Combination of Caffeine and Liver Albumin Plus Protects against Smoking-Induced Liver Injury in Rats

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Keywords

Caffeine · Liver Albumin Plus · Smoking · Alpha-fetoprotein · Hypoxia-inducible factor

Abstract

Background: A number of studies have revealed the hepatoprotective effect of coffee and tea. However, the role of caffeine on smoking-induced liver injury is not well elucidated. Liver Albumin Plus (LAP) is a liver supplement given in different liver diseases; to our knowledge, its role in smoking-induced liver injury is not clear. **Objectives:** This study aimed to find out the protective effect of caffeine and LAP alone and in combination in attenuation of smoking-induced liver injury. **Methods:** Thirty male albino rats were divided into a control group and a smoking group; the smoking group was then subdivided into a smoking group, a smoking + caffeine group, a smoking + LAP group, and a smoking + caffeine + LAP group. At the end of the experimental study, blood samples were collected for assessment of liver enzymes, alpha-fetoprotein (AFP), and interleukin 6, and livers were excised. Biochemical analysis of hydrogen

peroxide (H₂O₂), superoxide dismutase (SOD), and hypoxia-inducible factor (HIF) as well as histological examination were done. **Results:** The results showed that smoking elevated liver enzymes, AFP, H₂O₂, and HIF and decreased SOD; histologically, deterioration of the liver was observed. On administration of caffeine, significant ($p < 0.05$) improvement in all measured parameters and preserved liver histological structure were observed, while intake of LAP alone showed some improvement. In combination, all liver parameters were improved and histological structure was preserved in contrast to each drug alone. **Conclusion:** It is better to give a combination of caffeine and LAP with cigarettes smoking to attenuate smoking-induced liver injury.

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Introduction

Caffeine (1,3,7-trimethylxanthine) is contained in many foods and beverages, such as coffee and tea. It is widely consumed as it acts as a central nervous system stimulator; moreover, it can be used clinically as a di-

uretic, analgesic, and muscle relaxant [1]. Studies mentioned that caffeine may increase the production of free radicals that in turn increase lipid peroxidation [2]. Oxidative stress leads to oxidative modification of cellular proteins, such as receptors and transport systems [3]. However, caffeine was found to protect against cellular damage via the production of useful antioxidant products [4]. Caffeine nowadays is known for its hepatoprotective effect against toxic agents on the liver, as detected via improvement in liver functions [5]. Liver Albumin Plus (LAP) is an available medication in the market as it is widely used as a liver support medication in many liver diseases; however, few studies have been performed on LAP. A study performed on polynitroxyl albumin plus found that it may protect against liver injury caused by reactive oxygen species (ROS) as in cases of liver ischemia reperfusion via its antioxidant and anti-inflammatory effect [6]. Smoking causes a variety of hazardous effects on organs which are toxic, immunological, and oncogenic in nature. Moreover, smoking produces chemical substances that have cytotoxic effects, such as necroinflammation and fibrosis. Additionally, smoking increases the production of proinflammatory cytokines such as interleukin 1 (IL-1), IL-6, and tumor necrosis factor alpha (TNF α), leading to liver cell injury [7]. It was found that cigarette smoking was significantly associated with increased liver enzymes [8]. However, hepatologists have conventionally paid little attention to the injurious effects of cigarette smoke. In the United States, tobacco use was responsible for 467,000 deaths in 2005 [9]. According to Ghaly et al. [10], cigarette smoke exposure can result in degenerative changes in hepatocytes, cellular infiltration, periportal fibrosis, and congestion of both the central and portal veins. Evidence provided by Azzalini et al. [11] showed that cigarette smoke causes oxidative stress and apoptosis in the liver. Tobacco is known for its oncogenic effect in several organs, a number of studies showing that cigarette smoking is associated with an increased incidence of hepatocellular carcinoma in cirrhotic patients [12]. Hypoxia-inducible factor (HIF) is a transcription factor that regulates a wide range of genes involved in cellular responses to hypoxia. HIF is a heterodimeric complex consisting of a β -subunit and an oxygen-sensitive α -subunit. Increased expression of HIF is observed in many liver diseases [13]. A common feature of liver diseases is tissue hypoxia due to an imbalance between demand and supply [14], as oxygen consumption by hepatocytes and infiltrating inflammatory leukocytes is dramatically increased [15]. Interestingly, increased

production of ROS is a common phenomenon in pathological liver conditions, and in turn they have also been shown to stabilize HIF [16]. The aim of this study was to find out the hepatoprotective effect of caffeine alone and in combination with LAP against the toxic effects of smoking. The focus of this paper was to predict the toxic and pathophysiological effects on human outcomes as animals can be used to predict human response to drugs and other chemicals.

Materials and Methods

Thirty male albino rats weighing 120–150 g were obtained and inbred, with veterinary care, in the Modern Sciences and Arts University animal house. Female rats were not included in this research due to the variable nature of female data caused by hormonal fluctuations associated with the female reproductive cycle. The animals were housed in wire mesh cages at room temperature with normal light-dark cycles and maintained on standard rat chow diet and tap water during the 4-week experimental period. They were allowed to acclimatize to their environment for 1 week before the start of the experiments, which were conducted in accordance with the ethical guidelines of the Faculty of Medicine, Cairo University. Animals were divided randomly into a control normal group (control group, $n = 6$) and cigarette smoke-exposed rats (smoking group, $n = 24$), the latter being subdivided into a cigarette smoke only-exposed group (smoking group, $n = 6$), a cigarette smoke-exposed group with administration of caffeine (smoking + caffeine, $n = 6$) in which caffeine was given at a dose of 100 mg/kg/day orally to the end of the experimental study [17], a cigarette smoke-exposed group with intake of LAP (smoking + LAP, $n = 6$) in which LAP was given at a dose of 16 mg/day/rat orally to the end of the experimental study, and a cigarette smoke-exposed group with administration of caffeine and LAP in the above-mentioned doses (smoking + caffeine + LAP, $n = 6$).

At the end of the experimental period, blood samples were collected and rats were then sacrificed. Liver tissues were collected and sectioned for biochemical analysis, preserved in foil, and kept frozen at -80°C ; the remaining tissues were preserved in formalin 10% for histological analysis.

Cigarette Smoking Protocol

Rats were exposed to cigarette smoke at a dose of five cigarettes/day to the end of the experimental period (4 weeks); a 1-h interval was left between the burning of two cigarettes [17].

Biochemical Measurements

(1) Liver enzymes (aspartate transaminase, alanine transaminase, and alkaline phosphatase) using commercially available kits (BioMed, Cairo, Egypt). (2) Serum IL-6 (assessed via Elisa kit supplied by MyBio Source, USA), according to the manufacturer's instruction. (3) Serum alpha-fetoprotein (AFP) using commercially available kits (BioMed). (4) Detection of liver hydrogen peroxide (H_2O_2) and superoxide dismutase (SOD) by polymerase chain reaction kits (BioMed). (5) Detection of liver HIF via Western blot kits supplied by Bio Basic Inc., Markham, ON, Canada.

Table 1. Assessment of ALT and AST

Group	Parameter	
	ALT	AST
Control	13.67±1.12	14.17±0.6
Smoking	62.83±4.17 ^a	52.5±1.12 ^a
Smoking + caffeine	32±0.93 ^{a, b}	30.17±0.79 ^{a, b}
Smoking + LAP	31.67±0.88 ^{a, b}	24±0.97 ^{a-c}
Smoking + caffeine + LAP	21.33±0.8 ^{a-d}	20±1.37 ^{a-d}

Values are expressed as mean ± standard deviation for 6 rats per group. ALT, alanine transaminase; AST, aspartate transaminase; LAP, Liver Albumin Plus. ^a Significant difference from the control group at $p < 0.05$. ^b Significant difference from the smoking group at $p < 0.05$. ^c Significant difference from the smoking + caffeine group at $p < 0.05$. ^d Significant difference from the smoking + LAP group at $p < 0.05$.

Histological Analysis

Liver tissue was fixed in a solution of 10% neutral buffered formalin, embedded in paraffin, sectioned at a thickness of 5 µm, and stained with hematoxylin and eosin for regular examination. The sections were analyzed using an Olympus BX51 microscope equipped with camera, with ×100 magnification in all examined groups.

Statistical Analysis

Results are expressed as mean ± standard deviation. Statistical significance was performed by one-way analysis of variance, used to make an analysis and sort out significant statistical differences, followed by Tukey-Kramer posttest. All groups showed normally distributed values and equal variances. The sample size was based on previous studies.

Results

Biochemical Results

Liver Enzymes. Liver enzymes were significantly elevated in the smoking group in comparison to the control normal nonsmoking group ($p < 0.05$). On administration of caffeine or LAP, significant improvement ($p < 0.05$) was detected in comparison to the smoking group. Combination of caffeine and LAP showed significant improvement in comparison to the smoking group and in comparison to each drug alone, but still elevation compared to the normal control group (Table 1).

Serum IL-6. IL-6 was significantly elevated in the smoking group in comparison to the control normal nonsmoking group ($p < 0.05$). On administration of caffeine or LAP, significant improvement ($p < 0.05$) was detected in comparison to the smoking group. Combination of

Table 2. Assessment of serum IL-6

Group	IL-6, pg/mL
Control	1.0±0.001
Smoking	7.57±0.41 ^a
Smoking + caffeine	3.73±0.06 ^{a, b}
Smoking + LAP	3.83±0.12 ^{a, b}
Smoking + caffeine + LAP	2.31±0.11 ^{a-d}

Values are expressed as mean ± standard deviation for 6 rats per group. IL-6, interleukin 6; LAP, Liver Albumin Plus. ^a Significant difference from the control group at $p < 0.05$. ^b Significant difference from the smoking group at $p < 0.05$. ^c Significant difference from the smoking + caffeine group at $p < 0.05$. ^d Significant difference from the smoking + LAP group at $p < 0.05$.

Table 3. Assessment of serum AFP

Group	AFP, ng/mL
Control	0.53±0.02
Smoking	0.96±0.03 ^a
Smoking + caffeine	0.68±0.01 ^{a, b}
Smoking + LAP	0.71±0.03 ^{a, b}
Smoking + caffeine + LAP	0.56±0.01 ^{b-d}

Values are expressed as mean ± standard deviation for 6 rats per group. AFP, alpha-fetoprotein; LAP, Liver Albumin Plus. ^a Significant difference from the control group at $p < 0.05$. ^b Significant difference from the smoking group at $p < 0.05$. ^c Significant difference from the smoking + caffeine group at $p < 0.05$. ^d Significant difference from the smoking + LAP group at $p < 0.05$.

caffeine and LAP showed significant improvement in comparison to the smoking group and in comparison to each drug alone, but still elevation compared to the normal control group (Table 2).

Serum AFP. AFP was significantly elevated in the smoking group in comparison to the control normal nonsmoking group ($p < 0.05$). On administration of caffeine or LAP, significant improvement ($p < 0.05$) was detected in comparison to the smoking group. Combination of caffeine and LAP showed significant improvement in comparison to the smoking group, and in comparison to each drug alone also normalization of its value was observed (Table 3).

Liver Expression Levels of H₂O₂ and SOD. A significant elevation in H₂O₂ and a significant decrease in SOD expression levels were detected in the smoking group in comparison to the control normal nonsmoking group

Fig. 1. Assessment of liver expression levels of H₂O₂ detected by polymerase chain reaction. Values are expressed as mean $\pm$ standard deviation. ^a Significant difference from the control group at $p < 0.05$. ^b Significant difference from the smoking group at $p < 0.05$. ^c Significant difference from the smoking + caffeine group at $p < 0.05$. ^d Significant difference from the smoking + LAP group at $p < 0.05$. H₂O₂, hydrogen peroxide; LAP, Liver Albumin Plus.

Fig. 2. Assessment of liver expression levels of SOD detected by polymerase chain reaction. Values are expressed as mean $\pm$ standard deviation. ^a Significant difference from the control group at $p < 0.05$. ^b Significant difference from the smoking group at $p < 0.05$. LAP, Liver Albumin Plus; SOD, superoxide dismutase.

Fig. 3. Assessment of liver expression levels of HIF detected by Western blot. Values are expressed as mean $\pm$ standard deviation. ^a Significant difference from the control group at $p < 0.05$. ^b Significant difference from the smoking group at $p < 0.05$. ^c Significant difference from the smoking + caffeine group at $p < 0.05$. ^d Significant difference from the smoking + LAP group at $p < 0.05$. HIF, hypoxia-inducible factor; LAP, Liver Albumin Plus.

Table 4. Assessment of liver expression levels of HIF detected by Western blot

Group	Parameter	
	H ₂ O ₂	SOD
Control	27.42±1.58	2.82±0.09
Smoking	110.95±0.79 ^a	0.74±0.08 ^a
Smoking + caffeine	58.68±1.21 ^{a, b}	1.96±0.09 ^{a, b}
Smoking + LAP	66.23±4.31 ^{a, b}	2.02±0.03 ^{a, b}
Smoking + caffeine + LAP	45.4±3.63 ^{a-d}	2.2±0.1 ^{a, b}

Values are expressed as mean $\pm$ standard deviation for 6 rats per group. H₂O₂, hydrogen peroxide; HIF, hypoxia-inducible factor; LAP, Liver Albumin Plus; SOD, superoxide dismutase. ^a Significant difference from the control group at $p < 0.05$. ^b Significant difference from the smoking group at $p < 0.05$. ^c Significant difference from the smoking + caffeine group at $p < 0.05$. ^d Significant difference from the smoking + LAP group at $p < 0.05$.

($p < 0.05$). On administration of caffeine or LAP, significant improvement ($p < 0.05$) was detected in comparison to the smoking group. Combination of caffeine and LAP showed significant improvement in comparison to the smoking group and in H₂O₂ in comparison to each drug alone, as results returned to normal values and there was no significance in comparison to the normal control group (Table 4; Fig. 1, 2).

Liver Expression Levels of HIF. Liver expression levels of HIF were significantly elevated in the smoking group in comparison to the control normal nonsmoking group ($p < 0.05$). On administration of caffeine or LAP, significant improvement ($p < 0.05$) was detected in comparison to the smoking group. Combination of caffeine and LAP showed significant improvement in comparison to the smoking group and in comparison to each drug alone (Table 5; Fig. 3).

Table 5. Liver expression levels of HIF

Group	HIF, pg/mL
Control	1.00±0.001
Smoking	6.56±0.25 ^a
Smoking + caffeine	2.55±0.1 ^{a, b}
Smoking + LAP	2.82±0.09 ^{a, b}
Smoking + caffeine + LAP	1.78±0.12 ^{a-d}

Values are expressed as mean ± standard deviation for 6 rats per group. HIF, hypoxia-inducible factor; LAP, Liver Albumin Plus. ^a Significant difference from the control group at $p < 0.05$. ^b Significant difference from the smoking group at $p < 0.05$. ^c Significant difference from the smoking + caffeine group at $p < 0.05$. ^d Significant difference from the smoking + LAP group at $p < 0.05$.

Histological Results

The normal control group showed normal liver histological structure (Fig. 4a); the smoking group showed cytoplasmic vacuolation, congested central vein, inflammatory cellular infiltrate, and areas of necrosis all over the examined sections (Fig. 4b). On administration of caffeine, improvement was observed as decreased central vein congestion and decreased inflammatory cellular infiltrate in the portal tract while the liver still showed areas of necrosis (Fig. 4c). Unfortunately, LAP was not effective in the treatment of smoking effects on liver tissue, as cytoplasmic vacuolation was extensive and the liver showed areas of necrosis and inflammatory infiltration (Fig. 4d). Combination of caffeine and LAP mostly acquired normal architecture with normal-appearing hepatocytes (Fig. 4e).

Discussion

Smoking has nowadays become a widespread habit. To our knowledge, smoking affects all body organs and deteriorates their functions. The aim of the present study was to investigate the effects of cigarette smoking on rat liver and to search for potential protective effects of caffeine, which is a common nutritional beverage, and the protective effect of LAP as a liver supplement therapy alone and in combination on smoking-induced liver injury. The results of the present study showed that exposure of rats to cigarette smoke daily for 4 weeks resulted in deterioration of liver condition, as observed via increased serum levels of liver enzymes and serum AFP, which could be an indicator of liver failure, as mentioned by Varshney et al. [18], who reported that elevated serum

AFP is an indicator of acute liver failure. The data in this study were supported by histological examination, as was observed via increased liver inflammation and necrosis. These results are supported by Salahshoor et al. [19], who mentioned that smoking causes hepatocyte and central vein enlargement as well as elevated liver enzymes. In the present study, we searched for the mechanism of liver injury in the cigarette smoking group and observed elevated serum IL-6 in the exposed group. This result was mentioned by Sliwińska-Mossoń et al. [20], who detected a significant elevation in serum IL-6 in smoking patients and an increased expression of IL-6 in pancreatic acini with associated pancreatitis. In addition, Moszczyński et al. [21] mentioned that smoking increases the production of proinflammatory cytokines (IL-1, IL-6, and TNF- α), resulting in liver cell injury. In addition, elevated liver oxidative stress was clear in the present study detected via elevated liver expression of H₂O₂ and decreased expression of SOD. It was mentioned by Battah et al. [22] that the smoke-exposed group showed increased mitochondrial numbers in hepatic cells and widening of their intermembrane space with loss of mitochondrial cristae; these findings pointed out that smoking displays cellular damage and oxidative stress resulting in ultrastructural liver changes. This increased oxidative stress could also be shown by Avti et al. [23], who explained that decreased liver levels of SOD with smokeless tobacco lead to inefficient removal of oxygen free radicals from cells, resulting in overaccumulation of ROS. Moreover, overproduction of ROS resulted in inhibition of expression levels of SOD. HIF is commonly known to be increased in most liver diseases, such as alcoholic and nonalcoholic liver diseases as well as ischemic liver injury [13]. In the present study, elevated liver expression levels of HIF in the smoking group could be explained by the findings of Tannahill et al. [24], who declared that during inflammation, succinate is increased and functions as an HIF activator. In addition, Guzy et al. [25] noticed that ROS has been shown to stabilize HIFs and that increased production of ROS is commonly found in most pathological liver conditions. We conclude from previous data that elevated liver expression levels of HIF are a sign of liver injury and that they could be due to increased inflammatory conditions or overproduction of liver ROS. After the current study we think about using caffeine in attenuation of smoking-induced liver injury. Improvement was detected in the smoking group administered caffeine in the form of improvement of liver enzymes and AFP. These results are in accordance with the findings of Vitaglione et al. [26], who demonstrated that coffee consumption protects the liver

Fig. 4. Photomicrograph of liver sections stained with hematoxylin and eosin ($\times 100$). **a** Normal control group showing normal liver histology, average-sized central vein, and pink cytoplasm with central vesicular nucleus. **b** Smoking group showing cytoplasmic vacuolation, congested central vein, inflammatory cellular infiltrate, and areas of necrosis all over the examined sections. **c** Smoking + caffeine group showing that caffeine treatment reduces the effects of smoking on the liver, seen as decreased central vein congestion,

while the portal tract still shows inflammatory cellular infiltrate and areas of necrosis are still seen. **d** Smoking + LAP group: unfortunately, LAP was not effective in the treatment of smoking effects on liver tissue, as detected by extensive cytoplasmic vacuolation and areas of necrosis and inflammatory infiltration. **e** Smoking + caffeine + LAP group showed: great improvement was detected as liver tissue acquired normal architecture with normal-appearing hepatocytes. LAP, Liver Albumin Plus.

from damage by a high-fat diet. This effect could be mediated by a reduction in hepatic fat accumulation (through increased fatty acid β -oxidation), improvement of oxidative stress, and reduction of liver inflammation via decreased expression and concentrations of proteins and cytokines related to inflammation. The results are in accordance with the findings of Freedman et al. [27], who noticed, in a large prospective study of participants with advanced hepatitis C, that regular administration of coffee resulted in lower rates of disease progression. Moreover, the present study showed moderate improvement in liver histological results. We explain the improvement in smoking-induced liver injury on administration of caffeine to be through caffeine's anti-inflammatory and antioxidant effects, as observed via decreased IL-6 and decreased expression level of H_2O_2 . It was mentioned by Choi et al. [28] that treatment with coffee extract decreased the mRNA levels of IL-6 in mice stimulated by lipopolysaccharide. Moreover, Amer et al. [29] declared that caffeine provided an antifibrogenic, anti-inflammatory, and antioxidant effect associated with improvement of hepatic histological structure from thioacetamide-induced hepatotoxicity. They explained their results by caffeine reducing serum levels of IL-6. Moreover, they hypothesized that the antioxidant effect of caffeine could be due to its modulation of antioxidant enzymes that results in reduction of ROS. This study is one of the few studies carried out on the effect of LAP on smoking-induced liver

injury. Interestingly, in this study LAP intake in the smoking group improved all measured liver parameters; however, no improvement was seen in histological findings. In the present study, combination of caffeine and LAP showed much improvement in all measured parameters and also histological improvement.

In conclusion, combined administration of caffeine and LAP is able to attenuate smoking-induced liver injury.

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Statement of Ethics

Animal experiments conformed to internationally accepted standards and to the ethical guidelines of the Faculty of Medicine, Cairo University.

Disclosure Statement

The authors have no conflicts of interest to declare.

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