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Ameliorative effect of sesame lignans on nicotine toxicity in rats

Krishna Chattopadhyay [a,*], Srinath Mondal [b], Brajadulal Chattopadhyay [b], Santinath Ghosh [a]

[a] Department of Chemical Technology, University of Calcutta, 92 A.P.C. Road, Kolkata 700009, India
[b] Department of Physics, Jadavpur University, 188 Raja S.C. Mallick Road, Kolkata 700032, India

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ABSTRACT

Nicotine causes oxidative and genotoxic damages in the tissues leading to several diseases. Any strategy through natural diet that prevents or slows the progression and severity of nicotine toxicity has a significant health impact. This work is designed to investigate natural antioxidants that play effective protective role against nicotine-induced toxicity. Experiments were conducted on male albino rats by injecting nicotine tartrate (3.5 mg/kg body wt./day for 15 days) subcutaneously and thereby supplementing sesame lignans (0.1 g/100 g diet and 0.2 g/100 g diet) orally to them. Significant ($P < 0.01$) increase of total cholesterol, triglyceride, LDL-cholesterol, VLDL-cholesterol, decrease of HDL-cholesterol, decrease in antioxidant enzymes and increase in concentration of lipid peroxidative product has been observed in plasma due to nicotine toxicity. Significant ($P < 0.01$) decrease of total DNA contents and highly significant ($P < 0.001$) DNA damage of liver tissue is also observed on nicotine treatment. Sesame lignans minimizes the above mentioned effects. The nicotine-induced oxidative and genotoxic damages on the tissues can be effectively attenuated by sesame lignans supplemented diet.

1. Introduction

Nicotine, most acute acting pharmacological agent and major component of tobacco, plays a significant impact in the development of cardiovascular disorders (Bjartveit and Tverda, 2005), pulmonary disease and lung cancer (International Agency for Research on Cancer, 2004) and many other diseases (Jung et al., 2001). During the production of cigarette and chewing of tobacco, nicotine is at first being converted into two highly mutagenic nitrosamine, viz. N′-nitrosonornicotine (NNN) and 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK) and later metabolized into cotinine (Neogy et al., 2008). These derivatives also exhibit a wide spectrum of biological activity as compared to parent compound (Campain, 2004). Nicotine induces oxidative stress both *in vivo* and *in vitro* that causes a peroxidant/antioxidant imbalance in blood cells, blood plasma and tissues (Suleyman et al., 2002). The mechanisms of free radical generation by nicotine are still not very clear. It has been reported that nicotine disrupts the mitochondrial respiratory chain leading to an increased generation of superoxide anions and hydrogen peroxide (Kalpana and Menon, 2004). Oxidative stress occurs when there are excess free radicals and/or low antioxidant defense and results in chemical alteration of biomolecules causing structural and functional modification (Sreekala and Indira, 2009). Oxygen free radical (OFR) production has been directly linked to oxidation of cellular molecules, which may induce a variety of cellular responses through generation of secondary metabolic reactive species (Chiarugi, 2003). It is also established that metabolism of nicotine produces reactive intermediates capable of binding to proteins and DNA which increases the risk of hepatocellular carcinoma (Hukkanen et al., 2005). Nicotine also causes liver cell injury in human (El-Zayadi, 2006) and exerts genotoxic effect on rat liver (Bandyopadhyaya et al., 2008). Kleinsasser et al. (2005) have shown that nicotine expresses significant direct genotoxic effects in human target cells *in vitro*.

Sesame (*Sesamum indicum* L.) is one of the most important oil seed crops cultivated in Asia. India is the largest producer of sesame with approximately 27% of the total production in the world (Dhar et al., 2007). Different forms of sesame are very commonly used in our country. The superior oxidative stability of sesame oil is due to sesamol, which is present in a very small amount in the natural oil (Ide et al., 2001). Sesame lignans (sesamin and episesamin) are compounds commonly found in refined sesame oil. Episesamin, one of the important components of sesame lignans, is generated from an equivalent amount of sesamin by isomerization during the acid clay bleaching of oil (Fukuda et al., 1986).

Abbreviations: CAT, catalase; Gr., group; HDL-C, high density lipoprotein cholesterol; HPLC, high performance liquid chromatography; LDL-C, low density lipoprotein cholesterol; LPO, lipid peroxidation; MDA, malondialdehyde; OFR, oxygen free radical; ROS, reactive oxygen species; SOD, superoxide dismutase; SSB, single strand break; TBARS, thiobarbituric acid reactive substances; TC, total cholesterol; UV, ultra violet; VLDL-C, very low density lipoprotein cholesterol.

* Corresponding author. Tel.: +91 9433122559; fax: +91 03323519755.
E-mail address: kris_ami@yahoo.co.in (K. Chattopadhyay).

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Sesame lignans such as sesamin, episesamin, sesamolin have been implicated as having anti-tumorigenic (Hirose et al., 1992), estrogenic and/or anti-estrogenic (Collins et al., 1997; Shittu et al., 2006) and antioxidant (Shittu et al., 2007) properties. Moreover, the ROS scavenging moiety of sesame lignans may contribute important components which prevent body cells from the free radical injury (Jeng and Hou, 2005). Sesame lignans also affect lipid metabolism, inhibit cholesterol absorption from the intestine, and reduce 3-hydroxy-3-methyl-glutaryl CoA reductase activity in liver microsomes (Hirose et al., 1991), thus reducing the cholesterol biosynthesis. Ide et al. (2009) have shown that sesame lignans (sesamin, episesamin and sesamolin) affecting gene expression profile and fatty acid oxidation in rat liver. Among the sunflower, groundnut and sesame oils, sesame oil offers better protection over blood pressure, lipid profiles and lipid peroxidation and increases enzymatic and nonenzymatic antioxidants (Sankar et al., 2005). Shittu et al. (2007) have reported that sesame improves the storage capacity for the spermatozoa in the epididymis in a dose related manner.

Elucidation of the complex effect of nicotine has worldwide implications. Though effect of nicotine has been extensively investigated, large variations caused by several critical host factors still makes opening of different avenues of research on nicotine toxicity. Nutritional status alters the actions, potencies and detoxification of toxicants. Most studies did not take into account the association between diet, nicotine and DNA damage. Therefore the present studies were aimed to elucidate the impact of high and low doses of sesame lignans (natural antioxidant) on nicotine-induced oxidative damage and genotoxicity on the liver tissue of experimental rats.

2. Materials and methods

2.1. Extraction and purification of sesame lignans

Sesame lignans were prepared from refined sesame oil supplied by Vinayak Oils and Fats Ltd., Kolkata, India and purified by the method of Fukuda et al. (1986). Composition of sesame lignans was analyzed by HPLC in our laboratory by our standardized method (Dhar et al., 2007). The instrument was provided with binary HPLC pump1525 and UV detector 2487. The column was Novapac bonded C18 (size: 4.6 × 150 mm) having microparticulate silica of particle size 5 μm. A total of 20 μl of the sample solution was injected. The mobile phase was methanol/water (65:35 v/v) at a flow rate of 0.8 ml/min. The ultraviolet detection wavelength for sesamine and episesamine was 290 nm. Sesame lignans were 97.4% pure as observed in the HPLC chromatogram.

2.2. Animal experiment

The animal experiment was performed under the supervision of animal ethical committee of the Department of Chemical Technology, University of Calcutta, India. Male albino rats of Charles Foster strain (100–120 g body wt.) were housed in individual cages and fed the dietary oils and fresh water *ad libitum*. After maintaining the animals for 4 weeks, they were divided into four groups (Gr-A, Gr-B, Gr-C and Gr-D), having 10 rats in each group. Daily food consumption and weekly body weight gain was recorded. After determination of the effective dose (3.5 mg/kg body wt.), nicotine tartrate (Sigma Chemical Company, St. Louis, MO, USA) dissolved in 0.9% saline (w/v) was injected subcutaneously at a dose of 3.5 mg/kg body wt./day for 15 days to the experimental groups (Gr-B–D). Control group (Gr-A) was injected normal saline in a similar manner at the same time.

Basal diet was prepared according to the American Institution of Nutrition. The rats were fed experimental diet composed of fat free casein 18%, fat (experimental fat blends) 20%, starch 55%, salt mixture 4% (composition of salt mixture No. 12 in g: NaCl – 292.5, KH_2PO_4 – 816.6, $MgSO_4$ – 120.3, $CaCO_3$ – 800.8, $FeSO_4{\cdot}7H_2O$ – 56.6, KCl – 1.66, $MnSO_4{\cdot}2H_2O$ – 9.35, $ZnCl_2$ – 0.5452, $CuSO_4{\cdot}5H_2O$ – 0.9988 and $CoCl_2{\cdot}6H_2O$ – 0.0476), cellulose 3% and one multivitamin capsule per kg of diet. The diet was adequate in all nutrients. Two different doses of sesame lignans (sunflower oil:sesame lignans: 99.5:0.5 w/w and 99.0:1.0 w/w) were supplemented simultaneously in two experimental groups. The selection of sesame lignans doses were according to the previous studied as described by Dhar et al. (2007) where it has been observed that sesame lignans when added to the food on those particular doses, showed antioxidant properties.

The dietary fat blends were as follows:

Group-A: Sunflower oil (20% fat) diet (animals in this group referred as control and did not receive nicotine treatment).
Group-B: Sunflower oil (20% fat) diet (animals in this group referred as experimental and received nicotine treatment).
Group-C: Sunflower oil (20% fat):sesame lignans (99.5:0.5 w/w) (animals in this group referred as experimental and received nicotine treatment).
Group-D: Sunflower oil (20% fat):sesame lignans (99.0:1.0 w/w) (animals in this group referred as experimental and received nicotine treatment).

Rats were sacrificed under mild anesthesia, blood was collected and liver tissue was immediately excised, blotted, weighed and stored at deep-freeze temperature (−40 °C) for analysis.

2.3. Biochemical analysis

The lipid components such as TC (Allain et al., 1974), HDL-C (Warnick et al., 1985) and triglyceride (Werner et al., 1981) were estimated in plasma by using standard kits supplied by Ranbaxy Diagnostic Ltd., Mumbai, India. VLDL-C and LDL-C were calculated from the values of triglyceride, TC and HDL-C by Friedwald and Fredicksons formula (Friedewald et al., 1972). Plasma and liver lipid peroxidation was measured by the assay of thiobarbituric acid reactive substances (TBARS) according to the standard method (Chatterjee and Agarwal, 1998). The amount of MDA was calculated by taking the extinction coefficient of MDA to be $1.56 \times 10^5\ M^{-1}\ cm^{-1}$. Superoxide dismutase (SOD) and catalase (CAT) of liver were determined in rats of all the four groups (Beauchamp and Fridovich, 1971; Cohen et al., 1970). Protein concentration of the tissue was determined by Lowry et al. (1951). The entire biochemical assays were repeated at least three times.

2.4. DNA damage study

DNA was isolated from frozen liver tissues by slightly modifying the procedure adopted by Gupta (1984). About 50 mg of frozen rat liver tissues was thawed in 10 ml of 1% SDS containing 1 mM EDTA. The tissue was then homogenized at 4000*g* for 2 min. This cycle was repeated thrice and the homogenate was incubated at 38 °C for 30 min with proteinase K (500 μg/ml). Next 0.5 ml of 1 M Tris–HCl (pH 7.4) was added to the homogenate and extracted by centrifugation (4000*g* at 4 °C) successively with one volume each of phenol (5 min), 1:1 mixture of phenol/Sevag (chloroform/isoamyl alcohol, 24:1) (3 min), and Sevag (3 min). The phases were separated by centrifugation (14,000*g* at 4 °C, 20 min). To the homogenate, 0.1 volume of 5 M NaCl and one volume of absolute ethanol was added and kept at −20 °C for 30 min. DNA thus precipitated was recovered by centrifugation at 14,000*g*, 4 °C for 15 min. DNA precipitate was rinsed carefully in 70% ethanol twice to remove salt and then dissolved in 0.5 ml TE (pH 8.0). RNA was removed by incubation at 38 °C for 30 min with RNAse A (100 μg/ml) in 50 mM Tris–HCl (pH 4). After the extraction of this solution with Sevag, DNA was recovered from the aqueous phase as described above, and finally dissolved in 0.2 ml of TE (pH 8), and its concentration as well as absorbencies at A_{230}, A_{260} and A_{280} were estimated spectrophotometrically. The total DNA content thus obtained was shown in the table as the mean of all observations in respective subgroup along with SD.

2.5. Comet assay

The procedure for Comet assay was followed as described by Bandyopadhyaya et al. (2008). Preparation of two gel layers only instead of three layers performed the assay. The liver tissues (50 mg) were minced, suspended at 1 ml/g in chilled homogenizing buffer (0.075 M NaCl and 0.024 M EDTA) and gently homogenized at 600 g for 2 min. To obtain nuclei, the homogenate was centrifuged at 1000*g* for 10 min at 0 °C, and the precipitate was resuspended in 1 ml chilled homogenizing buffer. About 100 μL of 2% regular melting point agarose (Genei, India) was quickly layered on a pre-cooled fully frosted slide and covered with a cover slip and allowed to solidify. The nuclear preparation was mixed 1:1 (v/v) with 2% low melting point agarose (Genei, India). The coverslip was removed carefully and a second layer of 100 μL of the mixture was pipette out on the slide, covered with the cover slip again and allowed to gel at 4 °C for 15 min. The slide (without cover slip) was immerged in a chilled lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris–HCl, 1% Sarkosyl, 10% DMSO and 1% Triton X-100, at pH 10) and kept at 4 °C for 2 h. The slides were then placed on a horizontal gel electrophoresis platform and covered with chilled alkaline solution made up of 300 mM NaOH and 1 mm EDTA (pH 12.5). The slides were left in the solution in dark for 15 min and then electrophoresed at 4 °C in the dark for 15 min at 1 V/cm and approximately 250 mA. The slides were gently rinsed in neutralization buffer (0.4 M Tris–HCl, pH 7.5). Each slide was stained with 50 μL of 20 μg/ml ethidium bromide and covered with a cover slip. The photomicrograph of each slide was taken in Leica Fluorescent Microscope at the same magnification (40×).

Measurement of the comet head diameter, tail length, tail moment and percentage of DNA damages were followed the procedure as described by Helma and Uhl (2000). A total of 50 cells were screened per animal and examined in a fluorescence microscope (Leica 300-FX with 40× magnification). Since broken DNA fragment stream further from the nucleus than intact DNA, the difference between length