Original Research Article

Attenuation of TNF-α induced liver injury by cinnamon extract in rats.

Abstract

Background: Nowadays, alternative medicinal therapy is recommended for protection and management liver diseases. Cinnamon is popular flavoring ingredient, widely used in as additive worldwide.

Objective: The mechanism of hepato-protective activity of water (WE) or ethanolic extracts (EE) of cinnamon against carbon tetrachloride (CCl_4) induced lipid peroxidation and the hepatic injury was investigated in rats.

Materials and methods: Four groups of male rats were included in this study, Group1: control, Group II; CCl_4 intoxicated, Group III (CCl_4+ WE) and Group IV (CCl_4+ EE) of cinnamon.

Results and Discussion: Serum AST and ALT were elevated in rats induced by CCl_4 while oral administrated with 100 mg/kg of either (WE or EE), daily showed improvement in these enzymes. The levels of malondialdehyde (MDA), IL-6 and TNF-α ($P<0.001$) were elevated in response to CCl_4, while the activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were significantly decreased ($P<0.001$). Results showed that WE or EE improved liver functions and lower hydroxyproline content. The results obtained indicated that EE has potent hepatoprotective action more than water extract against CCl_4 by inhibiting release of inflammatory cytokines that enhance release of proteases and neutrophil that cause liver injury. In addition, these extracts exert a protective effect by lowering MDA level and induce the antioxidants capacity.

Conclusion: It is concluded that free radical-scavenging polyphenols contents inhibit production of inflammatory mediators and enhancing antioxidant capacity.

Keywords: Cinnamon, CCl_4, cytokines, antioxidants.

Author contribution

This work was carried out in collaboration between all authors. 'Author YAM, TAK, SSM, and KOA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author WMA, KSB,SSY, managed the analyses of the study. All authors read and approved the final manuscript.

Introduction

Liver contributes in huge numbers of the physiological process include metabolism, excretion storage, detoxification, and synthesis. Signal transduction is involved in living cell function [Gurdip et al., 2007], development, differentiation, apoptosis and cell death. Signaling molecules including hormones, neurotransmitters and growth factors [Lee et al., 2008]. Signals mediated by a growth factor involve binding to its receptor initiates a process that starts with the binding with membrane or intracellular receptor [8]. The amplified signal is then propagated to the nucleus, resulting in induction or repression of gene expression [Peschel et al., 2006]. Mitochondrial dysfunction was suggested to be related with many chronic diseases. Oxidative damage are often related with path physiology of many diseases Cinnamon is widely used as natural spices taken orally with food and enhance the thermogenesis [Anderson & Broadhurst, 2004; Murcia et al., 2004]. Cinnamon is one of the naturally occurring cannabinoid. The biological effect of Cinnamon due to it stimulate pain receptors and release of analgesic mediators.The second action explanation it to activate

catecholamine as adrenaline secretion and sympathomimetic effect and subsequently, increases blood pressure. This indicated that the thermo genesis effect by capsicum is mediated by β-adrenergic stimulation and reduction in energy expenditure.
Several studies revealed that supplementation of Cinnamon was effective in the treatment of some disease as improve glucose tolerance in experimental diabetic animals [Murcia et al., 2004]. The metabolic role of Cinnamon is attributed to its role as potentiate the interaction with insulin receptor and improve the action
In spite of tremendous advances in modern medicine no effective drugs are available, which stimulate liver functions and offers protection to the liver from the damage or help to regenerate hepatic cells (Chattopadhyay, 2003). In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders (Chatterjee, 2000) and quite often claimed to offer significant relief. The goal of the present study was to explore the mechanism of the antioxidant and hepatoprotective efficacy of water or ethanolic extracts of cinnamon against oxidative stress induced by CCl_4 in rats.

Experimental design

Animals

Sixty male rats weighing (100-120 grams) were included in the current study.The animals were kept at 27±2 ◦C. Standard diet and water are given *ad libitum*.

Preparation of cinnamon extract

The cinnamon powder was obtained from the local market at Jeddah, Saudi Arabia.
All reagents and solvents used in this study were punched from Aldrich Company until otherwise stated. The dried powder was defatted with petroleum ether (100 grams in 200 ml ether). The defatted material was extracted with 95% ethanol and then vacuum dried. One part of powder was extracted in boiling water then filtered and vacuum dried.

Determination of total phenolics

The content of phenolics in cinnamon extracts was determined according to the method described by Negi & Jayaprakasha (2003). The cinnamon extracts (100 mg) was dissolved in a 10 ml of a mixture of methanol: water (6:4 v/v). Cinnamon extracts (equivalent to 100 mg) in 0.2ml was mixed with 1.0 ml of ten-fold diluted Folin-Ciocalteu reagent. The absorbance was measured at 765nm after 30 minutes.

Hepatotoxicity and treated groups.

Animals were divided into four groups (n = 15). Group I (control). Group II (CCl4) rats were injected a single dose of CCl_4 in corn oil (1ml/kg B.W, s.c.) (Avijeet et al.,2008). Groups III and IV were administered orally by gastric tube 100 mg/kg of aqueous or ethanolic extracts, respectively, in the form of aqueous suspension once daily for 7 days, then animals were administered a simultaneously single dose of CCl_4 (1ml /kg B.W, s.c.) Blood was collected, serum was separated at 3500 rpm for 10 min.

Serum biochemical assay

Serum enzymes aspartate aminotransferase (AST) and serum glutamate pyruvate transaminase (ALT) were determined by commercial kits according to (Reitman & Frankel, 1957).

Estimation of MDA, SOD, and CAT in liver tissue

Liver homogenates (5% w/v) were prepared in cold 50mM potassium phosphate buffer (pH 7.4) using glass homogenizer in ice. The cell debris was removed by centrifugation at 5000 rpm for 15 at 4^0C using a refrigerated centrifuge. The clear portion for the estimation of

malondialdehyde (MDA) (Yagi and Rastogi, 1979), superoxide dismutase (SOD) (Kakkar et al., 1972) and catalase activities (Smna, 1972)

Assay of liver Hydroxyproline

According to Patiyal & katoch , (2006). Briefly, liver sections (0.2g) was hydrolyzed (in 6 mol/L HCl at 100 °C for 2 hours).Samples were incubated for 10 min in 0.05 mol/L chloramine-T at room temperature, followed by 15 min at 65 °C. The absorbance at 570 nm and resulting values compared to a Hydroxyproline standard curve. The Hydroxyproline content was expressed as ug /grams liver tissue.

Assay of inflammatory mediators (TNFα and IL-6)

The levels of inflammatory mediators (TNFα and IL-6) in liver homogenate were determined as described (De Laurentiis et al.,2010), using a specific rat ELISA. The ELISA kits were obtained from BD Biosciences, Pharmingen, San Diego, CA, USA. Determination of TNF-α and IL-6 were performed according to the manufacturer's instructions. ELISA reader. The levels of TNF-α and IL-6 were expressed as pg/mg protein.

Histopathological studies

Sections were prepared and then stained with hematoxylin and eosin dye.

Statistical analysis

Statistical analysis was performed on a PC using SPSS, V.13, (special package for social sciences). Data are presented as arithmetic mean ± S.D., The difference among means has been analyzed by one-way ANOVA. A value of $P < 0.05$ was considered as statistically significant.

Results and discussion

Phenolic compounds of cinnamon extracts.

Results obtained showed that total phenolic of water extract was 11.5% while ethanol extract was 15.5%. The hepatoprotective effect of water or ethanol extracts on CCl_4-intoxicated rats are shown in Table 1. In the CCl_4 intoxicated group serum AST and ALT were significantly increased as compared to control group ($p<0.001$). The elevated activities of serum AST and ALT were significantly reduced in the animals groups treated with either water or ethanolic extracts. Treatment with ethanolic extract showed highly significant activity ($P < 0.001$) than water extract. So, the ethanol extract treated group was superior to the water extract. Results obtained revealed an increase in the level of liver MDA in CCl_4- intoxicated rats compared to control group. Treatment with extracts significantly prevented this raise in levels. The activities of SOD and CAT have significantly reduced in the CCl_4-intoxicated group, while it was significantly elevated in pretreated groups with either extract. The ethanolic extract has shown more protective than water extract. As a result of CCl_4 administration, inflammation increases and the release of cytokines like IL-6 and TNF stimulated. Data in table 2 showed that the serum level of IL-6 and TNF were markedly increased as a result of CCl_4 hepatotoxicity compared with the normal control group ($P<0.01$). Rats given water or ethanol extract diets showed a significantly lower IL-6 and TNF levels ($P<0.05$) compared with untreated animals.

Cinnamomum Verum belongs to the family Lauraceae and possesses significant anti allergic, anti ulcerogenic, antipyretic and anesthetic activities (Kurokawa & Shiraki, 1998). The bark yields an essential oil containing cinnamaldehyde and eugenol. Several biological activities

such as peripheral vasodilator, antitumor, antifungal, cytotoxic and ant mutagenic activities have been attributed to cinnamaldehyde (Shaughnessy& DeMarini, 2001).

Carbon tetrachloride **(**CCl_4) is being used extensively to investigate hepatoprotective activity on various experimental animals (Bhathal et al., 1983). The free radical scavenging activity of water or ethanolic extracts of cinnamon were evaluated.

Serum AST and ALT activities were used as a marker of liver damage. $CC1_4$ produces an experimental damage (James & Pickering, 1976). The toxic metabolite $CC1_3$ radical is produced by cytochrome p_{450} which further reacts with oxygen to give trichloromethyl peroxy radical.

Thabrew et al., (1987) found that serum transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes. The ethanolic extract induced suppression of the increased ALT and AST activities.

CCl_4 produces free radical that not only directly cause damage to tissues, but also initiate inflammation. Kupffer cells produce subsequently proinflammatory cytokines and activate other non-parenchymal cells involved in liver inflammation. TNF-α is produced by resident macrophages after CCl_4 administration and subsequently stimulates the release of cytokines from macrophages and induces phagocyte oxidative metabolism and NO production (Morio et al., 2001). NO is a highly reactive oxidant and it can augment oxidative stress by reacting with ROS and forming peroxynitrite (Rodenas et al., 1995). Another mediator of CCl_4-induced hepatic inflammation which is induced by pro-inflammatory cytokines, leading to the formation of proinflammatory substrates from arachidonic acid (Planaguma et al., 2005). We observed increases in the serum level of TNF-α and IL-6, which were attenuated by cinnamon extracts.

In this study, WE or EE treated rats showed a significant restore liver functions compared to CCl4 group. Also, lowering collagen precipitation and hydroxyproline content that were observed in CCl4 rats. The histopathological examination of liver tissue support this observation and showed that these extracts have a significant antifibrotic action as indicated by the disappearance of collagen accumulation.

Reduced lipid peroxidation was revealed by a significant decrease in MDA level in water or ethanol extracts pretreated groups with simultaneously a significant elevation in SOD and CAT activities. Results obtained showed that ethanolic extract was more potent antioxidant than water extract. The antioxidant properties of cinnamon extracts are attributable to the ability of its phenolic constituents to quench reactive oxygen species. In conclusion, this study suggests that ethanolic extract of cinnamon has a potent hepatoprotective activity in CCl4-induced liver injury in rats.

Conclusion: These observations were documented by biochemical results that supporting the potential clinical use of cinnamon in the treatment of some hepatic disorders. Further studies will be carried out to determine the types of phenol compounds that attributed to its antioxidant property.

Consent is not applicable.

Ethical Approval:

As per international standard or university standard was written ethical approval has been collected and preserved by the author(s).

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Table (1): Serum aminotransferase enzymes (ALT and AST), lipid peroxide product (Malendialdlyde) and antioxidant enzyme activities; superoxide dismutase (SOD), catalase, and of all studied groups (Mean ±SD).

Animal groups / Parameters	Normal Control group	CCl_4 group	WE+ CCl_4	EE+ CCl_4
Serum ALT IU/ml	28.4±4.56	64.0±7.86	37.9±7.14	31.6±5.08
P_1 value	---	P<001	<0.001	<0.001
P_2 value	---	---	<0.001	<0.05
P_3-value	—	—	—	<0.05
Serum AST IU/ml	32.9±4.56	74.0±7.86	36.8±7.14	31.0±5.08
P_1 value	---	P<001	<0.001	<0.001
P_2 value	---	---	<0.001	<0.05
P_3-value	—	—	—	<0.05
MDA (mmol/mg/protein) Mean±SD	3.31± 0.14	8.14± 0.57	3.94± 0.27	3.30± 0.32
P_1 value	---	<0.001	<0.001	<0.001
P_2 value	---	---	<0.001	<0.01
P_3-value	—	—	—	<0.05
SOD (MU/mg protein) Mean±SD	316.8± 13.8	209.5± 34.0	312.7± 25.8	289.3± 23.2
P_1 value	---	<0.001	N.S	<0.001
P_2 value	---	---	<0.001	0.01
P_3-value	—	—	—	0.01
(nmol/min/mg protein) Catalase Mean±SD	8899± 2667.7	3192.0± 146.6	7582.6± 1482.5	5281.3± 935.3
P_1 value		<0.001	N.S	<0.001
P_2 value	—	—	<0.001	0.01
P_3-value			—	0.05

WE. Water extract EE. Ethanol extract

P_1 – comparison to normal control P_3- water E versus ethanol E

P_2 comparison to CCl_4 intoxicated group N.S= non significan

Table (2): Serum IL6, TNF- α and liver hydroxyproline content in the different studied groups (Mean±SD)

Parameters / Groups	IL-6 (ng/mgprotein)	TNF-α (ng/mgprotein)	Hydroxyproline (µg/g protein)
Normal Mean ± SE	220 ± 38	0.13 ± 0.013	73 ± 1.6
CCl_4 group Mean ± SE P^1 value	1102 ± 106 <0.0001*	2.54 ± 0.122 <0.001*	304 ± 16.5 <0.001*

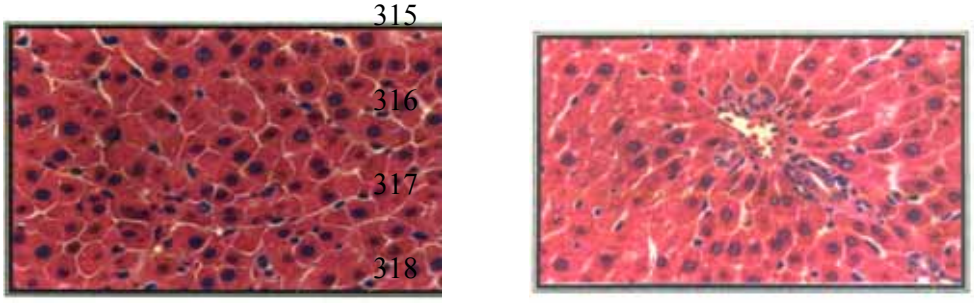
CCl_4 +WE Mean ± SE P^1 value P^2 value	687 ± 75 <0.001* <0.000*	0.92 ± 0.05 0.001* 0.000*	122 ± 4.3 <0.001* <0.000*
CCl_4 +EE Mean ± SE P^1 value P^2 value	390 ± 60 <0.001* <0.000*	0.82 ± 0.05 <0.001* <0.000*	122 ± 4.3 <0.001* <0.000*

WE. Water extract EE. Ethanol extract

P_1 – comparison to normal control P_3- water E versus ethanol E

P_2 comparison to CCl_4 intoxicated group N.S= non significan

Fig (1) Light micrographs of mice liver treated with CC14, without and with pretreatment with either water or ethanol extract of cinnamon compared with normal control. Representative sections from (a) normal control liver (b) rats intoxicated with $CC1_4$, showing extensive hepatocellular necrosis; and (c) pretreated treated with water E and (d) pretreated with ethanol extract showing absence of hepatocellular necrosis, magnification X 250.



a) control group b) CCl_4 group

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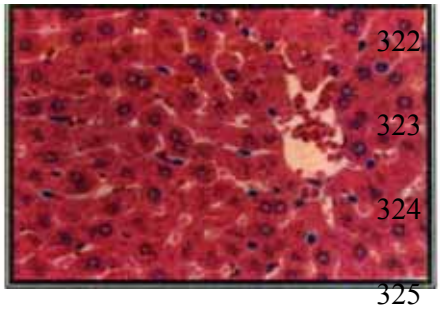

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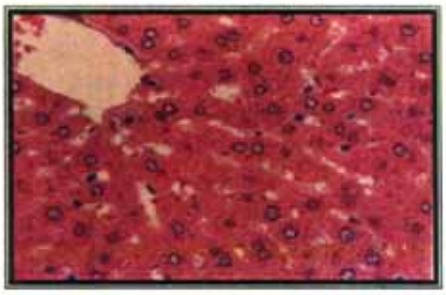

326 c) WE+ CCl_4 d) EE+ CCl_4