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EFFECT OF EXOGENOUS SELENIUM ON NICOTINE INDUCED HYPERLIPIDEMIA IN RATS

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Abstract : The effect of two different doses (1 µg Se/Kg and 50 µg Se/Kg Body wt) of selenium on nicotine induced hyperlipidemia was investigated in rats. Results revealed that nicotine intake caused an increase in concentration of cholesterol, triglycerides, free fatty acids, phospholipids and low density lipoprotein compared to control group. Coadministration of selenium along with nicotine reduced the levels of lipids compared to nicotine group. This reduction was due to reduction in the biosynthesis of lipids as evidenced by the reduced activity of HMGCoA reductase and lipogenic enzymes. Nicotine intake also reduced the absorption of selenium in the intestine. Histopathological studies revealed that selenium at a dose of 1 µg was more effective in reducing lipid levels and higher dose of selenium was toxic.

Key words : selenium nicotine hyperlipidemia HMGCoA reductase cholesterol

INTRODUCTION

Cigarette smoking causes harmful cardiovascular and atherogenic effects resulting from changes in lipid metabolism (1). There are numerous harmful substances found in the tobacco smoke and nicotine is one of the most toxic component in the tobacco smoke (2). Russel et al conducted studies on the human plasma nicotine levels after chewing nicotine gum and smoking cigarettes (3). Results showed that the average plasma nicotine concentration while taking 2-mg nicotine chewing-gum was only 10.8 ng/ml compared with 30.4 ng/ml two minutes after smoking a cigarette of nicotine content (3). We have observed in a previous study that the administration of nicotine at a dose of 0.6 mg for sixty days raised the plasma level of nicotine to 3.17×10^{-14} µg/ml and its metabolite cotinine to 3.64×10^{-14} µg/ml in experimental rats (4). Chronic smokers are at increased risk of developing atherosclerotic vascular disease, myocardial infarction, unstable angina and sudden death (5). Adverse effects of smoking on the cardiovascular system include a reduction in high-density lipoprotein (HDL) cholesterol, an increase in platelet reactivity and an increase in fibrinogen concentrations (5). These effects on systemic and coronary hemodynamics, lipid metabolism and

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hemostasis may contribute to the long-term adverse consequences of smoking (5). Habitual smoking increases plasma levels of glycerol as well as nor adrenaline, which is the main stimulating hormone of adipose tissue lipolysis (6).

Selenium is an essential trace element that is an integral part of many selenoproteins (7). It has been observed that selenium has an antiatherogenic action and suppresses peroxidation of lipids (8). Administration of selenium suppressed the amount of triglyceride, total cholesterol, free fatty acid and low density lipoprotein cholesterol (LDL-C) in the serum of rats fed with high cholesterol diet (9). Previous studies in our laboratory have also shown that selenium administration reduces alcohol and nicotine induced toxicity in the testis (4, 10). Our studies have also shown that coadministration of selenium and ascorbic acid provide protection against alcohol induced oxidative stress and hyperlipidemia (11, 12).

So the present studies were aimed to study the impact of a high and low dose of selenium on nicotine-induced hyperlipidemia in experimental rats.

MATERIAL AND METHODS

Animals

Male albino rats (Sprague Dawley strain) weighing between 90 and 110 g were divided into six groups of six rats each. Animals were housed in polypropylene cages. Cages were kept in a room that was maintained between 28 and 32°C. The light cycle was 12 h light and 12 h dark. Rats were fed with rat feed (Lipton India Ltd.). Food and water was given ad libitum. Animal experimentation was conducted in accordance with the institutional ethical committee guidelines for the conduct of the experiments on laboratory animals and as per the guide lines of CPCSEA, India.

Sodium selenite was purchased from M/s Sigma Aldrich Ltd. and nicotine was purchased from M/s Acros Organics Ltd., Belgium respectively. Sodium selenite and nicotine was administered as detailed below. Sodium selenite was freshly dissolved in distilled water and was given orally by gastric tube. Nicotine was dissolved in physiological saline (pH 7.4) and was given intravenously. The duration of the experiment was 60 days.

Experimental design

Group I – Control) :

Group II – Nicotine treated rats (0.6 mg nicotine/kg body wt);

Group III – High dose of selenium treated rats (50 µg sodium selenite/100 g body wt)

Group IV – Nicotine+High dose of selenium (0.6 mg nicotine/ kg, body wt + 50 µg sodium selenite/100 g body wt);

Group V – Low dose of selenium treated rats (1 µg sodium selenite/ 100 g body wt);

Group VI – Nicotine+Low dose of selenium (0.6 mg nicotine/ kg, body wt + 1 µg sodium selenite/100 g body wt)

At the end of the experimental period rats were fasted overnight and sacrificed. Blood, liver and kidney were removed to ice cold containers for various estimations.

Biochemical analysis

The liver, kidney and serum of experimental rats were extracted for lipid estimation according to the procedure of Folch et al (13). From the aliquot of extract cholesterol was estimated by the method of Abell et al (14). Triglyceride was estimated by the method of Van Handel and Zilversmith (15). Fatty acid was estimated by the method of Falholt et al (16). Phospholipid was estimated by the method of Zilversmith and Davis (17). HMG CoA reductase was estimated by the method of Rao and Ramakrishnan (18). Serum HDL and LDL+VLDL were separated by the heparin manganese precipitation method according to the procedure of Warnick and Albers (19). Glucose 6-phosphate dehydrogenase was assayed by the method of Kornberg and Horecker (20). Malate dehydrogenase was assayed by the method of Mehler et al (21). Tissue protein was estimated by the method of Lowry et al (22).

Absorption of selenium in the intestine was studied by the everted sac technique described by the Wilson and Wise man (23). For histopathological study liver fixed in Bouins fixative was embedded in the paraffin wax and sections were taken in the microtome. Sections were stained using haematoxylin and eosin. The pathological changes were examined using a sensitive light microscope.

Statistical analysis

The results were analyzed using a statistical programme SPSS/PC+, version 5.0 (SPSS Inc, Chicago, IL, USA). A one-way ANOVA was employed for comparison among the six groups. Duncan's post-hoc multiple comparison tests of significant differences among groups were determined, $P<0.05$ was considered to be significant.

RESULTS

The concentration of cholesterol (Table I), increased significantly in the liver, kidney and serum of nicotine treated rats compared to control group. Supplementation of selenium at both the levels (50 µg and 1 µg) decreased the concentration of cholesterol compared to nicotine group. Although co-administration of both the doses of selenium and nicotine reduced the cholesterol level, the reduction was more pronounced in the low dose group.

The concentration of HDL-cholesterol (Table I) was significantly reduced and the concentration of LDL+ VLDL-cholesterol was significantly enhanced in nicotine group compared to control. Selenium administration at both the levels increased the concentration of HDL-C and decreased the concentration of LDL+VLDL-C compared to nicotine group. Co administration of both the doses of selenium and nicotine significantly increased the concentration of HDL-C and decreased the concentration of LDL+VLDL-C; But the decrease was lesser in the high dose group in comparison with the low dose group.

TABLE I: Concentration of cholesterol.

Groups	Liver (mg/100 g wet tissue±SD)	Kidney (mg/100 g wet tissue±SD)	Serum (mg/100 ml serum±SD)	HDL-C (mg/100 ml serum±SD)	LDL+VLDL–C (mg/100 ml serum±SD)
I	358.25±39.57[a]	360.10±24.02[a]	75.50±2.76[a]	51.41±4.69[a]	24.09±2.08[a]
II	979.57±89.38[b]	484.89±44.25[b]	160.65±8.6[b]	18.72±1.20[b]	141.93±7.40[b]
III	297.18±26.24[c]	251.59±18.39	67.65±2.35	52.53±4.85[c]	15.12±1.20
IV	704.85±83.39[d]	351.27±32.06[d]	148.50±6.24[d]	22.38±2.15[d]	122.19±4.15[d]
V	329.58±33.21[e]	260.73±21.61[e]	72.59±3.82[e]	49.63±3.32[e]	22.96±1.50[e]
VI	559.75±51.09[f]	289.18±28.56[f]	139.23±6.04	36.31±3.09[f,g]	102.92±3.15[f,g]

[a]-P<0.05 between I and II group; [b]-P<0.05 between II and III group; [c]-P<0.05 between I and III group; [d]-P<0.05 between III and IV group; [e]-P<0.05 between II and V group; [f]-P<0.05 between IV and VI group. [g]-P<0.05 between V and VI group. Values expressed in mean±SD
Liver cholesterol (F-72.458), Kidney cholesterol (F-76.432), Serum cholesterol (F-106.858), HDL-C (F-13.442), LDL+VLDL–C (F-147.463).

The concentration of triglycerides (Table II) was enhanced significantly in the liver, kidney and serum of nicotine treated rats compared to control group. Administration of selenium at both the levels reduced the concentration of triglycerides compared to nicotine group. Co administration of both the doses of selenium and nicotine decreased the triglyceride concentration; but the decrease was less pronounced in the high dose group.

The concentration of free fatty acids (Table III), increased significantly in the liver, kidney and serum of nicotine treated rats compared to control group. Supplementation of selenium at both the levels decreased the concentration of free fatty acids compared to nicotine group. Although co administration of both the doses of selenium and nicotine reduced the free fatty acid level; the reduction was more in the low dose group.

The concentration of phospholipids (Table IV) was increased significantly in the liver, kidney and serum of nicotine treated rats

TABLE II: Concentration of triglycerides.

Groups	Liver (mg/100 g wet tissue±SD)	Kidney (mg/100 g wet tissue±SD)	Serum (mg/100 ml serum±SD)
I	370.20±35.19[a]	62.75±5.50[a]	6.27±0.57[a]
II	950.25±72.50[b]	206.55±22.78[b]	18.62±1.69[b]
III	254.58±22.34[c]	52.41±4.43[c]	3.55±0.33
IV	657.24±46.13[d]	157.86±19.35[d]	12.11±2.09[d]
V	339.20±30.09[e]	58.95±4.62[e]	4.51±0.41[e]
VI	534.52±52.66[g]	136.59±15.2[g]	9.14±1.06[g]

Foot notes same as in Table I; Values expressed as mean±SD.
Liver TG (F-243.97), Kidney TG (F-222.10), Serum TG (F-113.27).

TABLE III: Concentration of free fatty acids.

Groups	Liver (mg/100 g wet tissue±SD)	Kidney (mg/100 g wet tissue±SD)	Serum (mg/100 ml serum±SD)
I	280.23±23.20[a]	185.21±13.28[a]	2.153±0.19[a]
II	873.65±66.60[b]	473.93±34.95[b]	7.92±0.723[b]
III	154.68±12.36[c,h]	135.13±10.19[c,h]	1.45±0.10[c,h]
IV	601.34±40.23[d]	310.49±21.47[d]	5.15±0.470[d]
V	189.26±18.09[e]	148.43±12.07[e]	1.83±0.16[e]
VI	464.57±32.56[f,g]	220.91±17.07[f,g]	4.47±0.407[f,g]

Foot notes same as in Table I; [h]-P<0.05 between III and V group. Values expressed as mean±SD.
Liver Free fatty acid (F-243.97), Kidney Free fatty acid (F-203.91), Serum Free fatty acid (F-228.52).

TABLE IV: Concentration of phospholipids.

Groups	Liver (mg/100 g wet tissue±SD)	Kidney (mg/100 g wet tissue±SD)	Serum (mg/100 ml serum±SD)
I	2801.95±164.95[a]	2450.5±211.5[a]	155.80±14.2[a]
II	6029.98±185.82[b]	7766.47±708.76[b]	424.31±38.71[b]
III	1075.07±79.84[c]	1207.8±110.23[c]	121.16±11.05[c]
IV	4092.29±373.4[d]	5986.87±546.3[d]	273.25±24.93[d]
V	1402.13±127.95[e]	1944.15±177.43[e]	145.91±13.32[e]
VI	3811.56±347.84[g,h]	1269.12±188.6[f]	220.27±19.89[g]

Foot notes same as in Table I; Values expressed as mean±SD.
Liver phospholipid (F-229.43), Kidney phospholipid (F-111.977), Serum phospholipid (F-152.401)

compared to control group. Selenium administration at both the levels decreased the concentration of phospholipids compared to nicotine group. Co administration of both the doses of selenium and nicotine decreased the phospholipid level; but the decrease was less pronounced in the high dose group.

The HMG CoA/mevalonate ratio (Table V) was measured. Lower the ratio indicates higher enzyme activity. The activity of HMG CoA reductase was significantly increased in the liver and intestine of nicotine treated group compared to control group. Supplementation of selenium at both the levels slightly decreased the activity compared to nicotine group. Although co administration of both the doses of selenium and nicotine slightly decreased the activity; the reduction was comparatively higher in the low dose group.

TABLE V: Activity of HMG CoA reductase.

Groups	Liver (HMG/mevalonate)	Intestine (HMG/mevalonate)
I	7.90±0.72[a]	7.53±0.69[a]
II	5.25±0.47[b]	5.36±0.48[b]
III	13.49±1.23[c]	11.60±1.06[c]
IV	12.28±1.11	10.10±0.92
V	9.68±0.88[e]	9.56±0.87[e]
VI	8.18±0.74[f]	7.99±0.73[f]

Foot notes same as in Table I; Values expressed as mean±SD.
Liver HMG (F-68.350), Intestine HMG (F-43.508).

The activities of lipogenic enzymes such as glucose 6-phosphate dehydrogenase and malate dehydrogenase (Table VI) increased significantly in the liver of nicotine group compared to control. Supplementation of selenium at both the levels showed no significant change compared to control. Co administration of both the doses of selenium and nicotine decreased the activity; but the decrease was more pronounced in the low dose group.

TABLE VI: Activity of lipogenic enzymes.

Groups	G6PDH *(units/g protein)	Malate DH ~(units/g protein)
I	1.15±0.10[a]	1108.8±27.7[a]
II	12.28±5.2[b]	1844.7±40.5[b]
III	1.18±0.11[d]	1252.3±33.7[c]
IV	4.3±0.35	1312.4±36.4[d]
V	1.07±0.99[e]	1132.8±29.0[e]
VI	2.10±0.19[f]	1200.5±31.2[f]

Foot notes same as in Table I; Values expressed as mean±SD.
* Unit-Enzyme which causes increase in OD of 1/min/g protein.
~ Unit-Enzyme which causes increase in OD of 0.01/min/g protein.
Liver G6PDH (F-122.52), Liver Malate DH (F-158.732).

The absorption of selenium was significantly lower in the nicotine group compared to control. Selenium administration at both the levels increased the absorption compared to nicotine group. Although co administration of both the doses

TABLE VII : Intestinal absorption of selenium using se^{75}.

Groups	*Intestine (CPM/g tissue)*
I	$10.47\times10^{8}\pm0.95\times10^{8a}$
II	$5.28\times10^{8}\pm0.48\times10^{8b}$
III	$28.94\times10^{8}\pm2.64\times10^{8c}$
IV	$18.61\times10^{8}\pm1.71\times10^{8d}$
V	$15.49\times10^{8}\pm1.42\times10^{8e}$
VI	$12.46\times10^{8}\pm0.86\times10^{8f}$

Foot notes same as in Table I; Values expressed as mean±SD. Intestine (F-100.635).

of selenium and nicotine increased the absorption of selenium; the increase was more in the high dose group.

As shown in Fig. 1, histopathology of the liver of the control rats showed normal hepatic cell with central hepatic vein and portal triad. High dose of selenium treated group showed cirrhosis of the liver with degeneration of the central hepatic vein (Fig. 3). In the group IV also cirrhosis was observed (Fig. 4). Low dose selenium group had the hepatic structure similar to that of control (Fig. 2). In the nicotine treated group there was degeneration of hepatic cells with hyperchromatic vesicular nuclei (Fig. 5). In the group VI there was also degeneration of hepatic cells but it was less compared to that of nicotine (Fig. 6).

DISCUSSION

Nicotine plays an important role in the development of cardiovascular disease and lung cancer in smokers (24). Intravenous nicotine and smoking raise plasma free fatty acid (FFA) levels through enhanced lipolysis (2). Chronic administration of nicotine was found to produce enhanced synthesis of cholesterol, triglycerides, phospholipids and free fatty acids in the liver and testes (25). Our studies also confirmed the increased concentration of lipids and increased activity of key enzymes of lipogenesis in nicotine treated rats. ($P\leq0.01$).

It has been reported that exogenous selenium brings down the tissue lipid levels (26) and selenium deficiency can result in hypercholesterolemia and cardiovascular diseases. Consistent with the reports and also with our previous work we observed that nicotine induced hyperlipidemia was reversed by the administration of low dose selenium along with nicotine (24).

There was also enhanced lipogenesis in nicotine treated rats. Enhanced lipogenesis was due to enhanced HMG CoA reductase activity. This has been reported by Kavitharaj and Vijayammal (25). Co administration of low dose selenium and nicotine significantly decreased the activity of both lipogenic enzymes and HMG CoA reductase resulting in reduced lipid content. ($P\leq0.01$).

Studies have shown that smoking causes a reduction in HDL cholesterol and an increase in the concentration of LDL+VLDL (5). Our results are also in agreement with this. Co administration of low dose selenium and nicotine increased the concentration of HDL-C and decreased the concentration of LDL+VLDL-C. It has been reported that selenium supplementation is responsible for the up regulation of LDL-R activity as well as mRNA expression during hypercholesterolemia (27). Dhiangra and Bansal have also demonstrated that 1 ppm of selenium supplementation is responsible for the down regulation of apo B and

PLATE 1
Light microscopic of liver sections obtained using H & E.

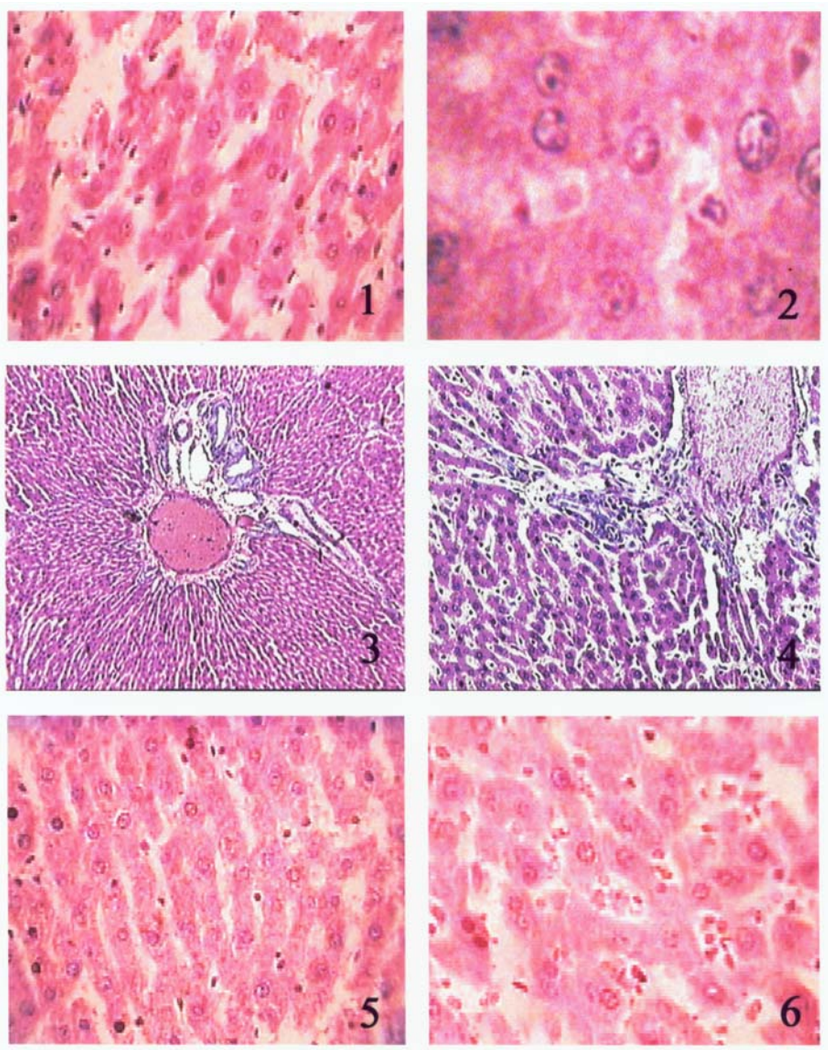

Fig. 1 : Microphotograph of liver of the control group (40X×10). This slide shows the structure of a normal liver lobule with pink cytoplasm and centrally placed nucleus.

Fig. 2 : Microphotograph of liver of the nicotine group (100X×10). Hepatic cells have undergone degeneration. Hyperchromatic vesicular nuclei are observed.

Fig. 3 : Microphotograph of liver of the high (se_{50}) dose selenium (10X×10). Liver cirrhosis has been observed with degeneration of the central hepatic vein.

Fig. 4 : Microphotograph of liver of the nicotine+se_{50} group (10X×10). Liver cirrhosis has been observed with more degeneration of central hepatic vein.

Fig. 5 : Microphotograph of liver of the low (se 1) dose selenium group (40X×10). The cells were almost similar to that of control.

Fig. 6 : Microphotograph of liver of the nicotine+ selenium 1 group (40X×10). There is degeneration of hepatic cells but it was less compared to nicotine group.

HMG CoA reductase expression during hypercholesterolemia (28).

One of the mechanisms of smoking induced toxicity is mediated through free radicals. Enhanced free radicals induce lipid peroxidation which in turn alters membrane permeability. So it was decided to study whether there is any alteration in the absorption of selenium on exposure to nicotine. More over there are reports of lower selenium in smokers (29). The se^{75} absorption by the intestine of nicotine treated group showed a decreased absorption. But this was reversed by the co administration of selenium along with nicotine. This shows that lower selenium content observed in the smokers may be due to reduced absorption of selenium in the presence of nicotine.

The effect of selenium is dose dependent (30). It is very toxic at high levels. Even though both the doses we studied reduced hyperlipidemia, histopathological studies revealed that Se at a dose of 50 µg was hepatotoxic.

Thus it can be concluded from both biochemical and histopathological studies that the co administration of selenium at a dose of 1 µg/100 g, body wt and nicotine offers protection against nicotine induced hyperlipidemia. Our studies confirmed that nicotine administration leads to hyperlipidemia as evidenced by increased concentration of lipids, decreased concentration of lipogenic enzymes and increased activity of HMG CoA reductase. Administration of low dose selenium and nicotine protects our organs from the deleterious effects of nicotine. The mechanism of action may be by affecting the absorption of nicotine and also by down regulating lipogenesis. These finding highlight the therapeutic potential of selenium supplementation in smokers.

We have observed in our previous studies that administration of nicotine along with selenium enhanced the metabolism of nicotine. This was evidenced by the low levels of cotinine in the plasma of group VI rats (4) Dawson et al also reported that the supplementation of the antioxidant lowers the urinary excretion of cotinine (31).

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