed cell death and it plays an important role in the protection of tissue homeostasis. Apoptosis is responsible from the loss of some special cells during the damages on various cell types in normal development as well as in the mature organism. The number of cells increases when apoptosis ratio is low, whereas the number of cells decreases as the apoptosis ratio increases thus causing an undesired tissue damage. The apoptosis ratio can be determined via various methods (11-13). The damaged cell is removed via apoptosis when a DNA damage occurs in the organism. Thus, the possibility for the cells with harmful mutations in their DNA to become cancerous is eliminated (14). Many recent studies have been carried out on apoptosis, the mechanism of which has been recently revealed, as well as on apoptosis genetics. The objective of this study is to examine whether BC has a protective effect against damage in lungs by administering rats with carbon tetrachloride.

Material and Method

Research groups

The study was carried out at the F.U. Experimental Animals Research Institute (FUDAM) with the consent numbered 129 of the Firat University Animal Experiments Ethics Council during the 27.11.2013 dated meeting with a meeting number of 2013-11. 28 male Wistar albino (n=28, 8 weeks) rats were used in the study. Light and dark periods of 12 hours were applied to rats. The rats were distributed into 4 groups according to their live weights. The groups were: (i) Negative Control (NC): Normal water consuming group to which no CCl_4 and BC is administered; (ii) Positive Control (PC): Normal water consuming group to which no CCl_4 is administered but BC is administered; (iii) CCl_4 Group: Normal water consuming and group to which CCl_4 is administered (1,5 ml/kg live weight, ip); CCl_4 + BC group: CCl_4 and BC administered group (1,5 ml/kg live weight, ip). The extract of boiled BC seeds was mixed into the drinking water of animals to be used as BC source (10 % w/v). The initial live weights of the animals were arranged to be equal. Live weights were recorded 3 times weekly throughout the study.

CCl_4 injection

CCl_4 injection was accomplished twice per week for four weeks intraperitoneally at 1,5ml/kg live weight injected together with olive oil at 1: 3 ratio (2).

Chemical matters and Black cumin

All chemicals used in the experimental work were obtained from Sigma-Aldrich (Germany), Bio-Rad (USA), BioShop (Canada) and Merck (USA). Dust form BC seeds to be boiled and mixed in the daily drinking waters of rats were acquired from Oz Gida Organik and Yoresel Urunler Ltd. Sti. (Elazığ).

Black cumin extract

The extract set up by mixing the ground BC seeds in water and boiling them was added to the drinking water of rats at a ratio of 10%. The water consumption of rats was recorded and recorded regularly.

Tissue homogenization

Lung tissue samples were separated into small parts and broken down inside lizis buffer (0,5 M Tris;

pH: 8, EDTA, β-Mercaptoethanol, Phenyl methyl sulphonyl fluoride [PMSF]) in mechanical homogenizator. These broken down tissue samples were centrifuged at 15000 rpm for 45 minutes. Supernatant was got and was stored at -80°C until usage time (2).

Analysis of DNA damage

The DNA samples obtained from tissues were dispersed in Tris-EDTA solution and analyzed in 1% gel via DNA agarose gel electrophoresis (2, 15). Images of the DNA bands were taken afterwards and similarities as well as differences were determined.

TUNEL method

Apoptosis in paraffin blocks, frozen cross sections, cells grown on cultured "plates" in the form of solutions or in lamellae can be determined using this method (16, 17). In our study, the damage that occurs in the DNA of lung tissue was determined via the TUNEL method.

Plasma MDA measurements

MDA determination of the plasma samples was carried out in accordance with the method developed by Ohkawa et al. (18). Samples (200 µl) were taken from the groups after which 200 µl of 8.1% SDS was added. 1.5 ml of 20% acetic acid (pH: 3.5) and 1.5 ml of 0.8% (pH: 3.5) TBA were added after which distilled water was added to make the volume 4 ml. The mixture was then left to wait in a 95°C boiling water bath for 1 h after which it was cooled and vortexed by adding 1 ml distilled water and 5 ml n-buthanol-pyridine mixture at a ratio of 15:1 (v/v). Following centrifugation at 4000 rpm for 15 min, the top organic layer was removed and measured spectrophotometrically at a wavelength of 532 nm. The results were recorded as nmol/ml.

Statistical analyses

All data were evaluated via SPSS 20 package software using variance analysis. One-Way ANOVA *post hoc* Tukey test was applied to determine the differences within the groups. The measurements were repeated at least 3 times to ensure the reliability of the statistical analyses after which they were recorded in the SPSS 20 package software and evaluation process was started.

Results

DNA damage results

When the DNA agarose gel image in Figure 1 is examined, greater fracturing (ladder pattern) in carbon tetrachloride group is observed in comparison with other groups. Whereas it is understood that these fractures are less in the CCl_4 + BC group. Hence, it can be interpreted that the DNA damage ratio in BC administered group with lung damage is less in comparison with the BC administered group with lung damage. Based on this, it can be stated that the antioxidant effect of BC decreases DNA damage.

Plasma MDA results

When the plasma MDA levels in Table 1 and Figure 2 are compared, no statistically significant difference is observed between groups ($p>0.05$); but it is observed that MDA level decreased in the CCl_4 + BC group in comparison with the control and more im-

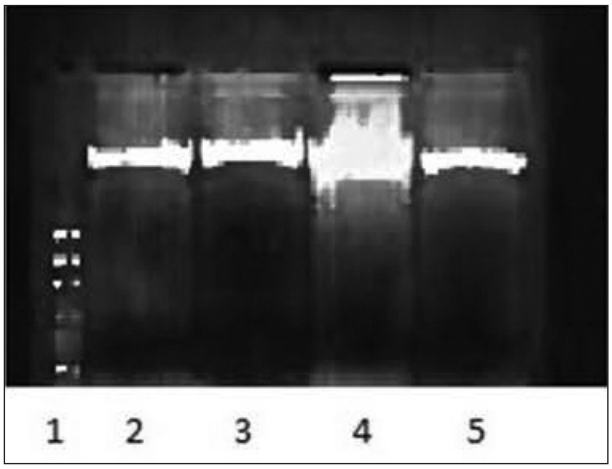

Figure 1. Cellular DNA damage in the control group and experimental group rats
Lines: (1) Marker; (2) NC; (3) PC; (4) CCl4; (5) CCl4 + BC

Table 1. MDA level between groups in the lung tissue

Groups	NC	PC	CCl_4	CCl_4 + BC
Plasma MDA (nmol/g)	3.11 ± 1.50[b]	3.80 ± 1.51[b]	8.66 ± 1.58[a]	6.33 ± 1.54[ab]

a-b: Differences between groups in the same column with different letters are statistically significant (p<0.05). One-Way ANOVA Post Hoc Tukey Test

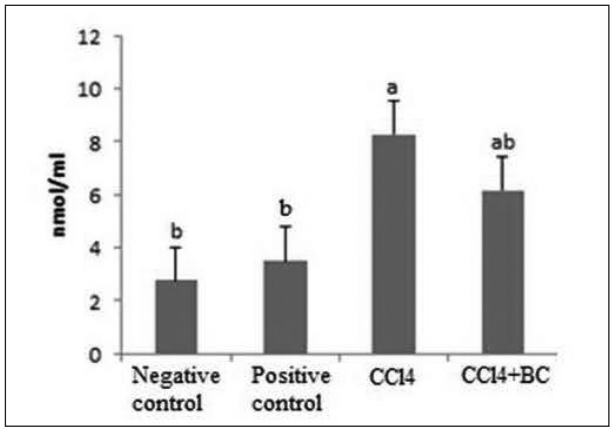

Figure 2. Comparison of the MDA levels in plasma between groups

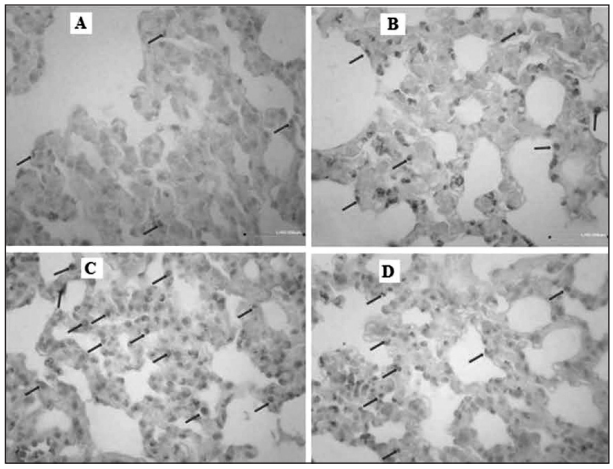

Figure 3. A) TUNEL positive cells (→) in the negative control group lung tissue. Scale bar: 50μm. B) TUNEL positive cells (→) in the positive control group lung tissue. Scale bar: 50μm. C). TUNEL positive cells (→) in the CCl4 group lung tissue. Scale bar: 50μm. D) TUNEL positive cells (→) in the CCl4 + Black cumin group lung tissue. Scale bar: 50μm.

portantly with the CCl4 group (p<0.05). The highest plasma MDA level observed in the CCl_4 administered group and a statistically significant difference ensured in comparison with other groups (p<0.05).

Histological results of TUNEL method

TUNEL method was used in the final stage of the study to determine apoptosis levels. The damage in the DNA has been determined in the lung tissue as a result of the acquired images. As a result of the examination of the TUNEL coloring under light microscope to determine the apoptotic cells; it has been observed that the apoptotic index (Tab. 2) is highest in the CCl_4 group when TUNEL positivity in the lungs is compared with the NC group. On the other hand, it is observed that BC shown its effect causing the apoptotic index in the CCl_4+BC group to decrease in comparison with the CCl_4 thus approaching normal levels (p<0.05) (Fig. 3 and 4).

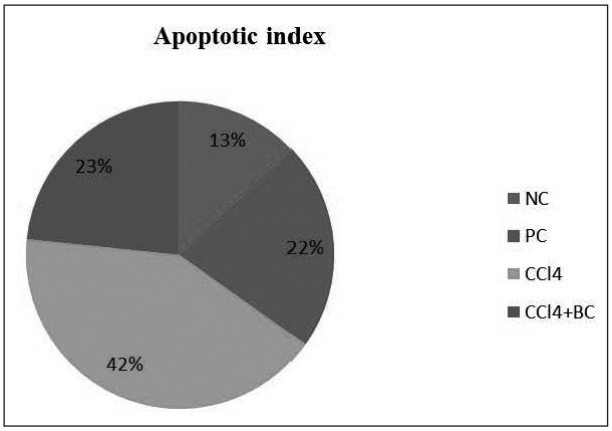

Figure 4. Apoptotic index graphic in lung tissue

Table 2. Apoptotik index (%)

Groups	NC	PC	CCl_4	CCl_4 + BC
Apoptotic index	8.5 ± 0.39[c]	14.41± 0.38[b]	27.48 ± 0.28[a]	15.35 ± 0.21[b]

a-c: Differences between groups in the same column with different letters are statistically significant (p<0.05). One-Way ANOVA Post Hoc Hochberg's GT2 Test.

Discussion

Many studies have been carried out in recent years with antioxidant plants due to their cell and tissue damage prevention properties. BC plant extract has various therapeutic effects such as bronchodilator, antitumor effects and has a strong antioxidant effect thanks to its free radical scavenging function (9). Ahmad et al. (2013) defined *Nigella sativa* as a miraculous lung protecting plant that eases respiration due to its therapeutic effects on trachea and the respiratory tract (19). A study has been carried out on rats following the CCl_4 based tissue damage and negative biochemical parameters observed, during which it has been determined after administering *Nigella sativa* seed extract to rats at a 10% ratio via drinking water that BC has significant therapeutic effects on the antioxidant defense system and the decrease of oxidative damage (20). Another study that examines the effects of BC on cadmium induced oxidative stress in rat blood put forth that the cadmium induced MDA level increase in plasma and erythrocytes can be decreased via BC treatment (21). When our results of DNA agarose gel electrophoresis images for CCl_4 induced lung damage in rats are examined (Fig. 1), it is observed that there are no DNA fractures in the NC and PC groups. The greatest number of DNA fracture was observed in the CCl_4 group and that the fractures in the CCl_4 + BC group are less in comparison with the CCl4 group. The fact that DNA fracture image is less in the BC administered damaged group in comparison with the damaged group not administered with BC indicates that DNA damage in less in the CCl_4 + BC group. This leads us to think that the active ingredients in BC can partially prevent DNA damage in damaged cells. Aycan et al. (2014) carried out studies in which they examined the therapeutic effects of the BC active ingredient on acetaminophen induced hepatoxicity, as a result of which they stated that the MDA level is greater at a statistically significant level ($p<0.05$) only in rats administered with acetaminophen in comparison with the other groups, that there is no statistically significant difference between control groups ($p>0.05$) and that the plasma MDA level of the group to which thymoquinone is administered together with acetaminophen decreased (22). Yaman and Balikci (2010) used the gentamin substance that has protective effects against negative bacterial infection in humans and animals but which causes nephrotoxicity at high doses to induce nephrotoxicity in rats and they examined the protective effects of *Nigella sativa* against the induced damage. They put forth in their studies that, the MDA level in the gentamicin administered group was higher at a statistically significant level ($p<0.05$) in comparison with the control group and the gentamicin and *Nigella sativa* administered group (23). When the plasma MDA levels of our study are examined (Tab. 1, Fig. 2), it was observed that there was no statistically significant difference ($p>0.05$) and that the highest plasma MDA level was observed in the CCl_4 group which differed from other groups at a statistically significant level ($p<0.05$). Whereas it can be stated for the CCl_4 + BC group that the plasma MDA level decreased and that it is due to the antioxidant effect of BC. Peng et al. (2014) stated that DNA fragmentation is characterized in the apoptotic tissue and that TUNEL method is widely used for determining apoptosis related DNA fragmentation. Peng et al. (2014) carried out a study in which they examined the reducing effects of metallothionines on paraquat (a chemical that kills plants) induced acute lung damage in rats via antioxidant and antiapoptosis mechanisms that no damage is observed in the control groups based on TUNEL method examinations and that only the apoptotic index ratio of paraquat induced rats is lower (24). Kanter et al. (2015) carried out a study in which they examined the effects of methylene blue on respiratory pneumonia in which they classified the rats into six groups as, containing salt solution (control), BIO (biosorb energy plus), HCl, salt solution + methylene blue, BIO + methylene blue and HCl + methylene blue. They determined as a result of the analysis they carried out after the surgical procedure based on TUNEL method examinations that the highest apoptosis ratio is observed in the HCl group ($p<0.05$), that the apoptosis levels of HCl + methylene blue and BIO groups have the highest apoptosis ratio after the HCl group and that the lowest apoptotic index occurs in the salt solution + methylene blue group (25). In addition, Aslan et al. (2015) defined *Nigella sativa* has protective effect on lung (26). When our TUNEL method results are examined, it is observed that the apoptotic index (Tab. 2) in the CCl_4

+ BC group decreased and approached normal values. It is observed that the highest apoptosis ratio is in the CCl_4 group and when compared with NC group there is a statistically significant difference ($p<0.05$). These results indicate that BC can be protective against lung damage (Fig. 3 and 4).

Conclusion

Based on the current data, it can be stated that BC is protective against lung damage in rats and that it protects the lungs with its antioxidant effects. In addition, we hope that these results will contribute to the relevant literature, that the data acquired will guide the way for further studies that will be carried out on humans.

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