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## Potential Amelioration of Curcumin Against Nicotine-induced Toxicity of Protein Malnourished Female Rats

<sup>1</sup>Surajit Sinha, <sup>1</sup>Moumita Maiti, <sup>2</sup>Krishna Chattopadhyay and <sup>1</sup>Brajadulal Chattopadhyay

<sup>1</sup>Department of Physics, Jadavpur University, Kolkata-700032, India

<sup>2</sup>Department of Chemical Technology, Calcutta University, Kolkata-700009, India

*Corresponding Author: Brajadulal Chattopadhyay, Department of Physics, Jadavpur University, Kolkata-700032, India*

### ABSTRACT

Nicotine plays an important role on reproductive disorders, cardio-vascular diseases, different cancers and destruction of defence system in human. Its effect in protein malnourished condition is very little understood. This investigation was designed to determine the nicotine-induced toxicity (subcutaneously injected at a dose of 2.5 mg kg<sup>-1</sup> b. wt. day<sup>-1</sup> for 21 days) on different tissues of female albino rats and corresponding ameliorative observations of curcumin (orally supplemented at a dose of 80 mg kg<sup>-1</sup> b. wt. day<sup>-1</sup> during the treatment) under protein malnourished condition. Nicotine causes more significant (p<0.001) increase of glucose level, depletion protein and alteration of triglycerides and cholesterol levels in serum. Disruption of liver function and alteration of phosphatase enzymes are more significantly (p<0.001) occurred in protein restricted condition due to nicotine treatment. Nicotine also decreases the activity of antioxidant enzymes, increases lipid peroxidation and affects the production of female hormones more significantly (p<0.001) in such condition. Nicotine disrupts the structural integrity of ovary and uterus that may hamper normal pregnancy. Curcumin effectively ameliorates the deleterious effects of nicotine in protein malnourished condition and may be used in the diet as potential blocker of nicotine to protect the health of malnourished female population.

**Key words:** Antioxidant, curcumin, gonadal hormones, nicotine, protein deficient

### INTRODUCTION

India ranks third among the most important tobacco producing countries next only to China and U.S.A. and India (NIOH, 2004). Along with the use of common tobacco forms consumed worldwide, such as cigarettes and pipes, the Indian population are also habituated with Chutta Beedi, (locally use hand made smoking elements) and chewing tobacco those are very common amongst them (Rahman *et al.*, 2003). Nicotine is the major constituent of tobacco (Wu *et al.*, 2002). It has also been implicated in pathologies such as delayed wound healing (Sorensen *et al.*, 2010), reproductive disorders (Windham *et al.*, 2005), cardiovascular disease (Adedeji and Etukudo, 2006; Frost-Pineda *et al.*, 2011) lung and oral cancer (Jung *et al.*, 2001) etc. Nicotine disrupts anti oxidant mechanism (Mary *et al.*, 2002) by enhancing Reactive Oxygen Species (ROS) production and thereby decreases antioxidant level causing peroxidative tissue damages (Ramesh *et al.*, 2007; Chattopadhyay and Chattopadhyay, 2008). The chemicals in tobacco smoke alter endocrine

function, perhaps at the level of the ovary which in turn effects release of female hormones. This endocrine disruption likely contributes to the reported associations of smoking with adverse reproductive outcomes, including menstrual dysfunction, infertility and earlier menopause (Windham *et al.*, 2005).

Nutritional factors play an important role in the metabolism and pharmacotoxicological activities of various drugs and xenobiotics (Campbell and Hayes, 1976). A dietary reduction in either quality or quantity of protein causes a depression of hepatic microsomal mixed function oxidase activities. According to Boyd (1969) these results have a direct application to the toxicity of drugs and chemical agents in persons whose dietary protein is low. The major nutritional disorders which are widely prevalent in all developing countries, are due to Protein Energy Malnutrition (PEM). This type of malnutrition affects all age groups and more so of poorer segments of the population. However, the repercussions are the strongest in children and women (Krishnaswami, 1987). Low dietary protein possesses a constraint on the biosynthetic activity, disposition and toxicity (Das *et al.*, 1996). Though it is already established that nicotine causes various damage in our body in normal dietary condition but the toxic effects of nicotine particularly in protein restricted dietary situation are still cause of concern.

Turmeric, cultivated in India, is not only used in Indian delicacies but also used extensively in Ayurveda, an ancient Indian medical system of preventive health care (Aggarwal *et al.*, 2007). Curcumin the main colouring component of turmeric possesses antitumeric (Abdel-Latif and Sadek, 1999), antihepatotoxic, antioxidant (Park *et al.*, 2000) and anti-inflammatory effect (Shehzad *et al.*, 2011). Curcumin also appears to be beneficial in preventing diabetes-induced oxidative stress in rats (Hussein and Abu-Zinadah, 2010). Reports say that administration of curcumin significantly lowers the biochemical marker enzymes, inhibits lipid peroxidation (Kalpana *et al.*, 2007) and enhances the antioxidant status (Kalpana and Menon, 2004). Recently, Tarasub *et al.* (2011) have shown that curcumin partially protects Cd-induced nephrotoxicity. Our study demonstrates that toxicity of nicotine on different tissues is aggravated under protein restriction condition and curcumin shows its potential to nullify those effects significantly under such condition.

## **MATERIALS AND METHODS**

**Chemicals:** Nicotine hydrogen tartrate and curcumin were purchased from Sigma Chemicals, St. Louis, USA. All other required chemicals were purchased from Genei. Bangalore, India and Spectrochem Pvt. Ltd. India. All the chemicals and reagents used were analytical grade.

**Animals and diet:** Thirty adult female albino rats of Wistar strain having body weights 120-130 g were procured at a time from the animal housing facility. The animals were acclimatized under standard conditions of temperature and humidity with 12 h light/dark cycles. They were maintained in accordance with the guidelines of the rule of Instructional Animal Ethics Committee of Jadavpur University, Kolkata, India (constituted as per the "Gazette of India "notification part II Sec. 3 (ii) 17 of the Ministry of Environment and Forestry, Government of India, dated 8th September 1998 for the "Prevention to cruelty to animal 1968"). They were housed to polypropylene cages and were fed standard pellet diet (Hindustan Lever Ltd., India) for 1 week and water ad libitum. Animals were divided equally into two groups (15 animals in each). One group was provided with normal protein diet (18% casein, 70% carbohydrate, 7% fat, 4% salt mixture and 1% vitamin mixture) and the other group with protein-restricted diet (5% casein, 83% carbohydrate,

7% fat, 4 % salt mixture and 1% vitamin mixture) according to Hawk *et al.* (1954). Each group was further divided into three subgroups (Control, Nicotine treated and Nicotine+Curcumin treated) having 5 animals in each. The animals in each group were maintained on their respective dietary regimen from 7 days before the treatment start till the completion of nicotine treatment.

**Mode of treatment:** After maintaining the animals in their respective dietary regimen for 7 days, nicotine tartrate (Sigma Chemical Company, St. Louis, MO, USA) dissolved in 0.9% saline (w/v) was injected subcutaneously to the rats of experimental (nicotine treated and nicotine+curcumin treated) subgroups at a dose of  $2.5 \text{ mg kg}^{-1} \text{ b. wt. daily}^{-1}$  for 21 days at 4 p.m. to avoid diurnal variation. The basis of dose selection was according to the previous study (Mandal *et al.*, 2004). The dilution of the drug was prepared in such a way that 0.5 mL of physiological saline contained the required dose of nicotine. Animals of nicotine+curcumin treated subgroups only received curcumin ( $80 \text{ mg kg}^{-1} \text{ b.wt. day}^{-1}$ ) orally at the same time (Bandyopadhyaya *et al.*, 2008). The animals in control subgroups of each group received subcutaneous injection of 0.5 mL physiological saline only at the same time.

**Sample collection:** After the last dose of injection received, animals were kept fasting overnight and sacrificed on the following morning by decapitation (Venkataraman *et al.*, 1984). Anesthetics have been reported to alter the hypothalamo-pituitary axis (Brown and Hegde, 1974). Blood was collected in sterilized tubes and serum was separated out after centrifugation and stored at  $-20^{\circ}\text{C}$  prior to further analysis. Liver, ovary and uterus were dissected, cleaned properly and stored for further investigations.

**Biochemical analysis:** Blood glucose was estimated through Contour T<sub>s</sub> (Bayer Health Care, Bayer Polychem, India Ltd.). Serum protein concentration was determined by Lowry *et al.* (1951). The lipid components such as Total Cholesterol (TC) (Allain *et al.*, 1974) and triglyceride (Werner *et al.*, 1981) were estimated in serum by using standard kits supplied by Ranbaxy Diagnostic Ltd., Mumbai, India. By the assay of thiobarbituric acid reactive substances (TBARS), serum and liver lipid peroxidation was measured (Chatterjee and Agarwal, 1998). The amount of MDA was calculated by taking the extinction coefficient of MDA to be  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ . Superoxide Dismutase (SOD) (Beauchamp and Fridovich, 1971), catalase (CAT) (Cohen *et al.*, 1970), Glutathione Peroxidase (GPx) (Levander *et al.*, 1983) and reduced Glutathione (GSH) (Davila *et al.*, 1991) content of liver and serum were determined in rats of all the four groups. Aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT) were measured by the method used by Reitman and Frankel (1957). The phosphatase activity in plasma was assayed according to the method of Basse *et al.* (1946).

**Estimation of steroidogenic hormones:** Estradiol is biologically the most active form of the naturally produced estrogen. The VIDAS Estradiol II and VIDAS Progesterone are two automated quantitative tests which were used for detection and measurement of the total  $17\beta$ -estradiol and progesterone respectively on the VIDAS instruments by using ELFA technique (Enzyme Linked Fluorescent Assay) (New and Used Lab Equipment-GMI Inc.).

**Histological study:** Cleaned tissues of ovary and uterus were fixed by using Bouin's fluid. After fixation, the tissues were washed several times by different graded alcohol to remove excess fluid

and then embedded in paraffin. Using of rotary microtome sliced the embedded tissues. The paraffin sections were then attached on the slide and washed by xylol before staining. The tissue section was then stained by using haematoxylin and eosin staining.

**Statistical analysis:** Whole experimental set up was repeated twice and data (n = 10) were averaged and given mean±SD. The statistical analysis of the data obtained from control, nicotine and nicotine+curcumin supplemented groups from both normal diet and protein restricted diet was performed by one way Analysis of Variance (ANOVA) along with all pair wise multiple comparison procedures (Holm-Sidak method) by using Sigma Stat (version 3.2). The significant levels of the observed data were determined at p<0.01 (significant) and p<0.001 (more or highly significant).

## RESULTS

Nicotine significantly (p<0.001) increased the blood glucose levels and decreased the serum protein at both dietary conditions (Table 1). Curcumin significantly (p<0.001) ameliorated the observed nicotine induced effects as shown in Table 1.

Both cholesterol and triglyceride of serum were significantly (p<0.001) increased due to nicotine treatment at both dietary conditions (Table 2). It was seen that the effect of nicotine was aggravated in protein-restricted condition. The amelioration of curcumin was more or less similar in both dietary conditions. Among the most sensitive and widely used liver enzymes are AST or

Table 1: Effect of nicotine and curcumin on blood glucose and serum protein

Group	Normal diet	Restricted diet
<b>Glucose (mg dL<sup>-1</sup>)</b>		
Control	76.92±4.22	77.15±6.90
Nicotine	116.38±6.34** (51.3↓)	110.03±6.26** (42.6↓)
Nicotine+curcumin	97.03 ±6.81*** (26.1↓)	97.01±6.03*** (25.7↓)
<b>Serum Protein (mg dL<sup>-1</sup>)</b>		
Control	6.46±1.31	6.13±1.30
Nicotine	4.47±0.43** (30.8↓)	3.75±0.55** (38.8↓)
Nicotine+curcumin	5.45±0.61** (15.6↓)	5.30±1.01** (13.5↓)

Values are given mean±SD (where n =10), \*Significant at p<0.01, \*\*More significant at p<0.001 with respect to control, †Significant at p<0.01, ††More significant at p<0.001 with respect to nicotine treated rats, the value with in the parenthesis are the percentage of increase (↑) or decrease (↓) with respect to the control value

Table 2: Effect of nicotine and curcumin on serum lipid component

Group	Normal diet	Restricted diet
<b>Cholesterol (mg dL<sup>-1</sup>)</b>		
Control	102.29±3.92	105.99±3.39
Nicotine	122.59±6.26** (19.8↑)	127.73±3.77** (20.5↑)
Nicotine+Curcumin	109.36±2.31††† (6.9↑)	115.01±5.56††† (8.5↑)
<b>Triglyceride (mg dL<sup>-1</sup>)</b>		
Control	53.78±2.79	53.30±3.67
Nicotine	70.66±2.52** (31.3↑)	72.56±2.55** (36.1↑)
Nicotine+Curcumin	59.84±1.69††† (11.2↑)	59.79±3.28††† (12.1↑)

Values are given mean±SD (where n =10), \*Significant at p<0.01, \*\*More significant at p<0.001 with respect to control, †Significant at p<0.01, ††More significant at p<0.001 with respect to nicotine treated rats, the value with in the parenthesis are the percentage of increase (↑) or decrease (↓) with respect to the control value

Table 3: Effect of nicotine and curcumin on liver function enzymes

Group	Normal diet	Restricted diet
<b>SGOT (IU L<sup>-1</sup>)</b>		
Control	84.76±4.16	90.95±6.04
Nicotine	106.62±5.98** (25.7↓)	117.64±7.46** (29.3↓)
Nicotine+curcumin	97.01±3.36** (14.4↓)	103.28±7.20*** (13.5↓)
<b>SGPT (IU L<sup>-1</sup>)</b>		
Control	64.47±4.62	73.19±5.19
Nicotine	82.14±4.80** (27.4↓)	96.17±4.48** (31.4↓)
Nicotine+Curcumin	72.25±3.82*** (12.0↓)	78.63±2.54*** (7.4↓)
<b>ALP (IU L<sup>-1</sup>)</b>		
Control	37.68±5.44	42.67±1.93
Nicotine	51.25±6.90** (36.0↓)	51.38±4.91** (20.4↓)
Nicotine+Curcumin	41.24±2.10*** (9.4↓)	49.53±3.76*** (16.0↓)
<b>ACP (IU L<sup>-1</sup>)</b>		
Control	10.04±1.25	9.89±0.56
Nicotine	15.80±1.42** (57.4↓)	17.42±2.59** (76.1↓)
Nicotine+Curcumin	12.46±1.22*** (24.1↓)	11.20±1.28*** (13.2↓)

Values are given mean±SD (where n =10), \*Significant at p<0.01, \*\*More significant at p<0.001 with respect to control, †Significant at p<0.01, †† More significant at p<0.001 with respect to nicotine treated rats, the value with in the parenthesis are the percentage of increase (↑) or decrease (↓) with respect to the control value

Table 4: Effect of nicotine and curcumin on superoxide dismutase

Group	Normal diet	Restricted diet
<b>SOD (n mol O<sub>2</sub> decomposed/min/100 mg protein)</b>		
Serum		
Control	9.72±0.78	9.03±0.44
Nicotine	6.35±1.03** (34.7↓)	6.05±1.13** (33.0↓)
Nicotine+Curcumin	8.53±0.49*** (12.2↓)	8.69±0.52*** (3.7↓)
Liver		
Control	3.42±0.56	3.57±0.33
Nicotine	2.04±0.37** (40.4↓)	1.88±0.63** (47.3↓)
Nicotine+Curcumin	3.16±0.24*** (7.6↓)	2.85±0.40*** (20.2↓)

Values are given mean±SD (where n =10), \*Significant at p<0.01, \*\*More significant at p<0.001 with respect to control, †Significant at p<0.01, †† More significant at p<0.001 with respect to nicotine treated rats, the value with in the parenthesis are the percentage of increase (↑) or decrease (↓) with respect to the control value

SGOT and ALT or SGPT. These enzymes were increased significantly (p<0.001) in serum of nicotine treated rats at both dietary conditions (Table 3). The increments of ACP were more significant (p<0.001) at protein-restricted condition. Where as higher concentrations of ALP was observed in normal diet fed rat (Table 3). Curcumin supplementation showed better attenuation against nicotine-induced effect on liver enzymes and ACP in protein restriction as reflected by Table 3.

Considerable (p<0.001) decreased SOD activity of serum and liver tissues both in both dietary conditions was seen in nicotine treatment (Table 4). Similar effect of nicotine was also observed in case of other antioxidant enzyme i.e., CAT (Table 5). Curcumin was found to be more active against nicotine-induced effect on antioxidant enzymes particularly in protein restriction condition (Table 4, 5).

Table 5: Effect of nicotine and curcumin on catalase

Group	Normal diet	Restricted diet
<b>CAT (n mol H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein)</b>		
Serum		
Control	8.89±0.71	8.62±0.56
Nicotine	5.69±0.55** (36.0↓)	5.33±0.64** (38.2↓)
Nicotine+Curcumin	7.46±0.43*** (16.1↓)	7.63±0.50*** (11.5↓)
Liver		
Control	28.78±2.83	28.00±2.26
Nicotine	19.68±1.47** (31.6↓)	18.79±2.24** (32.9↓)
Nicotine+Curcumin	23.44±2.11** (18.6↓)	26.06±2.21*** (6.9↓)

Values are given mean±SD (where n =10), \*Significant at p<0.01, \*\*More significant at p<0.001 with respect to control, †Significant at p<0.01, †† More significant at p<0.001 with respect to nicotine treated rats, the value with in the parenthesis are the percentage of increase (↑) or decrease (↓) with respect to the control value

Table 6: Effect of nicotine and curcumin on lipid peroxidation

Group	Normal diet	Restricted diet
<b>MDA (nmol mL<sup>-1</sup>)</b>		
Serum		
Control	5.18±0.40	4.75±0.54
Nicotine	8.26±1.11** (59.5↑)	8.26±0.66** (73.9↑)
Nicotine+Curcumin	5.96±0.41*** (15.1↓)	5.29±0.54*** (11.4↓)
Liver		
Control	18.36±1.32	19.41±2.64
Nicotine	26.20±1.84** (42.7↑)	28.20±2.58** (45.3↑)
Nicotine+Curcumin	22.99±1.91*** (25.2↑)	24.01± 1.88*** (23.7↑)

Values are given mean±SD (where n =10), \*Significant at p<0.01, \*\*More significant at p<0.001 with respect to control, †Significant at p<0.01, †† More significant at p<0.001 with respect to nicotine treated rats, the value with in the parenthesis are the percentage of increase (↑) or decrease (↓) with respect to the control value

Table 7: Effect of nicotine and curcumin on reduced glutathione of serum

Group	Normal diet	Restricted diet
<b>GSH (µg mg<sup>-1</sup> protein)</b>		
Control	37.19±2.77	40.85±2.06
Nicotine	28.16±2.21** (24.3↓)	30.11±3.01** (26.3↓)
Nicotine+Curcumin	32.00±1.75*** (14.0↓)	35.27±2.37*** (13.7↓)

Values are given mean±SD (where n =10), \*Significant at p<0.01, \*\*More significant at p<0.001 with respect to control, †Significant at p<0.01, †† More significant at p<0.001 with respect to nicotine treated rats, the value with in the parenthesis are the percentage of increase (↑) or decrease (↓) with respect to the control value

Nicotine more significantly (p<0.001) increased lipid peroxidation in restricted diet fed rats for which higher MDA levels were observed in serum and liver tissues of the rats (Table 6). Again amelioration of curcumin was more significantly (p<0.001) noted in both dietary conditions.

More significant (p<0.001) decreased of glutathione (Table 7) and significant (p<0.01) decreased of GPx (Table 8) in serum were seen due to nicotine treatment on rats of both dietary groups. Curcumin increased the glutathione and GPx levels as seen from Table 7 and 8.

Nicotine caused more significant (p<0.001) reduction of female sex hormones (17β-estradiol and progesterone) in protein-restricted condition as seen in Table 9. Curcumin was found to be more protective to 17β-estradiol than progesterone against nicotine-induced effect in both dietary conditions.

Table 8: Effect of nicotine and curcumin on glutathione peroxidase of serum

Group	Normal diet	Restricted diet
<b>GPx (nmole/min/mg protein)</b>		
Control	150.96±3.80	164.82±6.36
Nicotine	134.04±3.18* (11.2↓)	143.08±8.63* (13.2↓)
Nicotine+Curcumin	142.62±3.15** (5.5↓)	154.34±2.21** (6.3↓)

Values are given mean±SD (where n =10), \*Significant at p<0.01, \*\*More significant at p<0.001 with respect to control, †Significant at p<0.01, ‡ More significant at p<0.001 with respect to nicotine treated rats, the value with in the parenthesis are the percentage of increase (↑) or decrease (↓) with respect to the control value

Table 9: Effect of nicotine and curcumin on steroidogenic hormones

Group	Normal diet	Restricted diet
<b>17β-estradiol (pg mL<sup>-1</sup>)</b>		
Control	69.94±4.08	63.52±4.51
Nicotine	56.10±3.50** (19.8↓)	48.46±3.63** (23.7↓)
Nicotine+Curcumin	63.06±4.29*† (9.8↓)	57.33±3.17*** (9.7↓)
<b>Progesterone (ng mL<sup>-1</sup>)</b>		
Control	8.64±0.59	6.93±1.14
Nicotine	4.97±1.08** (42.5↓)	3.18 ± 0.76** (54.1↓)
Nicotine+Curcumin	6.34±0.67*** (26.6↓)	4.86±0.36*** (29.9↓)

Values are given mean±SD (where n =10), \*Significant at p<0.01, \*\*More significant at p<0.001 with respect to control, †Significant at p<0.01, ‡ More significant at p<0.001 with respect to nicotine treated rats, the value with in the parenthesis are the percentage of increase (↑) or decrease (↓) with respect to the control value

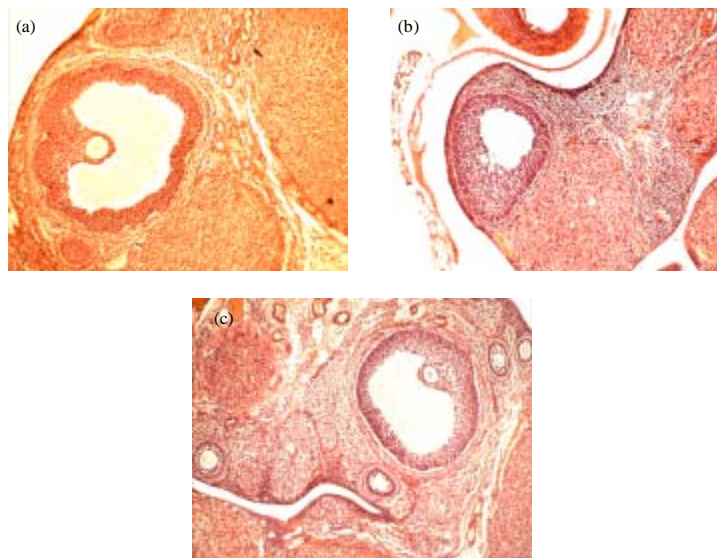


Fig. 1(a-c): Histological section of ovary (100x) of rat maintained with normal diet, (a) Control group, (b) Nicotine treated group, (c) Nicotine treated and curcumin supplemented group

Graafian follicles with an oocyte were observed in the ovarian cross section of both control groups of rats (Fig. 1a and 2a). There was no Graafian follicles were seen in nicotine treated



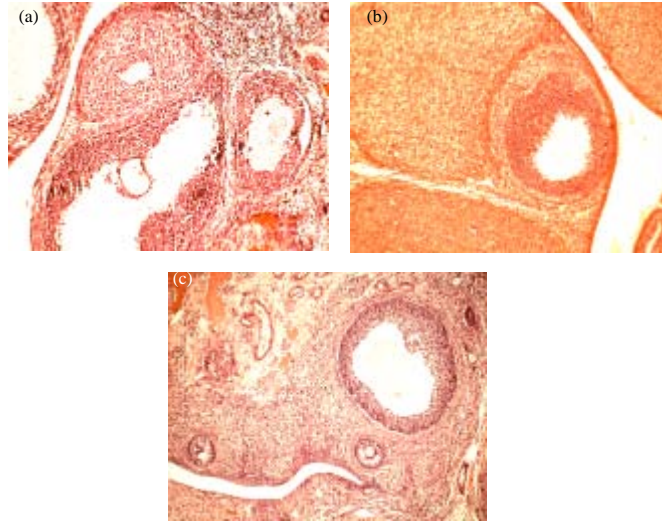


Fig. 2(a-c): Histological section of ovary (100x) of rat maintained with protein-restricted diet, (a) Control group, (b) Nicotine treated group, (c) Nicotine treated and curcumin supplemented group

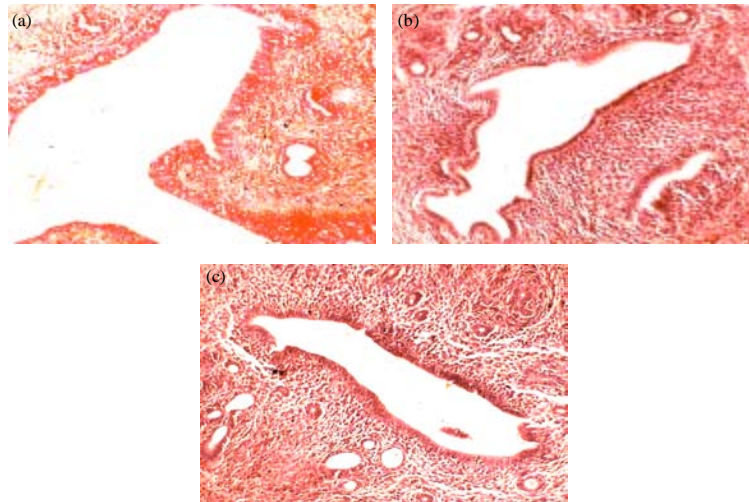


Fig. 3(a-c): Histological section of uterus (100 x) of rat maintained with normal diet, (a) Control group, (b) Nicotine treated group, (c) Nicotine treated and curcumin supplemented group

ovarian sections of both groups (Fig. 1b, 2b). Nicotine inhibits the normal growth of endometrium wall in the uterus as a result of which thinner endometrium wall is observed in the nicotine treated uterus section (Fig. 3b, 4b) than control groups (Fig. 3a, 4a). Curcumin treatment restores the normal structure of ovary (Fig. 1c, 2c) and uterus (Fig. 3c, 4c) to some extent in both dietary conditions.

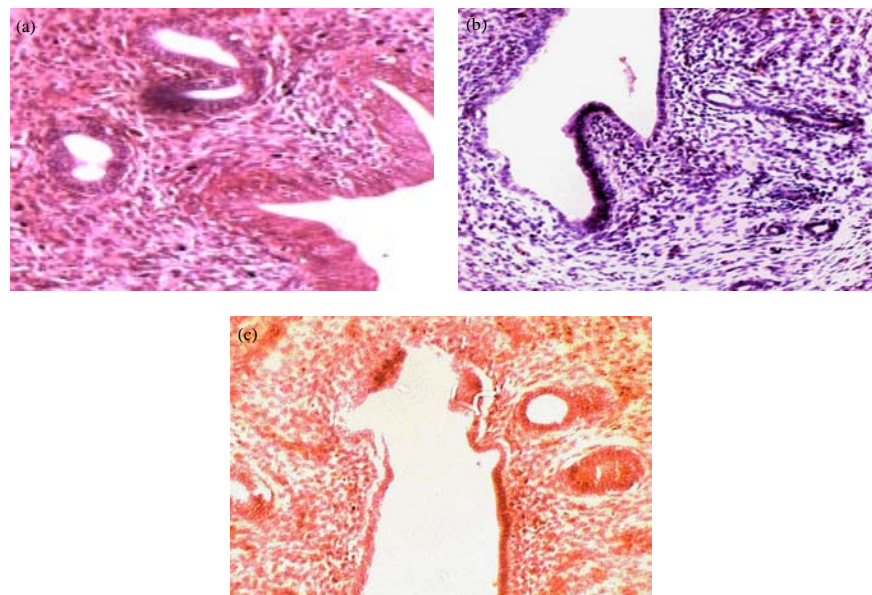


Fig. 4(a-c): Histological section of uterus (100 x) of rat maintained with normal diet, (a) Control group, (b) Nicotine treated group, (c) Nicotine treated and curcumin supplemented group

## DISCUSSION

In the present study a significant ( $p < 0.001$ ) elevation of blood glucose level is observed in both groups of animals exposed to nicotine insult (Table 1). Probably this elevation is due to the immediate response to tremendous physiological stress caused by nicotine or by impaired action of insulin. Nicotine induces the increased production of cAMP in blood (Al-Wadei *et al.*, 2009). Increased cAMP in blood stimulates gluconeogenesis and glycogenolysis (Xie *et al.*, 2000) as a result of which the blood glucose level is increased. Fujiwara *et al.* (2008) have shown that the curcumin possesses anti-diabetic property which is partly due to a reduction in hepatic glucose production caused by activation of AMP kinase and inhibition of G6Pase activity and PEPCK activity. The reduction of blood glucose level by curcumin may be due to its anti-diabetic effect which shows an additive inhibitory effect with insulin on both hepatic gluconeogenesis and glycogenolysis. This result corroborates with the finding of Sivabalan and Anuradha (2010). Nicotine decreases total protein concentration in serum but the decrement is higher in protein-restricted diet (38.8%) than normal protein diet (30.8%) fed rats as seen from Table 1. We have already reported that nicotine aggravates DNA damage under protein-restricted condition (Bandyopadhyaya *et al.*, 2008; Banerjee *et al.*, 2010). The reduced protein concentration in serum may be due to inhibition of protein synthesis caused by DNA damage as well as to low protein content supplied in the diet. The antagonizing effect of curcumin is due to the interaction of curcumin with nicotine as explained earlier (Banerjee *et al.*, 2010).

Cholesterol is an extremely important biological molecule that has roles in membrane structure as well as being a precursor for the synthesis of the steroid hormones and bile acids. Nicotine stimulates catecholamine synthesis which lipolysis adipose tissue resulting an increment in the level of serum cholesterol as well as of triglyceride (Balakrishnan and Menon, 2007). Our findings

of significantly ( $p < 0.001$ ) increased levels of serum cholesterol and triglyceride of the nicotine treated rats under both dietary conditions are in agreement with earlier studies (Balakrishnan and Menon, 2007; Chattopadhyay and Chattopadhyay, 2008). In curcumin treated group the level of cholesterol is decreased probably due to the increase in the CYP7A1 gene expression which is a rate limiting enzyme in the biosynthesis of bile acid from cholesterol as suggested by Kim and Kim (2010). Curcumin showed an obvious hypocholesterolemic effect that could be due to an effect on total cholesterol absorption (Hasimun *et al.*, 2011), degradation or elimination (Arafa, 2005). Triglyceride lowering effect is probably due to multiple inductions of fatty acid catabolism (Asai and Miyazawa, 2001).

Increased activities of AST and ALT by nicotine administration (Table 2) indicate liver tissue damage that is occurred due to loss of functional integrity of cells membranes (Balakrishnan and Menon, 2007; Wetscher *et al.*, 1995). These enzymes are normally contained within liver cells. If the liver is injured or damaged, the liver cells spill these enzymes into the blood, raising the enzyme levels in the blood and signaling the liver disease. Curcumin decreases the levels of the liver enzymes by preserving the structural integrity of the cell membrane (Al-Jassabi and Azirun, 2010). Acid phosphatase can free attached phosphate groups from other molecule during digestion. Basically it is a phosphomonoesterase. Nicotine increases the ACP and ALP by oxidative stress providing tissue injury and directly involved in the generation of reactive oxygen species (ROS) as explained by Mohammad and Ahmad (2010). In Curcumin treated group, both ACP and ALP levels are decreased probably due to improve in antioxidant status (Table 3).

Nicotine is a highly addictive alkaloid induced oxidative stress both *in vivo* and *in vitro* (Mahapatra *et al.*, 2009). It has been reported that the nicotine disrupts the mitochondrial respiratory chain leading to an increase generations of super oxide ions and hydrogen peroxide. (Yildiz *et al.*, 1998). Superoxide anion and hydrogen peroxide are the main sources of the nicotine induced free radical generation and depletion of the cellular antioxidant (Chakraborty *et al.*, 2010). Glutathione being an important cellular reductant involves in protection against free radicals, peroxides and toxic compounds (Grester, 1995). Therefore depletion of GSH not only impairs cell defense against toxic compounds but also results in enhanced oxidative stress and tissue damage (Sener *et al.*, 2007; Sreekala and Indira, 2009). Our observation shows that nicotine treatment more significantly ( $p < 0.001$ ) depletes GSH level of serum of both dietary groups indicating higher level of tissue damage (Table 7). GPx decomposes excess hydro peroxides including hydrogen peroxide ( $H_2O_2$ ). Decreased level of GPx also confirms that nicotine challenges the cell defense mechanism and disrupts antioxidative activities (Table 8). The ameliorative effect of curcumin is due to its scavenging or neutralizing free radicals activities that inhibits peroxidation of membrane lipids and maintains cell membrane interiority and functions (Ritukumari *et al.*, 2002).

Smoking has multiple effects on hormone secretion which is mediated by nicotine Kapoor and Jones (2005). Nicotine induces significant ( $p < 0.001$ ) reduction of  $17\beta$ -estradiol and progesterone levels in both experimental groups. It is observed that percentage decrease of plasma  $17\beta$ -estradiol and progesterone levels during protein restriction are greater than the normal diet fed groups (Table 9). Proteins are the building blocks for enzyme biosynthesis. It may be conjectured that low dietary level of protein causes a decrease in the synthesis of steroidogenic enzyme, resulting in lowering of steroid hormone levels at restricted diet control groups. This is further aggravated by exposure to nicotine.  $17\beta$ -estradiol level was decreased in nicotine treated group due to an anti estrogenic effect of nicotine which affected in hepatic estrogen metabolism (Michnovicz *et al.*, 1986; Kapoor and Jones, 2005). Smoking has a powerful effect on the 2-hydroxylation pathway of

estradiol metabolism leading to increased production of 2-hydroxyestrogens. These compounds have minimal estrogenic activity and are rapidly cleared from the circulation. Nicotine also decreases progesterone level by increasing PGF2 $\alpha$ , an important factor for luteolysis and also by increasing VEGF mRNA expression (Miceli *et al.*, 2005). Curcumin reduces the effect in nicotine treated group due to its high estradiol binding capacity (Zava *et al.*, 1998). In Curcumin treated group progesterone level is increased because curcumin inhibits microvascular endothelial cell angiogenesis through inhibition of COX-2 expression (Binion *et al.*, 2008).

By the histological studies, we have seen that there is marked decrease in growth of follicles in ovary under both normal and restricted dietary conditions due nicotine challenge. Graafian follicles with an oocyte are observed in the ovarian cross section in both normal groups of rats. But no graafian follicles are observed in the ovarian cross section when the animals are exposed in nicotine. This is probably due to the decrement of estrogen level in nicotine treatment. Growth of follicles are increased as well as oocyte appeared with normal form in the ovary after curcumin supplementation (Fig. 1) which indicates that curcumin restores the normal ovarian function by minimizing the toxic effect of nicotine which in turns increases estrogen levels (Table 9). According to Soares *et al.* (2007), heavy smoking disrupts the stability of cells in the lining of uterus differently which overall reduces general pregnancy rate. He also reports that there is a change of endometrium in heavy smokers. In our finding it is noted that endometrium of the uterus of nicotine treated rats becomes thinner than that of control groups of both dietary conditions (Fig. 2). Curcumin regains the normal structure and architecture of endometrium as a result of which the endometrium becomes thick. Many scientists have already shown that curcumin has a broad range of biological activities which are beneficial for our health (Foda *et al.*, 2007; Srivastava *et al.*, 2011). Our results are in line with the observations reported earlier.

## CONCLUSION

Low dietary protein induces a stress in the body. Stress and nicotine therefore synergistically heightens the immunological disorder in protein-restricted condition. Curcumin effectively antagonizes the deleterious effects of nicotine and be used in the diet as potential blocker of nicotine to protect the female population at malnourished condition.

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