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Ameliorative Effects of Broccoli (*Brassica oleracea*) on Incense Smoke-Induced Oxidative Stress and Hepatotoxicity in Albino Rats

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A B S T R A C T

Indoor air pollution caused by incense smoke is a significant health risk, especially in developing countries where incense consumption is heavily influenced by both home and religion. Therefore, this study was conducted to assess the potential of broccoli (*Brassica oleracea*) specific to its ameliorative effects on rats (Rattus norvegicus) exposed to incense smoke. The methodology involved three experimental groups designed by exposing each group of rats to incense smoke for an hour every day and providing broccoli extract orally to one of these three groups during a total of 28 days. Liver function was determined using ALT, AST, ALP, total protein, and albumin levels. In addition to using these methods to determine liver function, histological assessment via light microscopy of the liver was conducted to look for any signs of structural liver damage. Increased liver damage occurred within the incense-smoke-exposed group when evaluated via ALT, AST, and ALP levels as compared to the control group. In the incense smoke-exposed rats, there was decreased protein and albumin levels found; these results are consistent with impaired liver function. Additionally, significant liver damage and impairment of structure and function were substantiated histologically by necrosis, degeneration, vacuolization, dilation, and infiltration of inflammatory cells within the liver. Supplementation with broccoli resulted in significant improvements and reductions in rat liver damage. Evaluated using the same parameters (ALT, AST, ALP, total protein, and albumin) as the incense exposure group, rats in the broccoli-treatment group exhibited improvements toward normality. Histologically, cellular damage, inflammatory infiltrates, and structure were also significantly improved when compared to the incense smoke-exposed group. Due to the high quantities of antioxidants such as sulforaphane, flavonoids, and vitamins found in broccoli, it is reasonable to assume that the hepatoprotective effects of broccoli have occurred through the antioxidant potential of these compounds and through enhanced detoxification pathways through direct neutralization of oxidative stress. In conclusion, incense smoke exposure is associated with significant oxidative-stress-mediated hepatotoxicity, while broccoli demonstrates a significant protective effect against oxidative-stress-mediated toxicity, and should be further studied as a natural therapeutic alternative against environmental toxin exposures that cause liver damage.

Introduction

Indoor air pollution is now acknowledged as a significant environmental and public health problem, particularly in developing countries due to the widespread use of incense (agarbati) burning, especially by households, temples, and in cultural ceremonies. Continuous exposure to smoke occurs indoors where there is limited ventilation because of the large number of incense sticks that are burned daily. While the types of harmful substances produced are varied, a majority of the harmful substances produced by the burning of incense sticks consists of a complex mixture of harmful substances, including particulate matter ($PM_{2.5}$), carbon monoxide (CO), volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons (PAHs); all of which substantially contribute to the creation of indoor air pollution (Chen *et al*., 2020; Zhang *et al*., 2015). Studies have shown that the concentration of indoor air pollutants can be (4-10) times greater than outdoor air pollutant concentrations as a result of limited ventilation and continuous incense burning (Al-Rawas *et al*., 2009). Incense smoke produces fine particulate matter similar to the PM generated from tobacco smoking; both of these types of PM can penetrate deep into biological systems and produce systemic toxicity (Lung *et al*., 2003). The toxicants that are produced when incense is burned are readily absorbed into the bloodstream and distributed throughout the body, including the liver. The liver plays a key role in the detoxification and metabolism of xenobiotic compounds; therefore, the liver is at risk for damage from environmental contaminants (Hussain *et al*., 2014). Continuous exposure to incense smoke may overwhelm the liver's detoxification mechanisms leading to biochemical and structural changes in the liver tissue. Oxidative stress appears to be one of the major mechanisms of incense smoke-induced hepatotoxicity.Smoke inhalation is associated with increased levels of reactive oxygen species (ROS) which disrupt cellular homeostasis via damaging lipids, proteins, and nucleic acids (Donaldson *et al*., 2005) through lipid peroxidation, a major sign of oxidative stress, which leads to degrading cell membranes and impairing liver function. Studies on laboratory animals exposed to incense smoke have shown that oxidative stress markers including malondialdehyde (MDA) are significantly increased while antioxidant enzyme levels, including superoxide dismutase (SOD) and catalase (CAT) are significantly decreased (Hussain *et al*., 2019). Exposure to incense smoke has also shown significant histopathological changes in the liver. These changes include degeneration of hepatocytes, necrosis, inflammatory infiltration, and sinusoidal dilation (Bentayeb *et al*., 2018). If exposure is prolonged; chronic liver diseases, fibrosis, and carcinogenesis can occur. The need to identify protective mechanisms against the toxicity of incense smoke is urgent. Recently, natural antioxidants to reduce oxidative damage from environmental toxins have become a topic of investigation. One such natural antioxidant is broccoli (*Brassica oleracea*), a cruciferous plant high in bioactive compounds such as sulforaphane, glucosinolates, flavonoids and vitamins C & E. All of these compounds display strong antioxidant and anti-inflammatory properties, giving broccoli potential as a protective agent against toxic exposures (Zhang *et al*., 2015). Specifically, sulforaphane has been shown to stimulate phase II detoxification enzymes and enhance the cellular antioxidant defence system, thereby decreasing oxidative stress and damage to cells (Hafez *et al*., 2023). Several studies have reported that dietary supplementation with broccoli significantly decrease lipid peroxidation, enhance antioxidant enzyme levels, and improve tissue morphology in animal models of toxins. Despite these studies there are very few studies on the hepatoprotective qualities of broccoli in regards to the toxic effects attributed to the inhalation of incense smoke. Therefore, this study will examine the hepatotoxicity of incensed smoke exposure in albino rats and assess the potential of broccoli supplementation to ameliorate this effect. The results of this study will contribute significantly to the understanding and usefulness of natural antioxidants in the prevention of liver damage due to the exposure of environmental pollution and will be particularly useful for public health given the high incidence of indoor air pollution in certain areas.

Materials and Methods

3.1 Experimental Animals- Healthy adult albino rats (Rattus norvegicus), weighing 150–200 g, were used for the present study. The animals were maintained under controlled laboratory conditions at a temperature of 21 ± 0.5°C, relative humidity of 55 ± 5%, and a 12-hour light/dark cycle.

The rats were acclimatized for two weeks prior to the experiment and were provided standard pellet diet and water ad libitum. All experimental procedures were conducted in accordance with ethical guidelines for laboratory animal care (CPCSEA/IAEC).

3.2 Experimental Design

The experimental animals were randomly divided into four groups, each consisting of six rats (n = 6):

●Group I (Control): Normal diet, no exposure

●Group II (Incense Smoke): Exposed to incense smoke

●Group III (Incense Smoke + Broccoli): Exposed to incense smoke along with broccoli treatment.

Incense smoke exposure was carried out in a separate chamber for 1 hour per day (10:00 AM–11:00 AM) for a duration of 28 consecutive days. Broccoli was administered orally in the form of aqueous extract at (200–300 mg/kg body weight) throughout the experimental period.

3.3 Biochemical Analysis

At the end of each experimental interval (Day 1, 7, 14, 21, and 28), blood samples were collected from experimental animals via cardiac puncture under light anesthesia. The blood was allowed to clot at room temperature and centrifuged at 3000 rpm for 10 minutes to obtain clear serum, which was used for biochemical analysis.

The following liver function parameters were estimated using standard commercial diagnostic kits (Span Diagnostics/SRL) and analyzed with an automated biochemical analyzer:

●Alanine aminotransferase (ALT) by the Reitman and Frankel (1957) method

●Aspartate aminotransferase (AST) by the Reitman and Frankel method

●Alkaline phosphatase (ALP) by the King and Armstrong (1934) method

●Total protein by using the Biuret method

●Albumin and globulin by the Bromocresol Green (BCG) dye-binding method

●Albumin/Globulin (A/G) ratio

3.4 Oxidative Stress Markers

Liver tissues were excised, washed in ice-cold saline, and homogenized in phosphate buffer (pH 7.4). The homogenate was centrifuged and used for the estimation of oxidative stress markers:

●Malondialdehyde (MDA): Measured by TBARS method to assess lipid peroxidation

●Superoxide dismutase (SOD): Determined by inhibition of pyrogallol auto-oxidation

●Catalase (CAT): Assessed by decomposition rate of hydrogen peroxide

3.5 Histopathology-Liver tissues were fixed in 10% neutral buffered formalin for 24–48 hours. After fixation, tissues were dehydrated, embedded in paraffin, and sectioned at 4–5 µm thickness using a rotary microtome. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope

3.6 Statistical Analysis-Mean ± SD; ANOVA with $p < 0.05$ significance (By Tukey's post hoc test.)

Results

The effect of incense smoke exposure and broccoli supplementation on liver function parameters in albino rats is presented in Table 1.

A significant increase ($p < 0.05$) in serum levels of ALT, AST, and ALP was observed in the incense smoke-exposed group (Group II) compared to the control group, indicating hepatocellular damage and impaired liver function. The elevation in transaminase enzymes suggests increased membrane permeability and leakage of intracellular enzymes into the bloodstream due to toxic insult. the combined treatment group (Group III: Incense Smoke + Broccoli) demonstrated a significant reduction ($p < 0.05$) in ALT, AST, and ALP levels compared to the incense smoke group. However, the enzyme levels in this group remained slightly higher than control values, suggesting partial but significant recovery. Similarly, total protein and albumin levels were significantly decreased ($p < 0.05$) in the incense smoke group, reflecting impaired hepatic synthetic function. Broccoli supplementation restored these parameters towards normal levels in the combined treatment group, indicating improvement in liver functional capacity.

Table 1: Effect of Incense Smoke and Broccoli on Liver Function Parameters (Mean ± SD)

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Protein (g/dL)	Albumin (g/dL)
Control	35 ± 2	40 ± 3	80 ± 4	6.5 ± 0.3	3.8 ± 0.2
Incense Smoke	95 ± 5*	110 ± 6*	170 ± 8*	4.2 ± 0.2*	2.5 ± 0.2*
Smoke + Broccoli	55 ± 4#	65 ± 5#	110 ± 6#	5.8 ± 0.2#	3.4 ± 0.2#

* Significant increase compared to control ($p < 0.05$)

Significant improvement compared to incense smoke group ($p < 0.05$)

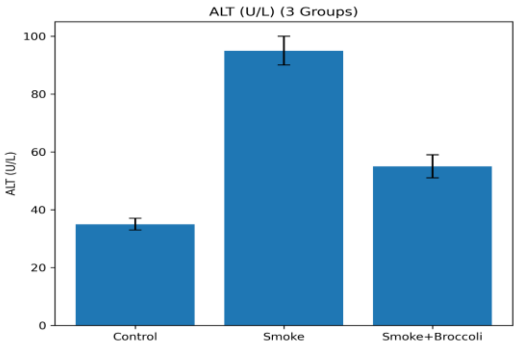

Figure 1. Effect of incense smoke exposure and broccoli supplementation on serum ALT levels (U/L) in albino rats.

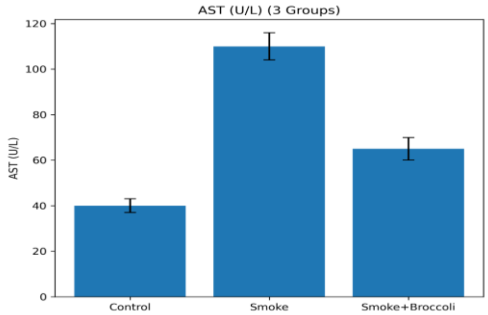

Figure 2. Incense smoke elevates AST levels, while broccoli supplementation reduces hepatotoxic effects.

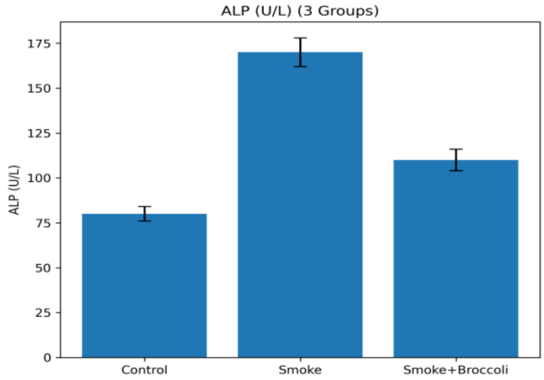

Figure 3. Effect of incense smoke exposure and broccoli supplementation on serum ALP levels (U/L) in albino rats.

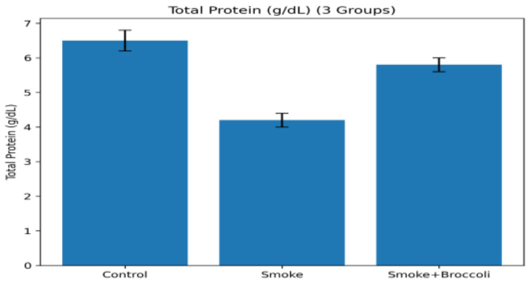

Figure 4. Incense smoke reduces total protein; broccoli restores levels toward normal.

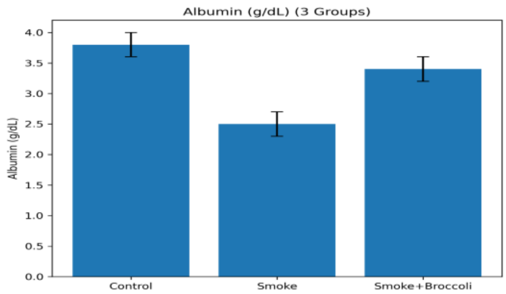

Figure 5. Effect of incense smoke exposure and broccoli supplementation on serum albumin levels (g/dL) in albino rats. Values are expressed as mean ± SD. Incense smoke significantly reduces albumin levels, and the combined treatment group, indicating hepatoprotective activity.

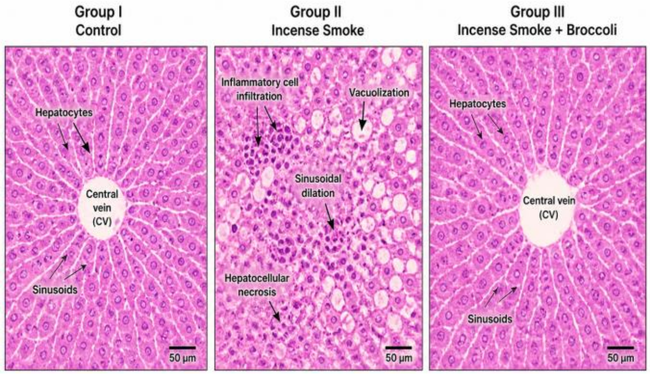

Figure 6. Photomicrographs of liver sections of albino rats stained with hematoxylin and eosin (H&E, 400×; scale bar = 50 µm) in three-group design. (A) Control group showing normal hepatic architecture with intact hepatocytes, central vein (CV), and sinusoids. (B) Incense smoke-exposed group showing hepatocellular necrosis, vacuolization, sinusoidal dilation, and inflammatory cell infiltration. (C) Incense smoke + broccoli group showing improved hepatic architecture with reduced cellular damage and near-normal histological appearance.

Discussion

Our research utilized a three group study design to examine for hepatotoxicity in the albino rat model, by comparing three experimental groups: Non-Exposed Control, Exposed to Incense Smoke, and Exposed to Incense Smoke and Broccoli are. The long-term exposure to the incense smoke produced significant hepatotoxicity (as indicated by highly significant elevations ($p < .05$)) of the serum levels of amino-alkane transaminases [alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)], compared to the Non-Exposed Control group. Increases in these transaminases elevated levels of ALT, AST and ALP are reliable indicators of damage occurring at the hepatocyte level; when these enzymes increase in serum following hepatocellular injury, it identifies hepatocellular membrane damage and subsequent leaking of intracellular contents into the systemic circulation (Hussain *et al*., 2014). 5These biochemical changes concur with published information regarding the toxicological composition of incense smoke, such as: fine particulate matter ($PM_{2.5}$), volatile organic compounds (VOCs), carbon monoxide (CO), and polycyclic aromatic hydrocarbons (PAHs). Incense smoke is metabolized in the liver to form reactive oxygen species (ROS), and thus produce oxidative stress and lipid peroxidation, which compromise the structural integrity of the cell (Chen *et al*., 2020; Zhang *et al*., 2015). In addition, the statistically significant decrease in the total protein concentration and the albumin concentration provide additional support that hepatic synthetic function was impaired as a result of incense smoke exposure. The albumin is produced exclusively by hepatocytes, therefore the observed reduction of serum albumin levels following chronic radiation from incense smoke represent a reduction in protein synthesis and loss of liver function (Bentayeb *et al*., 2018). The supportive histopathological results confirm and validate the aforementioned biochemical examination findings; the liver sections from the rats chronically exposed to incense smoke revealed evidence of hepatocyte degeneration and apoptosis, vacuolization of the cytoplasm, dilation of the sinusoids and infiltration of inflammatory cells - all of which are associated with hepatotoxicity resulting from toxin exposure. Previous studies have reported similar structural changes in tissues of animals exposed to combustion-generated vapors due to oxidative stress and inflammatory cascades (Hussain *et al*., 2019). The presence of vacuoles is representative of lipids associated with metabolic disturbance and the presence of inflammatory cells indicates activation of the immune response to injury to the tissue. In contrast, the rats who were chronically exposed to incense smoke and fed broccoli demonstrated statistically significant improvement among all parameters measured when compared to the group only exposed to incense smoke. Serum concentrations of ALT, AST, and ALP were significantly decreased, while total protein and albumin levels expressed in the incense smoke and the broccoli group approached the mean values expressed in the Non-Exposed Control group. The final histological examination of liver tissue yielded decreased necrosis and inflammation as well as nearly complete restoration of normal hepatic architecture, indicating successful repair of liver tissue. The hepatoprotective properties of broccoli (*Brassica oleracea*) are attributable to the abundance of bioactive phytochemicals, such as: sulforaphane, glucosinolates, flavonoids and antioxidant vitamins. Specifically, sulforaphane has been shown to induce the nuclear factor erythroid 2–related factor 2 pathway (Nrf2), which induces the production of endogenous antioxidant enzymes (e.g. superoxide dismutase) and phase II detoxification enzymes (e.g. glutathione S-transferases) (Hafez *et al*., 2023). Therefore, the activation of this pathway enhances the natural defense mechanisms of the cells through antioxidant production, decreases lipid peroxidation, and promotes structural integrity. In addition to the antioxidant effects of broccoli phytochemicals, the anti-inflammatory properties may reduce the inflammatory response to liver tissue damage, thus contributing to hepatoprotection. through the three-group design study, the toxic effects of incense smoke and the therapeutic value of broccoli have been well established. In addition, these studies provide evidence of oxidative stress as a primary mechanism by which incense smoke produces hepatotoxicity and demonstrate the ability of dietary antioxidant-rich foods to significantly reduce the associated effects. These data have important public health implications, especially in populations exposed to indoor air pollutants. Therefore, the inclusion of antioxidant-rich foods such as broccoli may be an easy and effective method for reducing liver damage caused by environmental toxins..

Conclusion

The results of this investigation direct evidence was the exposure to incense smoke causes significant liver damage in albino rats and that broccoli consumption has a protective effect against such damage.

Rats were continuously exposed to incense smoke and the substantial elevation ($p < 0.05$), in liver transaminase levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) level, indicates hepatic cellular damage and the cytoplasmic membrane permeability. Thus, this demonstrates that incense smoke disrupts normal hepatic function by damaging the integrity of hepatocytes. The elevation of these hepatic enzymes and the significant decrease of total protein and albumin levels in the incense smoke group indicate that the synthetic function of the liver has been impaired. Albumin is exclusively produced by the liver and therefore the reduced serum content of this protein provides a reliable assessment of the liver's synthetic function. Collectively, all of the biochemical changes presented herein demonstrate that incense smoke induces severe metabolic and synthetic impairment of liver function. Histopathological evaluation confirmed the biochemical changes with severe architectural damage and hepatocellular degeneration, necrosis, cytoplasmic vacuolization, sinusoidal dilation, and infiltration of inflammatory cells in the incense-exposed rats. This evidence indicates extensive liver injury caused primarily by the oxidative stress and inflammatory processes that occur as a result of the toxic components of the incense smoke. The broccoli group showed substantial improvement in biochemical and histological evaluations associated with exposure to incense smoke. The liver enzyme activities were substantially decreased and the serum levels of total protein and albumin were restored toward normal. The histological evaluations showed significantly decreased cellular damage, inflammation, and some restoration of hepatic architecture indicating recovery of the liver tissue. The protective effects of broccoli (*Brassica oleracea*) are attributed to the antioxidants it contains. Antioxidants such as sulforaphane, flavonoids, and vitamins, help to neutralize reactive oxygen species (ROS) within the cells and to enhance the body's own ROS defenses. The use of these antioxidants will play an important role in reducing the oxidative stress that occurs with incense smoke exposure, stabilizing cellular membranes, and aiding in the repair of damaged tissues. In summary, exposure to incense smoke has a significant effect on the functioning of the liver by inducing damage caused by an increase in oxidative stress and broccoli supplementation offers a cost effective and safe method to decrease the effects of oxidative stress on the liver. The results of this study suggest that the inclusion of vegetables containing antioxidants in our diet produces protection against environmental toxins and may lessen the health risks associated with poor air quality in the home.

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