

STUDIES ON THERAPEUTIC EFFICACY OF NANOCURCUMIN AGAINST NICOTINE INDUCED DAMAGE OF BLOOD CELLS

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ABSTRACT

Nicotine, after rapid absorption in human airways immediately goes to the blood resulting malfunction of the defence system. Studies on nicotine-induced toxicity of female population is still relevant because women are more susceptible to nicotine-induced complications. This study was an attempt to overcome the nicotine-induced genotoxicity of blood cells of female population by using nanocurcumin against nicotine-induced toxicity. Experiments were conducted on female rats exposed daily by effective dose of nicotine (2.5 mg/kg body weight) followed by supplementation of nanocurcumin (4 mg/kg body weight) for 21 days. Animals were eradicated after treatment period and several experiments were done from their blood. The molecular docking and *in vitro* interactions studies were explored to search the mechanism of action of nanocurcumin against nicotine-induced toxicity. Nanocurcumin showed its potential therapeutic efficacy against nicotine-induced complications. It increased haemoglobin (Hb) and DNA contents and reduced DNA damage very effectively ($p < 0.001$). Molecular docking predicted that nanocurcumin had stronger interaction to haemoglobin and DNA which protected those molecules from nicotine-induced toxicity. *In vitro* interaction studies also supported the molecular docking hypothesis. Nanocurcumin could be a promising therapeutic agent against nicotine-induced genotoxicities of blood cells and it could more effectively protect our health particularly, nicotine intoxicated female population.

Key words: Blood cells, DNA damage, Docking, Nanocurcumin, Nicotine.

INTRODUCTION

Nicotine, after consumption either through smoking or chewing tobacco leaves or taking snuffs, rapidly enters in the blood stream and causes disruption of the blood cells and defence system of our body. It causes several dreaded diseases like cardiac diseases [1], gastrointestinal cancer [2], pulmonary and oral cancer [3] etc. Women who are habituated in smoking, have a 25% greater increased risk of coronary heart disease than their male-smoker counterparts [4].

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Nicotine has been found to alter the endocrine function, which in turn affects release of female sex-hormones [5].

In general, women suffers more than man from nicotine-induced complications due to their low

inherent immunity [6, 7]. Scientist have already described the immunomodulation effect of nicotine and nanocurcumin in rat [8]. Studies on nicotine-induced toxicity and its amelioration of female population is still very much relevant for our society.

Nicotine reduces RBC counts and haemoglobin concentration in the blood due to which the body loses its ability for carrying oxygen to several organs in rats [9]. In fact, nicotine leads to shortness of breath and increases the pulse rate which creates a compensatory increment of the output of the heart leading to palpitations and chest pains [10]. In serious cases, the arms and legs may become swollen, and the individual may experience excessive sweating, heartburn, vomiting, bruises, and bloody stools [11]. Malenica et al. reported the aggravated effect of smoking on haematological parameter in healthy population [12]. Nicotine also enhances the reactive oxygen species (ROS) generation [5]. Enhanced ROS load and other toxic chemicals of nicotine cause peroxidative membrane damage of RBC [13]. It increases the viscosity of blood due to aggregation of RBC that results in impairment of blood flow. Nicotine also induces DNA damage in blood cells and various tissues due to its ROS generation activities [5, 14]. DNA damage inhibits M-CDKs (Mediator of Cyclin-dependent Kinases) which are a key component of progression into Mitosis [15]. It has also been observed that tobacco smoke induces aldehyde-DNA adducts in mice and humans, inhibits DNA repair activity and reduces repair proteins in mouse lung [16].

Curcumin derived from turmeric (*Curcuma longa*) possesses a wide range of pharmacological effects like antioxidant, anti-inflammatory and hepato-protective etc. [13, 14]. It protects DNA from damage and attenuates carboplatin-induced myelo-suppression by activating the DNA repair pathway in bone marrow cells [16, 17]. The promising therapeutic capabilities of curcumin is still restricted in use as therapeutic agent due to its poor aqueous solubility and limited bioavailability [18]. Encapsulated curcumin, liposomal curcumin etc. have been tried to increase its use in the recent past but due to large particle size, its effectiveness has been limited [19]. The concentration of curcumin is found to be very low in human blood because of its fast metabolic turnover in the liver and intestinal wall which results limited distribution in the tissues following oral dosing [20, 21]. Also, there are some reports concerning curcumin toxicity due to higher effective dose in the body [22]. Several studies were designed to formulate nanoparticles of curcumin through enhancing its bioavailability and bio-distribution activity for its therapeutic efficacy in drug delivery [23-28].

The current work was focused to evaluate the improved efficacy of nanocurcumin and its significant protective action against nicotine-induced damages of blood cells so that nanocurcumin could become the better replacement of curcumin for therapeutic uses in several diseases. An attempt was also made to find out the possible mechanism of action of nanocurcumin against nicotine-induced complications.

MATERIALS AND METHODS

Raw materials and Chemicals

Nicotine hydrogen tartrate, curcumin and powder haemoglobin (Hb) were purchased from Sigma Chemicals Company, St Louis, USA. All other analytical grade chemicals were supplied by SpectroChem Pvt. Ltd. India and Merck India. Nanocurcumin was prepared in our lab. The

preparation and characterization of nanocurcumin was elaborately discussed in the previous paper [24].

Diets and Treatments

A total of 36 female albino rats of Wistar strain (*Rattus norvegicus*), 60 - 75 days old, weighing 140-150 g were procured from the Animal housing facility and maintained according to 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). The animals were divided into 6 groups, each containing 6 animals as shown in Table 1.

Table 1: Animal groups and treatment

| Name of the group | Diet and treatment of animals for 21 days |
|---|---|
| Control (C) | Normal protein diet and received subcutaneous injection of 0.2 ml physiological saline only. |
| Nicotine treated (NT) | Normal protein diet and received subcutaneous injection with effective dose of nicotine (2.5 mg/kg body weight). |
| Nicotine treated and curcumin supplemented (NTCS) | Normal protein diet and treated with effective dose of nicotine (2.5 mg/kg body weight) followed by supplementation of effective dose of curcumin (80 mg/kg body weight) orally. |
| Nicotine treated and nanocurcumin supplemented (NTNCS) | Normal protein diet and treated with effective dose of nicotine (2.5 mg/kg body weight) followed by supplementation of effective dose of nanocurcumin (4 mg/kg body weight) orally. |
| Curcumin supplemented (CS) | These rats were fed with normal protein diet and supplemented with effective dose of curcumin (80 mg/kg body weight) orally. |
| Nanocurcumin supplemented (NCS) | These rats were fed with normal protein diet and supplemented with effective dose of nanocurcumin (4 mg/kg body weight) orally. |

Nicotine hydrogen tartrate salt was dissolved in normal saline water (2.5 g/mL) and required concentration of drug solution (based on the body weight of an animal) was added to distilled water to make the final volume of 0.2 mL which was injected subcutaneously to that particular animal. The effective doses of nicotine, curcumin and nanocurcumin were chosen from previous studies. Both curcumin and nanocurcumin dispersed in water were supplemented orally at 1 h after nicotine treatment once daily. After 21 days of treatment, animals were kept under fasting condition for 12 h and eradicated on the next day after mild anaesthesia. Blood samples were collected from the heart immediately after eradication and stored in both with or without anticoagulant (heparin) containing containers for the required experiments.

Haemoglobin (Hb) Estimation

The haemoglobin percentage in rat blood was determined by using Standard Sahli's Haemoglobin meter as described earlier [9].

Estimation of Total DNA Content

The total DNA content from 300 μ L of whole blood cells of each sample was estimated by using the protocol as described by Banerjee et al. [9]. The concentration and purity of the prepared DNA was determined spectrophotometrically by noting the absorbances at 230 nm (A_{230}), 260 nm (A_{260}) and 280 nm (A_{280}) respectively as describes earlier [9].

Comet Assay For DNA Damage

DNA damage study of whole blood sample was performed by comet assay as described by Bandyopadhyay et al. [29]. The measurement of the comet head diameter, tail length, tail moment and percentage of DNA damages were done accordingly. A total of 50 cells were screened per slide using a fluorescence microscope (Leica 300-FX with 20 x magnification of Objective). Quantification of DNA damage for each cell was determined by using the Perceptive Comet software version-4.

Molecular Docking

Molecular docking was performed to predict the mode of the interaction of DNA and haemoglobin with nicotine and nanocurcumin using GEMDOCK, a program for computing a ligand conformation and orientation relative to active site of the receptor and UCSF CHIMERA 1.13.1, an extensive molecular modeller system used for docking study. Protein Data Bank file for tumour suppressor P53 complex with DNA (PDB ID: 1TUP) and haemoglobin (PDB ID: 1IRD) was used as receptor molecule and nicotine (PubChem CID:89594) and nanocurcumin (PubChem CID:969516) was taken as ligand molecule for docking. The chemical structure of nanocurcumin was same as that of curcumin revealed by Bhawana et al. [30]. So the same PubChem ID of curcumin was taken into consideration for further studies.

Interaction Studies of Nicotine vs. Haemoglobin and Nanocurcumin

Solutions of nicotine hydrogen tartrate (10 mM) and haemoglobin (3 mg/ml normal saline) were prepared. Haemoglobin solution was taken in a quartz cuvette (1 mL) and its absorbance spectrum was recorded (300 nm – 500 nm) by using an UV-Visible spectrophotometer against normal saline (blank). Different concentrations of nicotine solution were added to the haemoglobin solution (final concentration of nicotine was varied from 50 to 500 μ M in the solution), mixed well and the spectrum of each mixture was taken similarly against the blank which contained normal saline with respectively added nicotine. The concentration of nicotine in the interaction between

nicotine vs. Hb was varied till the absorbance peak of Hb was suppressed completely. Next, freshly synthesized nanocurcumin was added gradually to nicotine (500 μM) + Hb solution (final concentration of nanocurcumin was varied from 10 to 50 μM in the solution), mixed well and absorbance spectrum was recorded at 300 nm – 500 nm against the blank containing normal saline with respective concentration of added nicotine and nanocurcumin to the solution.

Interaction Studies of Nicotine vs. DNA and Nanocurcumin

A solution of whole blood DNA (30 $\mu\text{g}/\text{mL}$ in TE buffer) was prepared. DNA solution (1 mL) was taken in a quartz cuvette and its absorbance spectrum was recorded (240 nm – 320 nm) against TE buffer by using an UV-Visible spectrophotometer. Different concentrations of nicotine solution were added to the DNA solution (final concentration of nicotine was varied from 50 to 250 μM in the solution), mixed well and the spectrum of each mixture was taken similarly against the blank containing respective concentration of nicotine. The concentration of nicotine in this case was till the absorbance peak of DNA was suppressed sufficiently by nicotine. Next, freshly synthesized nanocurcumin was added gradually to nicotine (250 μM) + DNA solution (final concentration of nanocurcumin was varied from 10 to 50 μM in the solution), mixed well and absorbance spectrum was recorded at 240 nm – 320 nm against the blank which contained TE buffer with respectively added nicotine and nanocurcumin.

The binding constant between haemoglobin and nicotine molecular interaction was calculated by Binding-Isotherm plot using the following equation [31].

$$1/\Delta A_c = 1/A_m + [1/A_m \times K] \times 1/C$$

Where, ΔA_c was the change of the absorbance intensity of the Hb molecule, A_m was the initial maximum absorbance intensity of the Hb molecule, C was the concentration of the nicotine (quencher) molecule and K was the binding constant of the nicotine with the Hb molecule. A graph was plotted between $1/\Delta A_c$ vs. $1/C$. The binding constant K, of the quencher was determined from the value of the intercept ($1/A_m$) and the slope ($1/A_m \times K$) of the curve $1/\Delta A_c$ vs. $1/C$.

STATISTICAL ANALYSIS

The experimental setup was repeated twice and all data were averaged over N = 12 animals, and given mean + S.D. Significance levels were determined by using ANOVA, where * implied significant ($p < .01$) and ** implied highly significant ($p < .001$) of the data when compared with the data of nicotine treatment. Similarly, # implied significant ($p < .01$) and ## implied highly significant ($p < .001$) of the data when compared with the data of nicotine + curcumin treatments.

RESULTS

Nicotine decreased the concentration of haemoglobin whereas, native curcumin and nanocurcumin both increased the haemoglobin concentration of the blood in the supplemented condition (Table 2). The total DNA contents in the whole blood cells was drastically reduced by nicotine as seen in nicotine treated rats (Table 2). Nanocurcumin showed significant ($p < 0.001$) ameliorative effect against nicotine on DNA content than native curcumin ($p < 0.01$). Nicotine treatment caused intense DNA damage ($> 39\%$) of the whole blood cells where as the DNA damage in control group was 2.76% only (Table 3). The DNA damage caused by nicotine was effectively reduced by curcumin ($>20\%$) and significantly by

nanocurcumin (>30%) (Table 3). Supplementation of curcumin or nanocurcumin alone did not produce any negative effect on Hb and DNA content of blood cells rather they showed healthy condition of blood cells (Tables 2 and 3).

In-silico docking of nicotine vs. haemoglobin molecule (Fig. 1A) and nanocurcumin (here curcumin) vs. haemoglobin molecule (Fig. 2A) clearly depicted the formation of complex structures of nicotine-haemoglobin molecule and nanocurcumin-haemoglobin molecule respectively. Out of 10 ligand conformations, the best binding energy between nicotine vs. haemoglobin was found to be around (-) 70.98 kcal and that of curcumin vs. haemoglobin be (-) 97.41 kcal respectively. In-silico docking of nicotine vs. nanocurcumin (here curcumin) suggested that binding energy between nicotine vs. nanocurcumin was (-) 51.29 kcal (Fig. 5A). Similarly, molecular docking experiment between p53 tumour suppressor DNA and nicotine showed the formation of nicotine - DNA complex molecule (Fig. 3A) and nanocurcumin (here curcumin) vs. DNA interactions showed complex structure of nanocurcumin – DNA molecule (Fig. 4A) respectively. Here also the best binding energy between nicotine-DNA interaction was determined as (-) 69.48 kcal and that of between curcumin vs. DNA was around (-) 86.35 kcal.

Table 2: Haemoglobin and Total DNA content in whole blood of animals in different group

| Parameter | Groups | | | | | |
|--|---------------|-------------------------|--------------------------|----------------------------|-------------------------|-------------------------|
| | C | NT | NTCS | NTNCS | NC | NCS |
| Haemoglobin (g/dL⁻¹) | 13.3 ± 0.3 | 9.8 ± 0.6 (26.0↓) | 12.2 ± 1.3 (8.1↓)** | 12.4 ± 0.5 (6.6↓)**# | 12.9 ± 0.2 (3.0↓) | 13.2 ± 0.2 (1.0↓) |
| Blood DNA (µg/300µL) | 113.95 ± 2.70 | 68.56 ± 1.52 (39.8↓) | 90.77 ± 5.02 (20.3↓)* | 107.85 ± 4.42 (5.4↓)**# | 114.72 ± 3.33 (1.0↑) | 116.67 ± 2.51 (2.4↑) |

The whole experimental setup was repeated twice and all data of each group was the averaged over N = 12 animals, and given mean + S.D. Significance levels were determined by using ANOVA, where * implied significant (p<.01) and ** implied more significant (p<.001) of the data when compared with the data of nicotine treatment. Similarly, # implied significant (p<.01) and ## implied more significant (p<.001) of the data when compared with the data of nicotine + curcumin treatments. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control.

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

| Parameter | Groups | | | | | |
|-------------------------------------|-------------|---------------|----------------|------------------|-------------|-------------|
| | (C) | (NT) | (NTCS) | (NTNCS) | (NC) | (NCS) |
| % DNA Damaged | 2.76 ± 0.30 | 39.97 ± 0.81 | 16.99 ± 1.60** | 9.20 ± 0.22**## | 2.32 ± 0.54 | 2.11 ± 0.48 |
| Tail moment (Arbitrary unit) | 70.5 ± 1.6 | 691.3 ± 10.60 | 245.5 ± 9.30* | 162.00 ± 3.30**# | 21.02 ± 3.4 | 20.6 ± 4.90 |

The whole experimental setup was repeated twice and all data of each group was the averaged over N = 12 animals, and given mean + S.D. Significance levels were determined by using ANOVA, where * implied significant ($p < .01$) and ** implied more significant ($p < .001$) of the data when compared with the data of nicotine treatment. Similarly, # implied significant ($p < .01$) and ## implied more significant ($p < .001$) of the data when compared with the data of nicotine + curcumin treatments. The data within the parenthesis represent the average percentage of increase (↑) or decrease (↓) relative to the control.

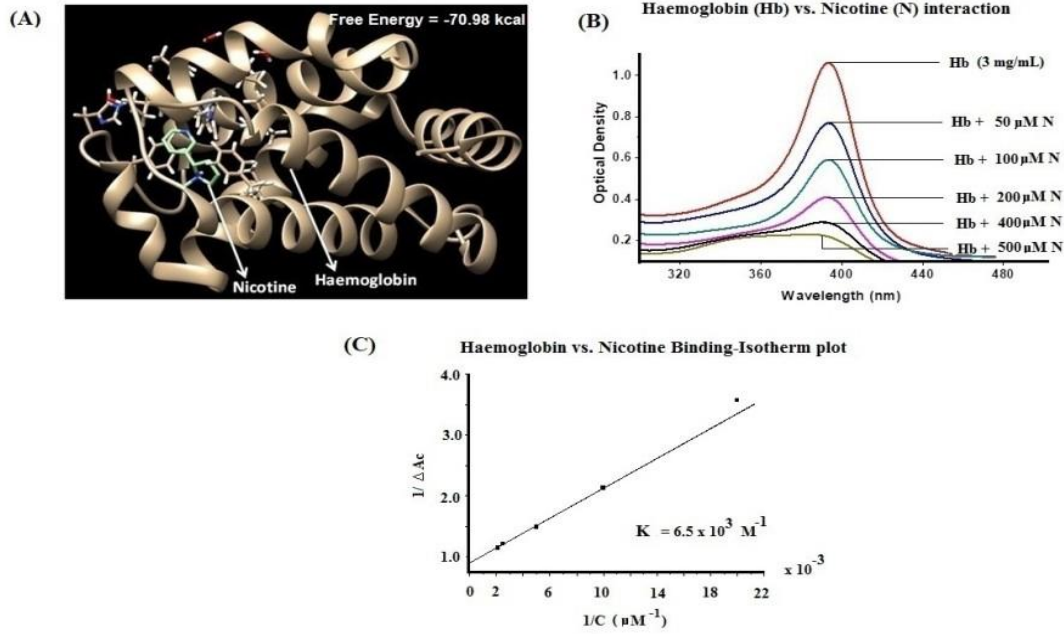


Figure 1 : Interaction studies between nicotine vs. haemoglobin (Hb).

Inset A shows the Molecular docking interaction between nicotine vs. Hb.
 Inset B shows the UV-Vis spectra of interaction between nicotine vs. Hb.
 Inset C shows the Binding -Isotherm Plot between nicotine vs. Hb.

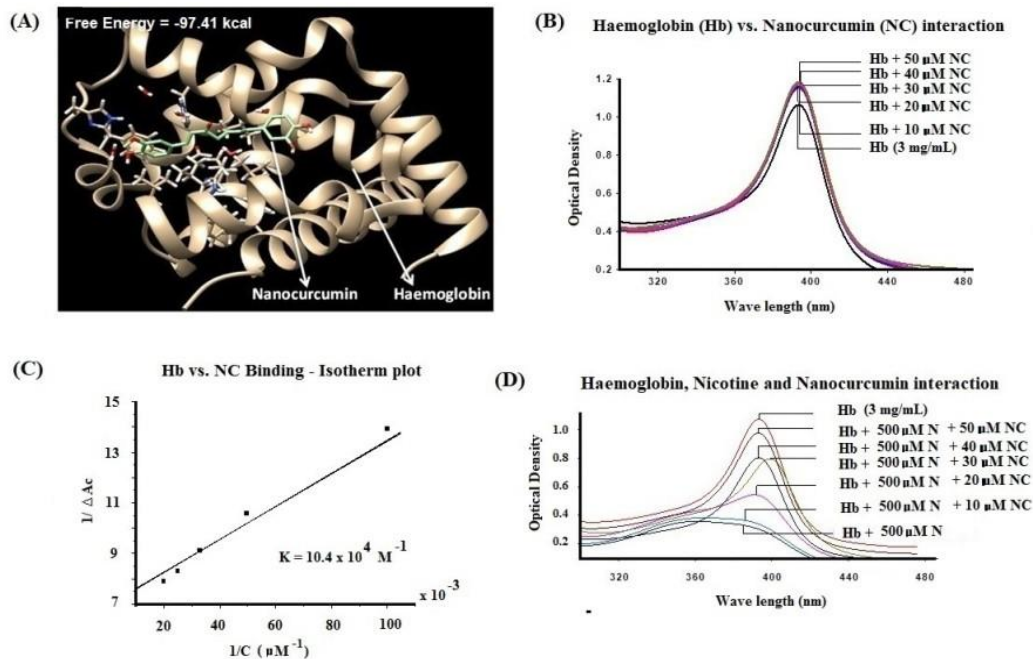


Figure 2: Interaction studies between nanocurcumin vs. haemoglobin (Hb).

Inset A shows the Molecular docking interaction between nanocurcumin vs. Hb.
 Inset B shows the UV-Vis spectra of interaction between nanocurcumin vs. Hb.
 Inset C shows the Binding -Isotherm Plot between nanocurcumin vs. Hb.
 Inset D shows the UV-Vis spectra of interaction between nanocurcumin vs. nicotine + Hb.

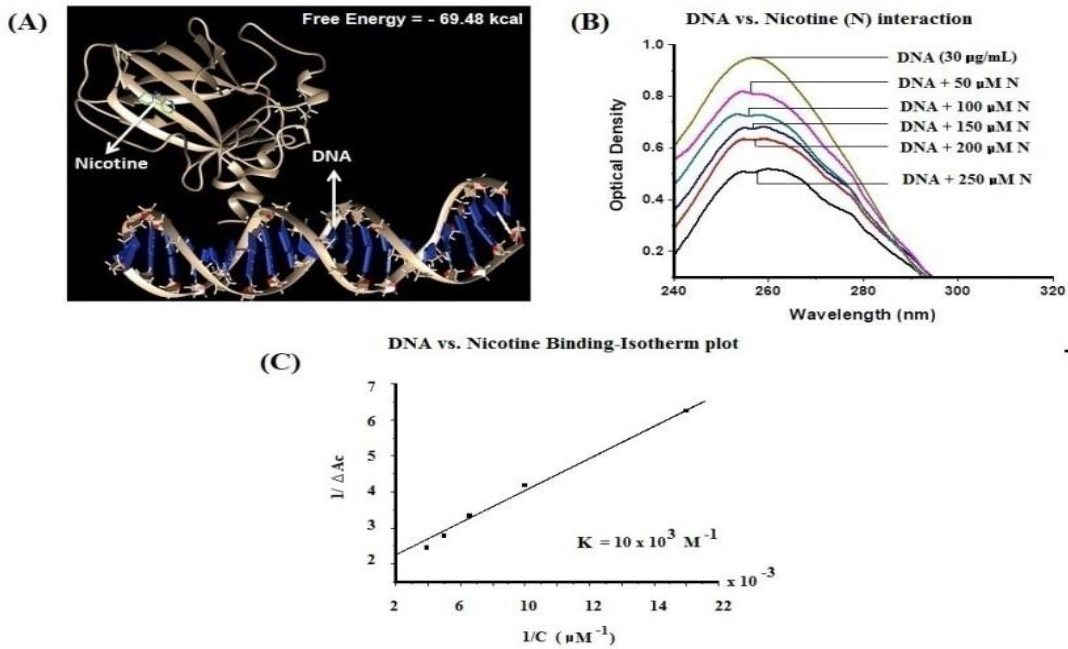


Figure 3: Interaction studies between nicotine vs. DNA.

Inset A shows the Molecular docking interaction between nicotine vs. DNA.

Inset B shows the UV-Vis spectra of interaction between nicotine vs. DNA.

Inset C shows the Binding -Isotherm Plot between nicotine vs. DNA.

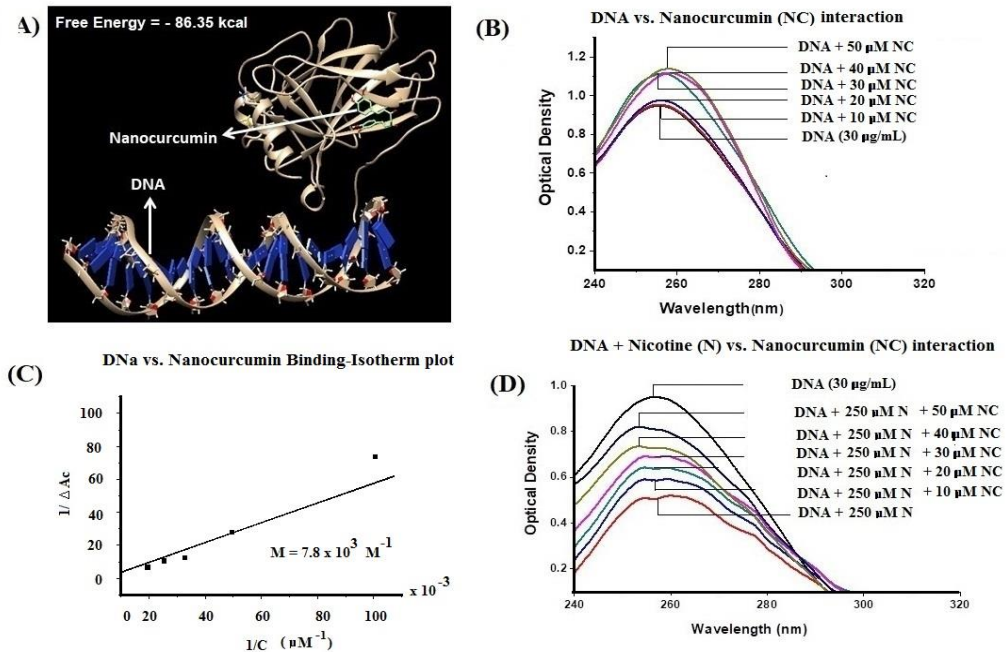


Figure 4: Interaction studies between nanocurcumin vs. DNA.

Inset A shows the Molecular docking interaction between nanocurcumin vs. DNA.
 Inset B shows the UV-Vis spectra of interaction between nanocurcumin vs. DNA.
 Inset C shows the Binding-Isotherm Plot between nanocurcumin vs. DNA.
 Inset D shows the UV-Vis spectra of interaction between nanocurcumin vs. nicotine + DNA.

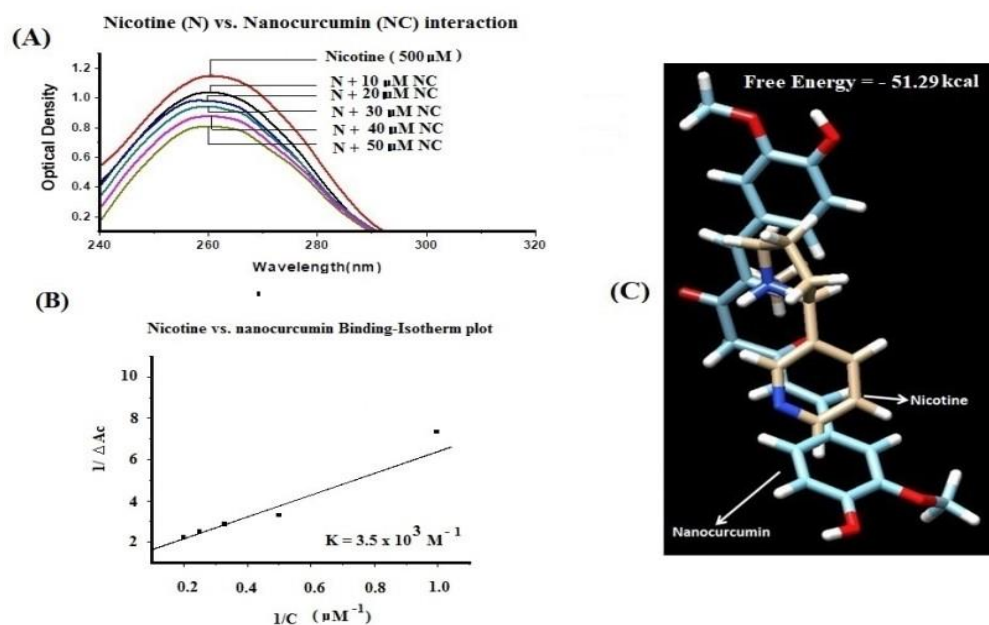


Figure 5: Interaction studies between nicotine vs. Nanocurcumin.

Inset A shows the UV-Vis spectra of interaction between nicotine vs. Nanocurcumin.
 Inset b shows the Binding-Isotherm Plot between nicotine vs. NANOCURCUMIN.
 Inset c shows the Molecular docking interaction between nicotine vs. nanocurcumin.

UV-Visible spectral studies between nicotine vs. haemoglobin interaction showed that nicotine bound with Hb (binding constant, $K = 6.5 \times 10^3 M^{-1}$) and completely suppressed the characteristics absorbance peak of Hb molecule at 400 nm (Fig. 1B). Whereas, nanocurcumin showed higher binding affinity (binding constant, $K = 10.4 \times 10^4 M^{-1}$) to Hb (Fig. 2B) and nullified the toxic effect of nicotine resulting reappearance of the characteristics absorbance peak of Hb at 400 nm. From UV-Visible spectral analysis between nicotine vs. DNA interaction (Fig. 3B) similarly showed that nicotine bound with DNA (binding constant, $K = 10 \times 10^3 M^{-1}$) and deformed the structural integrity of DNA resulting reduction of the characteristics absorbance maxima of DNA at 260 nm as well as produced multi absorbance maxima in the spectrum of DNA. Nanocurcumin showed similar binding affinity (binding constant, $K = 7.8 \times 10^3 M^{-1}$) with DNA (Fig. 4B) and reduced the toxic effect of nicotine on DNA due to which the characteristics absorbance spectrum of DNA was reappeared (Fig. 4B). Nanocurcumin also showed binding affinity to nicotine ($K = 3.5 \times 10^3 M^{-1}$).

Nanocurcumin did not show any negative effect on the characteristics absorbance maxima of Hb and DNA both rather it increased the absorbance maxima of Hb and DNA as seen from Fig. 2B and Fig. 4B respectively.

DISCUSSION

The broad range of pharmaceutical activities of curcumin is still not converted into clinical benefits because of its limited bioavailability and undesirable pharmacokinetics [18, 32]. Increased bioavailability of curcumin using a novel dispersion technology system has been demonstrated by Briskey et al. [33]. Increased efficacy of curcumin by formulation of nanocurcumin against nicotine-induced toxicity at cellular levels in rats is reported earlier [24]. The present study is another attempt to explore the superior efficacy of nanocurcumin against nicotine-induced genotoxicity on whole blood cells of female rats.

The study shows that nicotine causes a significant decrease in haemoglobin concentration in blood at normal condition. Earlier, Thomas and Lumb [34] have shown that binding of oxygen with haemoglobin is affected by carbon monoxide exerted from tobacco smoke, due to its greater binding affinity (>300 times) than that of oxygen. Banerjee et al. [9] have reported that nicotine reduces the RBC counts due to peroxidative membrane damage of erythrocytes resulting decrease in haemoglobin content of blood. In nicotine-haemoglobin docking, the amino residues such as TYR 42, ASN 97, PHE 98 and LEU 101 of haemoglobin molecule showed hydrophobic interaction with nicotine molecule and thus strengthen the nicotine haemoglobin complex structure. Similarly, in nanocurcumin-haemoglobin docking, nanocurcumin molecule can nicely accommodate into the haemoglobin's active site by forming one hydrogen bond with the residue His 58, SER 102, SER 133 of the protein. Besides this hydrogen bond, nanocurcumin also exhibited few hydrophobic interactions with the amino acid residues such as LYS 61, VAL 62, LEU 83, HIS 87, PHE 98 of haemoglobin molecule. The binding free energy between haemoglobin vs. nicotine was higher than that of nanocurcumin vs. haemoglobin. From the above docking experiment, it is clear that nanocurcumin binds more tightly with the haemoglobin molecule than nicotine did.

This study shows that nanocurcumin maintains the haemoglobin content of RBC cells against nicotine-induced stress due to its higher binding affinity to haemoglobin ($K = 10.4 \times 10^4 \text{ M}^{-1}$) than that of nicotine ($K = 6.5 \times 10^3 \text{ M}^{-1}$). It binds with Hb and resists nicotine to bind with Hb because of its higher binding affinity. Also, nanocurcumin possesses a binding affinity ($K = 3.5 \times 10^3 \text{ M}^{-1}$) to nicotine due to which it binds with the nicotine and reduces the free available nicotine molecules to interact with the RBC and causes less membrane damage. This may explain the increase of Hb concentration of the nicotine treated rat which were supplemented with nanocurcumin. Our study thus is in agreement with the earlier studies which show the efficacy of nanocurcumin against nicotine-induced toxicity [24] and confirms that nanocurcumin is more effective to increase the haemoglobin concentration of the blood and shows better protective efficacy towards haemoglobin [9].

Nicotine (N2) formed a hydrogen bond with SER 241 residue of p53 tumour suppressor protein. Other amino acids such as SER 240, ARG 248, ARG 249, ARG 273, VAL 274 etc. of p53 protein associated with DNA showed hydrophobic interaction with the nicotine molecule that strengthen the stability of nicotine-DNA complex. Likewise, in nanocurcumin-DNA docking, nanocurcumin molecule can nicely accommodate into the active site of p53 protein associated with DNA by forming one hydrogen bond with the residue GLN 165, ASN 247 of the protein.

Besides this hydrogen bond, nanocurcumin also exhibited few hydrophobic interactions with the amino acid residues such as ARG 248, CYS 176, HIS 179 of p53 protein associated with DNA molecule. The binding free energy between nicotine-DNA was determined as - 69.48 kcal, which was higher than that of nanocurcumin vs. DNA (- 86.35 kcal). This concludes that nanocurcumin binds more rigidly with the p53 protein associated DNA molecule than nicotine did and therefore nanocurcumin-DNA complex is more stable than nicotine-DNA complex. From the docking experiment of nicotine nanocurcumin interaction, it may be assumed that nanocurcumin might compete with nicotine while binding with a protein (Hb) and/or DNA and ameliorate nicotine-induced genotoxicity in whole blood cells.

The total DNA yield per 300 μ L of blood (109.38 μ g) under normal condition is also in good agreement with the value obtained previously [9]. The reduction of total DNA contents was due to the oxidative stress caused by nicotine. The decrease of total DNA concentration of the blood cells was more effectively ($p < 0.001$) resisted by nanocurcumin due to which the total DNA concentrations of the blood cells was almost restored to normal level in nanocurcumin supplemented condition as compared to that of native curcumin supplemented condition (Table 2). The observed minimal DNA damage of control blood cells was 2.76% (Table 2) which is acceptable, whereas, the average DNA damage in nicotine-treated rat was seen significantly higher (40% ; $p < 0.001$) in comparison to control (Table 3). Sanner and Grimsru [35] have shown that nicotine induces chromosomal aberration, sister chromatid exchange and single-strand DNA strand breaks due to oxidative stress. Banerjee et al.[9] have reported that nicotine increases the formation of free radicals and reactive oxygen species (ROS) resulting increased DNA damage in blood cells and liver tissues of female rats. They have also shown that curcumin effectively interacts with nicotine and DNA and also reduces the oxidative stress in nicotinic condition resulting less DNA damage. Here, it is observed that nanocurcumin increases the DNA content of whole blood cells against nicotine-induced toxicity. It has similar binding affinity to DNA ($K = 7.8 \times 10^3 \text{ M}^{-1}$) as nicotine ($K = 10.0 \times 10^3 \text{ M}^{-1}$). It therefore binds with DNA and resists nicotine to damage DNA. The binding affinity of nanocurcumin with nicotine is an added advantage to protect DNA from nicotinic attack. This study therefore suggests that nanocurcumin is an effective bio-molecule that can protect blood DNA against nicotine-induced genotoxicity.

From our wet lab study it is seen that nicotine has a strong interaction with Hb/DNA haemoglobin due to which the absorption peak of Hb/DNA is suppressed (Fig. 2 and Fig. 4). Nanocurcumin also shows very strong interaction with Hb (binding affinity of nanocurcumin is 10 times higher than that of nicotine when interacts with Hb) as well as it has an interaction with nicotine. These results of these interactions thus corroborate the molecular docking results and explain the protective action of nanocurcumin in Hb/DNA against nicotine treated condition. In presence of nanocurcumin, free nicotine molecules become less available to interact with Hb/DNA, as nicotine is out competed by nanocurcumin and this results in regain of structural integrity of Hb/DNA as seen from spectral analysis. The effective amelioration of nanocurcumin against nicotine-induced genotoxicity of whole blood cells of female rats thus may be described from our experimental results.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest of any kind related to this work.

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