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ORIGINAL ARTICLE

Antioxidant Effect of Caffeic Acid on Oxytetracycline Induced Lipid Peroxidation in Albino Rats

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Abstract Caffeic acid is a well-known phenolic compound widely present in plant kingdom. The aim of this study was to investigate the possible protective effect of caffeic acid (CA) against oxytetracycline (OXT) induced hepatotoxicity in male Albino Wistar rats. A total of 30 rats weighing 150–170 g were randomly divided into five groups of six rats in each group. Oral administration of OXT (200 mg/kg body weight/day) for 15 days produced hepatic damage as manifested by a significant increase in serum hepatic markers namely aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin and increased plasma and hepatic lipid peroxidation indices (TBARS and hydroperoxide). The present finding shows that the levels of enzymatic antioxidants namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were significantly decreased in OXT intoxicated rats. Upon oral administration of caffeic acid (40 mg/kg body weight/day) there were decreased hepatic marker activities, bilirubin and lipid peroxidation and increased enzymatic antioxidants in OXT + Caffeic acid group compared to Normal + OXT group($P < 0.05$). Our study suggests that caffeic acid has antioxidant property and hepatoprotective ability against OXT induced toxicity.

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Introduction

Oxytetracycline is a type of antibiotic called a tetracycline. It is commonly used antibiotic for the treatment of Anthrax, Chlamydia, Cholera, Lyme disease, Typhus, Relapsing Fever, Tularaemia, Malaria, Plaque, Syphilis, Respiratory infection, Mycoplasma, Rickettsiae, Streptococcal infection and Acne. High doses of OXT is generally regarded as toxic, they produce a fairly large number of adverse effects, some of which can be life threatening. Several lines of evidence shows that OXT produces severe microvesicular steatosis of the liver in human and it has been reported that excessive dose of OXT produce hepatic damage [1].

Caffeic acid is an active component in the phenolic propolis extract and also in a wide variety of plants. Recent experimental evidence suggests that caffeic acid is a potent antioxidant [2] and might have beneficial health impact in vivo [3, 4]. Caffeic acid has several biological and pharmacological properties, such as antiviral [5], antioxidants [6], anti-inflammatory [7], anticarcinogenic [8], and immunomodulatory activites [9]. It has been shown that caffeic acid inhibits both lipoxygenase activity and suppresses lipid peroxidation [10]. Caffeic acid completely blocks the production of reactive oxygen species (ROS) and xanthine/xanthine oxidase system [11].

There are a lot of antioxidants that are introduced to minimize actions of reactive oxygen species. For example, phenolic compounds can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes [12]. According to recent research,

caffeic acid was a superior antioxidant compared with *p*-coumaric and ferulic acids, in inhibiting LDL oxidation [13, 14] but also quenching radicals [15] and singlet oxygen. Caffeic acid always behaves as potent antioxidants.

The present study was undertaken to investigate, the possible antioxidant effects and hepatoprotective role of caffeic acid against changes induced by oxytetracycline toxicity in Albino Wistar rats.

Materials and Methods

Experimental Animals

Male albino Wistar rats, weighing of 150–170 g, from an inbred colony were used for the present study. Rats were housed in a polypropylene cage with locally procured paddy husk (*Oryza sativa*) as bedding throughout the experiment at Central Animal House, Rajah Muthiah Medical College (RMMC), Annamalai University. All possible measures were taken to prevent experimental rats against infections.

The animals were kept under 12 h light/12 h dark cycles at room temperature and maintained on pellet diet from Karnataka Agro Food Corporation Limited, Bangalore and water ad libitum. Animal care and handling were performed according to the guidelines set by the World Health Organization (WHO), National Institute of Nutrition, Indian Council of Medical Research, Hyderabad. The Departmental Animal Ethical Committee approved the present study.

Source of Chemicals

Caffeic acid was purchased from Sigma Chemical Company (St. Louis, MO, USA). Oxytetracycline procured from Pfizer, India. All other chemicals and biochemicals used for the experiments were of analytical grade obtained from local firms.

Experimental Design

Rats were randomized and divided into five groups (n = 6 in each group). Rats in Group 1 were untreated (control); Group 2 normal rats received caffeic acid (40 mg/kg body weight/day) in aqueous suspension daily using intra-gastric tube for 15 days. Group 3 rats received OXT (200 mg/kg body weight/day i.p.) in 0.5 ml sterile physiological saline intra-peritonially for 15 days. Group 4 rats received OXT (200 mg/kg body weight/day i.p.) along with caffeic acid (20 mg/kg body weight) for 15 days and Group 5 rats received OXT (200 mg/kg body weight/day i.p.) along with caffeic acid (40 mg/kg body weight) for 15 days.

At the end of the experimental regimen, the animals in different groups were sacrificed by decapitation. Blood was collected in two different tubes, i.e. one with anticoagulant, for plasma separation and another without anticoagulant for sera separation. Plasma and sera were separated by centrifugation and used for various biochemical estimations.

Analytical Methods

Activities of Serum Hepatic Markers

The activities of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and the levels of total bilirubin were assayed spectrophotometrically according to the standard procedures using commercially available diagnostic kits (Sigma Diagnostics (I) Pvt. Ltd., Baroda, India).

Liver Homogenate for Biochemical Investigations

A portion of liver was weighed, perfused with saline and homogenized in chilled potassium chloride (1.15%) using a homogenizer. The homogenates were centrifuged at 800 g for 5 min at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500*g* for 20 min at 4°C to get the post mitochondrial supernatant which was used to assay SOD (superoxide dismutase), CAT (catalase), GPx (glutathione peroxidase) and lipid peroxidation (TBARS and Hydroperoxides) activities.

Assay of Lipid Peroxidation

The lipid peroxidation indices namely TBARS [16] and lipid hydroperoxides [17] were estimated in plasma and liver homogenate.

Determination of Enzymic Antioxidants

The activities of superoxide dismutase [18], catalase [19] and glutathione peroxidases [20] were estimated in tissue homogenate using spectrophotometer. Tissue protein levels were determined by the Lowry procedure, with bovine serum albumin as a standard [21].

Statistical Analysis

All data were expressed as mean ± standard deviation. The biochemical parameters were analyzed by one-way analysis of variance (ANOVA) using SPSS version 9.0 (SPSS, Cary, NS, USA) and the group means were accompanied by Duncan's Multiple Range Test (DMRT), values were considered statistically significant when $P < 0.05$ [22].

Table 1 Effect of CA and OXT changes in the activities of hepatic markers in serum of normal and experimental rats

Groups	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	LDH (IU/l)	Bilirubin (mg/dl)
Normal	59.84 ± 4.13[a]	28.94 ± 1.72[a]	90.8 ± 6.17[a]	105.43 ± 6.31[a]	0.47 ± 0.03[a]
Normal + CA (40 mg/kg)	62.69 ± 3.3[a]	31.09 ± 2.13[a]	93.91 ± 5.5[a]	114.2 ± 9.71[a]	0.44 ± .0.04[a]
Normal + OXT (200 mg/kg)	94.71 ± 6.0[b]	51.11 ± 3.24[b]	136.06 ± 9.9[b]	172.97 ± 14.04[b]	0.99 ± 0.05[b]
OXT (200 mg/kg) + CA (20 mg/kg)	81.61 ± 4.44[c]	40.17 ± 2.18[c]	123.45 ± 7.02[c]	153.32 ± 9.18[c]	0.85 ± 0.06[c]
OXT (200 mg/kg) + CA (40 mg/kg)	67.57 ± 3.81[d]	32.42 ± 1.52[d]	110.05 ± 9.25[d]	123.29 ± 7.79[d]	0.56 ± 0.05[d]

Values are mean ± SD of six rats for each group; *CA* caffeic acid, *OXT* oxytetracycline

Values not sharing a common superscript letters (a–d) differ significantly at $P < 0.05$ (DMRT)

Table 2 Changes in enzymatic antioxidants in liver tissue and lipid peroxidation level in plasma and liver tissue of normal and experimental rats

Groups	Superoxide dismutase (U[#]/mg protein)	Catalase (U[#]/mg protein)	Glutathione peroxidase (U[#]/mg protein)	TBARS		Hydroperoxides	
				Plasma (mM/dl)	Liver (mM/100 g tissue)	Plasma (mM/dl)	Liver (mM/100 g tissue)
Normal	6.38[a] ± 0.38	71.16[a] ± 4.23	6.09[a] ± 0.42	0.16[a] ± 0.01	0.93[a] ± 0.05	9.29[a] ± 0.72	95.24[a] ± 8.10
Normal + CA (40 mg/kg)	6.34[a] ± 0.44	74.88[a] ± 5.18	6.16[a] ± 0.33	0.15[a] ± 0.01	0.89[a] ± 0.07	8.64[a] ± 0.64	89.48[a] ± 7.61
Normal + OXT (200 mg/kg)	3.43[b] ± 0.22	48.65[b] ± 3.09	2.23[b] ± 0.14	0.32[b] ± 0.02	1.64[b] ± 0.13	16.47[b] ± 1.5	142.78[b] ± 11.59
OXT (200 mg/kg) + CA (40 mg/kg)	5.23[c] ± 0.23	63.44[c] ± 2.82	5.40[c] ± 0.29	0.18[c] ± 0.01	1.15[c] ± 0.09	10.25[c] ± 0.57	113.06[c] ± 9.18

Values are mean ± SD of six rats for each group; *CA* caffeic acid, *OXT* oxytetracycline

Values not sharing a common superscript letters (a–c) differ significantly at $P < 0.05$ (DMRT)

[#] Units of enzyme activity expressed

SOD, one unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1 min; CAT, μ moles of hydrogen peroxide consumed/min/mg protein; GPx, µg of glutathione consumed/min/mg protein

Results and Discussion

The changes in the activities of serum hepatic markers in normal and experimental rats are shown in Table 1. In the OXT treated group, serum AST, ALT, ALP, LDH and the level of bilirubin were significantly increased as compared to those in the normal rats. When rats administered with OXT and caffeic acid, a significant decrease in elevation of serum AST, ALT, ALP, LDH and in the level of bilirubin were found. These results showed that 40 mg/kg body weight/day of caffeic acid were more effective than 20 mg/kg body weight/day. So 40 mg/kg body weight/day of caffeic acid was used for further studies. The activity of enzymic antioxidants and the levels of lipid peroxidation in normal and experimental rats are presented in Table 2. The levels of TBARS and hydroperoxides were significantly increased whereas the levels SOD, CAT and GPx were significantly decreased in rats treated with OXT.

In addition, upon administration of caffeic acid (40 mg/kg body weight/day), there were decreased hepatic marker activities, bilirubin, lipid peroxidation and increase in depleted levels of SOD, CAT and GPx were observed in OXT + Caffeic acid group compared to Normal + OXT group ($P < 0.05$). Thus the above results clearly showed a significant protective effect by maintaining the levels of enzymic antioxidants in OXT toxicated rats.

The increased activities of serum AST, ALT, ALP, LDH and bilirubin levels are the most sensitive markers employed in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage [23]. The increased specific activity of the above hepatic enzymes clearly indicates the abnormalities in liver function. In this context, we observed a significant increase in the levels of these biochemical markers in OXT treated rats.

Caffeic acid used in the present study seems to maintain the structural integrity of membrane against OXT challenge. This was evident from the significant normalization of serum AST, ALT, ALP, LDH and bilirubin levels in caffeic acid administered OXT treated rats. The above findings could be correlated with previous studies, which reported that treatment with ferulic acid [24], tetrahydrocurcumin and quercetin [25] significantly reduced the levels of serum markers in drug induced hepatotoxicity [26].

In the present study, the increased levels of serum and hepatic lipid peroxidation products and decreased

antioxidant levels in OXT treated rats might be reflective of increased lipid peroxidation levels in plasma and tissues. Treatment with caffeic acid offered significant protection from free radical derivatives, as evident from significant decrease in the levels of TBARS and hydroperoxide in plasma and liver tissue.

It has been hypothesized that caffeic acid protects the rats from oxidative stress by scavenging the free radicals. Clinical complication in oxidative stress related disease might be due to the dysfunctioning of key antioxidant enzymes. SOD, CAT and GPx are among those enzymes which metabolize endogenous reactive oxygen species and free radicals.

In the present study, the decreased levels of SOD, CAT and GPx were observed in OXT treated rats. The ability of caffeic acid to increase the activities of antioxidant enzymes in OXT treated rats implies that caffeic acid reactivates the antioxidant defense system, thereby increasing the capacity of detoxification through the enhanced scavenging of free radicals. Low levels of antioxidants have been implicated as a risk factor for the development of liver toxic injury [27].

An antioxidant should be efficient and protective, preferably by inhibiting the lipid peroxidation process, where most of the oxidative damage occurs. Normally the phenolic compounds act by scavenging free radical and quenching the lipid peroxidation [28]. As presented here caffeic acid has the ability to scavenge the free radicals [29] and attenuate the lipid peroxidation as indicated by increased levels of enzymic antioxidants in the liver [30].

Our study suggests that caffeic acid can protect the OXT induced liver toxicity. This may be attributed to the antiradical activity of caffeic acid which inhibits lipid peroxidation and enhances the antioxidant defense against OXT induced oxidative damage in tissues.

In conclusion, the phenolic propolis extract (caffeic acid) in this animal model may thus be its free radical scavenging activity and its ability to protect cellular molecules from oxidative damage. Furthermore, the observations suggest that caffeic acid can prevent lipid peroxidation and its enhancement of antioxidant defense. Thus contributing to protection against the OXT induced to liver injury.

Accordingly it remains within the future scope to see the effect of non enzymic antioxidants and the extent of oxidatively damaged DNA using comet assay, against OXT induced hepatic toxicity and a large clinical trial might be needed.

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