IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 14, Issue 1 Ver. I (Jan – Feb 2019), PP 14-21
www.Iosrjournals.Org

Role of Cinnamon Extract in the Protection against Amoxicillin/Clavulanate-Induced Liver Damage in Rats

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Abstract: *Amoxicillin/clavulanate (AC), which is effectively used in the treatment of several number of bacterial infections, may cause hepatotoxicity. Cinnamon extract contains natural products which showed antioxidant, anti-inflammatory and anti-bacterial properties. In the present study, two doses of AC, therapeutic (30 mg/kg) and double therapeutic (60 mg/kg), were orally given to rats alone or in combination with cinnamon (200 mg/kg) for 10 consecutive days, to test the potential protective impact of cinnamon extract against AC-induced hepatic injury. Obtained results showed significant increases in serum levels of alanine aminotransferase (ALT), aspartate aminotransferase(AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT) and total bilirubin in rats treated with AC. Hepatic contents of malondialdehyde (MDA), protein carbonyl (PC), hydrogen peroxide (H_2O_2) and nitric oxide (NO) were also markedly increased following administration of AC. On contrary, treatment with AC produced significant decreases in the hepatic levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase(GPx), glutathione reductase (GRd), glutathione-s-transferase (GST) and reduced glutathione (GSH). The drug was also found to induce upregulation of pro-apoptotic p53 and caspase-3 proteins expression, while it downregulated the expression of the anti-apoptotic protein Bcl-2 in the liver of treated rats. AC-induced adverse effects in all investigated biochemical indices seemed to be dose-dependent. However, administration of cinnamon extract along with AC to rats reduced liver injury, oxidative stress and apoptosis caused by treatment with AC alone. It could be concluded that cinnamon extract may be useful in the protection against AC-induced liver damage in rats.*

Key words: *Amoxicillin/clavulanate, cinnamon, liver, oxidative stress, antioxidants*

Date of Submission: 21-12-2018 Date of acceptance: 06-01-2019

I. Introduction

Amoxicillin is a semi-synthetic penicillin (Fig. 1), which has been effectively used as antibiotic in the treatment of various bacterial infections. It possesses a potent anti-bacterial effect against all of gram negative as well as most of the gram positive bacteria.[1] The drug has been combined with clavulanic acid (Fig. 1), an inhibitor of bacterial 3-lactamases, to decrease antimicrobial resistance.[2,3]

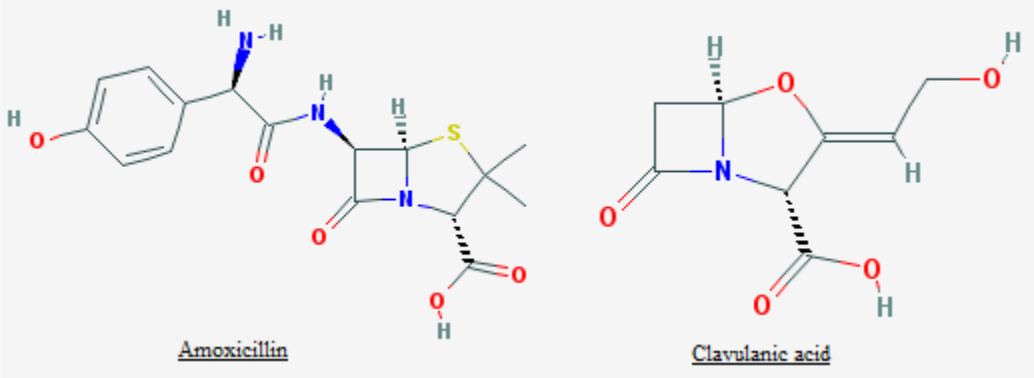

Figure 1: Chemical structure of amoxicillin and clavulanic acid.

Although AC has become one of the most widely prescribed antibiotics,[4] the administration of the drug might be associated with liver injury, which appeared to be primarily due to the clavulanate component.[5] Previous studies showed marked increases in the activity of serum transaminases, ALP and total bilirubin following treatment with AC.[6,7] However, liver injury pattern which associated with AC was classified to be cholestatic, hepatocellular and mixed, with signs of hypersensitivity in some cases.[8,9]

It has been suggested that oxidative stress plays a central role in the pathogenesis of AC-induced liver injury. In previous studies, administration of AC in rats increased lipid peroxidation and reduced levels of GSH,

SOD, CAT and GSH-dependent enzymes in the liver.[10,11] Also, treatment of isolated chicken primary hepatocytes with lipopolysaccharide/amoxicillin clavulanate potassium (LPS/AC) produced increases in reactive oxygen species and lipid peroxidation, and decrease in the activities of antioxidant enzymes. Moreover, LPS/AC increased the apoptotic cells and biomarkers of cell death in the treated cells.[12]

Cinnamon (*Cinnamomum zeylanicum*) is a tropical evergreen tree belonging to the family Lauraceae. The bark of various cinnamon species is one of the most important and popular spices used worldwide for both cooking, and traditional and modern medicines.[13] Cinnamon has strong neuroprotective, hepatoprotective, cardioprotective and gastroprotective effects due to its potent antioxidant and anti-inflammatory properties.[14,13] The antioxidant activity and the beneficial health effects of cinnamon bark is attributed to the presence of polyphenolic component like proanthocyanidins.[15] Furthermore, cinnamon extract exerted anti-apoptotic effect through modulating the changes in apoptotic markers induced by chemicals such as cisplatin.[16] On the other hand, cinnamon and its oils, like AC, showed anti-bacterial activity.[17]

Based on the ability of cinnamon to exert antioxidant, anti-inflammatory and anti-bacterial actions, this study was undertaken to examine, the potential protective impact of ethanolic extract of cinnamon bark against AC-induced hepatotoxicity in male albino rats.

II. Materials and methods

1. Animals

Forty-two healthy male Wistar rats weighing 150-170 g were used. They were obtained from animal house of the Biological Products & Vaccines (VASERA), Cairo, Egypt. Rats were left for two weeks for acclimatization before starting the experiment. They were kept in plastic cages, fed on basal diet and given water *ad-libitum*. All care and procedures adopted for the present investigation were accordance to the approval of animal ethics committee of Mansoura University, Egypt.

2. Drug and doses preparation

Amoxicillin/clavulanic acid (625mg) was purchased from local pharmacy at Mansoura city, Egypt in the form of film-coated tablets manufactured by GlaxoSmithKline®. The tablets were grinded till forming powder, then suspended in distilled water (w/v). Drug suspension then was given daily to the rats according to the selected doses (30 and 60mg/kg).

3. Preparation of cinnamon extract

Cinnamon barks were purchased from local aromatherapy market at Mansoura city, Egypt. The cinnamon barks were washed well, dried and about 100 g was grinded to form powder. The powder of cinnamon barks was extracted with 2 liters of 70% ethanol in bottle for 72 hours. The extract solution was filtrated and the solvent (ethanol) then was evaporated at room temperature for 5 days extract. The residues of extract was collected, weighted and dissolved in DMSO (10%). The solution was then kept in refrigerator and given daily to the rats using gastric tube in a dose of 200 mg/kg.

4. Experimental design

After two weeks of acclimatization, rats were divided randomly into seven groups of six rats per each, as follows: group (1) control; group (2) DMSO 10%; group (3) cinnamon extract, 200 mg/kg[18]; group (4) AC_{30}, 30 mg/kg[11]; group (5) AC_{30}+cinnamon, as groups 3 and 4; group (6) AC_{60}, 60 mg/kg; and group (7) AC_{60}+cinnamon, as groups 3 and 6.

All chemical solutions used for treatment were introduced to rats by gavage once a day for 10 days. At the end of the experiment, overnight fasted rats were anesthetized by ketamine/xylazine (10 ml/kg, ip), and blood samples were collected by cardiac puncture using vacuum tubes. The tubes were then centrifuged at 3000 rpm for 20 min to separate sera which were labeled and kept at -20 ^{0}C until biochemical analysis. Tissue samples were obtained from the right lobe of the liver cleaned, weighed and stored at -20 °C. Later, tissue samples were removed from deep freezer and homogenized in normal saline to form 10% (w/v), and the homogenates were centrifuged (1000 rpm) for 5 min to separate the supernatant which used for biochemical assays.

5. Biochemical investigations

The level of hepatic malondialdehyde (MDA) was measured by the method of Ohkawa *et al*.,[19] Hepatic content of PC was determined according to the method described by Dalle-Donne *et al*.[20] Hepatic content of H_2O_2 was estimated by the method of Aebi.[21] Level of NO in liver tissue was determined on the basis of procedure of Montogomery and Dymock.[22] The concentration of GSH in the liver was estimated by the method of Beutler *et al.*[23] Activities of SOD, CAT, GPx, GRd and GST in the liver were evaluated by the methods of Nishikimi *et al*.,[24] Aebi,[21] Paglia and Valentine,[25] Goldberg and Spooner,[26] and Habig *et al.*[27] respectively.

Activities of ALT, AST, ALP, GGT and total bilirubin concentration in serum were estimated following to methods described by Murray,[28] Wenger *et al*,[29] Gendler,[30] Levitt and Levitt,[31] Schultz[32], and Koller and Kaplan.[33] Hepatic apoptotic markers p53, caspase-3 and Bcl-2 were evaluated by flow cytometric analysis according to the method reported by Gong *et al*.[34]

6. Statistical analysis

Differences among groups were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. All values were expressed as mean ± SE, and *p*-values equal or less than 0.05 were considered significant.

III. Results

Table (1) shows that, treatment of male rats with AC in doses of 30 and 60 mg/kg for 10 consecutive days caused dose-dependent significant increases in the activities of ALT, AST, ALP and GGT; and level of total bilirubin in the serum, as compared to control group. However, treatment with cinnamon extract (200 mg/kg) along with AC for the same period led to reduce elevated serum levels of measured biomarkers of liver injury, as compared to AC-treated groups alone.

In table (2), treatment of rats with AC (30 and 60 mg/kg) produced dose-dependent significant increases in the hepatic content of MDA, PC, H_2O_2 and NO, in comparison with results of control group. Meanwhile, combined treatment with cinnamon extract and AC (30 and 60 mg/kg) significantly lowered the raised hepatic concentrations of MDA, PC, H_2O_2 and NO, when compared to AC-treated groups alone.

As described in table (3), hepatic levels of GSH, SOD, CAT, GPx, GRd and GST were markedly decreased, in a dose-dependent manner, in rats administered AC, in comparison with control. In rats treated simultaneously with cinnamon extract and AC, the activity of mentioned antioxidant enzymes along with the level of GSH in the liver were markedly elevated, in comparison with groups treated with AC alone.

Table (4) and figure (2) exhibit that, treatment of male rats with AC (30 and 60 mg/kg) for 10 consecutive days caused dose-dependent marked increases in the pro-apoptotic proteins expression p53 and caspase-3, accompanied with a significant decrease in the anti-apoptotic protein Bcl-2, as compared to control group. However, combined treatment with cinnamon extract and AC displayed remarkable reduction in the pro-apoptotic proteins expression caspass-3 and p53, parallel to marked increase in the anti-apoptotic protein Bcl-2, in comparison with results of rats administered AC alone.

It has been noticed that, treatment with cinnamon extract alone or DMSO alone had no significant adverse effects on all investigated parameters (Tables 1, 2 and 3).

Table 1: Effect of cinnamon extract on AC-induced adverse changes in the liver injury markers in male rats.

	Con	DMSO	Cin	AC 30	Cin + AC 30	AC 60	Cin + AC 60
ALT (u/l)	24.99 ± 1.20	24.83 ± 1.23	24.85 ± 1.24	67.25 ± 2.11^{a}	33.93 ± 1.83^{ab}	83.02 ± 1.70^{a}	36.38 ± 1.90^{ac}
AST (u/l)	81.93 ± 1.74	82.15 ± 2.28	81.32 ± 2.52	152.4 ± 4.14^{a}	94.12 ± 4.13^{ab}	184.2 ± 2.68^{a}	94.83 ± 2.69^{ac}
ALP (u/l)	44.20 ± 2.56	46.20± 2.997	45.40 ± 1.98	89.82 ± 2.63^{a}	54.27 ± 3.07^{ab}	117.5 ± 3.52^{a}	58.50 ± 1.91^{ac}
GGT (u/l)	10.90 ± 0.44	10.75 ± 0.55	10.52 ± 0.64	26.28 ± 1.10^{a}	14.35 ± 0.79^{ab}	31.10 ± 1.62^{a}	15.28 ± 0.72^{ac}
T.B (mg/dl)	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.51 ± 0.02^{a}	0.20 ± 0.01^{ab}	0.69 ± 0.03^{a}	0.22 ± 0.01^{ac}

- C= Control, DMSO = Dimethylsulfoxide, Cin = Cinnamon, AC = amoxicillin/clavulanic acid
- Values were expressed as mean ± SE (n = 6 for each group)
- a, b and c = significantly difference at $p \leq 0.05$ comparing to control, AC 30mg and AC 60mg groups respectively.

Table 2: Effect of cinnamon extract on AC-induced deleterious changes in markers of oxidative tissue damage in the liver of male rats.

	Con	DMSO	Cin	Co-A 30	Cin + Co-A 30	Co-A 60	Cin + Co-A 60
MDA (nmol/g)	68.04 ± 1.35	67.11 ± 2.02	64.60 ± 2.54	129.70 ± 2.50^{a}	74.28 ± 1.90^{ab}	150.4 ± 1.64^{a}	82.22 ± 3.77^{ac}
PC (nmol/g)	33.54 ± 1.65	33.46 ± 1.75	33.00 ± 1.77	62.21 ± 1.69^{a}	43.69 ± 2.07^{ab}	78.86 ± 1.63^{a}	45.78 ± 2.25^{ac}
H_2O_2 (mM/g)	9.29 ± 0.23	9.39 ± 0.39	9.21 ± 0.25	26.95 ± 1.09^{a}	11.53 ± 0.86^{ab}	31.83 ± 1.56^{a}	12.46 ± 0.91^{ac}
NO (µmol/g)	139.5 ± 2.34	139.1± 2.51	138.5 ± 2.88	241.3 ± 2.93^{a}	147.5 ± 1.83^{ab}	265.0 ± 2.51^{a}	154.4 ± 3.12^{ac}

- C= Control, DMSO = Dimethylsulfoxide, Cin = Cinnamon, AC = amoxicillin/clavulanic acid

- Values were expressed as mean ± SE (n = 6 for each group)
- a, b and c = significantly difference at $p \leq 0.05$ comparing to control, AC 30mg and AC 60mg groups respectively.

Table 3: Effect of cinnamon extract on AC-induced adverse changes in antioxidant parameters in the liver of male rats.

	Con	DMSO	Cin	Co-A 30	Cin + Co-A 30	Co-A 60	Cin + Co-A 60
GSH(mg/g)	4.11 ± 0.08	4.06 ± 0.08	4.18 ± 0.08	2.29 ± 0.06^{a}	3.77 ± 0.07^{ab}	1.22 ± 0.08^{a}	3.59 ± 0.07^{ac}
SOD(u/g)	52.03 ± 1.72	52.79 ± 0.77	55.32 ± 1.38	28.27 ± 1.55^{a}	45.95 ± 0.95^{ab}	20.46 ± 1.20^{a}	42.76 ± 1.22^{ac}
CAT(u/g)	45.87 ± 1.05	45.77 ± 1.24	47.51 ± 0.96	17.89 ± 0.63^{a}	40.93 ± 1.76^{ab}	10.45 ± 0.83^{a}	38.53 ± 1.55^{ac}
GPx (u/g)	50.90 ± 1.31	49.67 ± 0.98	51.58 ± 1.09	24.02 ± 1.39^{a}	46.25 ± 1.50^{ab}	15.36 ± 1.07^{a}	42.75 ± \1.38^{ac}
GR (u/g)	6.09 ± 0.16	6.08 ± 0.07	6.23 ± 0.09	3.53 ± 0.13^{a}	5.45 ± 0.07^{ab}	2.89 ± 0.17^{a}	5.22 ± 0.17^{ac}
GST (u/g)	87.94 ± 1.66	88.65 ± 1.67	91.03 ± 1.64	48.35 ± 2.05^{a}	77.16 ± 1.83^{ab}	32.11 ± 1.92^{a}	73.08 ± 1.68^{ac}

- C= Control, DMSO = Dimethylsulfoxide, Cin = Cinnamon, AC = amoxicillin/clavulanic acid
- Values were expressed as mean ± SE (n = 6 for each group)
- a, b and c = significantly difference at $p \leq 0.05$ comparing to control, AC 30mg and AC 60mg groups respectively.

Table 4: Effect of cinnamon extract on AC-induced apoptosis in the liver of male rats.

	Con	DMSO	Cin	Co-A 30	Cin + Co-A 30	Co-A 60	Cin + Co-A 60
p53 (%)	18.13 ± 0.75	17.10 ± 0.81	17.13 ± 1.13	45.37 ± 1.33^{a}	24.50 ± 1.55^{ab}	57.20 ± 1.03^{a}	32.80 ± 1.65^{ac}
Caspass-3 (%)	22.23 ± 0.98	22.13 ± 0.71	22.60 ± 1.46	56.33 ± 1.34^{a}	29.40 ± 1.51^{ab}	61.37 ± 1.95^{a}	40.70 ± 2.25^{ac}
Bcl-2 (%)	66.93 ± 0.89	66.97 ± 1.68	67.20 ± 1.96	35.1 ± 2.37^{a}	61.17 ± 1.13^{ab}	28.53 ± 1.19^{a}	52.33 ± 1.74^{ac}

- C= Control, DMSO = Dimethylsulfoxide, Cin = Cinnamon, AC = amoxicillin/clavulanic acid
- Values were expressed as mean ± SE (n = 6 for each group)
- a, b and c = significantly difference at $p \leq 0.05$ comparing to control, AC 30mg and AC 60mg groups respectively.

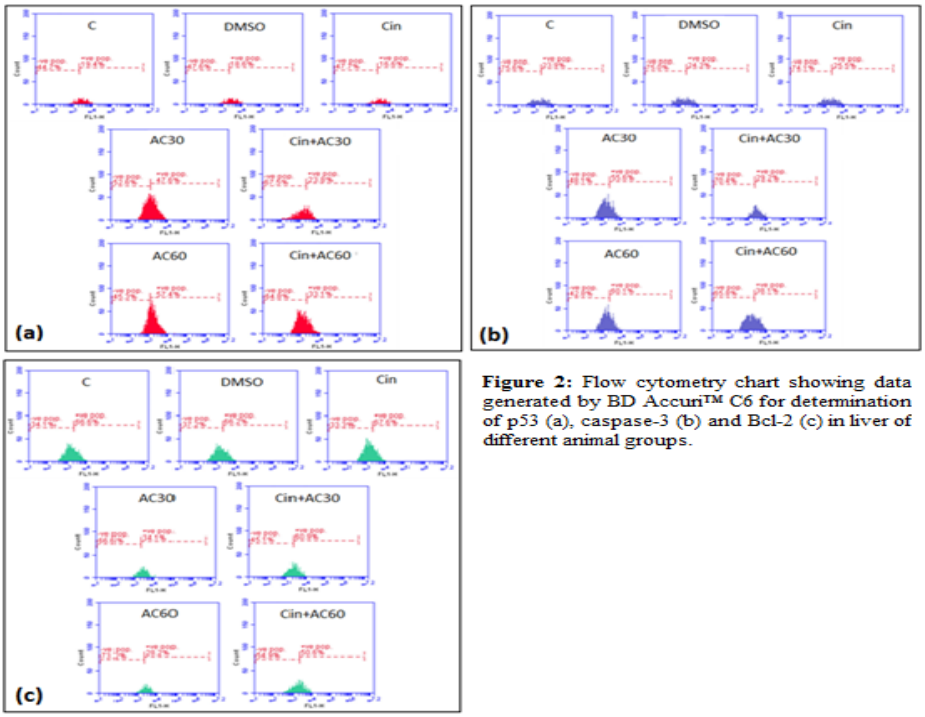

Figure 2: Flow cytometry chart showing data generated by BD Accuri™ C6 for determination of p53 (a), caspase-3 (b) and Bcl-2 (c) in liver of different animal groups.

IV. Discussion

AC is an antibiotic which is highly prescribed for the treatment of various bacterial infections. However, there is an increasing number of evidence demonstrated that it is the most common drug involved in drug-induced liver injury.[35] In the present study, we provided an additional support that treatment with AC may be associated with the risk of hepatotoxicity. Furthermore, the study introduced, for the first time, an evidence indicates the ability of cinnamon extract to play a role in the protection against AC-induced hepatotoxicity.

Obtained results showed that AC administration in rats produced dose-dependent increases in the activities of ALT, AST, ALP and GGT; and levels of total bilirubin in the serum, indicating hepatocellular injury and possibly jaundice. ALT and AST are hepatocyte cytoplasmic enzymes, and leakage of them into the blood stream, especially ALT, indicates hepatocellular membrane disruption. On the other hand, both ALP and GGT are known as a cholestatic liver enzymes, which they are found along the canalicular membrane of hepatocytes. So, increased serum activity of ALP and GGT, in addition to the level of total bilirubin, could reflect hepatobiliary disorder and cholestatic injury. Present finding of AC-induced hepatocellular injury in rats is in agreement with several previous published studies. deLemos *et al*[36] reported that AC treatment in human patients is associated with jaundice and elevated activity of hepatic enzymes in the serum. In animal studies, El-Hosseiny *et al*[11] recorded significant elevation in the serum AST, ALT, ALP, GGT, total bilirubin in AC-treated rats.

Elevated serum levels of diagnostic biomarkers of liver injury following administration of AC indicated hepatocellular membrane damage, and suggested the implication of lipid peroxidation and oxidative stress in the pathogenesis of AC-induced injury. In the present study, administration of AC in rats induced dose-dependent increases in the liver contents of reactive molecules H_2O_2 and NO accompanying with enhanced products of lipid peroxidation (MDA) and protein oxidation (PC). In parallel, the drug, dose dependently, reduced the hepatic levels of antioxidant molecules including GSH, SOD, CAT, GPx and GRd. It has been known that, the disturbance in the ordinary ratio between prooxidants and antioxidants leads to induction of oxidative stress.[37] So, increased production of free radicals and reduction of the detoxifying capability of the liver in the present study can cause oxidative damage to vital molecules in the cells such as proteins, lipids and DNA leading to degenerative disorders including destruction of cell membranes with subsequent release of biomarkers of liver injury.

Both H_2O_2 and NO which were increased in the liver of rats treated with AC are very toxic to the cell and may cause cell death. It has been reported that, the toxicity of H_2O_2 is attributed to its ability to oxidize proteins, membrane lipids and DNA by the peroxide ions.[38] Furthermore, accumulation of H_2O_2 within the cell can generate the $OH^{\bullet}$ in the presence of metal ions and $O^{\bullet-}{}_2$ ($O^{\bullet-}{}_2 + H_2O_2 \rightarrow OH^{\bullet} + OH^{-} + O_2$), which is a far more damaging molecule to the cell.[39] NO has a multitude of potentially toxic effects, but many of these are probably mediated by oxidation products rather than by NO itself. The major oxidative product of NO is the highly reactive peroxynitrite ($ONOO^-$), which results from the reaction between NO and $O^{\bullet-}{}_2$. $ONOO^-$ can react with most biological molecules causing damage to DNA, proteins and lipids. This leads to disruption of the functions of these vital molecules with the production of serious pathological consequences.[40]

The generation of free radicals in normal cell occurred under tight homeostatic control by antioxidants. However, when these reactive radicals levels exceed the antioxidant capacity of the cell, a harmful condition known as oxidative stress take places. The body possesses a highly qualified defense system of enzymatic and non-enzymatic antioxidants which used to eliminate the free radicals and reactive metabolites of chemicals such as drugs and toxins. The first line of defense system against reactive oxygen species is the SOD enzyme which catalyzes the conversion of $O^{\bullet-}{}_2$ to H_2O_2.[41] Cytoprotection against oxidative stress induced by H_2O_2 is carried out by CAT enzyme which converts H_2O_2 to H_2O and O_2.[42]

It has been established that GSH can participate in different detoxification reactions involved conjugation with electrophilic compounds, which are catalyzed by GST. On the other hand, GSH, as a potent endogenous antioxidant, may scavenge free radicals and reduce peroxides such as H_2O_2. Therefore, GSH supply the cell with multitude defenses against reactive oxygen species and toxic products.[43] However, the presence of GSH itself in not sufficient to prevent the cytotoxicity of reactive metabolites, and the GSH-dependent enzymes which participate in the first and second lines of defence against mediators of oxidative stress should be present. In effect, GSH and GSH-dependent enzymes, GPx, GRd and GST, represent a coordinately regulated defence against oxidative stress.

GPx is a main cytosolic selenium-dependent enzyme which protect the cell against attack of hydroperoxide. It acts in association with GSH forming oxidized glutathione (GSSG) and the reduction product of the hydroperoxide.[44] GPx also uses GSH to remove H_2O_2 with the production of GSSG. For regeneration the GSH, another important enzyme called GRd can reduce the GSSG using NADPH as a reductant.[45] Importantly, the ratio of GSH/GSSG is a good measure of oxidative stress,[46] as the depletion of GSH may lead to accumulation of free radicals which induce cytotoxicity.

The GST are structurally highly diverse enzymes which protect against the breakdown products of macromolecules during oxidative stress such as reactive α,β-unsaturated carbonyls, epoxides and hydroperoxides. They also detoxify noxious electrophilic metabolites of xenobiotics which are produced intracellularly following exposure to various chemicals-induced toxicity.[47] The major reactions which are catalyzed by GST are the conjugation of GSH with various reactive substrates, that take place in phase II metabolism.[48] Beside this, a number of GST exhibit GPx-like activity towards organic hydroperoxides, since the enzyme catalyzes the reduction of certain substrates such as cholesteryl and phospholipid hydroperoxides using GSH as a reductant.[49] Collectively, the activity of GPx, GRd and GST together with GSH molecule represent one of the most important determinants of cellular protection against oxidative stress, and the reduction of these antioxidant parameters could contribute to AC-induced hepatic damage.

Apoptosis is a process of programmed cell death, which occurs under both physiological and pathological conditions. However, free radicals-induced oxidative stress were found to play a very important role in mediation of apoptosis. The free radical can attack the membrane phospholipids, and in mitochondria, this leads to loss of mitochondrial membrane potential causing release of intermembrane proteins, such as cytochrome c, to the cytoplasm. Such protein can trigger caspase-3 activation leading to DNA breakage, nuclear chromatin condensation and cell apoptosis.[50] The Bcl-2 family includes pro-apoptotic (Bax and Bid) or anti-apoptotic (Bcl-2 and Bcl-xl) proteins can regulate the mitochondrial apoptotic pathway. Bcl-2 and Bax have opposite effects on cell death: Bcl-2 inhibits or delays cell death, whereas Bax accelerates apoptosis.[51] On the other hand, p53 is an apoptotic protein which has the ability to activate transcription of various pro-apoptotic genes that stimulate apoptosis typically via the mitochondrial pathway.[52] It also modulates cell death through death receptors pathway by stimulating Fas transcription.[53]

In the present study, oral administration of AC (30 and 60 mg/kg) produced marked increases in the apoptotic markers, p53 and caspase-3 in the liver of treated rats. In contrast, AC caused significant decrease in the hepatic content of the anti-apoptotic marker, Bcl-2. The apoptotic effect of the drug was found to be dose dependent. AC-triggered apoptosis in the liver of the treated rats could be attributed to the production of oxidative stress and increased the generation of reactive molecules such as H_2O_2 and NO. Current finding of induction of apoptosis by AC is consistent with previous published studies. In recent *in vitro* study, treatment with LPS/AC was found to induce apoptosis in cultured hepatocytes. This was indicated by increased levels of proteins of caspase-3, caspase-9 and Bax; and release of cytochrome c from mitochondria into the cytoplasm in parallel with decreased level of Bcl-2.[12]

Great attention has recently been exerted regarding the medicinal plants as they contain components of high biological activities such as polyphenols of antioxidant properties that could counteract oxidative damages induced by various agents. Among these plants which contain polyphenols is cinnamon that may be effectively used in cytoprotection against agents-induced toxicity.[54] In the current study, we demonstrated the ability of cinnamon ethanolic extract in the protection of the liver of albino rats against AC-induced cytotoxicity. Obtained results showed that administration of cinnamon extract led to decreases in AC-induced rise in serum levels of hepatic diagnostic markers, ALT, AST, ALP and total bilirubin, in comparison with results of groups treated with AC alone. This finding suggested the ability of cinnamon to protect against AC-induced hepatocellular injury by improving the structural integrity of liver cells as it contains high amount of antioxidant phenolic compounds. In this line, Eidi *et al*[55] reported that treatment of rats with cinnamon extract markedly reduced the toxic effect of CCl_4 on the serum markers of liver injury (ALT, AST and ALP). Also, administration of cinnamon oil prevented paracetamol-induced elevation in serum activities of ALT, AST and ALP in rats.[56]

Considering the effect of cinnamon extract on the markers of oxidative stress, present results demonstrated ability of cinnamon to lower AC-induced elevations in the hepatic content of MDA, PC, H_2O_2 and NO in the treated rats. This observed protective effect of cinnamon extract against AC-induced oxidative stress could be attributed to its antioxidant activity and ability to scavenge the free radicals, due to its contents of flavonoid and phenolic compounds. In this line, Morgan *et al*[57] reported that, oral administration of cinnamon aqueous extract 2 h before bisphenol A (BPA) and octylphenol (OP) exposure caused significant decrease in the levels of lipid peroxidation product in the brain, kidney and testis of rats. In another study, treatment with cinnamon reduced the production of MDA in the liver of rats treated with paracetamol.[56]

Importantly, phytochemical studies on cinnamon bark revealed the presence of flavonoid and phenolic compounds which can mainly contribute to cinnamon hepatoprotective activity due to their antioxidant activity.[54] It has been reported that, flavonoids can interfere with certain free radical-producing systems, and can also increase the function of the endogenous antioxidants. Flavonoids can prevent injury caused by free radicals in various ways. One way is the direct scavenging of free radicals by flavonoids due to reactivity of their hydroxyl group, which results in inactivation of radicals.[58] Taken in consideration the protective impact of flavonoids against NO as a reactive radical-induced injury, it has been reported that NO molecules are scavenged directly by flavonoids. Moreover, as flavonoids can scavenge the free radicals, it prevents more

reactions between $O^{\bullet-}_2$ and NO resulting in the blockage of production of $ONOO^-$ which ultimately leads to reduction of oxidative damage.[59]

Increased production of free radicals, and the subsequent oxidative tissue damage during injury induced by any factor may result in consumption and depletion of the endogenous antioxidant compounds. Under such condition, exogenous supplement with antioxidants such as flavonoids may have an additive effect to the endogenous scavenging compounds. In the present study, administration of cinnamon extract, which possesses antioxidant activity, along with AC markedly increased the hepatic levels of GSH, GPx, GRd and GST, when compared to the results of rats treated with AC alone. The ability of cinnamon to ameliorate the hepatic levels of these antioxidants could contribute to the protective impact of this plant extract against AC-induced oxidative stress. In previous studies, treatment with cinnamon extract resulted in marked increase in the hepatic activity of SOD and CAT in rats intoxicated with CCl_4.[55] Also, administration of cinnamon oil significantly increased the activity of GPX and GST in the liver of rats treated with paracetamol.[56] In recent study, cinnamon extract was found to increase serum activities of SOD and GPx in rats exposed to ischemia/reperfusion-induced injury.[60]

Interestingly, present study exhibited that treatment of rats with cinnamon extract suppressed AC-induced apoptosis, as indicated by down-regulation of the levels of pro-apoptotic proteins, p53 and caspase-3; and up-regulation of the level of anti-apoptotic protein, Bcl-2, in the liver. Similar *in vitro* study showed that, cinnamon suppressed accumulation of Bax in mitochondria, release of mitochondrial cytochrome c, caspase-3 activation and DNA fragmentation induced by cisplatin, suggesting the ability of cinnamon to protect against cisplatin-induced apoptosis.[16] The anti-apoptotic effect of cinnamon extract might be attributed to the ability of plant extract to protect the liver against oxidative stress induced by AC.

V. Conclusion

1. Obtained results of rats treated with AC provided an additional evidence that treatment with AC may have potential hepatotoxicity. So, it is recommended, in human therapy with AC, to monitor the diagnostic markers of liver injury in the blood serum.
2. Administration of cinnamon extract along with AC caused considerable protective effect against AC-induced hepatic injury, oxidative stress and apoptosis in albino rats. This hepatoprotective effect of cinnamon could be attributed largely to its antioxidant activity and its ability to scavenge free radicals.

Declaration of interest:

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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