Metabolic and Behavioral Effects of Nicotine in Swim Stressed Mice

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Absract: Nicotine, in the form of tobacco smoking, is the most commonly abused drug throughout the world. It contributes to the harmful tobacco smoking habit leading to high morbidity and mortality throughout the world. The drug has addictive properties and causes drug dependence. Apart from these effects, nicotine alters a number of metabolic pathways such as lipid profile and glucose homeostasis leading to increased risk of cardiovascular diseases. Present study investigated the metabolic and behavioral effects of nicotine in stressed mice. For this purpose adult male mice were subjected to chronic nicotine treatment(3.08 mg/100 ml in drinking water) for 3 weeks followed by forced swim test (FST) and serum glucose, lipid profile and tryptophan were investigated. When swim stressed mice were compared with chow control, it was found that serum glucose ($P<0.001$), total cholesterol ($P<0.001$), triglycerides ($P<0.01$), and LDL cholesterol ($P<0.01$) were increased. Similarly glucose concentration ($P<0.05$), total cholesterol ($P<0.05$), triglycerides ($P<0.01$) and LDL cholesterol ($P<0.05$) were increased in drug treated swim stressed mice. However HDL remained unaltered in both groups. Serum tryptophan was decreased ($P<0.01$) in swim stressed and nicotine treated swim stressed mice. During FST, swimming behavior was significantly increased at the cost of climbing with no change in immobility in nicotine treated mice as compared to controls. It is concluded that nicotine worsens lipid profile and glucose homeostasis in stressful situations thereby increasing the risk of cardiovascular diseases in chronic smokers and the drug induced behavioral alterations may be related to the serotonergic pathway.

Keywords: Nicotine, forced swim test, lipid profile, glucose, tryptophan, antidepressant.

INTRODUCTION

Epidemiological studies indicated that smokers have an increased risk of depression. Furthermore, the more they smoke, the greater their risk or vice versa depression has become a risk factor for nicotine dependence [1, 2]. FST is an animal model of depression in the rodent. The forced swim test is used for evaluating antidepressant efficacy of drugs. Substances thought to have antidepressant effects decrease the duration of behavioral immobility during the test. The water immersion causes mainly psychical stress (fear of drowning and suffocation) and consequently also physical stress (vigorous activity to come out). It has been demonstrated that stress contributes to unfavorable concentrations of lipoproteins that may lead to cardiovascular diseases [3]. Studies in non-human primates showed that stress induces increased concentration of the atherogenic low density lipoproteins and decreased concentration of the antiatherogenic high density lipoproteins (HDL) [4]. Tobacco smoking has always been associated with stress. Smokers increase the intensity of smoking in stressful situations and relief from the symptoms produced by stress is considered to be an important reason for smoking. Moreover smoking rates are higher in people suffering from anxiety-related disorders [5] and smokers often report an anxiolytic effect of short-term nicotine exposure [6]. Studies have also demonstrated that cigarette smoking may arise or increase as a result of self-medication of depressive symptoms. [7, 8] The antidepressant effect of nicotine has been associated with serotonergic system. It has been demonstrated that nicotine has bidirectional relationship (both anxiogenic and anxiolytic) with serotonergic system. Nicotine increases 5-hydroxy-tryptamine (5-HT) release in the cortex, striatum, hippocampus, dorsal raphé nucleus, hypothalamus, and spinal cord [9]. Despite the antidepressant effects of nicotine, it is not recommended by physicians to treat depression due to its addictive properties. Cigarette smoking is reported to be associated with an increase in the risk of death from coronary artery disease. Nicotine and other toxic substances from tobacco smoke are absorbed through the lungs into the blood stream and are circulated throughout the body. These substances alter blood coagulation [10], blood lipid and lipoprotein concentrations [11, 12] and narrow or damage the blood vessel walls which allow plaque to form at a faster rate than they would in a non smoker [13]. It has been shown that oral administration of nicotine raises plasma total cholesterol and low-density lipoprotein cholesterol, and lowers high-density lipoprotein cholesterol in normal dietary condition [14, 15]. The present study was undertaken to evaluate

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behavioral and metabolic effects of chronic nicotine treatment in animal model of depression.

MATERIAL AND METHODS

Animals and Treatments

Locally bred adult male Albino mice (20-25 gm body wt.) were housed one mouse per cage under natural light dark cycle at 25°C ± 2°C room temperature and were maintained on lab chow and water *ad libitum*. Mice were divided into three groups, each group had five mice. Two groups of mice (untreated controls and FST controls) received tap water and 3rd group received Nicotine hydrogen tartrate (3.08 mg/ml) in 100 ml of drinking water for 21 days. On last day of treatment, the untreated control mice remained in their cages while the other group (FST controls) was subjected to forced swim test for five minutes. Similarly the chronic nicotine treated mice were also subjected to forced swim test for five minutes. After forced swim test all the mice were killed by decapitation and serum was isolated for analysis.

Forced Swim Test (FST)

Animals were subjected to forced swim test to create a model of depression. Animals were placed in a cylindrical glass tank (46cm tall x 20cm in diameter) of 21°C to 22 °C, water filled to a depth of 30cm. The water depth of 30cm allowed the rats to swim or float without hind limbs touching the bottom of the tank. Mice were placed in the water tank for 5 minutes. Control animals (untreated) were not subjected to force swimming. [16, 17].

Behavioral Analysis

Behavior during the test swimming session was scored using a time-sampling method [18] every five seconds; one of three behaviors was recorded. Immobility was scored when the animal was making the minimum movements necessary to stay afloat. Swimming was scored when the animal actively swam around the tank, making movements greater than those necessary to stay afloat. Climbing was scored when the animal made vigorous thrashing movements with its forepaws, usually directed against the sides of the tank Behavioral results are shown as the total number of counts for each behavioral category.

Analysis of Serum Parameters

Serum glucose concentration was determined by O-Toludine method [19]. Lipid profile was performed by using Randox kit (Randox Laboratories Ltd.). Serum tryptophan was determined by spectroflourimetric procedure [20].

Chemicals and Drugs

Nicotine hydrogen (+)-tartrate was purchased from Sigma chemical Co. All the other chemicals were of highest purity analytical grade.

Statistical Analysis

Statistical analysis was performed using student's t-test.

RESULTS

Figure **1** shows that serum glucose levels were significantly increased (P< 0.001) in swim stressed group which further increased (P<0.05) after nicotine treatment. Figure **2** shows that serum total cholesterol (P<0.001), triglycerides (P<0.01), and LDL-C (P<0.01) were significantly elevated in FST mice. Similarly, serum total cholesterol (P< 0.05), triglycerides (P< 0.01), and LDL-C (P<0.05) and were significantly elevated in nicotine treated swim stressed mice when compared to swim stressed mice. However serum

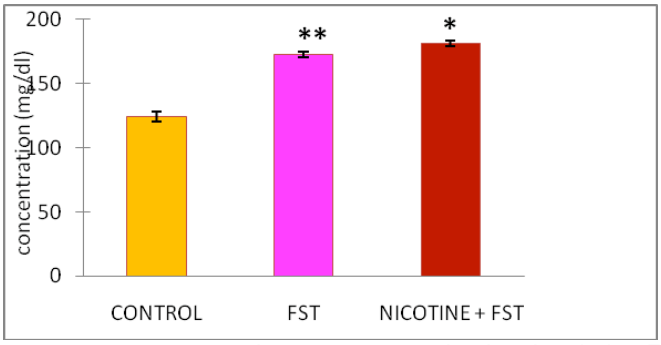

Figure 1: Effect of Chronic nicotine treatment on serum glucose concentration in mice during FST. Values shown are mean ± sem. Chronic nicotine treated mice were compared with FST controls and FST controls were compared with untreated controls. The significance of the difference is indicated by *P<0.05, **P<0.001.

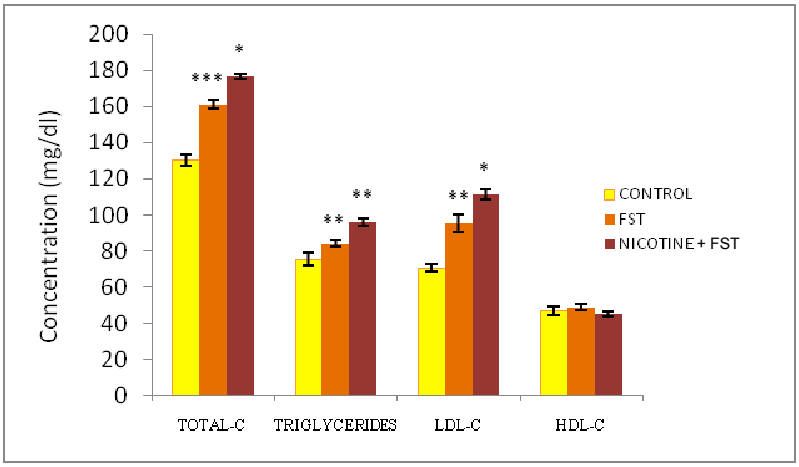

Figure 2: Effect of nicotine treatment on lipid profile in mice during FST. Values shown are mean ± SEM. Chronic nicotine treated mice were compared with FST controls and FST controls were compared with untreated controls. The significance of the difference is indicated by *P<0.05, **P<0.01, P<0.001.

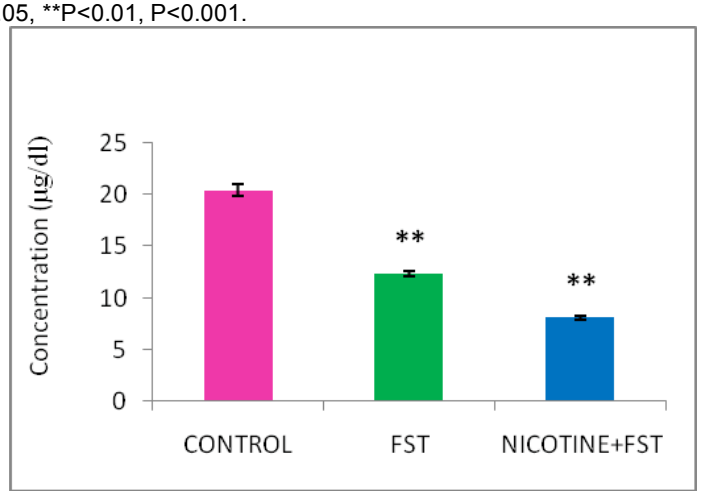

Figure 3: Effect of nicotine treatment serum total tryptophan in mice during FST. Values shown are mean ± SEM. Chronic nicotine treated mice were compared with FST controls and FST controls were compared with untreated controls. The significance of the difference is indicated by **P<0.01.

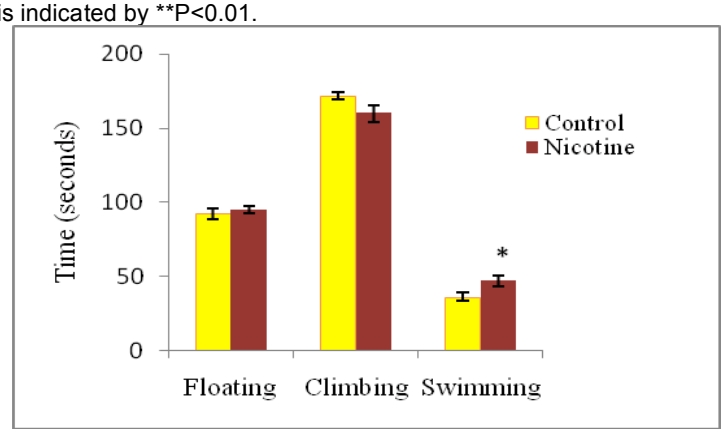

Figure 4: Effect of nicotine treatment on floating, climbing and swimming in mice during FST. Values shown are mean ± SEM. Parameters in Chronic Statistical analysis was performed using student's t-test *P<0.05.

HDL-C remained unaltered in both groups. Figure **3** shows that serum total tryptophan concentration decreased in swim stressed mice (P<0.01) which further decreased in nicotine treated swim stressed mice (P<0.01). Figure **4** shows that nicotine increased swimming behavior (P<0.05) of mice as compared to controls.

DISCUSSION

The behavioral and metabolic effects of nicotine were studied in forced swim mice. It was found that nicotine increased swimming behavior of mice without affecting climbing and immobility time in FST (Figure **4**). We have found that chronic nicotine treatment failed

to reduce immobility time in FST paradigm that nullifies its antidepressant property. Previous studies on behavioral effects of nicotine have reported both depressive [21, 22] and antidepressive [23-26] effects. These differences in the depressive and antidepressant behavioral effects of nicotine could be due to the difference in conditions such as dose, number of administrations, previous exposure to nicotine, time after use, animal model used, strain and gender difference etc .The wide diversity of behavioral effects mediated by nicotine in human smokers and animal models has been suggested to be mediated through selective activation of different nicotinic acetylcholine receptors subtypes [27] and serotonergic system [28].

Tryptophan (TRP), an essential aminoacid, is the precursor for the neurotransmitter serotonin (5HT). It has been suggested that a 5HT deficit underlies depression. [29]. Tryptophan exists in plasma in two forms i.e. free tryptophan and albumin bound tryptophan. Major portion of the plasma tryptophan is protein-bound [30]. Tryptophan is carried across the blood-brain barrier by the large neutral amino acids (LNAA) transport system so it must compete with valine, leucine, isoleucine, tyrosine, phenylalanine and methionine for access to the carrier-binding site [31]. The relation between serum tryptophan and brain tryptophan is not clear and there has been some disagreement between different research groups as to whether plasma free TRP or TRP:LNAA ratio predict uptake of tryptophan in the brain. In a study on the effect of different dietary composition, it was reported that the TRP: LNAA and free TRP:LNAA ratios are the better predictors of brain TRP [32]. If plasma TRP levels change while other LNAA remain constant, the rate of TRP transport into the brain will also change. Since the other LNAA compete with TRP for transport, a change in their concentrations will equally influence TRP entry into the brain, even if plasma TRP remains constant. The rate-limiting step of serotonin synthesis is the hydroxylation of TRP to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase. In depressed patient reduction in plasma tryptophan and its availability may contribute to abnormalities in brain 5-HT function. Increased brain serotonin levels improve the ability to overcome stress, while a decline in serotonin activity is associated with depression. In our experiment serum total tryptophan decreased in FST treated mice. These findings are consistent with the previous ones reported by Bano and Dawood [33]. The nicotine treatment further decreased the tryptophan levels as compared to FST controls (Figure **3**). Previously Badawy *et al.* have reported an increase in serum total tryptophan following nicotine treatment, this difference may be due to FST treatment to our mice [34]. In a study, it has been reported that restraint stress but not foot shock stress decreased plasma tryptophan levels in rats pretreated with nicotine with increased 5HT turnover rate (5HIAA/5HT ratio) in both stresses [35]. In another study, it has been demonstrated that nicotine injections (0.05 mg/kg) increased 5HT turnover in swim stressed mice [36] suggesting the role of both nicotinic and serotonergic systems in antidepressant actions of nicotine. In our experiment, although serum total tryptophan has reduced predicting reduction in 5HT levels.

Glucose is the main energy source for all tissues and it is derived from food intake, synthesis in the body and the breakdown of glycogen which is a form of glucose that the body stores in the liver. Insulin and glucagon regulate blood glucose levels. Insulin lowers the glucose concentration in the blood; glucagon elevates it, while hormones from the adrenal and pituitary backup glucagon function in stressful conditions. We have found that forced swim stress significantly elevated serum glucose in mice (Figure **1**). This finding is consistent with the previous one reported by Radahmadi *et al.* who reported that forced swim stress increased serum glucose levels however it did not caused diabetes in rats [37]. Dutour *et al,* have reported that an acute psychological stress may play a role in the glycemic instability of some patients with type I diabetes [38]. It has also been proposed that stress makes one more prone to diabetes. If one is in a pre-diabetic phase or possesses hereditary background, psychical stress would be able to provoke diabetic state. It has also been suggested that acute stress induced hyperglycemia results due to increased gluconeogenesis and glycogenolysis without alteration in glycolysis. Such an experience might lead to insulin resistance because prolonged hyperglycemic condition is known to cause insulin resistance [39]. Serum glucose levels were further elevated in nicotine treated stressed mice as nicotine also increases blood glucose level (Figure **1**) [40]. These results can be explained by the fact that nicotine administration causes significant decrease in circulating insulin levels.

Cigarette smoking has been associated with an increased risk of coronary heart diseases. The most important mechanism is the alteration of lipoproteins concentrations in the blood [11]. We found that FST increased total cholesterol, triglycerides and LDL cholesterol; however serum HDL remained unaltered

(Figure **2**). These findings are consistent with previous findings [41, 42] that have reported alterations in lipid profile following different stressful procedures. The physiological mechanism of stress induced changes in lipid levels remains unclear. It appears like that the hypothalamic–pituitary–adrenal (HPA) axis contributes to the stress induced cholesterol changes [43]. Bagby *et al.* suggested that, in stress conditions [44], adipose tissue lipoprotein lipase (LPL) activity decreases because plasma insulin levels decrease and catecholamine concentrations increase, whereas muscle LPL activity increases in response to elevated levels of glucocorticoids and catecholamines. According to this model, the increased adipose tissue lipolysis provides a rise in circulating fatty acids, which primarily go to muscle where they are used as an energy substrate, but some are directed to the liver and incorporated into triglycerides and VLDL. In this case, the excess of hepatic triglycerides would cause an increase in the release of VLDL in blood. The chronic nicotine treatment further elevated triglycerides, total and LDL cholesterol levels. These findings were consistent with those reported earlier by Latha *et. al,* [45] and later by Chattopadhyay and Chattopadhyay [12]. Serum HDL cholesterol concentrations remained unaltered. These findings were consistent with those reported by Abd el Mohsen *et al.* [41]. However it has also been found that smoking or chronic exposure to nicotine decreases serum HDL cholesterol concentrations [46]. It has been suggested that nicotine exerts hyperlipidemic effects particularly by increasing the synthesis and secretion of triglyceride-rich lipoproteins. Also the activity of lipoprotein lipase in extrahepatic tissues and plasma lecithin cholesterol acyl transferase activity are significantly lowered by nicotine [47]. It has been shown that nicotine promotes cholesterogenesis by increasing the activity of HMG-CoA reductase and lipogenic enzymes. The uptake of circulating triglyceride rich lipoproteins (chylomicrons and VLDL) are also decreased by nicotine by decreasing the activity of extrahepatic lipoprotein lipase. Hepatic degradation of cholesterol to bile acids is also decreased by nicotine [48]. Also nicotine stimulates sympathetic adrenal system leading to increased secretion of catecholamines resulting in increased lipolysis and increased concentration of plasma free fatty acids (FFA) which further result in increased secretion of hepatic free fatty acids and hepatic triglycerides along with VLDL- C in the blood stream [49]. It has been suggested that both stress or nicotine treatment stimulates the release of epinephrine and norepinephrine in the circulation within a few minutes and their combination might enhance their lipolytic effect [50].

In summary, chronic nicotine treatment fails to alleviate depression like symptoms, worsen disturbed lipid profile and glucose homeostasis in forced swim mice that can be related to increased risk of cardiovascular illness and depression among chronic smokers under stressful life situations. These harmful side effects overweigh the euphoric properties of nicotine. Further clinical studies on plasma TRP/LNAA ratio and cortisol levels in tobacco users (predictive of brain tryptophan and 5HT metabolism) are required to evaluate the possible role of serotonergic pathway in nicotine dependence. Whether nicotine use causes depression or relieves depression in human needs investigation.

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