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Hepatoprotective and immunomodulatory properties of aqueous extract of *Curcuma longa* in carbon tetra chloride intoxicated Swiss albino mice

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ABSTRACT

Objective: To evaluate the hepatoprotective and immunotherapeutic effects of aqueous extract of turmeric rhizome in CCl_4 intoxicated Swiss albino mice. **Methods:** First group of mice (n=5) received CCl_4 treatment at a dose of 0.5 mL/kg bw (i.p.) for 7 days. Second group was fed orally the aqueous extract of turmeric at a dose of 50 mg/kg bw for 15 days. The third group was given both the turmeric extract (for 15 days, orally) and CCl_4 (for last 7 days, i.p.). The fourth group was kept as a control. To study the liver function, the transaminase enzymes (SGOT and SGPT) and bilirubin level were measured in the serum of respective groups. For assaying the immunotherapeutic action of *Curcuma longa* (*C. longa*), non specific host response parameters like morphological alteration, phagocytosis, nitric oxide release, myeloperoxidase release and intracellular killing capacity of peritoneal macrophages were studied from the respective groups. **Results:** The result of present study suggested that CCl_4 administration increased the level of SGOT and SGPT and bilirubin level in serum. However, the aqueous extract of turmeric reduced the level of SGOT, SGPT and bilirubin in CCl_4 intoxicated mice. Apart from damaging the liver system, CCl_4 also reduced non specific host response parameters like morphological alteration, phagocytosis, nitric oxide release, myeloperoxidase release and intracellular killing capacity of peritoneal macrophages. Administration of aqueous extract of *C. longa* offered significant protection from these damaging actions of CCl_4 on the non specific host response in the peritoneal macrophages of CCl_4 intoxicated mice. **Conclusions:** In conclusion, the present study suggests that *C. longa* has immunotherapeutic properties along with its ability to ameliorate hepatotoxicity.

1. Introduction

The liver is the main organ responsible for the biosynthesis, uptake and degradation of proteins and enzymes. Liver function may therefore be reflected to some extent on the levels and/or the activities of these circulating biochemical compounds. The immune system is increasingly found to be involved in the development of several chronic illnesses, for which allopathic medicine has provided limited tools for treatment and especially prevention. In that context, it appears worthwhile to target the immune system in order to modulate the risk of certain chronic illnesses. Meanwhile, natural health products (NHPs) are generating renewed interest, particularly in the prevention and treatment of several chronic diseases. Herbal drugs are known to posses immunomodulatory properties and generally act by stimulating or suppressing both specific and non-specific immunity[1].

Turmeric is the dried rhizome powder of *Curcuma longa* (*C. longa*), a perennial herb of the Zingiberaceae family. *C. longa* is cultivated extensively in Asia (India and China). The major chemical principles of turmeric are curcuminoids, which impart characteristic yellow colour to it.

Previous works have shown that *C. longa* inhibited the growth of activity of some bacteria and fungi[2,3]. Turmeric extract was found to possess *in vitro* free radical scavenging, ROS scavenging ability and cell proliferation activity in cell line[4]. Curcumin seems to regulate the immune function of mice in a dose-dependent fashion as curcumin treatment enhanced the phagocytosis of peritoneal macrophages and differentially regulates the proliferation of splenocytes[5]. The effect of curcumin in Alzheimer's disease is mediated through the downmodulation of cytokine (*i.e.*, TNF- α and IL-1 β) and chemokine (*i.e.*, MIP-1b, MCP-1, and IL-8) activity in peripheral blood monocytes and reduces

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amyloid-β plaque formation[6-8]. Curcumin treatment modulates cellular and humoral immune responses of infected mice and leads to a significant reduction of parasite burden and liver pathology in acute murine schistosomiasis mansoni[9]. Curcumin also downregulates matrix metalloproteinase (MMP)-2 and upregulates tissue inhibitor of metalloproteinase (TIMP)-1, two common effectors molecules involved in cell invasion[10]. In one study, it was reported that postnatal modulation of hepatic biotransformation system enzymes via translactational exposure of F1 mouse pups to turmeric and curcumin[11].

Curcumin has been found to modulate the growth and cellular response of various cell types of the immune system. Numerous lines of evidence suggest that curcumin can modulate both the proliferation and the activation of T cells. Curcumin inhibited the proliferation induced by concanavalin A (Con A), phytohemagglutinin (PHA), and phorbol-12- myristate-13-acetate (PMA) of lymphocytes derived from fresh human spleen[12]. The curcumin can suppress the PHA-induced proliferation of human peripheral blood mononuclear cells (PBMCs) and inhibit IL-2 expression and NF-AB[13]. In another report, curcumin inhibited the activation of human V B CT cells induced cancer by phosphoantigens[14].

Anti-inflammatory activity of turmeric oil has been reported on pepper's model[15]. The volatile oil of *C. longa* was effective in anti-inflammatory and anti-hyaluronidase action[16]. They suggested the antioxidative effect as evidenced by inhibition of diffusion capability of the hyaluronidase enzyme by the oil. Further the cytotoxic, anti-inflammatory and antioxidant activity of curcumin I, II and III from *C. longa* was also reported[17]. They observed cytotoxic activity against leukemia, colon cancer, CNS melanoma, renal and breast cancer. The present investigation was aimed to see whether the chemical principles of *C. longa* can be used for promoting immunotherapeutic causes as well as ameliorating hepatotoxic dysfunctions.

2. Materials and methods

2.1. Preparation of extracts

After drying at 37 ℃ for 24 h the plant material was ground into powder. Exposure to sunlight was avoided to prevent the loss of active components.

One liter of double distilled water was mixed with 200 g of powdered *C. longa* rhizome, filtered with nitrocellulose membrane and the extracted liquid was subjected to water bath evaporation to remove the water. For water bath evaporation, liquid extract material was then placed into a beaker and subjected to water bath evaporation at 70 ℃ temperature for 7-10 h daily for 2-3 days until a semisolid state of extracted liquid is obtained. The semisolid extract produced was kept in the deep freezer at -20 ℃ overnight and then subjected to freeze drying. Extract obtained by this method was be then weighed and stored at 22 ℃ in desiccators until further use.

2.2. Animals

20 mice weighing approximately (20±1.0) g were taken and these mice were divided into four groups of five mice each. The first group was kept as control. The second group consisted of CCl_4 at a dose of 0.5 mL/kg bw (i.p.) from the 8th day to 15th day of the experiment. In the third group, the mice were fed with extract of turmeric at a concentration of 50 mg/kg bw (orally) by feeding needle for 15 days. In the fourth group, the mice were given both turmeric extract (for 15 days, orally) and CCl_4 (for last 7 days, i.p.). Animal experiments were in accordance with the instructions for the care and use provided by the institution at which the research was carried out.

2.3. Collection of blood

Blood was collected from the retro-orbital plexus of the animals and serum isolated from the blood samples. The animals were sacrificed on the 16th day of treatment. Peritoneal macrophages were isolated from the sacrificed mice.

2.4. Determination of serum bilirubin level

The formation of pink-colored azo-bilirubin by the reaction between bilirubin and a diazo reagent was utilized. In practice, one estimation was done with addition of alcohol and the other without alcohol. The former yields total bilirubin and latter conjugated bilirubin[18].

2.5. Estimation of serum glutamate oxaloacetate transaminase (aspartate transaminase) and serum glutamate pyruvate transaminase (alanine transaminase) levels in serum

For each enzyme, 0.1 mL of non-hemolyzed serum was mixed respectively with 0.5 mL of glutamic-oxaloacetic transaminase (SGOT or AST) substrate and 0.5 mL glutmatic-pyruvate transaminase (SGPT or ALT) substrate and incubated for 1 h at 37 ℃. Then 0.5 mL of 2, 4-dinitrophenyl hydrazine solution was added to the respective test tubes and allowed to stand for 15 min at room temperature. Then, 5 mL of 0.4 (N) NaOH was added, mixed and kept at room temperature for 20 min. The intensity of the developed color was read at 540 nm after setting the instrument to zero density with water. The decrease in density represents the decrease in α-ketoglutarate from which the activity was calculated[19].

2.6. Isolation of peritoneal macrophages

On the 13th day, all the mice were injected with 50 μL of 3% starch (i.p.). After two days they were sacrificed by cervical dislocation. Following this 5 mL of ice-cold RPMI-1640 were injected (i.p.) in all the dead mice and the injected area was lavaged softly. A small perforation was made to withdraw the RPMI-1640 containing peritoneal fluid. The process was repeated to accumulate the remaining fluid by aspirating and collecting in plastic centrifuge tubes. The samples were then centrifuged for 30 min at 3 500 rpm. All the samples were then washed in RPMI-1640 twice and the pellet were collected separately and incubated for 2 h at 37 ℃. The supernatant was decanted and the adhered macrophages present in the micro centrifuge tubes were resuspended in 1 mL RPMI-1640. A portion of entire cell samples (100 μL) isolated from all the mice, were then aspirated and smeared on a glass slide and incubated for 1 h, fixed with 4.1% formaldehyde, kept for 30 min before staining with Giemsa