



EFFECT OF NICOTINE ON HEMATOLOGY, LIPID PROFILE AND LIVER ENZYMES IN ADULT MALE MICE (*MUS MUSCULUS*)

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Article Info	ABSTRACT
<p>Received 22/10/2013 Revised 16/11/2013 Accepted 18/12/2013</p> <p>Key words: Nicotine administration, Hematological parameters, Lipid profile, Liver enzymes, Mice.</p>	<p>The study was designed to observe effects of nicotine on various hematological parameters, lipid and hepatic profile of adult male mice. Mice (n=60) were divided into two groups; control and experimental. Subcutaneous injections of 1 mg/kg body weight of nicotine were given to the experimental (n= 40) and saline solution to the control (n= 20) group. Hematological parameters were assessed by using hematological analyzer and biochemical analyses were done by Chemistry analyzer. The WBCs, PLT, HCT, MCV, Cholesterol, triglycerides and LDL-C were found significantly higher ($p<0.05$) in an experimental group by comparing with control group while the significant decrease was observed in RBCs count, Hgb, MCH and MCHC, HDL-C and HDL-C/LDL-C ratio in the experimental group. Significant increase in the liver enzymes ALT, AST and ALP were observed, whereas total bilirubin (mg/dl), albumin and total protein showed significant decrease in the experimental group. It is concluded that nicotine administration in mice resulted in deleterious effects on various hematological parameters and biochemical parameters.</p>

INTRODUCTION

Nicotine is thought to be the main component present in the smoke of tobacco which works as a developmental neuro-toxicant and largely accounts for most of the deleterious effects (Slotkin, 2004). Various studies have been done on humans, animals and in a number of various types of cell systems to largely examine the actions of nicotine [1].

Changes in the hematological parameters due to the inhalation of nicotine may be an important reason for various vascular diseases. Inhalation of considerable concentration of nicotine, cause alternations in various hematological parameters, including WBC, MCV, RBC, Hct, Hgb, monocyte, eosinophil, neutrophil and lymphocyte counts [2]. Liver is the major site of nicotine metabolism and the metabolites of nicotine are immersed in the liver [3,4].

Elevations of ALP are the symptoms of diseases of the liver. The relationship between smoking and serum lipids has been observed in various studies showing that plasma HDL level are lower in smokers as compared to non-smokers while the levels of triglyceride, cholesterol, LDL and VLDL levels were higher in smokers as compared to non –smokers. Smoking is strongly linked with the risk of atherosclerosis and coronary heart disease (CHD) [5].

As the genetic knowledge of mice grow rapidly far beyond than that of rats, mouse models are becoming more prevalent in the scientific literature and for this reason mice are chosen to conduct various experiments to study the pharmacological effects of nicotine [7,8]. Hence, the main aim of the present study was to study the influence of nicotine on various hematological parameters, lipid profile and liver enzymes in adult albino mice.

MATERIAL AND METHOD

Animals

A total of 65 adult male albino mice of age one month old were collected from Veterinary Research Institute (VRI) Lahore with the body weight range from

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19-27(g). The Institution of Animals Ethical Committee of Lahore College for Women University approved the animal studies. The animals were kept at animal house of Lahore College for Women University, Lahore and fed on standard pelleted diet for mice and tap water.

Experimental Design

The animals were acclimatized for a period of one month prior to experiment in animal house of Lahore College for Women University, Lahore. During acclimatizing period 5 mice died. All experiment was conducted on adult male albino mice when the weight reached to 30-39g (2 months old). To decrease the effects of circadian rhythm all treatments were done between 9 to 10 a.m. These animals were kept in wire cages in groups of 20 animals per cage. All the animals were kept under similar management and feeding conditions throughout the experiment and maintained on a 12-h light/12-h dark cycle with ambient temperature of 20-22°C. The relative humidity given was 60%. During acclimatizing period of one month out of 65 animals 5 mice were died. After acclimatization period mice were indiscriminately distributed into two groups, Control group {(n=20) without nicotine treatment} and experimental group {(n=40) Nicotine treated (1 mg/ kg of body weight)}. By giving nicotine injections subcutaneously mortality was not arisen within the 6 weeks of experimental period.

Mode of Treatment

The body weight of each adult male mouse was measured before giving them injections on the first day of experiment. An average weight of 33g was obtained. First group containing 20 mice was nominated as control in which equal volume of 0.1mL of normal saline was administered subcutaneously. Second group containing 40 mice was given effective dose of 1 mg/kg of body weight of nicotine hydrogen tartarate subcutaneously injected in the scruff of the neck daily for 6 weeks at 10 a.m. every day. Each adult male mouse of experimental group received 0.1mL subcutaneous dose of nicotine daily.

Sample Collection

For the assessment of various hematological parameters blood samples were collected in EDTA tubes by performing cardiac puncture directly from the ventricle of the heart after anesthetizing the animal. To avoid hemolysis, sampling is performed with moderate suction. For the assessment of lipid profile and liver enzymes blood is collected in heparinized tubes and centrifuged at 5000 rpm for 10 min for the serum separation and stored in eppendorfs at -20 °C till the assessment.

Assessment of Hematological Parameters

For this purpose the blood was immediately collected in EDTA tubes and the samples were immediately run on hematological analyzer (Model “KX-21 Sysmex”, Germany) to assess various hematological parameters including WBC count, RBC count, Hgb

concentration, Hct, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PLT).

Assessment of Biochemical Parameters

The lipid components such as total cholesterol, LDL-C, HDL-C and triglyceride in serum obtained from the blood of mice, determined by using chemistry analyzer. The liver enzymes AST, ALT and ALP and other biochemical parameters bilirubin, total proteins, and albumin in serum were also determined by using chemistry analyzer

Statistical Analysis

Values of various hematological parameters and BW, TC, HDL, LDL, AST, ALT, ALP, triglycerides, bilirubin, albumin and total protein were given as mean \pm Standard Error Mean (S.E.M). The comparisons between the parameters of control group and experimental group were statistically analyzed by using Independent Sample Student “t” Test. All the statements of significance are based on the 0.05 level of probability at 95% confidence interval. All graphs were obtained on Microsoft Excel Starter 2010 and statistical analysis was done on SPSS Version 13.0.

RESULTS

Physical Performance

The body weights of all the adult male mice (n=65) were measured on the first day of the acclimatizing period (1 month). The mean body weight was 19.95 ± 0.04 (g). The body weight of all adult male mice (n=60) was recorded again at the end (30th day) of the acclimatizing period. The mean value was 26.51 ± 0.06 (g). During the whole acclimatizing period the gain in body weight was approximately 6.56 (g) (Fig: 1). All the animals which were used in the experiment were very healthy and physically active. During the 6 weeks of experimental period it has been observed that the food intake as well as body weight of experimental mice was decreased as compared to the control group (Fig: 2 & Fig: 3)

Hematological parameters

The values of various hematological parameters including WBC count, RBC count, Hgb concentration, Hct, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PLT) assessed in the blood of control and experimental group are shown in Table 1. In nicotine treated adult albino mice, the *t-test* showed significant increase in WBCs count, PLT count, HCT and MCV in experimental group as compared to the control group after administration of nicotine ($p < 0.05$) and the dose of nicotine 1mg/kg body weight was found to be very effective. However, the result of *t-test* statistics indicated significant decreases in RBCs count, Hgb concentration, MCH and MCHC after administration of nicotine in experimental group ($p < 0.05$) as compared to control group.



Lipid Profile

The mean \pm SEM value of cholesterol level (mg/dl), Triglycerides, HDL-C, LDL-C and HDL/LDL-C ratio is presented in Table 2. The result of *t*-test statistic indicated the significant increase in cholesterol level, triglycerides level, LDL-C level after administration of nicotine in experimental group ($p < 0.05$) as compared to control group and significant decrease in HDL-C and HDL-C/LDL-C ratio.

Liver Enzymes

The mean values of liver enzymes (AST, ALT,

ALP), total bilirubin, Albumin, and total protein is presented in table 3. The result of *t*-test statistic revealed that significant increase ($p < 0.05$) in ALT, AST, ALP after administration of nicotine in experimental group as compared to control group. The mean \pm SEM value of total bilirubin (mg/dl), Albumin and total protein in experimental group and control group was presented in table 2. The result of *t*-test statistic revealed the significant decreased in bilirubin concentration, albumin, total protein after administration of nicotine in experimental group as compare to control group.

Table 1. Hematological parameters in adult male mice with and without administration of nicotine dose. The values are expressed as mean \pm S.E.M.

Hematological Parameters	Control Group	Experimental Group
WBC count ($10^3/\mu\text{L}$)	6.54 ± 0.26	$12.50^* \pm 0.26$
RBC count ($10^6/\text{mm}^3$)	8.19 ± 0.15	$5.85^* \pm 0.13$
HGB (g/dL)	15.55 ± 0.15	$11.21^* \pm 0.11$
HCT (%)	55.69 ± 0.79	$66.75^* \pm 0.55$
MCV (fL)	49.90 ± 0.27	$54.06^* \pm 0.22$
MCH (pg)	17.17 ± 0.17	$12.60^* \pm 0.14$
MCHC g/dL	32.75 ± 0.36	$28.28^* \pm 0.29$
PLT count (10^3 platelets/ μL)	1208.25 ± 18.47	$1596.45^* \pm 9.55$

Table 2. The mean \pm SEM values of liver enzymes lipid profile and other biochemical parameters in adult albino mice with and without administration of nicotine.

Parameters	Control Group	Experimental Group (After 42 Days)
ALT (IU/l)	92.00 ± 1.21	$104.37^{**} \pm 1.45$
AST (IU/l)	148.45 ± 1.13	$166.50^{**} \pm 1.08$
ALP (IU/l)	72.70 ± 1.41	$79.80^{**} \pm 1.54$
Total Bilirubin (mg/dl)	0.62 ± 0.02	$0.43^* \pm 0.02$
Albumin (g/l)	38.87 ± 0.41	$30.15^* \pm 0.93$
Total protein (g/l)	53.15 ± 1.61	$45.45^* \pm 1.15$
Cholesterol (mg/dl)	67.10 ± 4.97	$138.60^{**} \pm 2.95$
Triglycerides (mg/dl)	136.05 ± 1.48	$149.32^{**} \pm 1.61$
HDL-C (mg/dl)	54.30 ± 4.79	$39.35^* \pm 2.32$
LDL-C (mg/dl)	58.15 ± 4.64	$124.77^{**} \pm 2.64$
HDL-C/ LDL-C ratio	0.91 ± 0.013	$0.32^{**} \pm 0.022$

Independent sample *t*-test indicates significant increase in values in experimental group from control group ($^{**}p < 0.05$) and significant decrease in value in experimental group from control group ($^*p < 0.05$)

Figure 1. Body weight gain (g) mean \pm S.E.M in adult male mice (n=65) during acclimatizing period of one month.

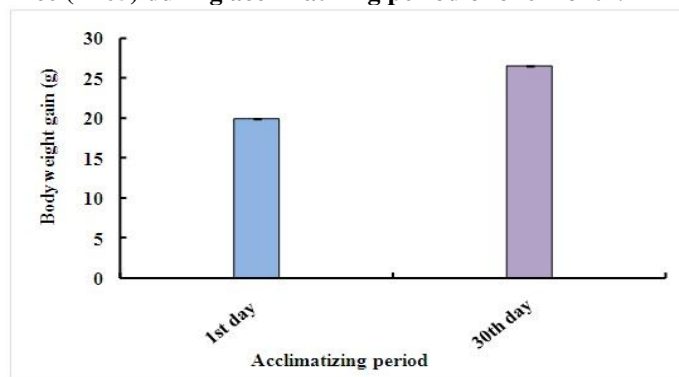


Figure 2. Graph showing decreasing trend of food intake (g/cage/day) in experimental group as compared to control group during 6 weeks of nicotine administration.

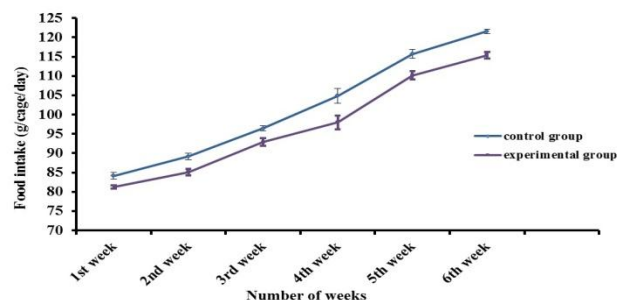
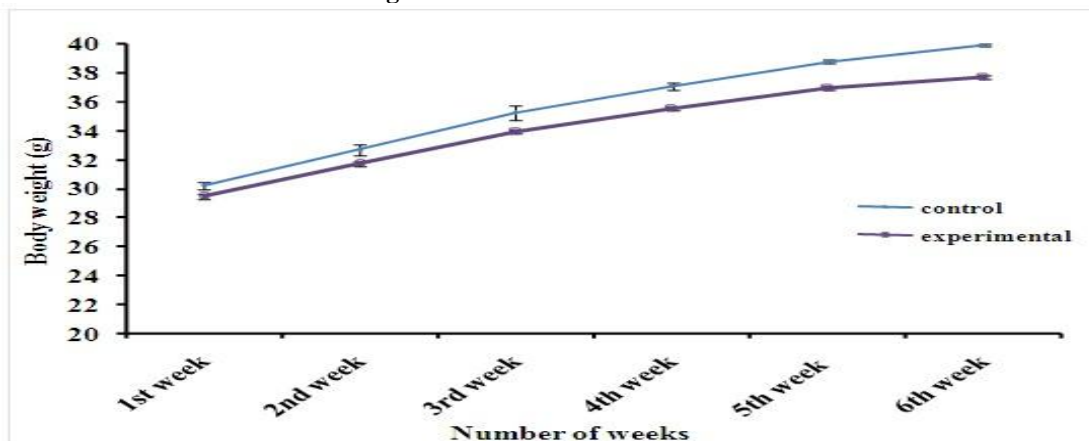


Figure 3. Graph showing decreasing trend of Body weight (g) in experimental group as compared to control group during 6 weeks of nicotine administration



DISCUSSION AND CONCLUSION

The present study was undertaken to study the effects of nicotine on various hematological parameters. Herxheimer *et al.* (1967) [8] showed that nicotine produces the same hemodynamic changes as cigarette smoking. Rausch *et al.* (1989) and Schwartz *et al.* (2005) [9,10] reported that nicotine causes many changes in blood cells as it simply diffuses into the cells. This study confirmed that administration of nicotine to adult male mice significantly altered various hematological parameters including WBC, RBC, Hgb concentration, HCT, MCV, MCH, MCHC and PLT.

Our results showed a significant ($p < 0.05$) decrease in the body weight and food intake of nicotine-treated adult male mice as compared to control mice. The work of Audi *et al.* (2006) [11] showed that administration of nicotine to rats caused a significant decrease in their body weight and food intake. The decrease in food intake and body weight caused by nicotine administration might be due to neuro-regulatory substances which effect food intake mechanism [12]. A significant increase in WBCs count and decrease in RBCs count ($p < 0.05$) was observed. Present results are similar to the results of Corre *et al.* (1971) [13]. The elevated WBCs count in our study is in agreement with the findings of other investigators including [14-18]. Geng *et al.* (1996) [19] demonstrated that one of the major effects of nicotine on the physiology of body is that it greatly suppresses the function of immune system and due to this reason the number of WBCs increased in the body to strengthen the immune system.

It is documented that nicotine inhibits the function of erythrocytes, fibroblasts and macrophages. The work of Siana *et al.* (1992) [20] clearly showed that the administration nicotine causes the diminished proliferation of red blood cells and as a result the RBCs count decreases. Low erythrocytes count may lead to a number of physiological disorders that may affect the efficiency of various enzymes.

In the present study it was observed that nicotine administration resulted in significantly ($p < 0.05$) decreased

hemoglobin level. Similar results to our study were found by the work of Zafar *et al.* (2003) [21]. In our study the HCT level was found to be significantly ($p < 0.05$) higher in experimental animals as compared to control subjects. Ogston *et al.* (1970) [22] showed that nicotine inhalation results in high level of HCT. One of the explanations for the apparent acute effect on the HCT level is that nicotine results in an increase in MCV.

MCV values were found to be significantly ($p < 0.05$) higher in our experimental group. Okuno (1973) [16] demonstrated in his study that nicotine caused an increase in MCV. A significant ($p < 0.05$) decrease in both MCH and MCHC was another finding of this study. Decrease in both values was due to decrease in hemoglobin level which was demonstrated in our study.

The PLT count in our present study was found to be significantly ($p < 0.05$) higher in experimental mice. Literature reports on the effects of nicotine on PLT count seem to be controversial. De-Gactano *et al.* (1990) [23] showed that nicotine caused platelet and leukocyte activation, and this resulted in the stimulation of platelet function. The work of Nowak *et al.* (1987) [24] demonstrated that increase in PLT in heavy smokers might be due to vascular damage caused by smoking. A study performed by Alster & Wennmalm (1981) [25] suggested that nicotine has no effect on PLT. Cahao *et al.* (1983) [26] and Renaud *et al.* (1984) [27] suggested that administration of nicotine increased PLT aggregation. Hoss *et al.* (1986) [28] found that PLT and WBCs have high affinity non-cholinergic binding sites for nicotine. The study of Cyer *et al.* (1976) [29] and Nicod *et al.* (1984) [30] showed that elevations in blood nicotine level caused an activation of PLT via enhancing the levels of epinephrine. Wanzel *et al.* (1958) [31] and Ponzer *et al.* (1970) [32] in his study demonstrated that nicotine enhances the concentration of free fatty acids and this result an increased aggregation and stickiness of PLT. Changes of hematological parameters, including increased PLT count and reactivity, increased fibrinogen concentration and blood viscosity due to inhalation of



nicotine in smokers was found to be the major reason for various diseases of heart.

A significant increase in cholesterol (mg/dl) level (mg/dl) in experimental group as compared to the control group ($p < 0.05$) was observed and our findings were similar to the previous studies. Annida and Venugopal, (2007) [33] described in their study that the level of free fatty acids, cholesterol and triglycerides increased in plasma of male albino Wistar rats treated with nicotine subcutaneously. The presences of hypercholesterolemia and triglyceridemia in heavy smokers increased level of cholesterol and it is documented to the increased activity of 3-hydroxy-3-methyl-glutaryl CoA reductase and increased incorporation of labeled acetate into cholesterol. Chattopadhyay, (2008) [34] also indicated that the

administration of nicotine in adult albino rats caused a significant increase of total cholesterol, triglycerides.

A significant increase in triglycerides (mg/dl) level in experimental group from the control group ($p < 0.05$). Our results were according to the previous studies. Higher level of triglycerides is occurred due to the presence of nicotine that decrease the activity of lipoprotein lipases and these enzymes involved in the uptake of circulating triglycerides rich lipoprotein and VLDL by the extra hepatic tissue. Chromaffin cells of adrenal medulla synthesis catecholamine by the stimulation of nicotine and adipose tissues lipolysis is carried out by catecholamine, which in turn increase the levels of cholesterol, triglycerides and also increased fatty acids [35].

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