

Protective Effects of Turmeric Extracts Against CCl₄-Induced Liver Injury in Rats

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Abstract

Background: This study highlights the effect of turmeric extracts on CCl₄-induced liver injury by follow up the hematological and biochemical parameter in treated rat group.

Objective: Estimate the antioxidant activity of Curcumin and its hepatic protective activity, also to assign the oxidative stress, and antioxidant markers and some hematological parameters in CCl₄ treated rat groups. Besides that, this study aims to evaluate the effect of curcumin extract against various pathogenic bacteria growth.

Materials and methods: three groups of rats were used, each group having ten albino rats. The first group represent the control group, which received a normal diet and intraperitoneal injection with oil (0.5 ml/kg b.w. (body weight)). The second group represented the CCl₄ 1ml/kg b.w. (1:1 in olive oil) model. The third group received CCl₄ 1 ml/kg body weight (1:1 in olive oil) and turmeric 200 mg dissolved in water and given to rates by gavage for four weeks (March to April 2015).

Results: The model produced oxidative stress and arising in the level of AST, ALP (Aspartate aminotransferase, Alanine aminotransferase respectively), and direct bilirubin and lowering GSH (glutathione), liver SOD (superoxide dismutase) levels. The treated group showed a significant lowering of AST, ALT, ALP (alkaline phosphatase), direct bilirubin and increasing GSH, liver SOD levels. Also ameliorated inflammation caused by CCl₄ treatment via decreasing WBC (White Blood Cells), RBC (Red Blood Cells), and PLT (Platelets). These results referred to a good evidence, that is, turmeric could be used as a source of antioxidant, hepatic protective activities and a good antibiotic agent against some pathogenic bacteria. The watery extracts of Turmeric powder with different concentrations in ml inhibited the growth of the pathogenic *E. coli*, *Staphylococcus aureus*, *Bacillus subtilus*.

Conclusions: Turmeric extracts was protective against oxidative damage in CCl₄ induced liver injury in rats. Additionally, the alcoholic turmeric extract was with higher antimicrobial activity than watery extract.

Keywords: Turmeric, CCl₄ – Induced liver injury

Introduction

Medicinal plants are an important part of health care. Large varieties of plants (more than 1200) are available with known therapeutic effects [1]. Approximately 70–80% of people depend on medicinal plants to cure different human ailments including viral diseases [2].

Natural antioxidants could protect the body against some toxin such as the adverse effects of CCl₄ [3]. Along with the detection of the side effects of synthetic drugs, there are many trends to applicate medicinal plants as alternatives to synthetic ones [4]. Curcumin is a yellow component of the spice turmeric gained from the rhizome of *Curcuma longa* Linn, which is a herb spread mainly throughout tropical and subtropical regions. Curcumin has strong anti-inflammatory, antioxidant, anti-mutagenic and anti-carcinogenic properties [5]. Curcumin can inhibit lipid peroxidation, and recover chemically-induced oxidative stress (OS) [6], also rise the activities of xenobiotic detoxifying enzymes in both the liver and kidneys [7]. Liver diseases considered as one of the most reason of morbidity and mortality around the world. The activity of drug-induced liver is a most reason of hepatic dysfunction [8]. In a different types of liver disorder, the initiation and progression of hepatic injury could be contributing by a mechanism of oxidative stress. A deficiency of antioxidants or an excess of reaction species derived from oxygen and nitrogen causes cell injury [9]. Among environmental toxins, carbon tetrachloride (CCl₄) devoted most of the conducted studies to itself [10]. Oxidative stress, including a promoted generation of ROS (reactive oxygen species) has been included etiology of many human diseases. The benefit of antioxidant lies in neutralize ROS. Natural dietary having antioxidant could be important [11]. Curcumin has many pharmacological properties involving induction of tumor cell apoptosis, antioxidant activity, protection against lipid peroxidation, reduction of metalloproteinase expression and suppression of protein kinase activation [12]. Curcumin was with antioxidant [13] anti-carcinogenic [14] and antimicrobial activities [15]. Moreover, Curcumin as a good antioxidant, it can inhibit the erythrocyte membranes, lipid peroxidation in rat liver microsomes and brain homogenates [16] via scavenging hydroxyl radicals and superoxide anions [17].

The research aimed to study the antioxidant activity of Curcumin and its hepatic protective activity, also to assign the oxidative stress, and antioxidant markers and some hematological parameters in CCl₄ treated rat groups. Beside that, this study aims to evaluate the effect of curcumin extract against various pathogenic bacteria growth.

Materials and methods

Materials

Plant preparation

Turmeric (*Curcuma longa* Linn) sample was collected from the local market of Erbil. Dry turmeric was ground into powder by a blender and suspended in water then given to rats.

Experimental animals

Thirty male of albino rats (*Rattus norvegicus*), weighing about 250 – 350gm were used. The animals were given normal rat diet chow, and housed in plastic cages

bedded by wooden chips, and kept in a room under controlled temperature of 24 ± 3 °C, 12/12 hours light/dark schedule in an animal house of Biology department, College of Science, Salahaddin University-Erbil. The normal chow ingredients included (wheat 66.6%, soya 25.6%, lime stone 1.5%, oil sun flower 4.4%, methionine 0.158%, salt 0.63%, Lysine 0.24%, choline chloride 0.062% and trace elements 0.05%).

Experimental Design

The experimental animals (rats) divided into three groups, each group consists of 10 rats. It carried out in four weeks as the following: The first group (control rats), this group were given olive oil intraperitoneally (0.5 ml/kg body weight). The second group (CCl₄ treated rats), this group were given CCl₄ intraperitoneally 1 ml/kg body weight (1:1 in olive oil). The third group (Turmeric group), this group were given CCl₄ intraperitoneally 1 ml/kg body weight (1:1 in olive oil) and turmeric 200 mg/kg dissolved in water and given to rats by gavage. All groups stay for four weeks and exposed to the said procedure daily.

Methods

Tissue preparation

Anesthesia, dissecting of the liver

Ketamine hydrochloride 80 mg/Kg (Trittau, Germany) and Xylazine 12 mg/Kg (Interchem, Holland) were used to anesthetized the rats. After removing the liver, it divided into two equal parts, one of these parts cut into small pieces (less than 0.5 cm three thicknesses), and kept in formalin. The other part stored at refrigerators until homogenized to estimate the SOD and GSH.

Tissue homogenate

Cold saline used to wash liver. Pieces of each tissue used for homogenization by 20mM cold phosphate buffer saline (pH 7.4). The liver tissues homogenized (10% w/v) using handheld glass homogenizer (18). Homogenates were centrifuged for 10 minutes at 6000 rpm. The supernatants were collected and stored at -80 °C until assay.

Estimation of glutathione in liver tissue

The procedure of Moron et al [19] was followed with some modification. 1gm of liver tissue was taken and homogenate by using handled homogenizer in 10 ml of cold tris buffer solution. One ml of tissue homogenate added to 0.25ml of 25% trichloroacetic acid, then centrifugation for 5 minutes at 3000rpm, 0.2 ml of supernatant was taken in a test tube, and adding one ml of 0.15M imidazole solution then adding 1.7ml of distilled water and 0.1ml of 5.5 DTNB (di thio bis-2 (nitro benzoic acid) solution. Finally, absorbance was taken at 412nm after 3 minutes of adding DTNB. The concentration of GSH was calculated according to the absorbance of blank (B), test (T) and standard (S) solutions by the following equation:

$$\text{GSH conc. } (\mu\text{mol/mg of tissue}) = \frac{\text{T-B}}{\text{S-B}} * \text{conc. Standard} * 100 \quad (1)$$

Determination of liver tissue superoxide dismutase

To remove the red blood cells, 0.9% NaCl (sodium chloride) were used to wash the samples of liver. Then the tissue has been dried and weighed, then homogenized in 200μl of buffer solution (0.05 M potassium phosphate and 0.1 mM

EDTA, pH 7.8) followed by centrifugation at 15000xg for 30 min. at 4 °C. The SOD (superoxide dismutase) determined after separating the supernatant. The determination of SOD concentration was by the assay kit of Elabscience (Elabscience, WuHan P.R.C). By using the competitive-ELISA method. The concentration of SOD in the samples is then determined by comparing the OD of the samples to the standard curve (Figure 1).

Blood collection

The collected blood divided into two parts, one of these parts undergo centrifugation and the sera were stored at -80 °C (Sanyo – Ultra-Low Temperature, Japan) until assayed. And the other part of blood sample collected in EDTA tube for measuring the hematological parameters.

Hematological analysis

White blood cell (WBC) count, lymphocytes (LYM) and platelet (PLT) count were calculated by using hematology auto-analyzer (Sysmex model: K-1000, Japan).

Determination of Liver Function Parameters

Aspartate Aminotransferase (ASP), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT) and bilirubin were determined by using full automated analyzer (COBAS Integra 400plus-roche, Germany).

The Antibacterial Effects of Watery and Alcoholic turmeric powder Extracts

The lowest inhibitory concentration of turmeric powder was carried out to inhibit the visible bacterial growths, which were isolated after incubation for one night. The inhibition value was decided based on the inhibition and growth noted on the agar plate which carried out as following: turmeric powder in different weight (0.01, 0.02, 0.1, 0.2 and 0.5) gm were added to freshly prepared bacterial growth media in 250ml conical flask, which is containing nutrient agar (100) and these media poured in sterilized Petri dish with 1 ml of suitable dilution and incubated at 3 °C for 24 hr. Triplicate tests were carried out and the mean value were calculated [20].

Inoculums was prepared by using a sterile swab, streaked on Muller-Hinton agar plate and left o dry. (5) mm wells were hollowed out in agar by using a sterile cork borer, (50)ml of the tested extracts compound were added separately in each well, incubated at 3 °C for one day; inhibition zone were measured and recorded in millimeter after subtraction well diameter (5mm).

Statistical analysis

One-way analysis of variance followed by Newman-Keuls post hoc test comparison procedures were used to compare between means of different groups. Data are represented by Graphpad prism program, version 6.01, and the mean \pm standard error ($M \pm SE$). $P < 0.05$ was considered statically significant.

Results

Effect of Turmeric extract on liver function tests in carbon tetrachloride treated rats.

Table (1) shows the effects of Turmeric on liver function tests in CCl_4 treated rats, the model produced oxidative stress and arising in the level of AST, ALP and direct bilirubin while the treated group showed a significant lowering of AST, ALT, ALP and direct bilirubin ($P \leq 0.001$) compared to CCl_4 treated rats.

Results of the current data showed the increase in ALP, AST, ALT and bilirubin levels in CCl_4 treated groups are in agreement with Girish et al [21]. The mechanism of hepatic damage by CCl_4 is well documented and reported that Cytochrome P450 enzymes metabolized CCl_4 to (CCl_3), which reacts with

molecular oxygen to produce trichloromethylperoxy. This radical makes a covalent bond with sulfhydryl groups of GSH, which results in their depletion, then lipid peroxidation takes place. The lipid peroxidation starts a series of reactions which leads to liver necrosis. Liver damage is detected by measuring the activities of liver function marker enzymes like ALT, AST and ALP, which are released into the blood from damaged cells. They are also indicators of liver injury [22].

This study showed that extract of turmeric could prevent the toxicity caused by induced CCl₄ in the liver by significantly reduction of AST, ALT, ALP and direct bilirubin levels, these results are in agreement with Barta et al [23] they achieved that the normalization of the liver enzymes values in rat with the curcumin which induce accelerated regeneration of liver cells by reducing the leakage of these enzymes into the blood stream. This results indicated that turmeric significantly prevented the increased liver function marker enzyme activity. The protective effects may be the result of repairing and preserving the structural integrity of the hepatic cells injury caused by CCl₄.

Effect of Turmeric extracts on some hematological parameters in carbon tetrachloride treated rats

Table (2) shows the effect of turmeric on the WBC, LYM, and PLT counts in CCl₄ treated rats. The results illustrate that WBC count significantly decreased ($P \leq 0.001$) with turmeric. Moreover, the number of LYM significantly decreased ($P \leq 0.05$) with turmeric when compared with the CCl₄ treated group. Furthermore, also (Table 2) shows the PLT count significantly decreased ($P \leq 0.05$) with turmeric when compared with CCl₄ treated rats. This study demonstrated that the rats treated with turmeric significantly decreased WBC, LYM, and PLT when compared with CCl₄ treated rats.

Effect of turmeric on the liver super-oxide dismutase and liver glutathione levels in carbon tetrachloride treated rats

As shown in the table (3) the level of liver GSH in turmeric groups significantly increased ($P \leq 0.001$), but there was no statistical difference of liver GSH level in control when compared to CCl₄ treated group. Also, liver SOD significantly increased in control ($P \leq 0.001$) and turmeric ($P \leq 0.01$).

Glutathione (GSH), the sulfa-containing and non-enzymatic tripeptide thiol can augment the free radical directly to be renal excreted and to get rid of the products of hepatic P450 activity and can also used to reduce glutathione peroxidase activity [24]. The current study demonstrated that turmeric stimulated the GSH level the same result was observed by Girish et al [21], achieved that antioxidant enzyme like SOD (superoxide dismutase), catalase and glutathione peroxidase by curcumin at higher level can decreased lipid peroxidation, so it is capable of scavenging oxygen free radical. The recent research indicated that the liver superoxide dismutase (SOD) increases significantly. This is in agreement with the findings of Li et al [25]. These results collectively supported the suggestion that treatment with *Curcuma longa* rhizome ethanolic extract could supply a good environment to protect hepatocytes from progressive injury by removing oxidative stress and increasing the level of hepatocellular antioxidant enzymes which scavenging the free radical results from TAA- toxicity. Antioxidant activity of turmeric is due to the phenolic compound which is presents as a constituents of the turmeric rhizomes.

As shown in the table (4) the alcoholic turmeric extract was more active against the pathogenic bacteria than the watery extract, and it may have a role in the treatment of some infectious diseases.

Table 1: Effect of CCl₄, turmeric on the liver function tests

Groups	S. ALP (U/L)	S. AST(U/L)	S. ALT(U/L)	S.D. Bilirubin (mg/dL)
CCl ₄	326±25.59	812.3±91.03	763.8±98.49	0.09625±0.006
Control	243.4±27	196.4±35.68	53.4±6.47	0.026±0.002
Turmeric	313.2±17.93	129.2±7.90	47.6±4.25	0.038±0.003

Table2: Effect of Turmeric on some hematological parameters in carbon tetrachloride treated rats

Groups	WBC *103/μL	LYM *103/μL	PLT*103/μL
CCl ₄	9.633±0.44	6.033±0.12	915.4±16.91
Control	8.4±0.8	4.55±0.15	532±117.5
Turmeric	7.4±1	5.833±0.47	911.5±42.47

Table 3: Effect of CCl₄, turmeric on the liver GSH, SOD levels.

Groups	GSH (μmol)	SOD
CCl ₄	13.33±0.7	0.03576±0.0112
Control	25.19±1.33	0.2804±0.03531
Turmeric	79.83±8.54	0.218±0.02083

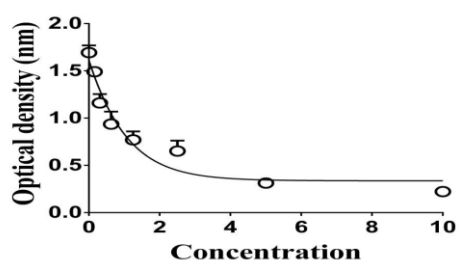


Fig.1 Standard curve of superoxide dismutase (SOD)

Conclusion

From the biochemical and physiological points of view, the model of CCl₄ caused several changes in the level of the oxidative parameters (decreasing of GSH) but the current plant was succeeded in attenuating these changes when added to the CCl₄ treated group. The model produced oxidative stress and rising in the levels of AST, ALT, ALP, direct bilirubin, while Tumeric edible powder lowered these levels and had shown the hepatic protective effect and ameliorated inflammation caused by CCl₄ treatment via decreasing of WBC and LYM count. Moreover, it decreased the thrombogenic activity of CCl₄ through decreasing of PLT count, and have shown the hepatic protective effect by increasing the liver SOD levels. This study finding support that the use of alcoholic tumeric extract was more active against the pathogenic bacteria than the watery extract and it may have a role in the treatment of some infectious diseases.

Table 4: The antibacterial Activity Tumeric watery and alcoholic extracts, inhibition zone measured in millimeter and percentage of inhibition

Types of bacteria	Concentration	Tumeric watery extracts	Tumeric alcoholic extracts
<i>E. coli</i>	0.01	7(44%)	13.5(86.5%)
0.02	6(43%)	7(43%)	13(87%)
0.1	50%	45%	100%
0.2	50%	46%	100%
0.5	60%	50%	100%
<i>Staphylococcus aureus</i>	0.01	8(46%)	14(86%)
0.02	11(66%)	10(65%)	13(87%)
0.1	68%	60%	100%
0.2	72%	60%	100%
0.5	80%	62%	100%
<i>Bacillus subtilus</i>	0.01	6(42%)	11(89%)
0.02	6 (44%)	6(42%)	13(90%)
0.1	50%	42%	100%
0.2	50%	44%	100%
0.5	60%	45%	100%

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