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Ameliorative Role of Dietary Supplemented Conjugated Linolenic Acid against Nicotine-Induced Toxicity in Rats

Krishna Chattopadhyay[1], Moumita Maity[2], Satyam Banerjee[2] and Brajadulal Chattopadhyay[2]*

[1]*Department of Chemical Technology, University of Calcutta, 92 A.P.C. Road, Kolkata 700009, India*
[2] *Department of Physics, Jadavpur University, 188 Raja S.C. Mallik Road, Kolkata 700032, India*

Abstract

Increasing consumption of tobacco in different forms harms almost every organs of our body. Nicotine is the culprit for various physiological repercussions arouse due to the uptake of tobacco. As nutritional status alters the actions, potencies and detoxification of toxicants, the present study was undertaken to evaluate the natural antioxidant efficacy of conjugated linolenic acid present in Karalla seed against nicotine-induced toxicity. Experiments were conducted on male albino rats (120–130 g body weight) by injecting nicotine tartarate (3.5 mg/kg body wt. /day for 15 days) subcutaneously and thereby simultaneously supplementing conjugated linolenic acid (0.5 and 1.0%) to their diets. Nicotine significantly altered serum and liver lipid profiles, lipid peroxidation and activities of antioxidant enzymes. It caused significant decrease of DNA contents ($P<0.01$) and DNA damage ($P<0.001$) of liver tissue. Conjugated linolenic acid has the ability to bind with DNA and protein similar to nicotine and thereby ameliorates nicotine-induced toxicity in rats. Thus intake of Karalla that contains conjugated linolenic in its seeds, in our daily diet can effectively attenuate nicotine-induced cellular and genetic damages.

K : Conjugated linolenic acid; DNA damage; Docking; Nicotine; Oxidative stress

A : α –ESA: α-Eleostearic Acid; CAT: Catalase; CLA: Conjugated Linoleic Acid; CLnA: Conjugated Linolenic Acid; GPx: Glutathione Peroxidase; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol; LPO: Lipid Peroxidation; MDA: Malonaldehyde; OS: Oxidative Stress; PDB: Protein Data Bank; GSH: Reduced Glutathione; ROS: Reactive Oxygen Species; SD: Standard Deviation; SSB: Single-Strand Breaks; SOD: Superoxide Dismutase; TBARS: iobarbituric Acid Reactive Substances; TC: Total Cholesterol; VLDL-C: Very Low Density Lipoprotein Cholesterol; HMG CoA reductase: Hydroxy-methyl-glutaryl-CoA Reductase; BSA: Bovine Serum Albumin

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Increasing uses of tobacco products is an alarming danger for health worldwide [1]. Nicotine the culprit component of tobacco causes oxidative damage in the tissues and nucleic acids leading to several diseases. Free radical-induced oxidative damage has been suggested to play a major role in the pathogenesis of smoke-related disorders [2]. During the smoking of cigarette and/or chewing of tobacco, nicotine is at rst being converted into highly mutagenic nitrosamine and later metabolized into cotinine [3]. Experiments have shown that chronic administration of nicotine causes increased lipid peroxidation products in serum and various tissues of rats, which is also dose dependent [4]. e increase of lipid peroxidative products is associated with decreased activity of endogenous antioxidants, catalase and superoxide dismutase [5]. 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 [6]. Kleinsasser et al. [7] have shown that nicotine expresses signi cant direct genotoxic e ects in human target cells *i i* . Nicotine also exerts genotoxic e ect on hepatic cells [8] and blood cells [9] of rat.

People of all over India and Asia o en eat bitter gourds (*M dica cha a ia*). e seed oil of such gourds contains 60% (wt:wt) α-eleostearic acid (α–ESA) and the esh contains a small amount of α-ESA. We are particularly interested in seed oils that contain conjugated linolenic acids (CLnA), which are the only conjugated fatty acids that can be prepared from natural sources in bulk. It has been reported that conjugated linolenic acid has a better anti-tumor e ect than conjugated linoleic acid (CLA) [10]. Koba et al. [11] have observed the e ect of dietary conjugated linolenic acid on body fat; serum and liver lipid levels with that of conjugated linoleic acid in rats. eoretically α-eleostearic acid consists of 33% cis and 66% trans molecular composition. is conjugated fatty acid is now regarded as natural antioxidant for its oxygen scavenging property [12].

e toxicity of nicotine is the subject of intense scienti c scrutiny. e potential damage caused by free radicals is normally minimized by a combination of biological antioxidant systems including enzymatic and non-enzymatic reactions [13]. Nutritional antioxidants that work against oxidative stress related diseases are always desirable and have gained immense interest recently. Naturally occurring therapeutic agents have always added advantage, as it has no negative side e ects on our health. Any strategy through natural diet that prevents or slows the progression and severity of nicotine toxicity has a signi cant health impact. As per knowledge is concerned, hardly reports are found that explore the ameliorative e ect of conjugated linolenic acid against nicotine-induced toxicity. is study illustrates that conjugated linolenic acid present in karela seed have potential antioxidative and antigenotoxic role against nicotine-induced toxicity. is naturally found ameliorative agent might have an extra edge over other available nicotinic therapeutic agents on health through daily dietary intake.

***Corresponding author:** Brajadulal Chattopadhyay, Department of Physics, Jadavpur University, 188 Raja S.C. Mallik Road, Kolkata 700032, India, Tel: +91-9433343917; Fax: +91-33-24138917; E-Mail: bdc_physics@yahoo.co.in

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Human serum albumin (PDB ID:1R4I), nicotine and comjugated linolenic acid were fed in these so wares for the experiments. Blast results indicated 73% homology between human serum albumin and *Ra egic* serum albumin protein sequences, thus human serum albumin was selected to monitor docking phenomena.

10) and kept at 4°C for 2 h. e 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). e slides were le 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. e slides were gently rinsed in neutralization bu er (0.4 M Tris–HCl, pH 7.5). Each slide was stained with 50 µL of 20 µg/ml ethidium bromides and covered with a cover slip. e photomicrograph of each slide was taken in Leica Fluorescent Microscope at the same magni cation (40x).

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 [27]. A total of 50 cells were screened per animal and examined in a uorescence microscope (Leica 300-FX with 40x magni cation). Quanti cation comet tail length (arbitrary unit) was as follows:

Comet tail length = (maximum total length)−(head diameter).

Quanti cation of DNA damage for each cell was determined by Image J so ware as:

Total DNA in comet=(Total comet area) x (mean DNA intensity)

Total DNA in comet head=(Total head area) x (mean DNA intensity)

% DNA damage=(Total DNA in comet) - (Total DNA in comet head) x 100

(Total DNA in comet)

Tail moment (arbitrary unit)=(% of DNA damage) x (tail length).

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Docking experiments were performed by using docking interactive following so ware: Hex 5.1 (http://so ware.informer.com/getfree-hex-5.1-so ware) and Chimera (http:// www.cgl. ucsf. edu/chimera/download.html) to monitor the interactions between nicotine and DNA, nicotine and conjugated linolenic acid, nicotine and albumin, conjugated linolenic acid and DNA, and conjugated linolenic acid and albumin. Protein data bank (PDB) les of p53 consensus sequence (GGGCATGCCTAGGCATGCC) of human DNA (PDB ID: 3KMD),

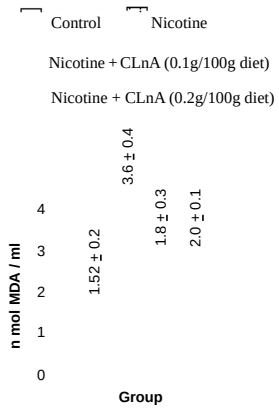

Figure 4: Liver lipid peroxidation of rat treated with nicotine and nicotine + conjugated linolenic acid (CLnA)
Values (nano mole melonaldehyde/ mg of tissue lipid) are shown as mean ± SD. *, Indicates P<0.01 (signifcant) and **, indicates P<0.001 (more signifcant) when compared with control. !, Indicates P<0.01 (signifcant) and !!, indicates P<0.001 (more signifcant) when compared with nicotine treated.

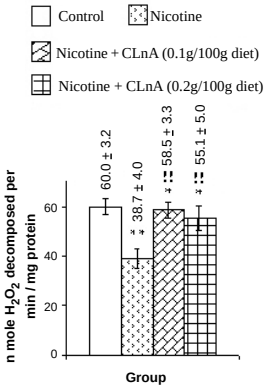

Figure 5: Liver catalase of rat treated with nicotine and nicotine + conjugated linolenic acid (CLnA) Values (nano mole hydrogen peroxide decomposed/ per minute/ mg protein) are shown as mean ± SD. *, Indicates P<0.01 (signifcant) and **, indicates P<0.001 (more signifcant) when compared with control. !, Indicates P<0.01 (signifcant) and !!, indicates P<0.001 (more signifcant) when compared with nicotine treated.

cholesterol, triglyceride (Figure 1) and VLDL-cholesterol (Figure 2). More signi cant (P<0.001) increase of LDL-cholesterol and signi cant reduction of HDL-cholesterol in plasma were also noted in nicotine treated rats (Figure 2). Supplementation of conjugated linolenic acid signi cantly (P<0.01) antagonized nicotine-induced toxic e ects (Figures 1 and 2). Concentration of lipid peroxidative product both in plasma (Figure 3) and liver (Figure 4) were increased more signi cantly by nicotine. It was noted that conjugated linolenic acid supplementation signi cantly ameliorated the nicotine-induced oxidative damage in rats and the e ect was more prominent when used at lower concentration of CLnA (sun ower oil: conjugated linolenic acid: 99.5:0.5 w/w) in the diet. More signi cantly (P<0.001) decreased activities of CAT (Figure 5), SOD (Figure 6) and GPx (Figure 7) were observed in liver of nicotine-treated rats as compared to control (untreated) rats. Dietary supplementation of conjugated linolenic acid showed increased activity of scavenging enzymes in liver (Figures 5-7). Reduced glutathione content in liver was also decreased due to nicotine treatment, which was attenuated by CLnA supplementation (Figure 8).

Figure 9 summarizes the observed values of total DNA contents of nicotine-induced liver tissue of rats. All these data were averaged over 20 animals. e total DNA contents in liver of rats were estimated as 1.82 ± 0.07 mg/g tissues. Nicotine treatment decreased 37% total DNA contents in rats liver tissues compared to the control, which was very much signi cant (P<0.001) as seen from gure 9. Supplementation of conjugated linolenic acid (0.1 g CLnA/100 g diet which is equivalent to sun ower oil: conjugated linolenic acid: 99.5:0.5 w/w) showed its antagonistic e ect on nicotine-induced reduction of total DNA content of liver tissue because the total DNA content was decreased only 9% in that case compared to its control. is implied that the total DNA content was increased more signi cantly (P<0.001) due to conjugated linolenic acid supplementation compared to nicotine treatment. Photomicrographs of the Comet assay of rat liver tissue DNA are shown in gure 10. Comet like pictures of hepatic DNA appeared due to nicotine treatment were normalizes to some extent on CLnA supplementation. Figure 11 shows the extent of the rat liver tissue DNA damage and gure 12 shows the comet tail moment due to nicotine and nicotine with conjugated linolenic acid supplementation. Nicotine signi cantly increased the percentage of DNA damage (50.6%) in liver tissues of rats compared to its control (7.2%). In comparison with the DNA damage caused by nicotine, conjugated linolenic acid (0.1 g CLnA

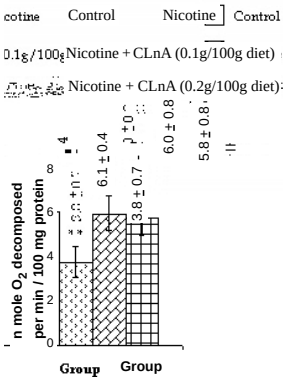

Figure 6: Liver superoxide dismutase of rat treated with nicotine and nicotine + conjugated linolenic acid (CLnA) Values are shown as mean ± SD where n mol O_2 means nano mole oxygen. *, Indicates P<0.01 (signifcant) and **, indicates P<0.001 (more signifcant) when compared with control. !, Indicates P<0.01 (signifcant) and !!, indicates P<0.001 (more signifcant) when compared with nicotine treated.

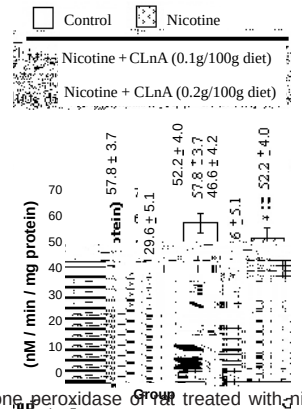

Figure 7: Glutathione peroxidase of rat treated with nicotine and nicotine + conjugated linolenic acid (CLnA). Values (mili mole/minute/mg of protein) are shown as mean ± SD. *, Indicates P<0.01 (signifcant) and **, indicates P<0.001 (more signifcant) when compared with control. !, Indicates P<0.01 (signifcant) and !!, indicates P<0.001 (more signifcant) when compared with nicotine treated.

/100 g diet) reduced the values to 31.8%. e increased value of tail moment (almost 24 times compared to control) by nicotine and the decreased value of tail moment (9.4 times for 0.1 g CLnA/100 g diet) by conjugated linolenic acid supplementation in nicotine-induced liver tissues were also con rmed the aggravated e ect of nicotine on DNA damage and ameliorative e ect of conjugated linolenic acid against nicotine treatment. Each data in this graph was the average of all observations from four animals in each group (for each animal 50 cells were screened).

DNA – nicotine docking indicated that nicotine could bind to the thymidine 6-consensus sequence of p53 gene (GGGCATGCCTAGGCATGCC) (PBD ID 3KMD) of human with a free energy change of – 163.74 kcal (Figure 13). CLnA showed its