Article

# *Brassica oleracea* var. italica extract reducing free radicals and inflammation initiated by an exposure to cigarette smoke

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## Abstract

*Introduction:* Herbal extracts are often administered to cigarette smokers to prevent excessive free radicals. These include *Brassica oleracea* var. Italica, known to contain high antioxidant flavonoids and selenium micronutrients. Therefore, this study aims to determine the efficacy of *Brassica oleracea* var extract. italica in reducing the free radicals and inflammation present in experimental animals exposed to cigarette smoke.

*Design and Methods:* This research was conducted based on an experimental method using a randomized controlled trial (RCT) for 21 days. The animals used were divided into six groups (negative control, positive control, and four treatments). Particularly, the positive control and treatment groups were exposed to cigarette smoke for 2 minutes, twice a day, at 50 PPM CO levels. The treatment groups were administered the extract at different doses (0.5 ml; 0.75 ml; 1 ml; 1.25 ml), before assessing the blood level of malondialdehyde and C-Reactive Protein.

*Result:* The results showed the tendency for exposure to smoke to increase the number of free radicals and stimulate inflammation responses in the body ($P<0,05$). In addition, a strong correlation between variables was established ($p=0.000$; $r=0.713$).

*Conclusions:* Broccoli extracts (*Brassica oleracea* L. var. Italica) administration has the potential to cause a decline in the two aspects, including free radicals and inflammation responses resulting from exposure to cigarette smoke.

## Introduction

Tobacco cigarette is one of the products consumed through smoking. In addition, there has been an increase in popularity despite the negative health effects. Numerous countries have explored various means to stop the use,[1] but these efforts have not yielded significant results. Based on previous reports, one of the serious potential problems of every smoker is the elevated risk of disease infections, which is implicated in around 6 million deaths, worldwide. Despite the practice of abstinence for several years, smokers are known to have a sustained higher risk of infection with earlier onset than non-smokers. These are strongly associated with 10 years of shorter life expectancy.[2] The total number of smokers worldwide is estimated to have reached 1 billion people and consist predominantly of men.[3] In addition, the prevalence in developed countries, including America is relatively high, at about 15% of the total population, and consists more of people in the productive age groups.[4] The number in developing countries, including Armenia, Laos and Indonesia, is comparably more, reaching over 50% of the total population.[5]

The increased mortality rate of smokers is evidence of the dangerous effects. Previous studies showed the development of various diseases, including lung cancer, respiratory tract, heart problems, and problems during pregnancy. Furthermore, about 40% of the total smoking population are known to die prematurely.[6] The increase in complications from diseases have been attributed to the free radicals present in cigarette smoke and are known to directly stimulate the body's inflammatory response.[7] Further exposure potentially instigates a decline in antioxidant levels and propagates immunosuppression.[8] Previous studies also highlighted the tendency for cigarettes to initiate endothelial dysfunction as a result of excessive free radicals and inflammation.[9]

Physiologically, free radicals play an important role in cellular functions related to the enzymatic defence system assumed to prevent further accumulation. The presence of excessive radicals causes cellular changes, and consequently oxidative stress.[10,11] The production of reactive oxygen species, including superoxide (O2 • -), peroxynitrite (ONOO • -), and hydroxyl (OH) is possible during metabolism.[12] The enzymatic antioxidants, in form of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and Catalase (CAT) function to neutralize these free radicals.[13] Other variants are possibly derived from food intake, including non-enzymatic antioxidants.[14] In addition, inflammation is another expression of the body's natural defence mechanism, which occurs in response to the entry of various foreign objects, including bacteria, viruses, allergens, toxic chemicals, and cigarettes. Pathogens or other hazardous substances continuously perpetuates chronic oxidative stress and consequently trigger protein oxidation, which releases pro-inflammatory cytokines and stimulates inflammatory responses all over the body.[15]

The intake of non-enzyme antioxidants is necessary to reduce excessive free radicals. These include polyphenols, a natural compound obtained from numerous fruits and vegetables, including broccoli. This constituent serves as a defence system from ultravi-

**Significance for public health**

*The increasing number of active smokers is expected to cause serious health problems. This habit has been implicated in the increase in free radicals entering the respiratory tract, which stimulate an inflammatory process in the body. This process triggers cell damage and death, eventually causing health problems. Moreover, Brassica oleracea var. italica is known to contain high antioxidants, hence, there is a postulation of potential capacity to neutralize free radicals and reduce body inflammations.*

olet radiation as well as various pathogens and also has anti-inflammatory properties with the potential to confer cellular protection against oxidative stress.[16] The broccoli plant (*Brassica oleracea* var. italica) is known to contain one of the polyphenol types termed flavonoids and considered to have higher amounts compared to other vegetables.[17,18] Therefore, this research aims to determine the effectiveness of broccoli in reducing free radicals and inflammation resulting from exposure to cigarette smoke.

## Design and Methods

This is an experimental research with a post-test control group design. This involved the use of male Wistar rats (Rattus novergicus) as samples during a 21 days experiment (No:139/KE/X/2021). The animals were subdivided into 6 groups (one negative control group, one positive control group, and four treatment groups). In addition, basic maintenance and care were provided with reference to the 3R principle (Replacement, Reduction, and Refinement).

Kretek cigarettes, predominantly consumed by Indonesians, was used in this study, and is characterized by 2.4 mg nicotine and 38 mg tar. The smoke exposure was carried out two times daily for 21 days (each for 2 minutes with 50 PPM Carbon Monoxide levels measured using a Carbon Monoxide Meter device).

The broccoli was extracted through a maceration process using 96% ethanol as the solvent. Furthermore, the preparations were obtained by evaporating the extracts with a vacuum rotary evaporator. Based on Laurence and Bacharach's framework, a flavonoid present in Broccoli extract (18 mg/ml) was measured and evaluated by comparing it with the adequacy daily rate of (8 mg/day). Hence, a dose of 0.5 ml; 0.75 ml; 1 ml; 1.25 ml were derived and used for the experiment.

The negative control (Group I) consisted of experimental animals administered a daily intake, while the positive control (II) were only exposed to cigarette smoke. In addition, each of the four treatment groups (Group III, IV, V, VI) comprised of experimental animals exposed to cigarette smoke and the different doses of Broccoli extracts (0.5 ml; 0.75 ml; 1 ml; 1.25 ml). Therefore, the ratio data of malondialdehyde and C-Reactive Protein for each group were obtained and analysed to ascertain the possible differences and relationships.

## Results and Discussions

The results showed the mean levels of malondialdehyde in each group. Table 1 highlights the highest values (11.23 ± 1.45) in Group II, while the lowest (3.36 ± 0.74) were demonstrated in I. Furthermore, all data obtained were subjected to a normality test ($p > 0.05$) to ensure a well-modelled dataset is used, based on a normal distribution. The homogeneity test ($p = 0.018$) identified the dataset as non-homogeneous ($p < 0.05$). Therefore, differences in both results were calculated using the T-Test. Table 2 showed differences in level within each group, although no significant differences were observed between II and III ($p = 0.898$) as well as between Group I and V ($p = 0.465$).

The free radicals contained in cigarette smoke are assumed to instigate tissue damage through various mechanical processes, and consequently trigger the peroxidation of lipids, proteins, and DNA. This damage reportedly reduces the antioxidant defence system and stimulates the release of pro-inflammatory cytokines.[19] In addition, an increase in the predominance of cellular damage from exposure to cigarette smoke that enters the respiratory tract directly elevates malondialdehyde levels in the blood. This compound is not considered a free radical, but the end product of lipid peroxidation.[20] The intrinsic characteristics include relatively strong stability and potential for use as an indicator of oxidative stress or body radical content.[21,22]

Table 3 shows the highest levels of C-reactive protein (6.58 ± 0.58) in Group II, while I had the lowest values (4.44 ± 0.74). Therefore, a normality test ($P > 0.05$) was performed on all the data obtained to determine if the dataset was well-modelled by a normal distribution. The homogeneity test ($p = 0.831$) showed the samples as homogeneous ($p > 0.05$). The differences in both results for each group were then calculated using the ANOVA test, followed by the Least Significance Different (LSD) assessment.

In addition, free radicals entering the body potentially stimulate the phagocytosis process of the body's immune system. This

**Table 1. Means of malondialdehyde between all groups.**

| Groups | | Means ± SD | Normality | Homogeneity |
|---|---|---|---|---|
| I | A group only given daily intake | 3.36 ± 0.74 | 0.754 | 0.018 |
| II | A group only exposed to cigarette smoke | 11.23 ± 1.45 | 0.367 | |
| III | A group given daily intake, cigarette smoke, and 0.5 ml of *Brassica oleracea* var. italica extract | 11.14 ± 1.62 | 0.580 | |
| IV | A group given daily intake, cigarette smoke, and 0.75 ml of *Brassica oleracea* var. italica extract | 5.60 ± 0.94 | 0.237 | |
| V | A group given daily intake, cigarette smoke, and 1 ml of *Brassica oleracea* var. italica extract | 3.87 ± 0.83 | 0.640 | |
| VI | A group given daily intake, cigarette smoke, and 1.25 ml of *Brassica oleracea* var. italica extract | 1.90 ± 0.70 | 0.552 | |

**Table 2. The results of T-test on malondialdehyde levels between all groups.**

| Groups | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| I | - | - | - | - | - | - |
| II | 0.000 | - | - | - | - | - |
| III | 0.000 | 0.898 | - | - | - | - |
| IV | 0.003 | 0.000 | 0.000 | - | - | - |
| V | 0.465 | 0.000 | 0.000 | 0.019 | - | - |
| VI | 0.038 | 0.000 | 0.000 | 0.000 | 0.006 | - |

process stimulates the secretion of pro-inflammatory cytokines, including interleukin-1, interleukin-6, and tumour necrosis factor-alpha (TNF-α), known to be responsible for inflammatory responses.[23,24,25] The reaction serves as a defence mechanism against various microorganisms or foreign objects, and there is a need to control this effect to avoid attacks by autoimmune diseases.[26] Furthermore, a gradual change in the cells surrounding the site is observed during chronic attacks, and potentially results in permanent damage.[27] These conditions are also possibly created after chronic cigarette exposure, which initiates a plaque build-up in the artery. This formation is consequently implicated in atherosclerosis, which leads to serious diseases, including cardiovascular and stroke.[28] The increase in free radicals is signalled by an elevation in malondialdehyde levels, which directly triggers inflammatory responses with negative health impacts.

The ANOVA analysis results showed differences in the levels of C-reactive protein in each group. These were further evaluated using the Least Significance Different (LSD) test. Table 4 showed a significant difference ($p < 0.05$) in Group I compared to others, while II demonstrated no substantial variation in contrast with III ($p = 0.917$) and Group IV ($p = 0.152$). However, no significant difference were observed between Group IV and III ($p = 0.182$), as well as V ($p = 0.139$) and VI ($p = 0.085$). The results also indicate no substantial variations between Group V and VI ($p = 0.794$). Table 5 depicts the relationship between increased malondialdehyde and C-reactive protein levels. This was ascertained using the Pearson test, and the results showed a strong correlation between both parameters ($r = 0.713$).

The external antioxidants or exogen antioxidants obtained from food possess different action mechanisms compared to enzymatic antioxidants, despite the intrinsic neutralizing capacity. These compounds are known to reduce free radicals in various ways, including through the protection of cells from lipid peroxidation (Vitamin E), as strong reducing agents to be potentially re-reduced by enzymes and glutathione (Vitamin C). Previous reports showed proficiency in boosting the immune system (β carotene) and polyphenol groups, known to act as both antioxidant and anti-inflammatory agents.[29,30,31] In addition, non-enzymatic antioxidants reportedly reduce muscle pain and physical strain resulting from oxidative stress reactions.[32] Broccoli (*Brassica oleracea* var. italica) contains various types of non-enzymatic antioxidants and high polyphenol, including flavonoids, which is estimated to reduce free radicals and inflammation caused by cigarette smoke exposure.[33,34] This vegetable also contains selenium, and is known to potentially increase various enzymatic antioxidants, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px).[35]

## Conclusions

The conclusion of this study is exposure to cigarette smoke increases free radicals and stimulates an inflammatory response in the body. Broccoli (*Brassica oleracea* var. italica) extracts plays an intrinsic role in reducing these negative impacts, and the effect is dose-dependent.



**Table 3. Means of C-reactive protein levels between all groups.**

| Groups | | Means ± SD | Normality | Homogeneity | P |
|---|---|---|---|---|---|
| I | A group only given daily intake | 4.44 ± 0.74 | 0.530 | 0.831 | 0.000 |
| II | A group only exposed to cigarette smoke | 6.58 ± 0.58 | 0.642 | | |
| III | A group given daily intake, cigarette smoke, and 0.5 ml of *Brassica oleracea* var. italica extract | 6.54 ± 0.50 | 0.384 | | |
| IV | A group given daily intake, cigarette smoke, and 0.75 ml of *Brassica oleracea* var. italica extract | 6.02 ± 0.52 | 0.758 | | |
| V | A group given daily intake, cigarette smoke, and 1 ml of *Brassica oleracea* var. italica extract | 5.44 ± 0.45 | 0.074 | | |
| VI | A group given daily intake, cigarette smoke, and 1.25 ml of *Brassica oleracea* var. italica extract | 5.34 ± 0.74 | 0.248 | | |

**Table 4. The results of the Least Significance Different (LSD) test between all groups.**

| Groups | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| I | - | - | - | - | - | - |
| II | 0.000 | - | - | - | - | - |
| III | 0.000 | 0.917 | - | - | - | - |
| IV | 0.000 | 0.152 | 0.182 | - | - | - |
| V | 0.014 | 0.006 | 0.008 | 0.139 | - | - |
| VI | 0.026 | 0.003 | 0.004 | 0.085 | 0.794 | - |

**Table 5. The results of the Pearson test between all groups.**

| Groups | P | Pearson Test |
|---|---|---|
| Malondialdehyde<br>C-Reactive Protein | 0.000 | 0.713 |

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