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EFFECT OF GREEN TEA ON COMPREHENSIVE METABOLIC PANEL AND HYPERLIPIDAEMIA DUE TO NICOTINE EXPOSURE IN WISTAR ALBINO RAT

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ABSTRACT

Background: Nicotine has a long and storied history in physiology and pharmacology. We investigated the effects of nicotine on comprehensive metabolic panel (CMP) and lipid profile within male Wistar albino rats and interaction of aqueous extract of green tea. **Methods:** Forty male wistar albino rats were divided into four groups of ten, controls without any treatment, green tea, nicotine, and green tea + nicotine. Over a 28 day period the control received daily subcutaneous does of saline (10 ml) and drank only water, green tea group received subcutaneous does of saline (10 ml) and drank only green tea, the nicotine group received subcutaneous does of nicotine in a saline carrier solution (10ml saline with 3mg/kg body weight of nicotine) and drank only water, and green tea + nicotine received subcutaneous does of nicotine in a saline carrier solution (10ml saline with 3mg/kg body weight of nicotine) and drank only green tea. At the end of the experiment period, blood samples were collected for biochemical analysis. **Results:** There were no differences between the control and green tea only groups for any parameter. The nicotine group had comprehensive metabolic panel and lipid profile concentrations significantly ($P < 0.05$) changes compare to control, and Green tea only groups. However using green tea with nicotine had reversed these changes. **Conclusions:** This study showed that injecting nicotine subcutaneously had effect on comprehensive metabolic panel and lipid profile which eliminated by aqueous extract of green tea on Wistar albino rats.

KEYWORDS: Green tea, Nicotine, Comprehensive metabolic panel, Lipid profile.

INTRODUCTION

It is well established that nicotine has a considerable influence on the increasing levels of lipids in the blood. And in other side nicotine has been recognized to result in oxidative stress by inducing the generation of reactive oxygen species (ROS) by various mechanisms.[1] The predominant effects of nicotine in the whole intact animal or human consist of an increase in pulse rate, blood pressure, and an increase in plasma free fatty acids, a mobilization of blood sugar, and an increase in the level of catecholamines in the blood.[2] In addition to the release of these hormones, chronic nicotine treatment has also been shown to activate tyrosine hydroxylase, the first and the rate limiting enzyme in catecholamine biosynthesis.[3] The other effects of nicotine at the cellular level are inhibition of cell proliferation, and suppression of apoptosis.[4] Mohamed and his group reported that a decrease in total serum protein content and in all the fractions of globulin in mice treated with two different concentrations of nicotine.[5] Nicotine, the major component of the cigarette smoke, plays an important role in the development of cardiovascular disease and lung cancer.[6] Smoking also results in higher levels of oxidized LDL, an atherogenic lipid that results as a consequence of exposure to oxidant gases in tobacco smoke.[7] Administered orally to healthy nonsmokers resulted in no changes in plasma concentrations of triglycerides, HDL, LDL cholesterol.[8]

Tea derives from the leaves of the plant *Camellia Sinensrs*, and is reported to contain nearly 4,000 bioactive chemical compounds, one-third of which is polyphenols.[9] Green tea consumption has been known to maintain and improve health.[10] Green tea is a type of tea made solely with the leaves of *Camellia sinensis*, which has undergone minimal oxidation during processing. In recent years many studies have shown that various types of tea have a wide range of physiological, biochemical and pharmacological effects due to the properties of their constituents.[11] It has also become increasingly common in weight loss supplements in the form of an extract.[12] Green tea is rich of catechins, and polyphenols with flavonoid structure, which are important antioxidant.[13] A group of researchers

concluded that green tea administration reduces albuminuria in diabetic patients nephropathy, and probably by diminishing podocyte apoptosis.[14] In other research showed that garlic reduces nicotine-induced hyperglyceamia and hyperlipidaemia in rats.[15] Even in our own previous publication we concluded that an green tea extract reduce the effects of nicotine on urea and uric acid concentration.[16] From here our study aims to identify if the nicotine exposure had harmful effects on comprehensive metabolic panel (total protein, albumin and globulin concentration, and albumin/globulin ratio) and lipid profile (triglycerides, cholesterol) of Wister albino rat and possibility of green tea extract to reduce this effect.

MATERIALS AND METHODS

Animals

This study was approval by Libyan Academy ethics review committee. Forty male Wistar albino rats (150-250 g body weight) were obtained from Biotechnology Research Center (BTRC), Twisha, Tripoli, Libya, and housed in the National Medical Research Center (NMRC), Al Zawiyah, Libya. The rats were kept in a controlled environment of 50-60% humidity at 25^0C with 12-hr light/dark period, and were treated gently. A standard rodent pellets consisting of a mixture of protein, fat, fibre, and ash were used to feed the rats. Diet and water supply were ad libitum. The rats were randomly distributed into four groups, each with ten animals, and those four groups were then randomly assigned to each experimental group as defined below.

Green Tea Aqueous Extract Preparation

Green tea aqueous extract was locally prepared from commercial best quality dried green tea (*Camellia sinensis*) leaves purchased one time trip from a local market, have been imported from East Asia. The aqueous extract of the tea was prepared daily by boiling 3 grams dry tea in 100 ml water for 5 minutes with subsequent standing for 20 min and cooling down to room temperature approximately 84% of the total antioxidant activity was solubilized within the first 5 minutes of brewing.[17] After separation of undissolved residue, the solution was used for experiments. The green tea was given orally to the green tea groups ad libitium.

Nicotine Preparation

Nicotine used was of analytical grade purity and were purchased mostly from (Sigma-Aldrich). Nicotine 3 mg/kg was prepared by mixing 40 mg of nicotine in 10 ml normal saline. Solution was injected subcutaneously daily; the experiment was carried out for four weeks (28 day period).[18]

Experimental Design Groups

Control group: injected subcutaneous daily with 10 ml normal saline and was under same room conditions, drank only water ad libitium. Green tea group: injected subcutaneous daily with 10 ml normal saline and was under same room conditions, drank only green tea ad libitium. Nicotine group: injected subcutaneous daily with 3 mg/kg body weight of nicotine and was under same room conditions, drank only water ad libitium. Green tea + nicotine group: injected subcutaneous daily with 3 mg/kg body weight of nicotine and was under same room conditions, drank only green tea ad libitium. There was no animal mortality during the experimental period.

Blood Collection and Laboratory Procedures

On day 29, rats were anaesthetized with ketamine hydrochloride (10mg/kg). Blood samples were collected through a cardiac puncture, and centrifuged at 3,000 rpm for 10 min by Hettich universal centrifuge, serum collection plastic tubes (Additive clot activator and silicone coated interior, 10.0 ml volume) were used, these tubes supplied from BD Vacutainer®. The resultant serum was collected and stored at -80^0C until analysed. Comprehensive metabolic panel (total protein, albumin and globulin concentration, and albumin/globulin ratio) were determined using a UV-visible spectrophotometer (Ce 3021, 3000 series, Cecil instrument, Cambridge), lipid profile (triglycerides, cholesterol) were determined using Cobas analyser (c111, Roche Diagnostic Ltd, Germany).

Data Analysis

The data are expressed as mean ± standard deviation and statistical analysis was performed using analysis of variance followed by Student's *t*-test with $P < 0.05$ being considered as statistically significant.

RESULTS

The daily observations showed no external or behaviour changes recorded during experiment with all groups eating and drinking similar amounts. The effects of the treatment of Wistar albino rats with green tea, nicotine and nicotine concurrent with green tea on serum concentration are shown Table 1. There were no differences between the control and green tea only groups for any parameter. The nicotine group had Comprehensive metabolic panel (total protein, albumin and globulin concentration, and albumin/globulin ratio) and lipid profile (triglycerides, cholesterol) significantly ($P < 0.05$) greater than the control, green tea only groups, and the green tea + nicotine groups shows significantly ($P < 0.05$) reverse effects compare with nicotine group.

Table 1: Effects of exposure to green tea, nicotine, and green tea + nicotine on comprehensive metabolic panel (CMP) and lipid profile (mean ± standard deviation within forty male albino rats (n = 10 per group)

Parameter	Control Mean ± SD	Green tea Mean ± SD	Nicotine Mean ± SD	Green tea + Nicotine Mean ± SD
CMP:				
Total protein (g/dl)	6.18 ± 0.59	5.87 ± 0.5	4.75 ± 033*	8.3 ± 0.93#
Albumin (g/dl)	1.41 ± 0.18	1.55 ± 0.14	2.75 ± 0.37*	1.82 ± 0.21#
Globulin (g/dl)	4.77 ± 0.46	4.32 ± 0.4	2.0 ± 0.25*	6.48 ± 0.74#
A/G (ratio)	0.3 ± 0.03	0.36 ± 0.02	1.41 ± 0.33*	0.28 ± 0.01#
Lipid profile: Triglycerides(mg/dl)	34.3 ± 4.3	36.0 ± 4.6	66.0 ± 18.5*	30.3 ± 4.1#
Cholesterol (mg/dl)	53.45 ± 7.89	45.3 ± 9.8	88.67 ± 10.97*	50.2 ± 14.3#

**Mean value was significantly different to that at control and green tea group (P < 0.05), SD = standard deviation*
#Mean value was significantly different to that at nicotine group (P < 0.05), SD = standard deviation

DISCUSSION

This study demonstrates that 3 mg/kg nicotine administered subcutaneously daily for 28 days in Wister albino rat altered both the comprehensive metabolic panel and lipid profile. This are consistent with previous reports that nicotine in cigarette stimulates the secretion of catecholamines resulting in increasing the rate of lipolysis and the increased concentration of plasma free fatty acids (FFA) which further result in increasing the releasing of hepatic FFAs and hepatic triglycerides to the blood stream.[19,20] Even more evidence that nicotine has been recognized as a major risk factor for the development of ischaemic heart disease and it may lead to alteration of the normal plasma lipoprotein pattern.[21] Abdul-Razaq report that serum Triglycerides, and Cholesterol were significantly higher in heavy smokers as compared to both moderate and non-smokers which support our funding.[22] In other group of researcher showed Total cholesterol showed a significant increase, while triglycerides showed highly significant increases after the second and third weeks of nicotine administration in adult male albino rats injected subcutaneously two times daily with 0.5 mg nicotine /kg body weight for 3 weeks.[23] Effraim *et al.* (2000) recorded that a significant increase in serum cholesterol and triglycerides levels after 7 weeks of treatment in rats injected i.p. (once daily) with 1 mg/kg body weight nicotine.[24]

In other hand our study showed that nicotine decrease total serum protein, increase the level of albumin, and decrease the level of globulin compare with control group, and green tea. Abu-El-Zahab and his group showed that nicotine induce highly significant decreases in total serum proteins and albumins were recorded after the 2nd and 3rd week in adult male white albino rats injected subcutaneously two times daily with 0.5 mg nicotine /kg body weight for 3 weeks, while, serum globulins remained unchanged during the three weeks of nicotine administration which consist with our funding.[25] There are several reason for these changes which are radicals giving rise to increased oxidative damage.[26] depletion of plasma antioxidants.[27] Finally cigarette smoke also induces oxidative stress and decreasing antioxidant defences, leading to lipid peroxidation.[28] Cunningham & Friend (1964) reported that, oral administration of nicotine to pigs led to increases in the proportion of protein in lean meat as compared to fat deposits and they suggested that nicotine in-creases the level of free fatty acids in the blood stream where they become a source of energy for protein formation.[29]

Our control group result show that albumin was less than globulin, this prove that smaller animals and birds function at a lower blood pressure from here they need less oncotic pressure to balance the low pressure, and less oncotic pressure need less albumin to maintain proper fluid distribution.[30] Beside higher globulin concentration is mostly related to increased immune competence. It has been established that the liver is the sole source for the synthesis of albumin, fibrinogen and most of Alpha and β globulins, while the immunoglobulin are formed in the lymphoid tissues by the plasma cell.[31] Accordingly, the liver affected by nicotine may suffer from dysfunctions and this may modify the synthesis and metabolism of proteins. This might explain the significant decrease observed in the total serum proteins in rats treated with 3 mg/kg/day with nicotine. The results are also in accordance with the work of Sershen et al., (1982) who found that, injection of nicotine produced inhibition of protein synthesis, due to exposure to cigarette smoke. They referred this effect to the decrease in body temperature.[32]

The changes in lipid profile in our study in nicotine group were supressed when green tea had been drunk for

same period by rats in fourth group. Bursill in 2007 found that increase consumption of green tea lowered serum cholesterol levels.[33] Imai & Nakachi, (1995) recorded that consumption of green tea was associated with decreased serum concentrations of total cholesterol and triglycerides.[34] Other investigators reported that consumption of tea plant leaves by rats for a long period of time decreased serum levels of triglyceride and total cholesterol.[35] Hasegawa et al., (2003) reported that powdered green tea lowered the plasma total cholesterol and total lipid as well as triglycerides contents.[36] Green tea reduces adipose tissue weight in animal models of obesity and has a pronounced effect on lipid metabolism in hyperlipidemia models.[37]

Our results suggest that green tea is a barrier against nicotine damage and that *camellia sinensis* antioxidant property helped to neutralise the oxidative damage and stress by the use of nicotine on comprehensive metabolic panel and lipid profile.

Competing of interest

The authors declare that there is no competing of interests regarding the publication of this paper.

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Ethical approval

The study was approved by the Institutional Animal Ethics Committee.

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