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Toxicological Impact of Incense Smoke Exposure on Pulmonary Function in Albino Rats and the Protective Role of Broccoli (*Brassica oleracea*)

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ABSTRACT

Indoor air pollution due to incense smoke is an environmental and public health hazard of major concern and is noticed prominently in developing countries and in deep-rooted cultural and religious rituals as incense burning. The current study was carried out for evaluation of toxicological impact of incense smoke on the pulmonary tissues and biochemical parameters of albino rats and to investigate the protective role of these changes by inclusion of broccoli (*Brassica oleracea*) in the diet. Assessment was made on the albino rat (*Rattus norvegicus*) which were divided into three groups: control, incense smoke exposed, and incense smoke + broccoli groups. Experimental animals were exposed to incense smoke for 1 hour/day for 28 days and broccoli extract was given by oral administration at the dose of 300 mg/kg body wt. A significant rise in serum aspartate aminotransferase (AST) and alkaline phosphatase (ALP) was seen in incense smoke exposed groups as compared with control and the total protein and albumin levels were significantly decreased, suggesting damages in tissues, which later was confirmed through the stress report. Histopathological examination of lung tissues showed severe pulmonary inflammation, congestion, thickening of alveolar lining, and reduction in the spaces of alveolar air in incense smoke group compared with restorative amelioration near normal with Pulmonary architecture in broccoli often choking the incidence. The study concludes that chronic exposure to incense smoke produces oxidative stress, inflammation and pulmonary toxicity in albino rats and broccoli supplementation results in considerable decline of biochemical and histological damage due antioxidant and antiinflammatory role of broccoli in diet

Introduction

The pollution of the environment is highly dangerous particularity for human health and animal life. Of all the types of pollution, indoor pollution has been observed to be the most dangerous since man spends a good deal of time inside houses, offices, and places of worship. Indoor pollutants have different origins, including tobacco smoke, cooking fuels, pesticides, cleaning agents, incense, and volatile organic compounds. Incense burning is common in eastern countries like India and, accustomed to continuous exposure to incense smoke, people there might suffer bad effects (Lin *et al*., 2008). Incense sticks are prepared using sticks of bamboo, powder of charcoal, odorous materials, sticky compounds, and powder of some herbs. These types of sticks when burnt give out an intricate combination of particulate matter (PM2.5 and PM10), Carbon monoxide, Sulphur di oxide, Nitrogen oxides, Benzene, Formaldehyde, Volatile Organic Compounds, and Polycyclic aromatic hydrocarbons, which is a cocktail of deleterious chemicals which the body finds easy to absorb deep into the system, to the lungs and tissues of the alveoli, resulting pulmonary inflammation. Increased oxidative stress and cell injury has been linked to incense exposure (Friborg *et al*., 2008). Because of gaseous exchange and supply of oxygen to the other tissues, the lungs are very delicate.Pulmonary tissues owe their endangered status to their direct contact with air. Direct inhalation of incense smoke can lead to bronchial epithelium irritation and inflammation, destruction of alveolar architecture and spirit of respiration. Lung *et al*., 2003 test showed that incense smoke leads to particulate ultrafine size granular atoms equivalent to that of tobacco smoke that further leads to oxidative and respiratory toxicity (Hussain *et al*,2014). Abnormal psyche changes were exhibited and emphasizing pulmonary pathology examination to the direct inhalation of incense smoke. Alavi *et al*., 2010 report on albino rats revealed degeneration of lungs and pleuritis, and showed that continued inhalation may lead to degeneration (exhibits histotoxic impact) and apoptosis (Hussain *et al*., 2014). The hyperstimulation of cytosolic triggering oxidin via inhalation of smoke has generated interest in incense smoke. Yamamoto *et al* (2021) studied that incense smoke inhalation leads to the disturbance of physical integrity of bronchial epithelium and asthmatic hyperresponsiveness, mediated by oxidative stress. raj *et al* (2021) reported elevation of AST, ALT, AP and also changes in the lipographic profile along with a neutrophilia increase after periodic exposure to incense smoke. Raj *et al* reported that this change shows serum physiopathological changes and also almost restores values to normal when offered natural honey. This corroborates the lungs may not solely be affected. Hussain etal (2014) pointed out that necrosis of the membranes starts within the first hour from inhalation and leads toward erosion/degeneration. Incense herbsmoke is slow to destroy as result of having insignificant penetration due to the cotad/mbereotia where the vapour of smoke travels on lung surfaces. The present study is aimed at investigating the toxicological impact of incense smoke exposure on pulmonary function in albino rats and modulate its activity by feeding them broccoli during incense smoke exposure.

Materials and Methods

Experimental Animals:-Healthy adult albino rats (*Rattus norvegicus*) weighing 150–200 g were used for the study. Animals were housed in wire cages under standard laboratory conditions at 21 ± 0.5°C with 55 ± 0.5% relative humidity and a 12-hour light/dark cycle. Rats were acclimatized for two weeks prior to experimentation.

Experimental Design- Animals were divided into three groups with six rats (n=6) in each group:

1.Control Group: Normal diet without exposure.

2.Incense Smoke Group: Exposed to incense smoke for 1 hour/day.

3.Incense Smoke + Broccoli Group: Exposed to incense smoke and supplemented with broccoli extract.

Exposure Procedure-Commercially available "Zed Black" incense sticks and cones were used. Experimental rats were exposed to incense smoke in a separate chamber for 1 hour daily for 28 days.

Broccoli Supplementation

Fresh broccoli extract was prepared daily and administered orally at a dose of 300 mg/kg body weight.

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 by the Bromocresol Green (BCG) dye-binding method

Histopathological Studies-Lung tissues were dissected, fixed in 10% formalin, processed by standard paraffin embedding techniques, sectioned at 5 µm thickness, and stained with hematoxylin and eosin for microscopic examination.

Statistical Analysis-Data were expressed as Mean ± SE. Statistical significance was determined using one-way ANOVA followed by Student's t-test. A p-value < 0.05 was considered significant.

Results

Prolonged exposure to incense smoke and treatment with broccoli produced notable changes in the blood levels of liver enzymes of the female albino rats. This has been represented in Tables 1 – 5. In comparison with the control of both treatment of incense smoke exposure visceral serum hepatic enzymes and proteins has been altered, however broccoli supplementation counteracts the effects returning the alterations in serum parameters of the two exposures being closer to normal. Serum ALT, AST and ALP levels also significantly increased on per day and reaching the peak on exposure to incense smoke on day 28. Increasing serum ALT, AST and ALP activities per day are also indicated. However taking those serum enzymes significantly reduced (P≤0.05) lower than the incense smoke exposed rat group. Total protein, albumin and globulin decrease significantly to lesser values were seen in rat groups treated with incense smoke with serum protein levels significantly (P≤0.05) improved upon supplementing with broccoli. The albumin/globulin ratio similarly dec, significantly lowered. Histological examination revealed normal lung architecture in the control group. Incense smoke exposure caused severe pulmonary damage including alveolar thickening, inflammatory infiltration, congestion, epithelial degeneration, and emphysematous changes. Broccoli supplementation markedly improved lung histology by reducing inflammation and restoring near-normal alveolar structure, indicating significant protective effects against incense smoke-induced pulmonary toxicity.

Table: 1 Liver Biochemical Analysis in Albino Rats Exposed to Incense Smoke and Broccoli Treatment

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Protein (g/dL)	Albumin (g/dL)
Control	35 ± 2	40 ± 3	82 ± 4	6.8 ± 0.3	4.1 ± 0.2
Incense Smoke	88 ± 5*	102 ± 6*	165 ± 8*	4.2 ± 0.2*	2.5 ± 0.1*
Incense Smoke + Broccoli	54 ± 4#	66 ± 5#	118 ± 6#	5.9 ± 0.2#	3.5 ± 0.2#

Values are Mean ± SE (n = 6).

* Significant difference compared to control group ($p < 0.05$).

significant recovery compared to incense smoke group ($p < 0.05$).

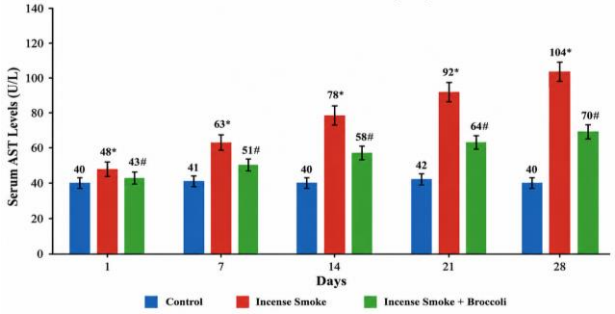


Fig: 1 Broccoli supplementation reduced incense smoke-induced elevation of serum AST levels in albino rats

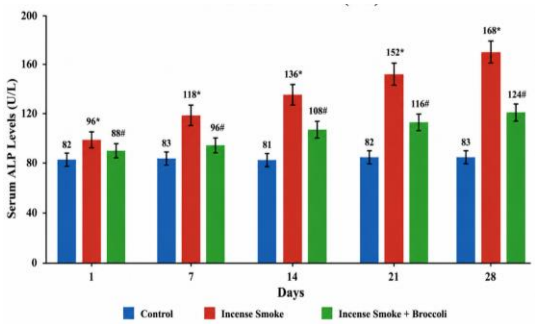


Fig: 2 Broccoli supplementation reduced incense smoke-induced elevation of serum ALP levels in albino rats.

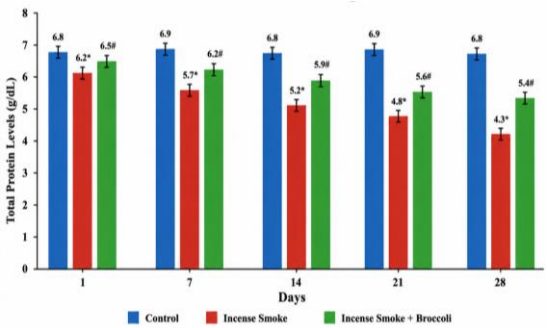


Fig:3 Broccoli supplementation improved reduced serum total protein levels in incense smoke-exposed rats.

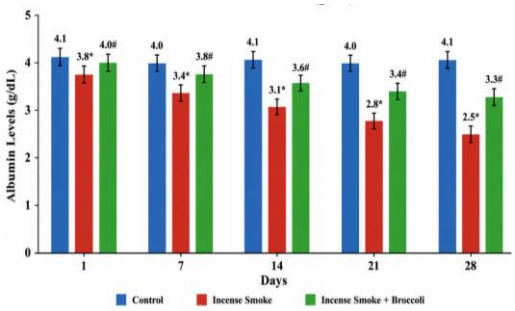
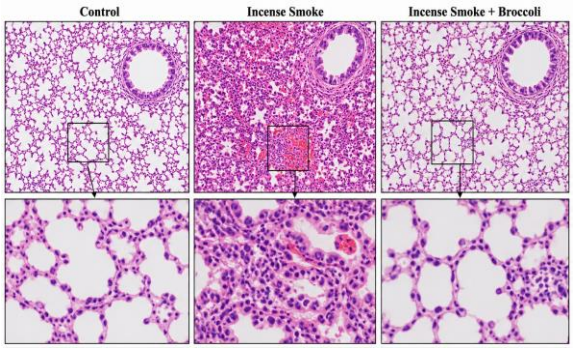


Fig:4 Broccoli supplementation improved reduced serum albumin levels in incense smoke-exposed albino rats.



H&E staining, Magnification: Top panal 100X, Bottom panel 400X

Fig: 5 Comparative lung histology showing incense smoke-induced damage and ameliorative effects of broccoli supplementation in albino rats.

Discussion

The findings of the present investigation clearly indicate that continuous exposure to incense smoke causes significant toxic effects on pulmonary tissues and alters important biochemical parameters in albino rats. The study also demonstrated that broccoli supplementation provided substantial protection against incense smoke-induced toxicity. Elevated serum AST and ALP activities along with reduced total protein and albumin levels observed in incense smoke-exposed rats suggest the development of systemic oxidative stress and tissue injury. Incense smoke is composed of harmful pollutants such as particulate matter, volatile organic compounds, carbon monoxide, formaldehyde, and polycyclic aromatic hydrocarbons. During combustion, these toxic substances are released into the surrounding atmosphere and enter the respiratory system through inhalation. Fine particulate matter generated from incense smoke can penetrate deeply into alveolar tissues, resulting in inflammatory reactions and oxidative damage (Lin *et al*., 2008). In the present study, progressive elevation in AST and ALP activities was recorded with increasing duration of smoke exposure. Such increases generally indicate cellular membrane damage and leakage of intracellular enzymes into circulation due to tissue injury. The decline in total protein and albumin levels in the incense smoke group may be associated with impaired protein synthesis and metabolic disturbances induced by toxic smoke constituents. Albumin is an important plasma protein synthesized by the liver, and reduction in its concentration reflects altered physiological and metabolic activities. Similar reductions in serum protein profile following exposure to toxic pollutants have been reported in earlier experimental studies (Hussain *et al*., 2014). Histopathological examination of lung tissues further supported the biochemical findings. The control group exhibited normal pulmonary architecture with intact alveoli and clear air spaces. In contrast, the incense smoke-exposed group showed severe pathological alterations including thickening of alveolar septa, inflammatory cell infiltration, congestion, epithelial degeneration, and partial destruction of alveolar spaces. These changes indicate pulmonary inflammation and impaired respiratory function resulting from chronic smoke inhalation. Similar histological alterations have previously been reported in animals exposed to incense smoke and other airborne pollutants (Alarifi *et al*., 2004). The pulmonary toxicity observed in the present study may primarily be due to oxidative stress caused by excessive production of reactive oxygen species. Oxidative stress damages lipids, proteins, and nucleic acids, ultimately resulting in cellular degeneration and inflammatory responses. Previous researchers have demonstrated that incense smoke exposure induces oxidative injury and inflammatory responses in pulmonary tissues through ROS-mediated mechanisms (Yamamoto *et al*., 2021). Broccoli supplementation significantly reduced the severity of incense smoke-induced toxicity. Animals treated with broccoli showed improvement in serum biochemical parameters and restoration of near-normal lung architecture. Reduced inflammatory infiltration and improved alveolar structure in broccoli-treated rats indicate the protective role of broccoli against pulmonary damage. These beneficial effects may be attributed to the antioxidant compounds present in broccoli, particularly sulforaphane, flavonoids, glucosinolates, and vitamins C and E. Sulforaphane is known to activate antioxidant defense pathways and enhance detoxification mechanisms within cells. It scavenges reactive oxygen species and protects tissues against oxidative injury. The antioxidant and anti-inflammatory properties of broccoli therefore help minimize tissue degeneration caused by incense smoke exposure. the present study suggests that chronic exposure to incense smoke can severely affect pulmonary health and biochemical functions in albino rats. However, dietary supplementation with broccoli may effectively reduce these harmful effects and provide protection against oxidative stress-mediated tissue injury.

Conclusion

The present study clearly demonstrates that chronic exposure to incense smoke has adverse effects on pulmonary tissues and biochemical parameters in albino rats. Continuous inhalation of incense smoke for 28 days caused marked alterations in serum liver enzymes and proteins as well as lung histology, indicating toxicity and oxidative stress. The statistically significant increase in serum AST and ALP levels implied cellular membranes damage and metabolic disturbances due to harmful effects of the smoke substances. The significant decrease in total protein, indicating impairment of physiological and biochemical status was also revealed by a decrease in albumin level. Histopathological examination of lung tissues confirmed the toxic impact of incense smoke exposure. Severe alterations included thickening of alveolar septa, inflammatory cell infiltration, congestion of blood vessels, degeneration of epithelial lining and destruction of air cell spaces. These structural changes indicate impaired respiratory function of the lungs and pulmonary inflammation due to the occurrence of indoor air pollutants produced during the burning of incense sticks, as is common practice in many countries. Finally, the role of antioxidant-rich “broccoli” (*Brassica oleracea*) offers protection against incense smoke toxicity. The study reveals that, the rats exposed to incense smoke for 28 days, prophylactically treated with broccoli, showed significant improvement in biochemical parameters and restoration of near normal pulmonary architecture. The potent anti-inflamatory action of broccoli may have enabled reduction of inflammatory responses, minimized tissue degeneration thereby improving serum protein levels. The protective effect of broccoli could be attributed to presence of radical neutralizing antioxidant compound of sulforaphane, flavonoids, glucosinolates and vitamins.

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