ORIGINAL ARTICLE

Protective Effects of Caffeic Acid Phenethyl Ester on Isoniazid and Rifampicin-induced Hepatic and Pancreatic Injury

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ABSTRACT

Objective: *To investigate the protective effects of caffeic acid phenethyl ester (CAPE) against isoniazid (INH)- and rifampicin (RFP)-induced hepatic and pancreatic damage.*

Methods: *Eighty adult rats were randomly divided into eight groups: control, INH, RFP, INH+RFP, INH+CAPE, RFP+CAPE, INH+RFP+CAPE, and CAPE. Both INH and RFP were orally administered for 30 days at a dose of 50 mg/kg/day. Caffeic acid phenethyl ester was intraperitoneally injected for 30 days (10 µmol/kg). Blood samples, hepatic and pancreatic tissues were obtained on day 30.*

Results: *Total oxidant status levels were significantly higher in INH and/or RFP-treated groups than those of control and CAPE groups, while total antioxidant status and paraoxonase levels were significantly reduced in INH-RFP groups compared with the group receiving CAPE. Histopathological deterioration was observed in RFP and INH groups in pancreatic and hepatic tissue. However, significant amelioration was observed in CAPE-treated groups.*

Conclusion: *Our findings suggest that CAPE may be a promising agent to prevent the side effects of INH and RFP treatment on hepatic and pancreatic tissues.*

Keywords: Caffeic acid phenethyl ester, isoniazid, liver, pancreas, rifampicin.

INTRODUCTION

Tuberculosis (TB) is an infectious disease causing significant morbidity and mortality worldwide. The causative agent, *Mycobacterium tuberculosis* (MTB), is endemic in the world's population, particularly in Africa, the West Pacific and Eastern Europe, but it may be encountered anywhere (1, 2). Treatment of TB is difficult, in part, because MTB avoids the immune system by residing inside macrophages and requires relatively long courses of antibiotic treatment (3, 4).

Isoniazid (INH) and rifampicin (RFP) are the first-line drugs for anti-TB therapy, but potential toxic reactions, particularly the hepatotoxicity from the use of these drugs, remain a significant problem in clinical treatment (5–7). Isoniazid is directly or indirectly metabolized to acetyl hydrazine and hydrazine by *N*-acetyltransferase and amidohydrolase. These INH metabolites have been implicated as hepatotoxins (8, 9). Similarly, RFP is also a potent hepatotoxin (10). Hepatotoxicity is increased significantly when INH is combined with RFP. However, some controversial results on this subject are also available in the literature (11).

Besides, pancreatitis is also a potentially serious toxic side effect of anti-TB drugs (12, 13). Moreover, due to the risk of recurrent acute pancreatitis, we may have to cease these medications permanently.

Although standard anti-TB treatment is generally 6 months with INH, RFP, pyrazinamide and ethambutol (ETM), it may be extended to more than a year. In anti-TB treatment, INH and RIF are the best valuable drugs. In the treatment of MTB, the alternative drugs are limited and some other drugs may be added to or replaced with the two main drugs (INH and RFP) resistant cases or in patients with specific toxic side effects. When anti-TB treatment is stopped due to the toxic side effects, TB may get worse (5–7).

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Previous studies have investigated oxidative stress and hepatotoxicity in patients receiving anti-TB treatment (14, 15). Peroxidation of endogenous lipids has been shown to be a major factor in the cytotoxic action of INH and RFP (6, 7). Some studies have shown the importance of fighting against oxidative stress to protect from liver damage (9, 10).

To minimize the negative effects of the anti-TB drugs, antioxidants may be added to the treatment. In many studies in the literature, different agents were used to decrease oxidative stress and inflammation (16, 17). Among them, CAPE is an active component in honeybee propolis extracts and is considered to have medicinal properties. In various studies, it has been used for its antioxidant, anti-inflammatory, anticarcinogenic, antiviral, antimutagenic and antidote activities (18–20). In addition, it has also been shown to protect the liver from ischaemia reperfusion injury, ribavirin and methotrexate-induced liver and pancreatic damage (21).

To the best of our knowledge, no study has been published in regard to the protective effects of CAPE on INH- or RFP-induced liver and pancreatic toxicity. In this study, we aimed to investigate the protective effects of CAPE against RFP-and INH-induced hepatic and pancreatic toxicity in terms of histopathological and biochemical changes.

MATERIALS AND METHODS

Animals

A total of 80 adult male Sprague–Dawley rats, 2 months old and weighing 180–200 g, were used. The animals were housed in appropriate cages under continuous observation in a quiet, temperature (21°C + 2°C)- and humidity (60 ± 5%)-controlled room, in which a 12/12-hour light–dark cycle was maintained. The animals were housed five per cage and provided with a standard commercial diet and water, *ad libitum*. All experiments were performed in accordance with the 'Principles of Laboratory Animal Care'. Experiments were approved by the Ethical Committee of Animal Research at Dicle University, Diyarbakır, Turkey

The rats were randomly divided into eight groups, each containing 10 rats as follows: control, INH, RFP, INH+RFP, INH+CAPE, RFP+CAPE, INH+RFP+CAPE, and CAPE. Isoniazid within saline was orally administered to animals for 30 days at a 50 mg/kg/day dose by gavage. Rifampicin within saline was administered for 30 days in a 50 mg/kg/day dose orally. Caffeic acid phenethyl ester was intraperitoneally injected for 30 days with a 10 μmol/kg dose. After completion of the treatment, all animals were anaesthetized with an intramuscular injection of ketamine HCL (50 mg/kg) (Ketalar, Parke-Davis, Karachi, Pakistan) and xylazine (10 mg/kg) intraperitoneally on the 30th day, and euthanized after surgical anaesthesia. Then, 5 cc of blood was taken from the cardiac cavities of the rats, and the liver and pancreas were quickly removed. The specimens were divided longitudinally into two equal sections. One section was used for biochemical analysis, and the other section was stored in 10% formalin for histopathological examination.

Histopathological evaluation

After tissue samples were kept for evaluation in the 10% formalin solution for 24 hours, a sample was taken from both the pancreas and liver for histopathological examination. The samples were embedded in paraffin blocks after routine histological tissue processing. Approximately 4-μm tissue cross-sections were also taken with a microtome and stained with haematoxylin-eosin (H&E). The preparations were evaluated and photographed using a light microscope (Olympus, BX53, Japan) at ×40, ×100, ×200 and ×400 magnifications by an expert pathologist. The pancreatic tissues were evaluated for oedema, presence of ductal dilatation, acinar cell degeneration and necrosis, perivascular and periductal inflammation. Findings were scored according to the following criteria: no pathological change in the tissue (0); low degree of change (1); medium degree of change (2); significant, extensive change (3) (22).

In the hepatic tissue, portal inflammation, sinusoidal congestion, cytoplasmic hypereosinophilia, and nuclear pyknosis in hepatocyte findings were scored according to the following criteria: no pathological change in the tissue (0); low degree of change (1); medium degree of change (2); significant, extensive change (3).

Biochemical evaluation

Each blood sample was immediately centrifuged at 4000 rpm + 4°C for 10 minutes and then transferred into an Eppendorf tube. These samples were then kept on ice and allowed to deep freeze at –80°C until the end of the experiment. The serum activities of amylase, and aspartate aminotransferase and alanine aminotransferase levels were measured using an autoanalyser (Hitachi Modular automatic analyzer, Hitachi, Tokyo, Japan). Serum total antioxidant status (TAS), total oxidant status (TOS) and paraoxonase-1 (PON1) were also studied in the rats. The specimens were harvested and stored at –80°C until assayed for TOS, TAS and PON-1 activities.

The liver and the pancreatic tissues of the rats were homogenized, and biochemical analyses were performed in accordance with the manufacturer's instructions. The PON1 activity in the cells was analysed using the photometric method, while TAS and TOS were analysed in the autoanalyser. Serum PON1 levels were measured spectrophotometrically by the modified Eckerson method (23). The TAS of supernatant fractions was evaluated by using a novel automated and colorimetric measurement method developed by Erel (24). The TAS results were expressed as nmol Trolox equivalent/mg protein. The TOS of supernatant fractions was evaluated by using a novel automated and colorimetric measurement method developed by Erel (25).

Statistical analysis

All statistical analyses were performed using statistical package for the social sciences (SPSS) software (ver. 15.0 for Windows; SPSS Inc., Chicago, IL, USA). Data are expressed as medians, minimums and maximums. For multiple comparisons, the Kruskal–Wallis test was used for comparisons among groups and the Mann–Whitney test was used if any statistical significance was found. A two-sided p value < 0.05 was considered to indicate statistical significance.

RESULTS

Serum biochemical results

The serum biochemical results are summarized in Table 1. According to the serum biochemical results, significantly increased amylase levels were observed in INH, RFP and INH-RFP groups when compared to the control group. In CAPE-receiving groups, significant improvements were observed in all of the aforementioned biochemical parameters. The serum TAS and PON-1 levels were significantly decreased, whereas the serum TOS levels were significantly increased in the INH and RFP groups, when compared to control groups. There was a significant decrease in the TOS serum levels in the CAPE-receiving groups when compared with INH, RFP and INH+RFP groups ($p = 0.002$, $p = 0.001$ and $p = 0.001$, respectively). Moreover, a significant increase in the serum TAS and PON-1 levels was observed in the CAPE-receiving groups when compared with INH, RFP and INH+RFP groups ($p = 0.002$, $p = 0.001$ and $p = 0.001$, respectively).

Biochemical results of hepatic and pancreatic tissue

The biochemical results of the tissues are summarized in Table 2. There were significantly decreased TAS and PON-1 levels and significantly increased TOS levels in the INH and RFP groups in the hepatic and pancreatic tissues when compared with the control group. The tissue TOS levels were observed to be significantly lower in the CAPE-receiving groups than in the INH, RFP and INH+RFP groups. In contrast, in the CAPE-receiving groups, tissue TAS and PON-1 levels were significantly higher when compared to the INH, RFP and INH+RFP groups.

Table 1: Serum biochemistry results (mean ± standard deviation)

Groups	**PON1** (U/L)	**TAS** (mmol Trolox equivalent/l)	**TOS** (μmol H_2O_2 equiv./l)	**Ast** (U/L)	**Alt** (U/L)	**Amylase** (IU/L)
Control (I)	92.60 ± 6.03	2.10 ± 0.58	34.44 ± 9.59	95.88 ± 23.27	58.0 ± 16.12	780.6 ± 62.31
CAPE (II)	89.65 ± 18.45	2.10 ± 0.27	48.90 ± 11.87	81.53 ± 13.63	66.37 ± 16.13	787.8 ± 172.1
RMP (III)	64.7 ± 13.0	1.11 ± 0.15	120.8 ± 28.68	189.4 ± 79.92	134.1 ± 40.25	798.4 ± 166.3
INH (IV)	66.34 ± 8.16	1.30 ± 0.45	123.1 ± 31.53	191.0 ± 16.07	149.5 ± 35.63	794.0 ± 93.79
INH+RMP (V)	56.57 ± 9.95	1.14 ± 0.49	205.9 ± 97.33	211.6 ± 63.57	189.8 ± 43.65	910.7 ± 80.18
INH+CAPE (VI)	81.76 ± 10.39	1.90 ± 0.33	84.53 ± 37.02	108.8 ± 32.29	70.95 ± 13.83	779.3 ± 146.4
RMP+CAPE (VII)	87.88 ± 17.06	1.84 ± 0.35	82.02 ± 14.80	107.9 ± 19.19	72.27 ± 22.04	782.5 ± 53.67
INH+RMP+CAPE (VIII)	88.15 ± 16.35	1.98 ± 0.29	45.60 ± 10.2	92.11 ± 30.23	73.7 ± 20.61	838.7 ± 114.3
I–III	< 0.001	< 0.001	< 0.001	0.002	< 0.001	0.048
I–IV	< 0.001	0.005	< 0.001	< 0.001	< 0.001	0.05
I–V	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001
III–VII	0.006	< 0.001	0.006	0.003	0.004	0.042
IV–VI	0.004	0.009	0.027	< 0.001	< 0.001	0.04
V–VIII	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

RMP = rifampicin; INH = isoniazid; PON1 = paraoxonase-1; TAS = total antioxidant status; TOS = total oxidant status; Ast = aspartate aminotransferase; Alt = alanine aminotransferase.

Table 2: PON1, TAS and TOS levels in pancreatic and hepatic tissue

Groups	PON1		TAS		TOS	
	Liver	Pancreas	Liver	Pancreas	Liver	Pancreas
Control (I)	0.85 ± 0.33	0.57 ± 0.18	0.51 ± 0.11	0.39 ± 0.18	17.27 ± 3.13	17.09 ± 5.65
CAPE (II)	0.58 ± 0.41	0.56 ± 0.25	0.59 ± 0.63	0.29 ± 0.14	16.10 ± 3.28	13.89 ± 5.78
RMP (III)	0.27 ± 0.05	0.52 ± 0.13	0.23 ± 0.09	0.24 ± 0.02	41.22 ± 5.91	22.39 ± 8.42
INH (IV)	0.27 ± 0.06	0.47 ± 0.14	0.12 ± 0.03	0.23 ± 0.08	33.52 ± 13.60	22.39 ± 8.47
INH+RMP (V)	0.38 ± 0.11	0.74 ± 0.25	0.27 ± 0.06	0.15 ± 0.02	31.01 ± 10.35	24.18 ± 7.20
INH+CAPE (VI)	0.75 ± 0.28	0.60 ± 0.19	0.45 ± 0.16	0.34 ± 0.07	22.49 ± 8.78	17.72 ± 8.71
RMP+CAPE (VII)	0.71 ± 0.25	0.44 ± 0.14	0.38 ± 0.17	0.38 ± 0.08	24.26 ± 8.30	20.96 ± 7.48
INH+RMP+CAPE (VIII)	0.54 ± 0.25	0.54 ± 0.25	0.34 ± 0.17	0.34 ± 0.16	23.14 ± 5.25	19.56 ± 5.99
I–III	< 0.001	0.234	< 0.001	0.06	< 0.001	0.234
I–IV	< 0.001	0.574	< 0.001	0.028	< 0.001	0.279
I–V	0.002	0.237	< 0.001	< 0.001	< 0.001	0.05
III–VII	< 0.001	0.126	0.101	0.001	0.001	0.762
IV–VI	< 0.001	0.203	< 0.001	0.001	0.03	0.36
V–VIII	0.218	0.105	0.436	< 0.001	0.04	0.143

RMP = rifampicin; INH = isoniazid; PON1 = paraoxonase-1; TAS = total antioxidant status; TOS = total oxidant status.

Histopathological evaluation

Liver

In histopathological examinations, the control and CAPE-receiving groups showed normal morphology (Fig. 1a). Low and medium degree portal inflammation were observed in the RFP and INH groups, while moderate portal inflammation was present in the INH+RFP group compared to the control group ($p < 0.001$, $p < 0.001$, $p < 0.001$, respectively). Cytoplasmic hypereosinophilia and nuclear pyknosis in hepatocytes were observed in the RFP, INH and INH+RFP groups ($p = 0.004$, $p = 0.001$, $p < 0.001$, respectively). The damage was more obvious in the INH+RFP group (Fig. 1b). While the degree of sinusoidal congestion was minimal in the INH and RFP groups, it was moderate in the INH+RFP group ($p = 0.084$, $p = 0.001$, $p < 0.001$, respectively). In the CAPE-receiving groups, these histopathological damages were ameliorated (Fig. 1c).

A significant amelioration was observed in the findings of portal inflammation and sinusoidal congestion in the INH+RFP+CAPE groups when compared to the INH+RFP group ($p = 0.05$, $p = 0.031$, respectively). Also, amelioration was observed in cytoplasmic hypereosinophilia and nuclear pyknosis, but this was not statistically significant ($p = 0.818$, $p = 0.422$, respectively)

Pancreas

While a normal histopathological appearance was present in the pancreatic cross-sections of the control and CAPE-receiving group (Fig. 2a), a significant deterioration was observed in oedema, acinar cell degeneration and necrosis, ductal dilatation, periductal and perivascular inflammation findings in INH, RFP and INH+RFP groups. Histopathologically, in the INH+RFP group, pancreatic damage was more noticeable than in RFP and in INH groups (Fig. 2b). A significant amelioration was observed in the oedema, acinar cell degeneration and necrosis, periductal and perivascular inflammation findings in the CAPE-receiving groups, particularly when we compared the INH+RFP group with CAPE+INH+RFP

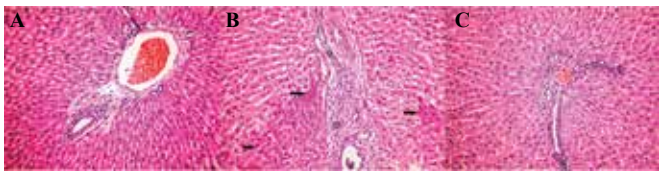

Fig. 1: (a) The control group showed normal morphology (HE, ×200). (b) INH+RIF- treated group showed moderate portal inflammation (star) and sinusoidal congestion. Cytoplasmic hypereosinophilia and nuclear pyknosis in hepatocyte was observed (arrow) (HE, ×200). (c) INH+RIF+CAPE-treated group showed prominent improvement in the hepatic injury. Minimal portal inflammation and sinusoidal congestion existed (HE, ×200).

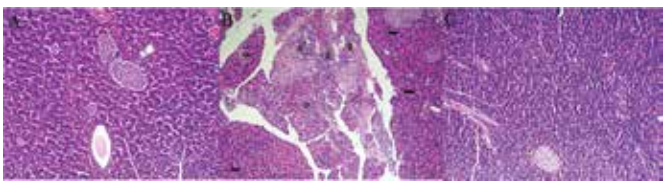

Fig. 2: (a) The control group demonstrated normal pancreatic structure (HE, ×100). (b) INH+RIF group showed severe oedema, moderate inflammation cell infiltration (star), ductal dilatation and acinar cell degeneration and necrosis (arrow) (HE, ×100). (c) INH+RIF+CAPE-treated group showed significantly reduced pancreatic oedema, ductal dilatation, inflammation and acinar necrosis (HE, ×100).

group (Fig. 2c) (p = 0.034, p = 0.001, p = 0.05, respectively).

DISCUSSION

Isoniazid and RFP are two of the most common drugs used in TB treatment. Although the toxic effects of INH and RFP on pancreatic and hepatic tissue are well known (1–3), there is insufficient knowledge in the literature regarding the degree of damage when these two drugs are used in combination. Furthermore, there is limited research on how to minimize these toxic effects. In the current study, the histopathological changes in hepatic and pancreatic tissues following the use of INH, RFP and the combination of these two drugs were evaluated and the protective effect of CAPE on hepatic and pancreatic toxicity of the drugs was documented both biochemically and histopathologically.

According to a meta-analysis published by Steele *et al*, combined treatment of INH and RFP cause hepatotoxicity more frequently than INH alone (10). However, it has been reported that Wistar rats treated with the combination of INH and RFP showed no liver injury in a study (11). We believe that the causes of these differences may be variation of the parameters and differences of the subjects, including rats used in studies, or the resulting different immune responses in rats. Perhaps further studies should be done to clarify this issue.

In experimental studies, it was shown that INH treatment can increase oxidative stress which may result in hepatotoxicity (6). In the current study, we showed a significant increase in oxidative stress markers with INH or RFP treatment, particularly more evident with their combination. However, in the CAPE-receiving groups, an increase in oxidative stress markers was not observed.

In the histopathological examination, an increase in hepatic portal inflammation, sinusoidal congestion, cytoplasmic hypereosinophilia and nuclear pyknosis in the hepatocyte was observed in the INH and/or RFP-treated groups. It was observed that the damage in the hepatic tissues increases when INH and RFP are combined. This histopathological damage is prevented by CAPE treatment. In an experimental study by Şahin *et al* (26), it was shown that oxidative stress in ocular tissue increased and histopathological damage occurred in rats treated with ETM and INH. However, retina ganglion cell loss and oxidative stress decreased with CAPE treatment. They also reported that the best position of CAPE on each superoxide dismutase (SOD) isoform succeeds the lowest binding energy score, means highest binding capacity, or lowest Kd, in comparison to INH and ETM in silico experiment. They suggested that CAPE treatment prevents the binding of INH and ETM to the active site of the SOD enzyme, and it has a role in regulating antioxidant enzyme activity, particularly SOD isoforms (26).

In experimental studies, the antioxidant and antihepatotoxic characteristics of CAPE were shown. Motor *et al* showed that liver damage and oxidant stress due to ribavirin treatment were prevented by CAPE treatment (21).

Albukhari *et al* studied the protective effects of CAPE against tamoxifen-induced hepatotoxicity. They reported that increase of the oxidative stress and lipid peroxidation are prevented and hepatic inflammation, such as widening in liver portal ducts, bile ductular proliferation, lymphocytic infiltration and hepatocyte degeneration, is decreased with CAPE treatment (12).

Recently, acute pancreatitis has been reported in patients undergoing INH and RFM treatment (13, 27). In order to prevent recurrent episodes of acute pancreatitis, it may be necessary to permanently cease medications, which may lead to progression of TB (13, 27). In our study, in pancreatic tissue, oedema, acinar cell necrosis, periductal and perivascular inflammation were observed in INH- and/or RFP-treated groups. These histopathological changes were prevented by CAPE treatment. The protective effect of CAPE was also reported in ribavirin-induced pancreas toxicity (21).

Koyu *et al* reported that CAPE treatment prevented vancomycin-induced pancreatic alterations such as interlobular ductal fibrosis, vascular wall fibrosis, extensive inflammation in surrounding blood vessels and interlobular connective tissue, vascular congestion, inflammation in intralobular ducts, and inflammation in adjacent adipose in rats (28). They also showed amelioration in oxidative status in the CAPE-treated group. Moreover, they reported that the pancreas is more sensitive to oxidative stress than other tissues.

In an experimental cerulean-induced pancreatitis model, massive oedema and inflammation were observed. However, in the CAPE-treated group, oedema and inflammation were significantly decreased, which was attributed to the anti-inflammatory and antioxidant effect of CAPE (29).

In the present study, we observed the toxic effects of INH and RFP on the hepatic and pancreatic tissues of rats. The CAPE treatment significantly prevented the toxicity of INH and RFP. We suggest that the antioxidant and anti-inflammatory effects of CAPE have prevented the damage, as reported previously.

A limitation of our study is that CAPE was administered in a single treatment regimen. As far as we know,

this is the first study to evaluate the effects of caffeic acid phenethyl esther on isoniazid and rifamicin induced hepatic and pancreatic injury. As a result of this experimental study, it was observed that INH and RFP have a toxic effect on hepatic and pancreatic tissues. This effect is more significant when INH and RFP are used in combination. This toxic effect significantly decreased with the application of CAPE. Therefore, these toxic effects that may take place during INH and RFP usage should be considered. Further experimental and clinical studies are warranted to investigate the protective role of CAPE in INH- and RFP-induced toxic effects.

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AUTHORS' NOTE

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