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Apple Cider Vinegar (A Prophetic Medicine Remedy) Protects against Nicotine Hepatotoxicity: A Histopathological and Biochemical Report

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Abstract Nicotine is the most abundant component in cigarette smoking and is involved in the pathogenesis of lung cancer and increases the risk of developing hepatocellular carcinoma and liver cirrhosis. Prevention of nicotine-induced lung cancer and liver damage may be achieved via decreasing nicotine-induced pathological effects. Nicotine is metabolized in the liver. Natural diet contains a variety of compounds e.g. apple cider vinegar (ACV) that exhibits protective effects against different toxins. This study aims to investigate the effects of nicotine on the liver using morphometrical, histopathological and biochemical parameters and study the protective effect of **ACV** against toxicity of nicotine. Three groups of the male albino rat were used: untreated control group, nicotine treated group (4 mg/kg/day) while the third group received both ACV (2ml/kg/day) and nicotine (4 mg/kg/day). Treatment was given for 30 days. There was a significant increase in the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) together with a damage and degeneration in the liver tissues of the nicotine treated groups. **ACV** administration to nicotine-treated rats showed near normal liver biochemical markers with reduction in the tissue damage associated with the nicotine administration. **ACV** administration to nicotine-treated rat ameliorated the decrease in the size of the hepatocytes nuclei. These results, along with previous observations, suggest that **ACV** may be useful in combating tissue injury resulting from nicotine toxicity. In prophetic medicine, Prophet Muhammad peace be upon him strongly recommended eating vinegar in the prophetic hadeeth: "vinegar is the best edible". **Conclusion:** These finding confirm that chronic nicotine administration causes harmful effects to the liver and suggest that **ACV** may be useful in combating tissue injury resulting from nicotine toxicity. Hence, the intake of **ACV** might suppress the toxicity and mutagenic activity of nicotine. ACV may protect against nicotine-induced carcinogenesis.

Keywords: *nicotine exposure, apple cider vinegar, liver, hepatotoxicity, rats*

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1. Introduction

Tobacco is the most widely used drug in the world and is among the main causes of illness and premature death in developed and developing countries. Epidemiological studies have shown a relationship between smoking and increased risks of cardiovascular disorders, lung cancer and pulmonary diseases. Inhibition of nicotine-induced pathological effects may protect against a wide panel of malignant diseases e.g. lung cancer. Moreover, it has been shown that cigarette smoking may accelerate the

progression of renal, pulmonary, and cardiac fibrosis [1,2,3,4]. The detrimental effects of smoking have been extensively investigated by studies regarding the direct administration of nicotine, a major pharmacologically active component of tobacco smoke in a variety of cell systems [5,6,7]. The predominant effects of nicotine in animals or human being include an increase in heart rate (10 to 20 beats/min), blood pressure (5 to 10 mmHg) and release of catecholamines and free fatty acids. Numerous experimental and clinical evidences have supported the key role of oxidative stress in the pathogenesis of organ disorders after nicotine exposure [8,9,10,11]. Indeed, nicotine significantly increased oxidative stress by enhancing the generation of reactive oxygen species and lipid peroxidation [12,13,14,15].

In addition, nicotine induced the depletion of the antioxidant defense systems through decreasing the level of catalase, superoxide dismutase and glutathione peroxidase [16,17]. Nicotine, once absorbed, is mainly metabolized by the liver to a number of major and minor metabolites [18]. The major metabolite is cotinine, the primary product of the oxidation pathway of nicotine biotransformation that has been used as a marker for nicotine intake [19,20]. Considering that the liver is the major site of nicotine metabolism, it has been expected that the liver is highly susceptible for the oxidative stress associated with the toxicity of nicotine [21]. In fact, many epidemiological studies have shown an association between smoking and accelerated progression of liver fibrosis in patients with a variety of chronic liver diseases such as primary biliary cirrhosis and chronic hepatitis C [22,23].

The experimental models of Azzalini et al. [24] have shown that smoking caused oxidative stress and exacerbated the severity of non-alcoholic fatty liver disease in obese rats. Moreover, nicotine during heavy smoking increased the risk of developing hepatocellular carcinoma (HCC) and liver cirrhosis [25,26]. Many studies had reported that nicotine administration at a concentration similar to those attained by cigarette smoking was hepatotoxic [27,28,29,30].

1.1. Apple Cider Vinegar (ACV)

ACV is used in salad dressings, marinades, vinaigrettes, food preservatives, and chutneys, among other things. It is made by crushing apples and squeezing out the liquid. Bacteria and yeast are added to the liquid to start the alcoholic fermentation process where the sugars are turned into alcohol. In a second fermentation process, the alcohol is converted into vinegar by acetic acid forming bacteria (acetobacter). Acetic acid and malic acid give vinegar its sour taste [31]. Unpasteurized or organic ACV contains mother of vinegar, which has a cobweb-like appearance and can make the vinegar look slightly congealed, being present at concentration of 5%, other constituents of vinegar include polyphenolic compounds, some vitamins, minerals, mineral salt, amino acids and organic acids [32,33].

2. Materials and Methods

2.1. Chemicals

The chemicals in the experiment were nicotine ((S)-3-(1-methyl-2-pyrroli-dinyl) pyridine) and ACV. Nicotine was supplied as colorless liquid, from Al-Nasr Company for pharmaceutics, Cairo, Egypt. The mean lethal dose (LD) for intraperitoneally nicotine to adult albino rat was reported to be 14.5 mg/kg [34]. **5%** ACV was supplied in the form of liquid and diluted by distilled water at ratio 1:5 and was given by nasogastric tube.

2.2. Animals

Thirty pathogen-free male wistar rats (10 weeks of age, 280-300 g) were obtained from Sohag university animal house (Sohag, Egypt) and were used in this study. Animals were fed commercially prepared diets and had free access to tap water. All rats were kept under the same experimental conditions, fed standard diet, and water was available *ad libitum*. Procedures involving the animals and their care were done following institutional guidelines and were approved by the ethical committee of Sohag university in compliance with the national and international laws and guidelines for the use of animals in biomedical research [35].

2.3. Experimental Design

The animals were divided into three experimental groups: Group I; no treatment (control group), Group II; nicotine-treated group (NI): each rat in this group was given nicotine (4 mg/kg/day) intraperitoneally (i.p) for 30 days, and group III nicotine- ACV treated group: each animal in this group was given i.p. nicotine (4 mg/kg/day) at the same dose as group II with ACV (2 ml/ kg body weight) for the same period. The rats were sacrificed by cervical dislocation at the end of the experimental period where the liver specimens were sampled and kept in aqueous bouin for histological and morphometrical examinations.

2.4. Histological Examination

The liver specimens were collected, the liver mass (LM) and liver volume (LV) were measured by the liquid displacement method of Scherle [36] where the organ was separated into several minor fragments kept for 48 h at room temperature in a fixative (freshly prepared 4% w/v formaldehyde in 0.1M phosphate buffer, pH 7.2), embedded in Paraffin (Sigma Co., St. Louis, MO, USA), sectioned at 3 µm thickness, and the sections were stained with hematoxylin–eosin (H&E) [37]. A number of photomicrographs were taken at known magnifications.

2.5. Image Analysis

The data were obtained using Leica Qwin 500 Image Analyzer Computer System (England). The image analyzer consisted of a colored video camera, colored monitor, hard disc of IBM personal computer connected to the microscope, and controlled by Leica Qwin 500 software. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. Using the measuring field menu, the area, percentage area and the standard measuring frame of a standard area equaling 7286.78 m^2 were chosen from the parameters. Using the measuring menu, the hepatocytes nuclei were measured in ten fields for each specimen

using the same magnification. The data obtained were subjected to statistical analysis using Duncan's Test.

2.6. Statistical analysis

All results are expressed as Mean ± SEM. Statistical significance of the difference between group means was performed by one-way ANOVA followed by Student's t-test.

3. Results

1- Morphological observation

Chronic nicotine administration caused a significant decrease on the absolute and relative weight of the liver. However, when ACV was supplemented with nicotine, a significant increase in weight of the liver was observed compared to the nicotine- treated group ($p<0.05$) (Table 1). Significant changes in the liver volume were observed when comparing the two treated groups with the control group (Table 2). The control group presented a liver volume of 11.79±0.10 mm^3 while group II presented a liver volume of 13.86±0.23 and 12.72±0.10 mm^3 for the group III. In nicotine-treated group, a significant decrease in the nucleus area of hepatocytes was obtained where the value was 91.55 ± 0.68 μm^2 ($P< 0.05$), as compared to the control group 108.58±14.11μm^2. On the other hand, daily ACV administration to nicotine- treated group III showed significant ameliorative effects on the size of nuclei of hepatocytes 98.76±7.07 μm^2 (Table 3).

Table 1. Effects of chronic nicotine administration alone or associated with ACV on the body weight, absolute liver weight and relative liver weight in rats

Parameter	Group I (control group)	Group II (nicotine treated)	Group III (nicotine &ACV treated).
Mean body weight	285±2.12g	265±3.11g	270±4.42g
Mean liver weight	8±3.22g	6.5±1.43g*	7.2±3.45g*
Mean relative liver\body weight	2.8%	2.4%*	2.7%*

Each value is mean ± S.D. $p<0.05$ nicotine group vs. nicotine-ACV group.
*$p<0.05$ nicotine treated, nicotine & ACV vs. control group.

Table 2. Liver volume of control, nicotine-treated rats and nicotine-treated rats receiving ACV supplementation

Group	Group I (control group)	Group II (nicotine treated)	Group III (nicotine &ACV treated).
Mean liver volume	11.79±0.10 mm^3	13.86±0.23 mm^3	12.72±0.10 mm^3

Differences with $p<0.05$ were considered to be statistically significant.

Table 3. Effects of nicotine and nicotine + ACV on the area of the nucleus in liver sections of rats.

Group	Group I (control group)	Group II (nicotine treated)	Group III (nicotine &ACV treated).
Nuclear size	108.58±14.11 μm^2	91.55 ± 0.68 μm^2	98.76±7.07 μm^2

Differences with $p<0.05$ were considered to be statistically significant.

2- Biochemical parameters of liver functions

The effect of chronic nicotine supplementation on biochemical indicators of liver function in serum is shown in Table 4. Compared with the controls, group II has a significant increase ($p < 0.05$) in serum ALT, AST and ALP levels. However, nicotine -ACV group showed a significant improvement in the levels of ALT, AST, ALP and LDH compared to values of control group.

Table 4. Liver functions of control, nicotine-treated rats and nicotine-treated rats that received ACV supplementation

Parameter	Group I (control group)	Group II (nicotine treated)	Group III (nicotine &ACV treated).
ALT	32.00 ± 1.73	59.12 ± 1.82^s	35.44 ±0.34 ^{n}
AST	99.32 ± 02.33	140.14 ± 02.81^s	102.39 ± 02.03
ALP	209.51 ± 6.61	258.56 ± 31.52	220.13 ± 03.52^n
LDH	598.56 ± 42.19	1009.17 ± 39.27 ^{s}	750.07 ± 32.21^n

ALT: Alanine aminotransferase, AST; Aspartate aminotransferase, ALP: Alkaline phosphatase and LDH: Lactate dehydrogenase. Data represent mean ± S.D from ten rats in each group.
^{s}p <0.05 nicotine group vs. control group.
^{n}p <0.05 nicotine-ACV group vs. control group.

3- Histopathological observations

In the control group (CG), it was possible to observe the preserved hepatocytes and normal capillaries without inflammatory cells (Figure 1). However in the nicotine treated group (Figure 2 & Figure 3), it was also possible to observe varied sizes of hepatocytes with uncertain cellular limits, accumulation of lipid droplets in the cytoplasm and diffuse microvesicular steatosis. Liver sinusoids and the central v. were full of red blood cells. While group III treated with nicotine (4 mg/kg) and apple cider vinegar (ACV) (2ml/ kg body weight) showed more or less normal liver structure, normal appearance of the hepatocytes with decreased vacuolization compared with the nicotine group. In addition, ACV-treated group restored more or less the normal size of the nucleus and the focal congested sinusoids (Figure 4).

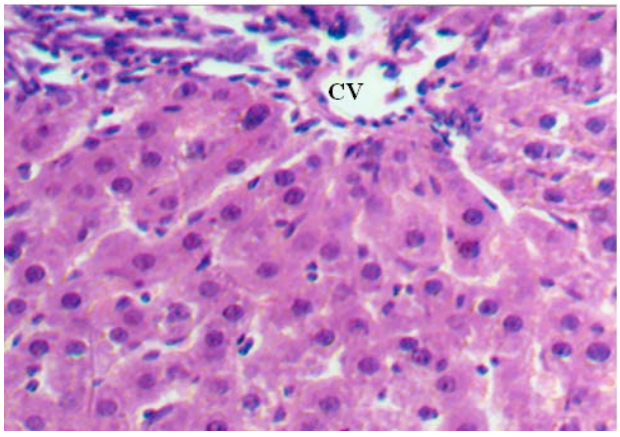

Figure 1. Micrograph of a liver section of control rats showed the normal hepatic trabecular arrangement with preserved hepatocytes and normal capillaries form (H&E X 200)

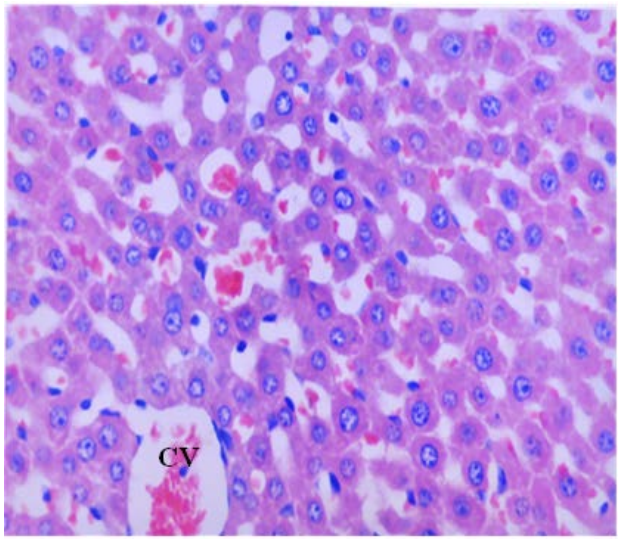

Figure 2. Micrographs of liver sections of nicotine-treated rats showed varied sizes of hepatocytes with uncertain cellular limits and accumulation of lipid droplets in the cytoplasm and diffuse microvesicular steatosis. The liver sinusoids and the central v. were full of red blood cells. (H&E, X200)

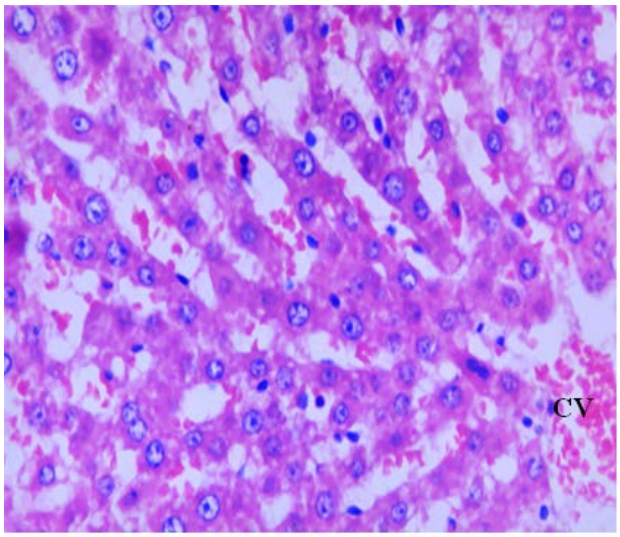

Figure 3. Micrographs of liver sections of nicotine-treated rats showed varied sizes of hepatocytes with uncertain cellular limits and accumulation of lipid droplets in the cytoplasm and diffuse microvesicular steatosis. The liver sinusoids and the central v. were full of red blood cells. (H&E, X200)

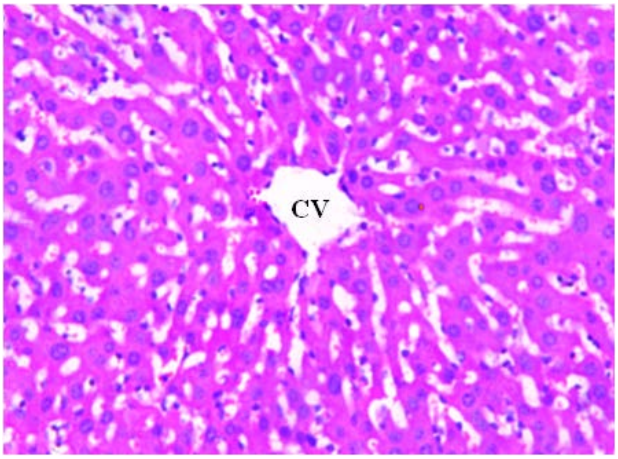

Figure 4. Photomicrograph of a liver section of a diabetic rat treated daily with ACV showing that the hepatocytes partly restored their normal configuration (X. 200)

4. Discussion

Tissue protection against nicotine-induced damage and toxicity is crucial for cancer prevention. Cigarette smoking is common in most societies worldwide and has been identified as injurious to human health. The effect of smoking on liver diseases has been studied and so many investigations have revealed its harmful effects [38]. Nicotine, a major toxic component of cigarette smoking is rapidly absorbed through the lung and is mainly metabolized in the liver [39]. Chronic administration of nicotine in rats reportedly induces cytochrome P-450, generates free radicals in tissues and exerts oxidative tissue injury [40]. So, individuals who consume alcohol frequently and smoke at the same time [41] suffer badly, which raises a question regarding the interactive effects of nicotine and ethanol on the antioxidant system in specific tissues of the body [42]. The liver is a major organ for drug biotransformation. Therefore, it is highly susceptible to the oxidative stress events associated with the toxicity of nicotine and ethanol [43]. The aim of this study was to evaluate the detrimental effects of chronic administration of nicotine alone or combined with apple cider vinegar on the serum biochemical parameters, antioxidant and histopathological changes in the liver of male Wistar rats.

In addition, the weight of the liver in the nicotine-treated group was significantly lower (Table 1) than the liver of the control group which may be due to the fat deposition in the cytoplasm of the hepatocytes causing enlarged liver volume (Table 2). This histopathological change was due to the formation of fatty liver (Figure 1-Figure 3). It could be due to the increased influx of fatty acids into the liver or to hyperlipidemia. In addition, it has been reported that the shifting in the redox state and NADH oxidation due to ethanol consumption contribute to hepatic metabolic abnormalities e.g. enhanced hepatic lipogenesis and steatosis [44,45,46]. However, the area of the nucleus in liver sections of rats treated with nicotine and ACV improved so much to near the values of the normal control (Table 3).

In this study, the assessment of serum biochemical parameters revealed a significant increase in LDH level in nicotine-treated animals (Table 4). LDH, a cytoplasmic marker enzyme is a known indicator of cell and tissue

damage by toxic compounds. The increase in LDH activity indicated that the cellular damage due to loss of functional integrity of the cell membranes. In fact, the oxidative tissue injury of hepatic membrane (after chronic exposure to nicotine) produced marked changes in the molecular organization of lipids leading to an increase in membrane permeability and to the leakage of cytoplasmic enzymes e.g. LDH. Thus, the enhanced LDH activity in nicotine-treated rats can be linked to the increased lipid peroxidation in rat hepatocytes (Table 4).

After chronic administration of nicotine plus ACV, we observed a significant decrease in the activity of indicators of liver function like AST, ALT, ALP and LDH. The decrease in the activities of these enzymes in serum was indicative for hepatocyte amelioration and refinement in liver functions. Thus, the improved liver function tests might be due to the combined exposure to nicotine and ACV compared to nicotine treated group (Table 4).

The mechanism of nicotine toxicity on the rat liver in this study was not fully understood, but the liver was the first organ directly injured with nicotine. Considerable evidences point to the role of oxidative stress in inducing DNA damage and cellular damage as possible causes of injury to the main organs of the body including the lungs [47], cardiovascular system [48], central nervous system [49], bone marrow cells [50], kidney [51] and testis [52].

On the other hand, ACV might attenuate the histological damage in rats liver (Figure 4) through its strong antioxidant properties [53]. Administration of ACV partly normalized the activity of hepatic enzymes and enhanced the level of non-enzymatic antioxidants. The degree of beneficial effects of ACV against toxicity of nicotine depends on the duration of treatment. Recent evidences *in vitro*, animal and human trial studies indicated the possibility that the consumption of ACV may reduce the risk of cancer among smokers [54]. The amelioration effect of ACV on nicotine toxicity may be attributed to its antioxidant properties [55] and the free radicals scavenging properties through decreasing lipid peroxidation and suppressing the induced oxidative damage; both caused oxidative damage in nicotine-treated animals [56]. The protective effects of ACV extract against nicotine toxicity may be due to the combination of several different mechanisms, including modulation of expressions of antioxidative systems, direct scavenging of free radicals, [55] reduction of the levels of several markers of oxidative stress, decreased lipid peroxidation and decreased DNA strand breakage induced by cigarette smoke in cultured human bronchial cells [56]. In prophetic medicine, Prophet Muhammad peace be upon him strongly recommended eating vinegar in the prophetic hadeeth: " vinegar is the best edible" [57].

5. Conclusion

ACV exerts significant tissue protective effects against nicotine-induced pathological effects that are involved in the pathogenesis of many diseases e.g. lung cancer. Here, ACV-induced protection may be attributed to its high contents of phenolic acid derivatives, antioxidant effects and scavenging activities in restoring the liver biomarkers and repairing the hepatocytes structure. Oral administration of ACV at 2 ml/kg b.w protected against nicotine-induced liver damage in rats. ACV may protect against nicotine-induced carcinogenesis.

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