RESEARCH ARTICLE

Efficacy of nano-curcumin on nicotine-induced genotoxicity and immunomodulatory disruption in protein-malnourished female rats

Somashree Biswas, Krishna Chattopadhyay, Brajadulal Chattopadhyay *

ABSTRACT

Background: Aggravated nicotine-induced DNA damage and immunomodulatory disruption are more prominent in female populations under proteinrestricted conditions. Females seem to be more susceptible to nicotine-induced complications due to their low innate immunity. Though the anti-inflammatory and anti-genotoxic properties of curcumin are able to overcome effectively nicotine-induced complications, still its therapeutic use is hindered due to its poor aqueous solubility and low bioavailability. Nano-curcumin appears to be a better therapeutic agent because of its increased aqueous solubility and higher bioavailability than curcumin. *Methods:* This study investigated the effects of nicotine (2.5 mg/kg body weight injected subcutaneously for three consecutive weeks) on genotoxicity and immunomodulatory disruption in female rats maintained under a protein-restricted diet (5% casein). It also measured the ameliorative efficacy of nanocurcumin (4 mg/kg body weight supplemented orally after one hour of nicotine exposure) against nicotine. *Results:* It is observed that nicotine decreases hemoglobin and DNA contents and causes severe DNA damage in blood cells under protein-restricted conditions. It disrupts the immune system and affects the endocrine functions of the female rats maintained under protein- a restricted diet. Nano-curcumin ameliorates the nicotine-mediated genotoxic effects significantly, maintains the female sex hormonal level effectively, and restores the normalcy of immune responses in protein-restricted diet rats more efficiently than curcumin. Conclusion: Nano-curcumin looks like a potential blocker of nicotine due to its enhanced bioavailability and acts as a prospective therapeutic herbal agent to protect the health of the protein-malnourished female population.

Keywords: Genotoxicity; Nano-curcumin; Nicotine; Protein malnutrition.

Indian Journal of Physiology and Allied Sciences (2024); DOI: 10.55184/ijpas.v76i01.211 **ISSN:** 0367-8350 (Print)

Introduction

The tobacco epidemic is one of the biggest public health threats the world has ever faced. It kills more than 8 million people a year, including around 1.2 million deaths from exposure to second-hand smoke[1] N′-Nitrosonornicotine, a tobacco-specific nitrosamine, is one of the strong carcinogens and is accused of the addictive potential of smoking. It is mediated by neuronal nicotinic acetylcholine receptors in the central nervous system.[2] Nicotine promotes endothelial cell migration, proliferation, and nitric oxide (NO) production *in-vitro*, mimicking the effect of other angiogenic growth factors.[3, 4] It also stimulates growth factors, fibroblast proliferation, collagen release, and expression of myofibroblast markers.[5] Cigarette smoke causes airway epithelial cell damage[6] and global epigenetic changes, including DNA methylation and chromatin remodeling, which negatively impact genes involved in physiologic lung repair and regeneration.[7, 8] Smoking yields chemicals with carcinogenic potential, and the metabolism of nicotine produces reactive intermediates capable of binding to proteins and DNA, which increases the risk of hepatocellular carcinoma.[9] The genotoxic effect of nicotine and its reactive metabolites induce chemical modifications of DNA (DNA adducts) and play a key role in chemically induced carcinogenesis. DNA adducts can lead to mutations during cell division and may ultimately disrupt the regular functioning of the systems regulating normal cell growth.

Department of Physics, Jadavpur University, Kolkata, India

***Corresponding author:** Brajadulal Chattopadhyay, Department of Physics, Jadavpur University, Kolkata, India, Email: bdc_physics@yahoo.co.in

How to cite this article: Biswas S, Chattopadhyay K, Chattopadhyay B. Efficacy of nano-curcumin on nicotine-induced genotoxicity and immunomodulatory disruption in protein-malnourished female rats. *Indian J Physiol Allied Sci* 2024;76(1):25-31.

Conflict of interest: None

Submitted: 01/01/2024 **Accepted:** 23/02/2024 **Published:** 31/03/2024

Recently, nicotine has been shown to increase the frequency of micronuclei in human gingival fibroblasts,[10] and DNA strand breaks in human spermatozoa.[11]

At the cellular level, the induction of autoimmunity is a manifestation of an imbalance between pathogenic effectors versus protective regulatory responses.[12] Nicotine-induced production of pro-inflammatory cytokines (IL-1β; IFN-γ from Th1 cells and IL-4 and IL-5 from Th2 cells) trigger the signaling pathway, which plays an essential role in the cellular survival, death, and various pathological states.[13-16] IFN-γ has antitumor, antiviral, and immunomodulatory activities. It coordinates both innate and adaptive immune responses.[17] IL-4, a cytokine produced by activated Th2 lymphocytes, mast cells, and basophiles, is involved in many immunologic processes, such as Th2 differentiation, class switching, and B cell proliferation.[18] Cigarette smoke exerts

Cytotoxic and both pro-inflammatory and anti-inflammatory effects on nasal epithelial cells, leading to increased reactive oxygen species (ROS) production, Toll-like receptor (TLR) 4 expression, and lipopolysaccharide (LPS).[19, 20] Changes in the redox status within the cell initiate the activation of redox-sensitive transcription factors, such as nuclear factor-κB (NF-κβ) and activator protein-1 (AP-1). NF-κβ plays a significant role in gene expression of pro-inflammatory mediators (IL-6 and TNF-α), linked to cigarette smoke exposure and altered cytokine production.[21, 22] The imbalance between oxidants and antioxidants resulting from exposure to tobacco smoke leads to oxidative stress and increases mucosal inflammation and inflammatory cytokines (IL-6 and TNF-α) expression. The primary role of TNF-αlies in the regulation of immune cells. It is an 11-enigmatic cytokine controlling signaling pathways towards cell proliferation and death. Complex-I and complex-II are the two complexes formed by TNF-α binding with TNF-R.[23] Complex-II induces the pro-apoptotic protein BAX that releases Cytochrome-C and reactive oxygen species (ROS) from the mitochondria. Cytochrome-C causes apoptosis, where ROS induces necrosis of the cell.[24] BCL-2 is localized to the outer membrane of mitochondria, where it plays an important role in promoting cellular survival and inhibiting the actions of pro-apoptotic proteins.[25]

Nicotine also alters the endocrine function, perhaps at the level of the ovary, which in turn affects the release of female hormones. Smoking is an established modifiable risk factor for a number of serious complications in pregnancy and maternal-fetal health. These complications include preterm delivery, intrauterine growth restriction, placental abruption, and prenatal mortality, *etc*. The endocrine disruption likely contributes to the reported associations of smoking with adverse reproductive outcomes, including menstrual dysfunction, infertility, and earlier menopause.[26]

Protein Energy Malnutrition (PEM) continues to be a major public health problem in developing countries and affects mostly all age groups like infants, young children, pregnant and lactating mothers and poorer segments of the population. Low dietary protein imposes a constraint on biosynthetic activity, disposition, and toxicity.[27] Though it is already proven that in normal dietary conditions, nicotine causes various damage to our body, the toxic effects of nicotine, particularly in protein-restricted dietary situations, are still a cause for concern. The therapeutic use of curcumin, a natural yellow polyphenolic pigment isolated from the rhizomes of the plant *Curcuma longa L*. (turmeric), in diet can inhibit the antigen-mediated activation of mast cells, IgE production, and airway inflammation. It shows a wide spectrum of biological and pharmacological effects, such as anti-inflammatory, antioxidant, antimicrobial, anti-hepatotoxic, hypolipidemic, and anticancer properties. Curcumin also has immunomodulatory and anti-allergic activities. Poor aqueous solubility, low bioavailability, poor absorption, and rapid excretion from our body are the basic drawbacks of curcumin against its use in clinical practice. Curcumin, in the form of nanoparticles, is more active than curcumin because of its more aqueous solubility and increased bioavailability. Nano-particles of curcumin are therefore proposed as nano-drugs for oral supplementation to increase their bio-availability against nicotine-induced toxicities under protein-restricted conditions.

Materials And Methods

Nicotine hydrogen tartrate and curcumin were purchased from Sigma Aldrich Chemicals Company, St. Louis, USA. The preparation and characterization of nano-curcumin was done in our laboratory, which had already been discussed elaborately elsewhere.[28] Spectrochem Pvt. Ltd. India and Merck India supplied all others analytical grade chemicals. PUREGENE-made ELISA Kits were supplied by Genetix Biotech Asia Pvt. Ltd. and were used for the detection of cytokines, apoptotic proteins, and steroidogenic hormones.

Protein Restricted Diet and Animals

Female albino rats of Wistar strain (*Rattus norvegicus)* having 130-150 gm body weight were procured from the Animal housing facility of Jadavpur University and maintained one week by feeding with the standard pellet diet (Hindustan Liver Ltd., India) and water ad libitum. The animals were fed a protein-restricted diet during the nicotine exposure period, which was three consecutive weeks. The protein-restricted diet contains carbohydrates (83%), protein(5%), fat (7%), salt mixture (4%), and vitamin mixture (1%).[29] The rats were maintained with a natural lighting schedule, *i.e.*, 12 hours light and 12 hours dark cycles, by following strictly the guidelines of the Institutional Animal Ethics committee of the Jadavpur University, Kolkata, India (Ref. No.: AEC/PHARM/1502/14/2015, Dated: 30/07/2015). There were 30 rats were taken and divided equally into five groups of six rats each.

Group –C: Animals in this group received no nicotine exposures and served as a control group.

Group–NT: Animals in this group were exposed to nicotine. Nicotine tartrate solution was subcutaneously injected at an effective dose of 2.5 mg nicotine/ kg body weight.

Group–NTCS: Animals in this group received an effective dose of nicotine followed by a supplemented effective dose of curcumin (80 mg/kg body weight), which was given orally after one hour of nicotine injection.

Group –NTNCS: Animals in this group received an effective dose of nicotine followed by a supplemented effective dose of nano-curcumin (4 mg/kg body weight), which was given orally after one hour of nicotine injection.

Group –NCS: Animals in this group received an effective dose of nano-curcumin (4 mg/kg body weight) only, which was given orally after one hour of nicotine injection.

After the completion of exposure for three consecutive weeks, all animals were kept fasting overnight and sacrificed the next day by decapitation.

Sample Collection

After decapitation, blood samples were collected from the heart immediately, divided into two parts, and stored with or without

an anticoagulant (Heparin). The serum was separated from the blood, kept in anticoagulant containers by centrifugation, and stored at - 20 °C for further analysis. The liver, kidney, and ovary were dissected out and stored in vacuum desiccators at -20°C to prevent auto-oxidation for future studies.

Hemoglobin Estimation

Percentages of hemoglobin estimation were done using the method described elsewhere.[30]

DNA Content Estimation

DNA contents from blood and tissues (liver, kidney, and ovary) were determined by following the protocol as described by Bandyopadhyay *et al*.[31] After the extraction of DNA, it was dissolved in TE buffer (pH 8) and its concentration and purity were measured by using a spectrophotometer at the range of 230, 260 and 280 nm.

Comet Assay

The whole procedure of comet assay was followed by a slightly modified technique as described by Bandyopadhyay *et al*.[31] The photomicrograph of each slide was taken in Leica Fluorescent Microscope at the 40x magnification, Model 300 FX. Measurements of the total comet tail length and mean DNA density, *etc*. were done by using the Perceptive Comet Assay IV software version 4.3. The parentage of DNA damage and tail moment were done accordingly. A total of 50 cells were screened per animal, and the data were averaged. Quantification of DNA damage for each call was as follows:

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

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

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

Tail moment = (Tail length) × (Mean DNA intensity in tail)

Cytokines and Apoptotic Molecules by ELISA

Various cytokines such as IL-6, IL4, TNF-α, and IFN-γ and apoptosis regulatory proteins (BCL-2 and BAX), which were induced by nicotine and nano-curcumin, were measured from the serum of protein-restricted rats. This process was conducted with the help of Quantikine Immunoassay Kits (Genetix Biotech Asia Pvt.Ltd.) as per the manufacturer's protocols. The quantitative sandwich enzyme immunoassay technique did this assay. The intensity of the color measure was proportional to the amount of rats IL-4 and IL-6 and bound in the initial step. After the completion of this assay, the plates were read for optical density at 450 nm and then compared with the standard curve.

Female Hormones by Radioimmunoassay

Un-conjugated forms of female hormones, viz., estradiol and progesterone hormones, were estimated with the help of an ELISA kit supplied by Genetix Biotech Asia Pvt. Ltd. as per the manufacturer's protocol. The concentration of the hormones in the serum of control and experimental rats was determined by interpolating the measured OD values at 450 nm from the standard curves.

Statistical Analysis

Each experiment was repeated twice, and data were averaged (N = 12) and tabulated as a mean ± standard deviation (SD). The statistical analysis of the data obtained from control, nicotine, nicotine+nano-curcumin, and only nano-curcumin supplemented animals was performed by one-way analysis of variance (ANOVA). The significant levels of the observed data were determined at $p < 0.05$.

RESULTS

Nicotine exposure caused a significant ($p < 0.001$) decrement in total hemoglobin content (25%) in the RBC of the animals under protein-restricted diet conditions. It was observed that both curcumin and nano-curcumin increased the hemoglobin concentration in RBC effectively (Table 1). Nicotine hampered DNA synthesis in the tissues of the rats' blood, liver, kidney, and ovary under a protein-restricted diet, as seen in Table 2. DNA contents in those tissues were reduced to more than 20%, which was significant ($p < 0.001$). The ameliorative action of nano-curcumin against nicotine in DNA synthesis was found to be significant ($p < 0.001$) than that of curcumin (Table 2).

Comet assay (Figure 1 and Table 3) showed that nicotine caused aggravated DNA damage (almost 50%) to the blood cells of female rats in protein-restriction conditions. Both curcumin and nano-curcumin resisted the deleterious action of nicotine against DNA damage. Table 3 shows that nano-curcumin possesses better ameliorative efficacy ($p < 0.001$) than curcumin in the case of repairing DNA damage.

The concentration levels of all the observed cytokine molecules (IL-4, IL-6, TNF-α, and IFN-γ) were increased due to the action of nicotine in female rats maintained under protein-restriction conditions (Figure 2). Results suggested that IL-4 increased 1.4 folds in nicotine-exposed and protein-restricted animals compared to control protein-restricted

Table 1: Hemoglobin levels of animals in different groups

	Groups				
Parameter	*(C)*	*(NT)*	*(NTCS)*	*(NTNCS)*	*(NCS)*
Haemoglobin (g/dL^{-1}) (n=12)	12.3 ± 0.34	9.16 ± 0.29** (25.52↓)	11.66 ± 0.18$^{*\#\#}$ (5.48↓)	12.3 ± 0.18$^{*\#\#}$ (0.0)	13.4 ± 0.5 (8.94↑)

Data are Mean ± SD. * and$^{\#}$ indicate significant differences with C and NT groups, respectively.

Values in parenthesis indicate the percentage of increase (↑) or decrease (↓) relative to the control.

Table 2: Total DNA content in different tissues of animals in different groups

	Groups				
Parameter	*(C)*	*(NT)*	*(NTCS)*	*(NTNCS)*	*(NCS)*
Blood (µg/300 µL)	110.67 ± 5.31	77.67 ± 6.75 (29.81 ↓) *	87.36 ± 9.39 (21.06 ↓) *#	104.82 ± 4.08 (5.28 ↓) *#	113.94 ± 3.86 (2.95 ↑)
Liver (µg/mg)	17.0 ± 2.13	13.29 ± 1.60 (21.82 ↓) *	14.89 ± 0.51 (12.41 ↓) *#	16.16 ± 3.91 (4.94 ↓) *#	17.06 ± 1.41 (0.35 ↑)
Kidney (µg/mg)	17.66 ± 0.42	13.49 ± 1.54 (23.61 ↓) *	15.63 ± 0.23 (11.49 ↓)*#	16.41 ± 2.36 (7.07 ↓) *#	17.74 ± 0.75 (0.45 ↑)
Ovary (µg/mg)	19.72 ± 0.67	13.84 ± 0.36 (29.81 ↓) *	15.29 ± 0.24 (22.46 ↓) *#	18.44 ± 0.05 (6.49 ↓) *#	19.39 ± 0.04 (1.67 ↑)

Data are Mean ± SD. * and # indicate significant differences with C and NT groups, respectively.

Values in parenthesis indicate the percentage of increase (↑) or decrease (↓) relative to the control.

Table 3: DNA damage percentage and tail moment in whole blood of different groups

	Groups				
Parameter	*(C)*	*(NT)*	*(NTCS)*	*(NTNCS)*	*(NCS)*
% DNA damaged	13.36 ± 0.5	48.00 ± 4.2*	26.89 ± 2.17*	16.06 ± 0.5*#	13.12 ± 0.14
Tail moment arbitrary unit	80.93 ± 2.50	636.95 ± 5.04*	210.8 ± 6.42*	164.27 ± 8.44*#	19.44 ± 8.35

Data are Mean ± SD. * and # indicate significant differences with C and NT groups, respectively.

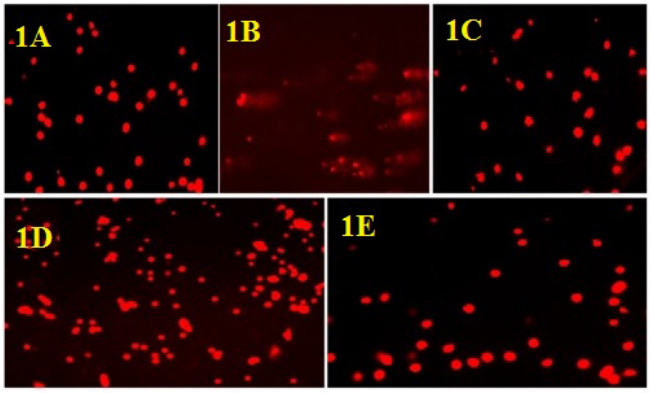

Figure 1: Photomicrographs of the comet assay of blood cell DNA of animals under protein-restricted conditions. (1A) represents the comet picture of blood cell DNA in protein-restricted diet-fed rats. (1B) represents the comet picture of blood cell DNA in the nicotine-exposed group. (1C) represents the comet picture of blood cell DNA in the nicotine + curcumin-exposed group. (1D) represents the comet picture of blood cell DNA in the nicotine+nano-curcumin-exposed group. (1E) represents the comet picture of blood cell DNA in the nano-curcumin-supplemented group.

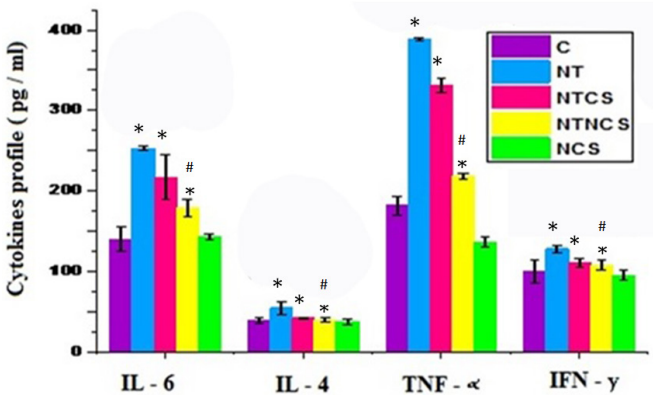

Figure 2: Cytokine profile determined by ELISA. Columns are Mean ± SD. * and # indicate significant differences with C and NT groups, respectively

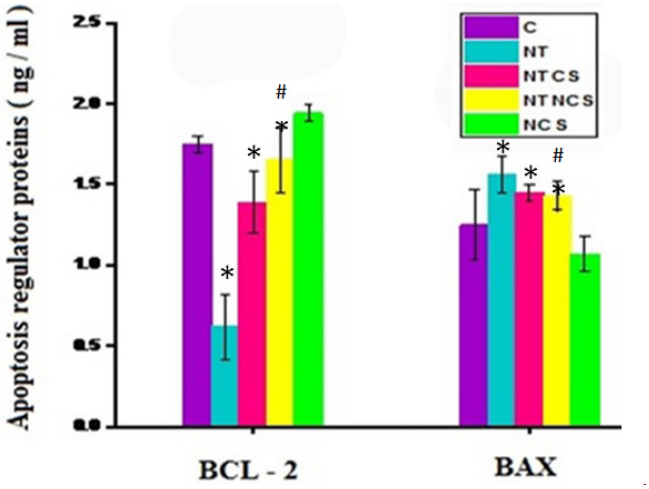

Figure 3: Apoptosis regulator protein determination by ELISA Columns are Mean ± SD* and # indicate significant differences between the C and NT groups, respectively

animals. Similarly, IL-6, TNF-α, and IFN-γ were increased by 1.8, 2.1, and 1.3 folds, respectively, in nicotine-exposed and protein-restricted animals than in control protein-restricted animals. Curcumin supplementation reduced the concentration levels of all these cytokines, and the concentration levels of IL-4, IL-6, TNF-α, and IFN-γ became 1.1, 1.6, 1.8, and 1.1 folds, respectively, in nicotine-exposed and protein-restricted animals. Supplementation of nano-curcumin to nicotine-exposed and protein-restricted animals reduced those cytokine levels very significantly ($p < 0.001$), due to which the concentration levels of IL-4, IL-6, TNF-α, and IFN-γ b became 1.0, 1.3, 1.2 and 1.1 folds, respectively.

Table 4: Levels of steroidogenic hormone released in serum of rats under protein restricted dietary condition determined by ELISA

Steroidogenic hormone	*control*	*Nicotine*	*Nicotine + curcumin*	*Nicotine + Nanocurcumin*	*Nanocurcumin*
Estradiol (pg/mL)	79.62 ± 5.25	63.69 ± 13.57	71.11 ± 10.1	78.88 ± 5.09	84.06 ± 5.01
Progesterone (ng/mL)	11.59 ± 0.23	8.95 ± 0.04	9.49 ± 0.121	10.7 ± 0.19	13.28 ± 0.20

The concentration of anti-apoptotic molecule (BCL-2) was drastically reduced (2.8-fold) by nicotine exposure in female rats under protein-restricted conditions (Figure 3). The concentration level of BCL-2 became 1.2 folds ($p < 0.01$) by curcumin and 1.0 folds ($p < 0.001$) by nano-curcumin supplementation in the nicotine-exposed and protein-restricted diet-fed female rats (Figure 3).

Nicotine also caused a decrement of female sex hormones (estradiol by 1.3 folds and progesterone by 1.3 folds) in the female rats maintained under a protein-restricted diet compared to their respective controls (Table 4). Nano-curcumin almost nullified the action of nicotine and restored the normal concentration levels of estradiol and progesterone more significantly ($p < 0.001$) than curcumin in the nicotine-exposed and protein-restricted diet-fed female rats.

Discussion

Different forms of tobacco consumption are widely spread over socio-economically handicapped populations in developing countries, particularly in India, and therefore, this study is worthy of investigation. The study shows that nicotine causes a significant reduction ($p < 0.001$) of total hemoglobin concentration in the blood cells of female rats when maintained under protein-restricted diet conditions. This is in agreement with our previous study,[30] where it is shown that nicotine induces a low RBC count and affects the binding efficacy of hemoglobin with oxygen. Hemoglobin concentration is therefore becoming low in nicotine exposure. Numerous studies have demonstrated that low hemoglobin (Hb) is a strong prognostic indicator of poor disease control. The observed increased concentration of hemoglobin by curcumin or nano-curcumin is due to their protective efficacy against nicotine, as explained by Banerjee *et al*.[30] The more bio-available nano-curcumin shows enhanced ameliorative efficacy than curcumin against the nicotine-induced effect.

Nicotine causes suppression of cellular proliferation and increases DNA breakdown, resulting in increased cell death (Mohammed *et al*., 2004). The present finding supports the earlier studies[31] as nicotine causes a very significant ($p < 0.001$) reduction of DNA content in various tissues (blood, liver, kidney, and ovary) of the female rats under protein-restricted conditions (Table 2). Chattopadhyay and Chattopadhyay[32] have shown that nicotine enhances lipid peroxidation in female rats under a protein-restricted diet, which results in increased oxidative DNA damage and, therefore, the total DNA content in various tissues becomes less compared to their respective controls in such conditions (Table 2). Though curcumin and nano-curcumin both are able to minimize the toxic effect of nicotine against the DNA content of the tissues, nano-curcumin shows better efficacy against nicotine due to its more bioavailability, resulting in less decrement of total DNA content in various tissues of nicotine-exposed animals. It is reported that nano-curcumin possesses higher binding efficacy with DNA than nicotine.[33] Therefore, the nano-curcumin vs. DNA complex is more stable than the nicotine vs. DNA complex. Nano-curcumin thus protects DNA from nicotine, resulting in increased DNA concentration in various tissues.

The comet assay results, as shown in Figure 1, clearly indicate that nicotine can break DNA strands, which is why the comet-like appearance is seen in the picture. The comet assay is a well-established genotoxicity test for *in-vitro* testing of chemicals against biological macromolecules.[34] The retrenched head diameter, extended tail length, decreased head diameter *vs.* tail length ratio, and amplified percentage of DNA damage are seen to be significantly higher in nicotine-exposed protein-restricted animals. In the comet assay, it is observed that the percentage of DNA damage in the protein-restricted control group is 13.36.%. Some amount of DNA damage (< 10%) always occurs in normal conditions due to various physiological stresses. Protein restriction causes additional stress, due to which a slightly higher percentage of DNA damage is observed in our study. Figure 1 for comet assay clearly indicates that nicotine plays a significant role in genotoxicity to the blood cells of female rats in protein-restricted conditions. This may be due to the increased production of free radicals and reactive oxygen species (ROS) that attack all types of macromolecules, including DNA, as reported in an earlier study.[35] Curcumin significantly reduces DNA damage due to its anti-mutagenic, tumor-inhibiting, and anti-oxidant properties. Nano-curcumin supplementation ($p < 0.001$) significantly prevents nicotine-induced DNA damage in the protein-restricted group. It suggests that more bio-available nano-curcumin has better efficacy in combating nicotine and restoring DNA damage than in nicotine-stressed conditions.

ELISA test was performed to determine the concentration of cytokines (IL-6, IL-4, TNF-α, IFN-γ) in serums of rats exposed to nicotine, curcumin, and nano-curcumin under protein-restricted dietary conditions. The results of this study demonstrated that cytokine levels were influenced by nicotine. At the physiological level, IL-6 plays an important role in the cytokine network. However, excessive production of IL-6 in response to exposure to lipopolysaccharide has an inflammatory effect, resulting in injury.[36] IL-4 in extravascular tissues promotes alternative activation of macrophages into M2 cells and inhibits the classical activation of macrophages into M1 cells. It stimulates the activation of B-cells and the proliferation of T-cells, and it differentiates B-cells into

plasma cells.[37] TNF- α, along with IL-1beta, up-regulates the expression of VCAM-1 on hepatic sinusoidal endothelium *in-vivo*, which promotes cancer cell adhesion and liver metastasis. IFN-γ possesses immune regulatory property that alters the transcription of~30 genes producing a variety of physiological and cellular responses.[38] Activated T cells release macrophage-activating factor IFN-γ which activates macrophages to secrete many inflammatory proteins such as IFN-γ inducible protein (IP-10), IFN inducible T cell α – chemoattachment (I-TAC), and monokine induced by IFN-γ to orchestrate the inflammatory process. ELISA results for the cytokines of serum demonstrate that the cytokines are elevated rapidly after nicotine exposure in normal protein dietary conditions, and curcumin prevents that elevation, whereas nano-curcumin supplementation helps more effectively than curcumin to regain the normal level of cytokines as released under normal dietary conditions.

Apoptosis plays an important role in regulating a variety of diseases. In the case of schizophrenia, a neurodegenerative disease, an abnormal ratio of pro- and anti-apoptotic factors is observed.[39] BCL-2 is localized to the outer membrane of mitochondria, where it plays an important role in promoting cellular survival and inhibiting the actions of pro-apoptotic proteins. The pro-apoptotic proteins in the BCL-2 family, including BAX and BCL-2, normally act on the mitochondrial membrane to promote permeabilization and release of cytochrome C and ROS, which are important signals in the apoptosis cascade. These pro-apoptotic proteins are, in turn, activated by BH3-only proteins and are inhibited by the function of BCL-2 and its relative BCL-Xl.[40] ELISA test shows that nicotine exposure decreases the concentration level of anti-apoptotic BCL-2 molecules and increases the concentration of pro-apoptotic BAX molecules. The more bio-available nano-curcumin supplementation regains the normal concentration levels of both apoptotic molecules more significantly than that of curcumin released under protein-restricted dietary conditions.

Cigarette smoking has major effects on the reproductive potential of humans. Being pro-androgenic, smoking is also anti-estrogenic, which means it has a negative effect on estrogen levels in women.[41] Nicotine significantly reduces 17 β-estradiol and progesterone levels in female rats maintained on a protein-restricted diet. Nicotine has an anti-estrogenic effect of which affects the hepatic estrogen metabolism,[42] thereby reducing 17 β-estradiol levels in the serum of those female rats. It also induces a decrease in progesterone levels by increasing PGF-2α, an important factor for autolysis, and also by increasing VEGF mRNA expression. Both curcumin and nano-curcumin combat the anti-estrogenic effect of nicotine, and increased 17β-estradiol level and progesterone levels are noted when supplemented in rats maintained under a protein-restricted diet. This increment occurs because of curcumin, which inhibits microvascular endothelial cell angiogenesis through the inhibition of COX-2 expression.[43] Nano-curcumin shows a more significant ameliorative effect than curcumin, increasing the 17 β-estradiol and progesterone levels near normal levels in the serum of rats maintained under protein-restricted conditions.

Acknowledgement

The work was done with the support of the Biophysics Laboratory, Department of Physics, Jadavpur University, and Molecular and Cell Biology Laboratory, Department of Zoology, University of Kalyani. The UGC (NTA) NET JRF Fellowship support from UGC, Government of India (Ref. No. 210510536878, Dated: 12-03-2022) to Somashree Biswas is gratefully acknowledged.

References

1. Global Burden of Disease [database]. Washington, DC: Institute of Health Metrics; 2019. IHME, accessed 17 July 2021.
2. Bailador FD, Wonnacott. Nicotinic acetylcholine receptors and the regulation of neuronal signaling. *Trends Pharmacol Sci*. 2004;.25(6):317-24. DOI: 10.1016/j.tips.2004.04.006.
3. Cardinale A, Nastrucci C, Cesario A, *et al*. Nicotine: specific role in angiogenesis, proliferation and apoptosis. *Crit Rev Toxicol*. 2012;42:68–89. DOI: 10.3109/10408444.2011.623150.
4. Lee J, Cooke JP. Nicotine and pathological angiogenesis. *Life Sci*. 2012;91:1058–64. DOI: 10.1016/j.lfs.2012.06.032.
5. Ebrahimpour A, Shrestha S, Bonnen MD, et al. Nicotine modulates growth factors and microRNA to promote inflammatory and fibrotic processes. *J Pharmacol Exp Ther*. 2019;368(2):169–78. DOI: 10.1124/jpet.118.252650.
6. Solleti SK, Simon DM, Srisuma S, *et al*. Airway epithelial cell PPARγ modulates cigarette smoke-induced chemokine expression and emphysema susceptibility in mice. *Am J Physiol Lung Cell Mol Physiol.* 2015;309:L293–304. DOI: 10.1152/ajplung.00287.2014.
7. Bellaye PS, Kolb M. Why do patients get idiopathic pulmonary fibrosis? Current concepts in the pathogenesis of pulmonary fibrosis. *BMC Med*. 2015;13:176. DOI: 10.1186/s12916-015-0412-6.
8. Yang IV, Schwartz DA. Epigenetics of idiopathic pulmonary fibrosis. *Transl Res*. 2015; 165:48–60. DOI:10.1016/j.trsl.2014.03.011.
9. Benowitz NL, Hukkanen J, Jacob P. Nicotine chemistry, metabolism, kinetics and biomarkers. *Handb Exp Pharmacol*. 2009;(192):29-60. DOI: 10.1007/978-3-540-69248-5_2.
10. Sanner T, Grimsrud TK. Nicotine: Carcinogenicity and Effects on Response to Cancer Exposure – A Review. *Front Oncol*. 2015;5:196. DOI: 10.3389/fonc.2015.00196.
11. Arabi M. Nicotinic infertility: assessing DNA and plasma membrane integrity of human spermatozoa. *Andrologia*. 2004;36:305-10. DOI: 10.1111/j.1439-0272.2004.00623.x.
12. Rosenblum MD, Remedios KA, Abbas AK. Mechanisms of human autoimmunity. *J Clin Invest*. 2015;125(6):2228-33. DOI: 10.1172/JCI78088.
13. Gour N, Karp MW. IL-4 and IL-13 Signaling in allergic airway disease. *Cytokine*. 2015;75(1):68-78. DOI: 10.1016/j.cyto.2015.05.014.
14. Rebe C, GhiringelliF. Interleukin-1β and Cancer. *Cancers (Basel)*. 2020;12(7):1791. DOI: 10.3390/cancers12071791.
15. Pelaia C, Paoletti G, Puggioni F, *et al*. Interleukin-5 in the Pathophysiology of Severe Asthma. *Front Physiol*. 2019;10:1514. DOI: 10.3389/fphys.2019.01514.
16. Tirado-Rodriguez B, Ortega E, Segura-Medina P, Huerta-Yepez S.

TGF-β: an important mediator of allergic disease and a molecule with dual activity in cancer development. *J Immunol Res*. 2014; 2014:318481. DOI: 10.1155/2014/318481.
17. Mendoza JL, Escalante NK, Jude KM *et al*. Structure of the IFNγ receptor complex guides design of biased agonists. *Nature*. 2019;567:56-60. DOI: 10.1038/s41586-019-0988-7.
18. Moghbeli M, Khedmatgozar H, Yadegari M. *et al*. Cytokines and the immune response in obesity-related disorders. *Adv Clin Chem*. 2021;101:135-68. DOI:10.1016/bs.acc.2020.06.004.
19. Comer DM, Elborn JS, Ennis M. Inflammatory and cytotoxic effects of acrolein, nicotine, acetaldehyde and cigarette smoke extract on human nasal epithelial cells. *BMC Pulm Med*. 2014;14:32. DOI: 10.1186/1471-2466-14-32.
20. Pace E, Ferraro M, Di Vincenzo S, *et al*. Oxidative stress and innate immunity responses in cigarette smoke stimulated nasal epithelial cells. *Toxicology*. 2014;28:292-9. DOI: 10.1016/j.tiv.2013.11.004.
21. Macnee W, Tuder RM. New paradigms in the pathogenesis of chronic obstructive pulmonary disease I. *Proc Am Thorac Soc*. 2009;6:527-31. DOI: 10.1513/pats.200905-025DS.
22. Rahman I. Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. *J Biochem Mol Biol*. 2003; 36:95-109. DOI: 10.5483/bmbrep.2003.36.1.095.
23. Wullaert A, Loo GV, Heyninick K, Beyaert R. Hepatic tumor necrosis factor signaling and nuclear factor-kappaB: effects on liver homeostasis and beyond. *Endocr Rev*. 2007; 28(4):365-86. DOI: 10.1210/er.2006-0031.
24. Peng WC, Logan CY, Fish M, *et al*. Inflammatory cytokine TNFα promotes the long-term expansion of primary hepatocytes in 3D culture. *Cell*. 2018;175(6):1607-19.e15. DOI: 10.1016/j.cell.2018.11.012.
25. Qian S, Wei Z, Yang W, *et al*. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. *Front Oncol*. 2022; 12:985363. DOI: 10.3389/fonc.2022.985363.
26. Ethier AR, Mckinney Ty, Tottenham LS, *et al*. The effect of reproductive hormones on women's daily smoking across the menstrual cycle. *Biol Sex Differ*. 2021;12(1):41. DOI: 10.1186/s13293-021-00384-1.
27. Okwu GN, Ukoha AL. Studies on the predisposing factors of protein energy malnutrition among pregnant women in a Nigerian community. *Online Journal of Health and Allied Sciences*. 2007;6(3):1-9. Available at https://web-archive.southampton.ac.uk/cogprints.org/5927/1/2007-3-1.pdf
28. Chattopadhyay K, Samanta A, Mukhopadhyay S, *et al*. Potential amelioration of nicotine-induced toxicity by nanocurcumin. *Drug Dev Res*. 2018; 79:119-28. DOI: 10.1002/ddr.21424.
29. Hawk PB, Oser BL, Summerson WH. Practical physiological chemistry. Mc Grew Hill Book Co.1954. New York, London.
30. Banerjee S, Bandyopadhyay G, Chattopadhyay K, Chattopadhyay BD. Amelioration of nicotine-induced damage of blood cells in protein-malnourished female rats by curcumin. *Int J Pharmacol*. 2010;6(4):444-55. DOI: 10.3923/ijp.2010.444.455.
31. Bandyopadhyay G, Sinha S, Chattopadhyay BD, Chakraborty A. Protective role of curcumin against nicotine-induced genotoxicity on rat liver under restricted dietary protein. *Eur J Phrmacol*. 2008;588:151-7. DOI: 10.1016/j.ejphar.2008.04.023
32. Chattopadhyay K, Chattopadhyay B. Effect of nicotine on lipid profile, peroxidation and antioxidant enzymes in female rats with restricted dietary protein. *Ind J Med Res*. 2008;127:571-6. PMID: 18765876.
33. Samanta A, Chowdhury T, Chattopadhyay B, Chattopadhyay K. Studies the therapeutic efficacy of nanocurcumin against nicotine-induced damage of blood cells. *Int J Pharmaceu Res Innovat*. 2021;14:12-26. Available at https://www.whitesscience.com/wp-content/uploads/woocommerce_uploads/2021/02/IJIPRI_14_12-24_2021-hodcbn.pdf.
34. Premkumar K, Kavitha S, Santhiya ST. Ramesh AR, Suwanteerangkul J. Interactive effects of saffron with garlic and curcumin against cyclophosphamide-induced genotoxicity in mice. *Asia Pac J Clin Nutr*. 2004;13(3):292-4. PMID: 15331343.
35. Ray S, Chattopadhyay N, Mitra A, Siddiqi M, Chatterjee A. Curcumin exhibits antimetastatic properties by modulating integrin receptors, collagenase activity, and expression of Nm23 and E-cadherin. *J Environ Pathol Toxicol Oncol*. 2003;22(1):49-58. PMID: 12678405.
36. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol*. 2014;6(10):a016295. DOI: 10.1101/cshperspect.a016295.
37. Luzina IG, Keegan AD, Heller NM, Rook GAW, Shea-Donohue T, Atamas SP. Regulation of inflammation by interleukin-4: a review of "alternatives". *J Leukoc Biol*. 2012;92(4):753–64. DOI: 10.1189/jlb.0412214.
38. Castro F, Cardoso AP, Gonçalves RM, Serre K, Oliveira MJ. Interferon-gamma at the crossroads of tumor immune surveillance or evasion. *Front Immunol*. 2018;9:847. DOI: 10.3389/fimmu.2018.00847.
39. Glantz LA, Gilmore JH, Lieberman JA. Jarskog LF. Apoptotic mechanisms and the synaptic pathology of schizophrenia. *Schizophrenia Res*. 2006;81:47-63. DOI: 10.1016/j.schres.2005.08.014.
40. Hardwick JM. Soane L. Multiple Functions of BCL-2 Family Proteins. *Cold Spring Harb Perspect Biol*. 2013;5(2):a008722. DOI: 10.1101/cshperspect.a008722.
41. Jandikova, H, Duskova M and Starka, L. The influence of smoking and cessation on the human reproductive hormonal balance. Physiol Res. 2017;66(Suppl 3): S323-s31.
42. Adesky ND, Vaccari JPDR, Bhattacharya, P. *et al*. Nicotine alters estrogen receptor-beta-regulated inflammasome activity and exacerbates ischemic brain damage in female rats. *Int J Mol Sci*. 2018;19(5):1330. DOI: 10.3390/ijms19051330.
43. Binion DG, Otterson MF, Rafiee P. Curcumin inhibits VEGF-mediated angiogenesis in human intestinal microvascular endothelial cells through COX-2 and MAPK inhibition. *Gut*. 2008;57(11):1509-17. DOI: 10.1136/gut.2008.152496.

PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.