SJIF Impact Factor: 5.464

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 4.
Issue: 3.
Page N. 82-88
Year: 2020

Original Article www.wjahr.com

EFFECTS OF NICOTINE AND CHROMIUM (VI) CO-EXPOSURE ON THE FUNCTIONAL STATUS OF LIVER IN MALE ALBINO RAT

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Received date: 03 March 2020 **Revised date:** 24 March 2020 **Accepted date:** 14 April 2020

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ABSTRACT

Background: Nicotine is one of the major constituents of different types of smoking and is the more toxic part also. Thirty metals including chromium and some chromium compounds have been detected in tobacco smoke are causally associated with cancer in humans. **Objective:** In the present investigation, we evaluate the individual and combined effect of nicotine and chromium (VI) on the functional status of liver in animal. **Materials and Methods:** In this study, a group of male Wistar rats (80-100 g) were induced by intraperitonial injection of vehicle (0.9% NaCl), nicotine tartrate (0.2 mg / 100 g body weight / day), $K_2Cr_2O_7$ (0.8 mg / 100 g body weight / day) and, combined exposure of nicotine tartrate and $K_2Cr_2O_7$ at an interval of six hours for a period of 28 days. After the period of treatment the blood samples were collected to measure the liver function test related parameters. **Results:** It was showed that individual and combined exposure of nicotine and chromium (VI) marked increased the activities of ALP, AST, ALT, LDH and the levels of bilirubins were decreased. On the other hand, there were no significant alterations were observed in the level of total protein and albumin in response to individual and combined exposure of nicotine and chromium. **Conclusion:** The present study suggests that nicotine and chromium exhibited significant changes during individual exposure whereas co-exposure showed a marked alteration of the functional status of liver in male albino rats.

KEYWORDS: Nicotine, Chromium, Nicotine chromium co-exposure, Functional status of liver.

INTRODUCTION

The liver is the key organ regulating homeostasis in the body. It involved with almost all the biochemical pathway related to growth, fight against disease, nutrient supply, energy provision and reproduction. It is expected not to only perform physiological functions but also to protect against hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of chemoology in recent years, liver problems are on rise. Tobacco smoking is the most socially spread habit and is considered as one of the leading causes of premature death in developed as well as developing countries. Epidemiological studies have shown that cigarette smoking may accelerate the progression of renal, pulmonary, and cardiac fibrosis,[1] but whether it might cause organ damage in rather healthy tissues is an important question. The bad effects of nicotine on body function such as rise in heart rate, blood pressure, disturbed lipid profile, atherosclerosis and ischemic heart disease,[2] had been previously shown by direct administration of nicotine in human and animals.[3] Liver is considered to be the major site of nicotine biotransformation and nicotine exerts a number of adverse physiological effects on the liver.[4] Nicotine is absorbed through the lungs with smoking and is rapidly metabolized in the liver which induces three major adverse effects on the liver: Direct or indirect toxic effects, immunological effects, and oncogenic effects.[5] Smoking causes liver cell injury and exerts genotoxic effect of rat liver.[6]

Heavy metals are highly toxic compounds widely spread in the environment, due to their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals with high public health significance. [7] Chromium is released in the environment from industrial processes, wood preservation, pigment, plating, welding, leather tanning, manufacture of stainless steel, and metal finishing,[8] therefore, its level in the industrial waste consists of an important health concern related to the environmental contamination.[9] Furthermore, chromium has been reported for its potential genotoxicity, cytotoxicity,[10] and

carcinogenicity effects.[11] In the same way, its exposure has been linked to cell damages and DNA disruption.[12] The organs that are the most affected by chromium bioaccumulation are liver, kidney, and spleen. In fact, liver is one of the main toxicity targets as it is the biotransformation organ of the majority of xenobiotics.

It was noted that nicotine is one of the major constituents of different types of smoking and is the more toxic part also. Thirty metals have been detected in tobacco smoke, including nickel, arsenic, cadmium, chromium and lead. Arsenic and arsenic compounds and chromium and some chromium compounds are causally associated with cancer in humans, while nickel and cadmium and their compounds are probably carcinogenic to humans. So, the present study aims to evaluate the functional status of liver following individuals and co-exposure of nicotine and chromium (VI) in male albino rat.

MATERIALS AND METHODS

Chemicals: Potassium dichromate and other fine chemicals were purchased from Sigma Chemical Company, USA. All other chemicals and reagents were purchased from Sisco Research Laboratory Pvt Ltd (SRL), India, and were of analytical grade.

Maintenance of Animals: Male albino rats of the Wistar strain (80-100 g) were obtained, divided into four groups, each group contain six animal, housed in polypropylene cages under standard conditions of temperature (25 ± 2.8^{0} C) and humidity ($60 \pm 5\%$), with alternating 12 h light : 12 h dark cycles, and fed standard diet and water *ad libitum*. Animals were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India, and approved by the ethical committee of Vidyasagar University (West Bengal, India).

Treatment of Animals: Laboratory acclimatized rats were divided into four groups of six animals each and almost equal average body weight. The animals of first group was injected intraperitoneally (i.p.) with Cr (VI) as K2Cr2O7 at a dose of 0.8 mg per 100 g body weight per day (20% LD50) for 28 days, as described earlier. [13] The animals of second group were induced by subcutaneous injection with nicotine tartrate (dissolved in 0.9% physiological saline) at a dose of 0.2 mg / 100 g body weight per day for 28 days, as described earlier. [14] The animal of third group was injected both chromium and nicotine as the previous doses at six hours interval for 28 days. The animals of the remaining group was received only the vehicle (0.9% NaCl), served as control.

Collection of blood samples and preparation of serum: After the experimental period, rats were sacrificed by cervical dislocation. Then blood samples were drawn from hepatic vein immediately. Serum was obtained by centrifugation at 1500 × g for 15 min of blood samples taken without anticoagulant.

Analytical methods: Alkaline phosphatase (ALP) was measured in serum according to Kind and King (1954).[15] In serum, the activities of transaminases (SGOT & SGPT) were estimated according to Reitman and Frankel (1957).[16] Total and direct bilirubin was estimated in blood serum according to Lo and Wu (1983).[17] The serum albumin was estimated according to Doumas et al (1997).[18] Total protein was determined according to Lowry et al (using bovine serum albumin as standard.[19]

Statistical Analysis: All the parameters were repeated at least three times. The data were presented as mean ± SEM. By performing ANOVA test (using a statistical package, Origin 6.1, Northampton, MA 01060, USA), the means of control and treated group were compared by multiple comparison t-test having $P<0.05$ as a limit of significance.

RESULTS AND DISCUSSION

In the present study it was noted that the alkaline phosphatase (ALP), transaminases (AST & ALT) and lactate dehydrogenase activities were significantly increased in response to individual and co-exposur of nicotine and chromium. But it was also found that marked changes was observed in co-exposure rats [Figure-1(A-C); Figure-2(A)]. It was noted that inhibition of acid phosphatase, adenosine triphosphatase and succinic dehydrogenase after administration of trivalent and hexavalent chromium.[20] On the other hand, it was observed that significant increase in alkaline phosphatise activity due to lead intoxication.[21] The chromium and other heavy metals have been reported to raise the level of amino transferases. The serum AST activity was significantly higher in animals injected with chromium than cobalt, zinc and manganese, while serum ALT activity were higher in cobalt than in chromium, zinc and manganese.[22] It was reported that ALT and AST activities are higher in tannery workers as compared to workers in the shoe factory. [23] Liver performance indices such as ALP, ALT, and AST are widely used to evaluate the liver damage.[24] Necrosis or cell membrane damage can trigger the release of these enzymes into the blood circulation. [24] It seems that the increased level of serum enzymes indicate cellular leakage, structural damage, and performance dysfunction of membrane markers in the liver due to nicotine administration.[25]

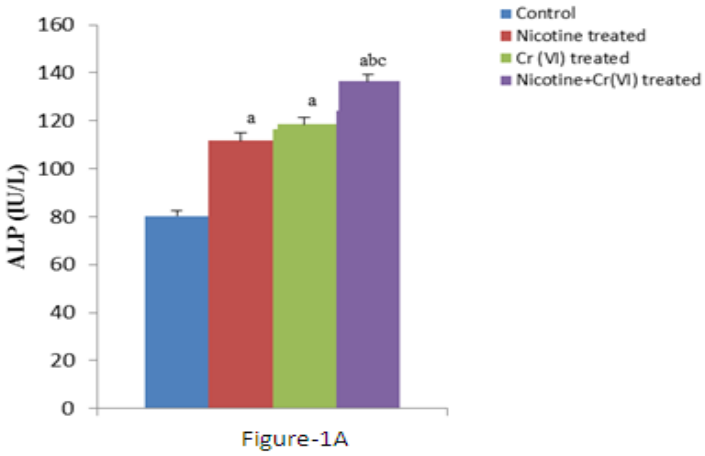

Figure-1A

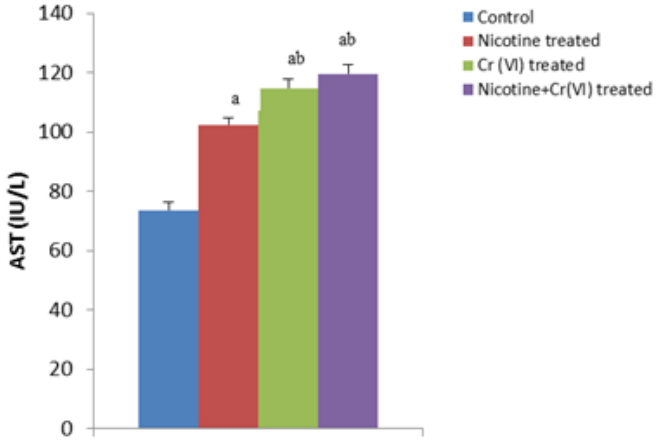

Figure-1B

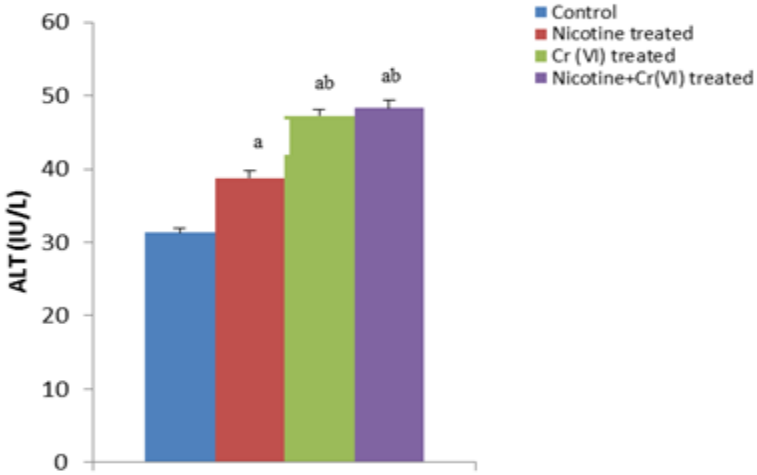

Figure-1C

Figure-1 (A-C): Shows the changes of ALP, AST and ALT activities in different experimental groups of rat. Data represents Mean ± SE.
'a' indicate significant difference (P<0.05) when compared with control.
'b' indicate significant difference (P<0.05) when compared with nicotine treated group.
'c' indicate significant difference (P<0.05) when compared with chromium treated group.

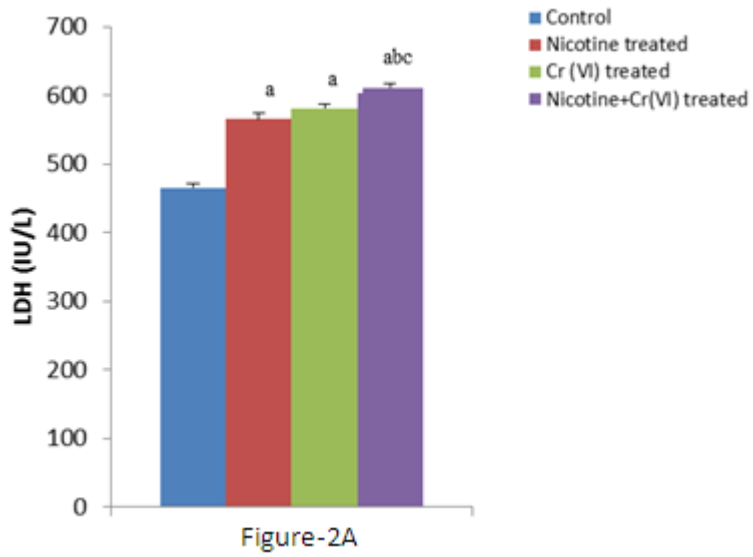

Figure-2A

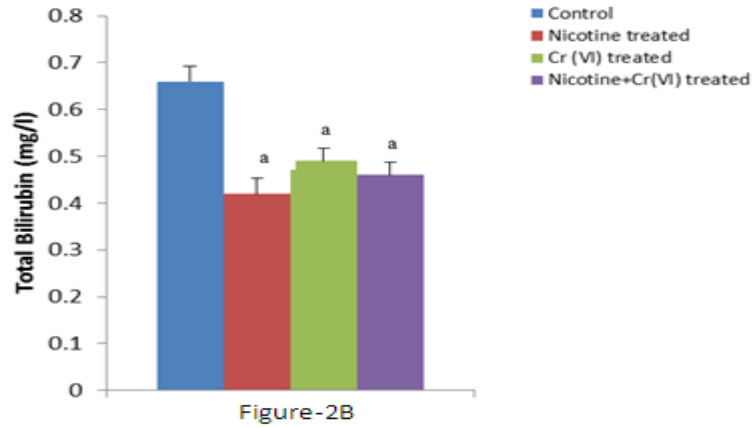

Figure-2B

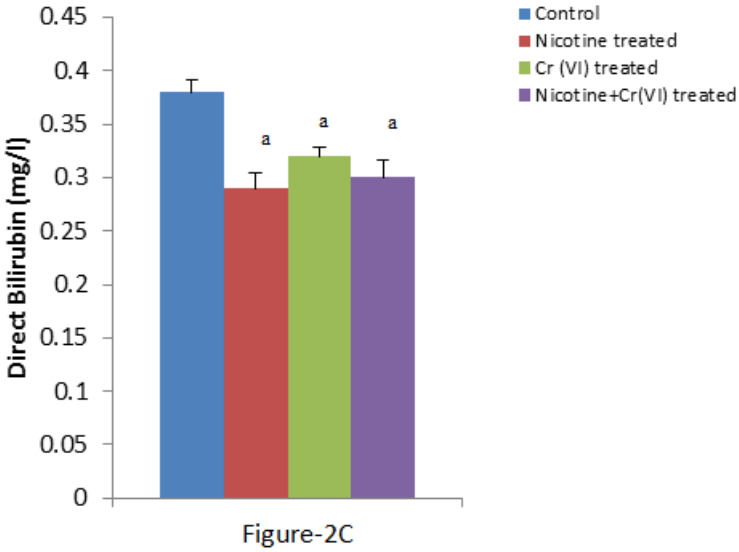

Figure-2C

Figure-2 (A-C): Shows the changes of LDH activity and the levels of Total bilirubin & Direct Bilirubin in different experimental groups of rat.
Data represents Mean ± SE.
'a' indicate significant difference (P<0.05) when compared with control.
'b' indicate significant difference (P<0.05) when compared with nicotine treated group.
'c' indicate significant difference (P<0.05) when compared with chromium treated group.

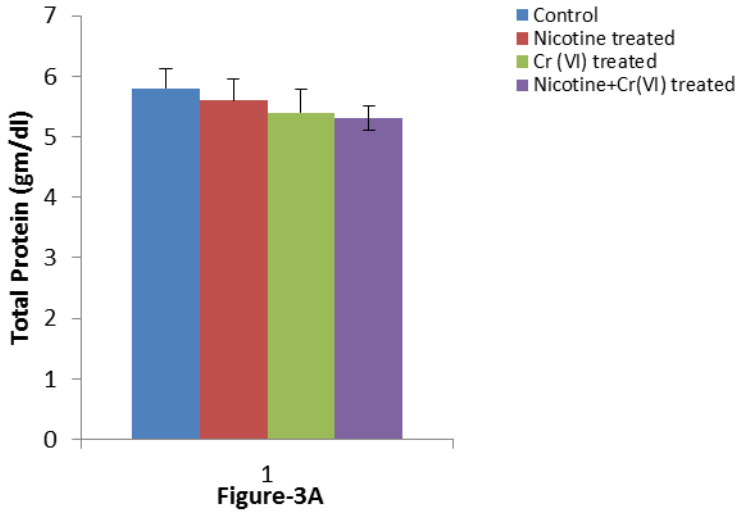

Figure-3A

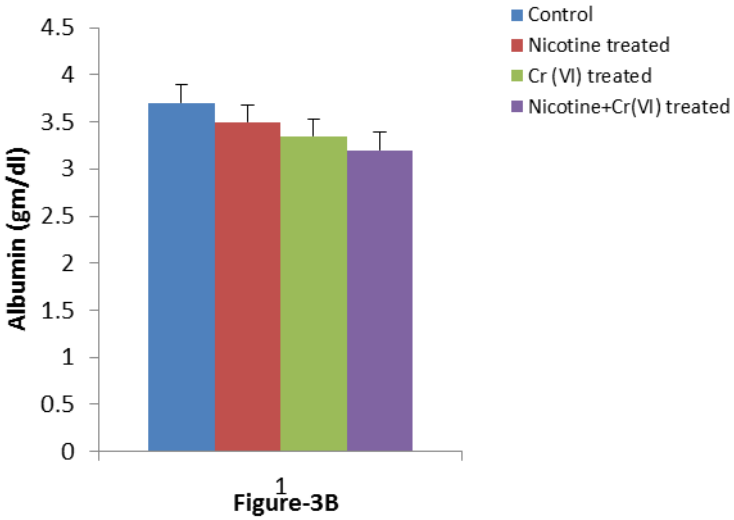

Figure-3B

Fifure-3 (A-B): Shows the changes of Total protein and albumin content in different experimental groups of rat. Data represents Mean ± SE.
'a' indicate significant difference (P<0.05) when compared with control.
'b' indicate significant difference (P<0.05) when compared with nicotine treated group.
'c' indicate significant difference (P<0.05) when compared with chromium treated group.

Bilirubin is the major end product of haemoglobin degradation. It also provides an exceedingly valuable tool for diagnosing both heamolytic blood diseases and various types of liver diseases. [26] It was reported that the 6-12 time increased dose of chromium picolinate resulted in anemia, hemolysis, liver dysfunction and renal failure. [27] From a recent study, the duration of smoking was found to be a more important determinant of serum bilirubin concentration than the number of cigarettes smoked per day.[28] So, smoking duration was an important determinant of serum bilirubin concentration together with smoking amount. Bilirubin, which is a bile pigment, is generally regarded as a waste product of heme catabolism. However, it has been suggested that bilirubin might play an important role as an antioxidant, and its role as an antioxidant in vitro has recently been identified.[29,30] A number of recent *in vitro* studies have shown that bilirubin is more efficient than α-tocopherol at inhibiting low-density lipoprotein (LDL) oxidation and is a more efficient protector of human ventricular myocytes than either vitamin C or vitamin E.[31,32] Cigarette smoke contains a large quantity of free radicals, resulting in endothelial injury.[33] LDL oxidation appears as an early event of atherosclerosis and increases with cigarette smoking.[34] In the present study a significantly decrease in the total and direct bilirubin were observed in individual and co-exposure group of rats (Figure-2B & 2C). This result indicates that accumulation of chromium and nicotine may cause the liver impairment.

Chen et al (2001) have not shown any significant effect on serum albumin and total protein after dietary chromium supplementation.[35] The possible variation

may relate to different levels of chromium exposure and liver toxicity.[36] Exposure to tobacco smoke may disturb protein synthesis due to its influence on liver function.[37] In addition, Cd present in tobacco smoke damages renal function and leads to higher proteinuria.[38] The difference in total protein loss between smoking and non-smoking pregnant women may be a signal of inappropriate liver function or of accelerated protein degradation processes in smokers due to the higher Cd concentration from tobacco smoke. Comparatively low level (not significantly) of albumin and total protein (Figure-3A & 3B) in treated rats may be due to decreased protein synthesis in response to individual and co-exposure of nicotine and chromium in mal albino rats.

To our knowledge this is the first study to suggest possible synergism between nicotine and chromium co-exposed animals. However, this was established based only on few variables. Thus there is need to have a more detailed study to establish mechanism. There were significant alterations of the status of liver function but the mechanisms behind these changes are not known. It can thus be concluded that both nicotine and chromium if given individually or in combination are toxic at the present dose and duration. Further studies are needed to be done in this direction to study the exact mechanism behind synergism between nicotine and chromium on the status liver functions.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Science & Technology (DST), Government of West Bengal, for sanctioning the Research Grant.

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