

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

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Abstract

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 in 120430 (05 d) b 15 (05 d) b 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.

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

Abbreviations: -ESA: -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: Thiobarbituric 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

Introduction

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 first 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]. The 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 significant direct genotoxic effects in human target cells in vitro. Nicotine also exerts genotoxic effect on hepatic cells [8] and blood cells [9] of rat.

People of all over India and Asia often eat bitter gourds (*Momordica charantia*). The seed oil of such gourds contains 60% (wt:wt) -eleostearic acid (-ESA) and the flesh contains a small amount of -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 effect than conjugated linoleic acid (CLA) [10]. Koba et al. [11] have observed the effect of dietary conjugated linolenic acid on body fat; serum and liver lipid levels with that of conjugated linoleic acid in rats. Theoretically -eleostearic acid consists of 33% cis and 66% trans molecular composition. This conjugated fatty acid is now regarded as natural antioxidant for its oxygen scavenging property [12].

The toxicity of nicotine is the subject of intense scientific scrutiny. The 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 effects on our health. Any strategy through natural diet that prevents or slows the progression and severity of nicotine toxicity has a significant health impact. As per knowledge is concerned, hardly reports are found that explore the ameliorative effect of conjugated linolenic acid against nicotine-induced toxicity. This study illustrates that conjugated linolenic acid present in karela seed have potential antioxidative and antigenotoxic role against nicotine-induced toxicity. This naturally found ameliorative agent might have an extra edge over other available nicotinic therapeutic agents on health through daily dietary intake.

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

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 bromides and covered with a cover slip. The photomicrograph of each slide was taken in Leica Fluorescent Microscope at the same magnification (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 fluorescence microscope (Leica 300-FX with 40x magnification). Quantification comet tail length (arbitrary unit) was as follows:

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

Quantification of DNA damage for each cell was determined by ImageJ software as:

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

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

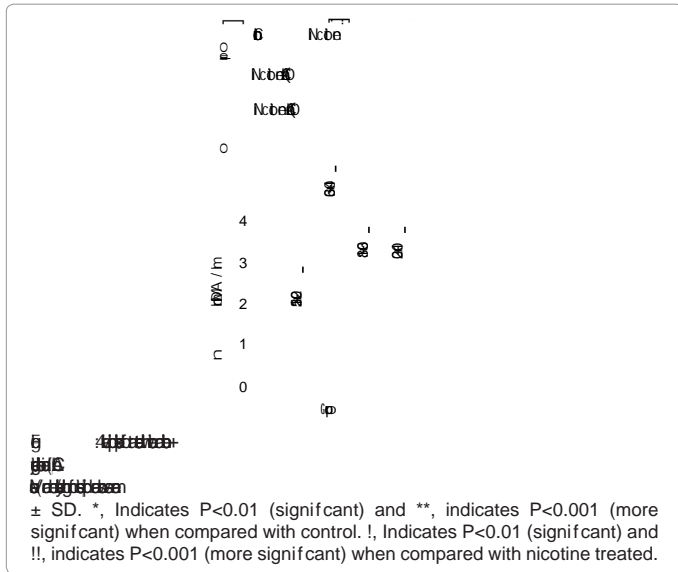
% DNA damage = (Total DNA in comet) - (Total DNA in comet head) x 100

(Total DNA in comet)

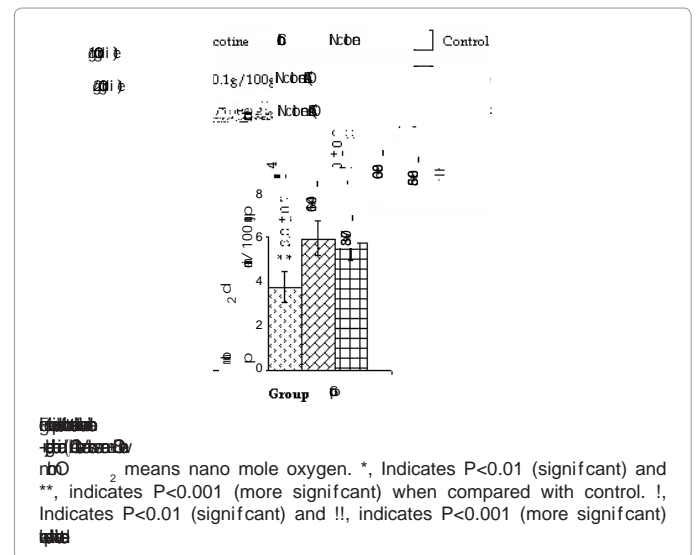
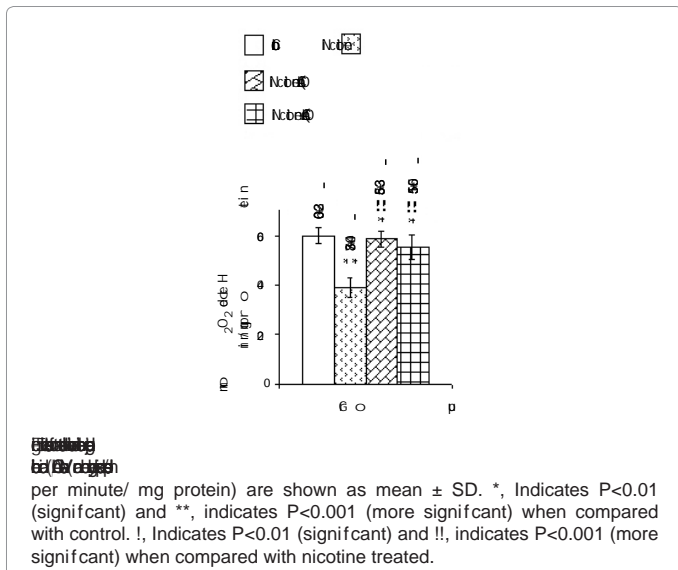
Tail moment (arbitrary unit) = (% of DNA damage) x (tail length).

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Docking experiments were performed by using docking interactive following software: Hex 5.1 (<http://software.informer.com/getfree-hex-5.1-software>) 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) files of p53 consensus sequence (GGGCATGCCTAGGCATGCC) of human DNA (PDB ID: 3KMD),



nicotine-induced liver tissue of rats. All these data were averaged over 20 animals. The total DNA contents in liver of rats were estimated as $1.82 \pm 0.07 \text{ mg/g}$ tissues. Nicotine treatment decreased 37% total DNA contents in rats liver tissues compared to the control, which was very much significant ($P < 0.001$) as seen from Figure 9. Supplementation of conjugated linolenic acid (0.1 g CLnA/100 g diet which is equivalent to sun flower oil: conjugated linolenic acid: 99.5:0.5 w/w) showed its antagonistic effect 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. It is implied that the total DNA content was increased more significantly ($P < 0.001$) due to conjugated linolenic acid supplementation compared to nicotine treatment. Photomicrographs of the Comet assay of rat liver tissue DNA are shown in Figure 10. Comet like pictures of hepatic DNA appeared due to nicotine treatment were normalized to some extent on CLnA supplementation. Figure 11 shows the extent of the rat liver tissue DNA damage and Figure 12 shows the comet tail moment due to nicotine and nicotine with conjugated linolenic acid supplementation. Nicotine significantly 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



cholesterol, triglyceride (Figure 1) and VLDL-cholesterol (Figure 2). More significant ($P < 0.001$) increase of LDL-cholesterol and significant reduction of HDL-cholesterol in plasma were also noted in nicotine treated rats (Figure 2). Supplementation of conjugated linolenic acid significantly ($P < 0.01$) antagonized nicotine-induced toxic effects (Figures 1 and 2). Concentration of lipid peroxidative product both in plasma (Figure 3) and liver (Figure 4) were increased more significantly by nicotine. It was noted that conjugated linolenic acid supplementation significantly ameliorated the nicotine-induced oxidative damage in rats and the effect was more prominent when used at lower concentration of CLnA (sun flower oil: conjugated linolenic acid: 99.5:0.5 w/w) in the diet. More significantly ($P < 0.001$) 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).

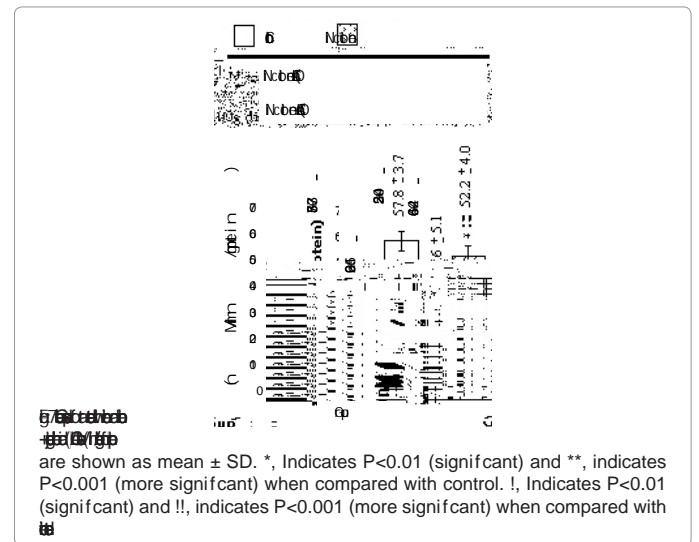


Figure 9 summarizes the observed values of total DNA contents of

/100 g diet) reduced the values to 31.8%. The 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 confirmed the aggravated effect of nicotine on DNA damage and ameliorative effect 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) (PDB ID 3KMD) of human with a free energy change of -163.74 kcal (Figure 13). CLnA showed its
