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Hepatoprotective effect of methanolic *Tanacetum parthenium* extract on CCl4-induced liver damage in rats

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ABSTRACT

The purpose of this study was to investigate the effects of Tanacetum Parthenium Extract (TPE) on Lipid peroxidation, antioxidant enzymes, biochemical factors, and liver enzymes in the rats damaged by Carbon Tetrachloride (CCl4).

54 male Wistar rats were divided into 9 groups each consisting of 6 rats. Two of the groups were control groups (normal and damage control groups), 4 of them were exposure groups which were respectively administered with 40, 80, and 120 mg/kg of TPE and silymarin for 14 days before being damaged by CCl4, and the other 3 groups were post-treatment groups which received 80 and 120 mg/kg of TPE and silymarin 2, 6, 24, and 48 h after being injected with CCl4. At the end of the study, biochemical factors, serum liver enzymes, malondialdehyde level, antioxidant enzymes, and liver morphology were assayed.

Pre- and post-treatment with TPE could significantly decrease ALT, AST, ALP, TG, LDL, TC, and glucose levels and increase HDL, and albumin levels and catalase, SOD, and GPx activities compared to the CCl4-damaged control group.

The results of this study are indicative of the antioxidant activity of TPE, its potential hepatoprotective effects, and its probable therapeutic properties for laboratory animals damaged by CCl4.

1. Introduction

Liver is one of the main organs involved in the metabolism of drugs and toxic chemicals and is the first target organ for almost all chemicals [1,2]. Most of the xenobiotics enter the body through gastrointestinal tract and after absorption enter the liver through portal vein. The liver has a high concentration of toxin-metabolizing enzymes which can convert xenobiotics to compounds with low toxicity and excrete them. However, sometimes toxic substances are converted to active metabolites during metabolism which can exacerbate liver damage and cause changes in the macroscopic structure of, or damage to, specific molecules such as bile acid transporters, families of nuclear receptors, intracellular lipids, proteins, and nucleic acids [3]. Improper performance of these molecules activates some secondary paths which finally lead to planned events such as apoptosis, necrosis, autophagy, mitochondrial defects, and immune responses [4]. Moreover, the entire cellular function is also disrupted by cytolytic activities and the destruction of membranes and transmembrane transport mechanisms.

CCl_4 is the most recognized chemical substance used in developing models of liver and kidney damage [5,6]. Therefore, CCl_4-induced liver damage is one of the best ways of inducing damage by xenobiotics and also one of the common methods of screening hepatoprotective or liver treatment drugs. The metabolism of CCl_4 begins with the formation of trichloromethyl and proxy chloromethyl free radicals via the activity of oxygenase system of cytochrome P450 in endoplasmic reticulum. The trichloromethyl radical reacts with various important biological substances such as fatty acids, proteins, lipids, nucleic acids, and amino acids [7,8].

Therefore, the antioxidant activity of the body and the inhibition of the production of free radicals are important in preventing CCl_4-induced hepatopathies. In CCl_4-induced liver damages, the balance between Reactive Oxygen Species (ROS) production and antioxidant defense system is disturbed due to oxidative stress which disrupts cellular functions through some events and causes liver damage and necrosis. Despite the considerable advancements in medicine and modern pharmacology, drugs used for the treatment of liver damages have many side effects and exacerbate the disease. Therefore, it is necessary to find new drugs to replace those with many side effects [9,10]. Non-Alcoholic Fatty Liver Disease (NAFLD) includes a wide spectrum of liver damages from simple fatty liver to steatohepatitis, steatonecrosis, and non-alcoholic steatohepatitis. The aims of treatment in NAFLD are weight loss for obesity, reduction of lipid-lowering agents for

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dyslipidemia, modifying antioxidants and probiotics for oxidative stress, cytoprotective agents for apoptosis, and anti-tumor necrosis factor (TNF-α) for proinflammatory cytokines. Thus, it seems necessary to find a compound that has hepatoprotective and antihyperlipidemic effects without causing other side effects [11–13].

TPE with the common name of 'Feverfew' is a plant belonging to the family of Asteraceae which is used for the treatment of various diseases such as arthritis and migraine in traditional medicine. This plant contains various antioxidant compounds such as sesquiterpene lactone and various flavonoids [14]). Therefore, this study was conducted with the aim of assaying the effects of TPE on lipid peroxidation, antioxidant enzymes, biochemical factors, and liver enzymes in the rats damaged by CCl_4.

2. Materials and methods

2.1. Chemical substances

Commercial kits of superoxide dismutase and glutathione peroxidase were bought from Randox Company (Crumlin, UK) and those of AST, ALP, ALT, TG, TC, Urea, HDL, and glucose were purchased from Pars Azmoon Company (Tehran, Iran). Chemical substances including hydrogen peroxide, methanol, thiobarbituric acid, bovine serum albumin, coomassie blue, CCl_4, ferric sulfate, ferric chloride, sodium acetate, and butanol were purchased from Merck Company (Germany). TPTZ and ketamine were bought from Fluka Company and Alphasan Company (Netherlands), respectively.

2.2. Plant collection and extraction

The plant samples were collected at the humidity level of 45–60% early in May from Khodafarin, Arasbaran Zone, East Azarbayjan Province, Iran which is situated at the altitude of 2100 m above sea level. After confirmation of the genus and species, which was done using valid identification keys by the herbarium experts of the Research Center for Agriculture and Natural Resources of East Azarbayjan Province with the herbarium code of 2411, the samples were prepared for extraction. The aerial parts of the plant were dried separately in shadow at the ambient temperature of 20–25 °C and then powdered using a mortar. After that, they were soaked in methanol 70% for 7 days. The obtained extract was percolated and then condensed by rotary evaporator. After the evaporation of alcohol, the extract was deposited in a freeze dryer at main drying and final drying phases for 2 weeks to become powder and then kept in a freezer.

2.3. The design of the study

2.3.1. Selection of animals

In this study, male Albino Wistar rats with body weights of 180 ± 20 g were purchased from Faculty of Veterinary Medicine, Tehran University. They were kept in standard conditions, that is, the temperature of about 22 °C, 12-h light/dark cycle, and in a bed of straw. This study was approved by the Ethics Committee of Ardabil University of Medical Sciences and the identification code received for it from the Clinical Trials Registry of Islamic Republic of Iran is 'IR.ARUMS.REC.1394.67'.

2.3.2. Induction of liver damage

In order to induce liver damage in rats, CCl_4 was solved in olive oil with the ratio of 1:1 and from the obtained mixture 1.5 mg/kg was injected to them intraperitoneally [15].

2.3.3. Grouping of the rats

54 rats were randomly assigned to 9 groups each consisting of 6 rats.

Group 1 (NC): This group was the normal group in which the rats received distilled water via gavage for 14 days and were injected with 1.5 mg/kg of just olive oil (the solvent of CCl_4) on the 14th day.

Group 2 (CC): This group was the exposure group in which the rats received distilled water via gavage for 14 days and were injected with 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day.

Group 3 (bTP_{40}): This group was the pretreatment group with the dose of 40 in which the rats were administered with 40 mg/kg TPE for 14 days and received 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day.

Group 4 (bTP_{80}): This group was the pretreatment group with the dose of 80 in which the rats were administered with 80 mg/kg TPE for 14 days and received 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day.

Group 5 (bTP_{120}): This group was the pretreatment group with the dose of 120 in which the rats were administered with 120 mg/kg TPE for 14 days and received 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day.

Group 6 (bSC_{100}): This group was the positive control group with the standard drug silymarin. The rats in this group were administered with 100 mg/kg silymarin for 14 days and received 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day.

Group 7 (aTP_{80}): This group was the post-treatment group with the dose of 80 in which the rats were administered with 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day and received 80 mg/kg TPE via gavage 2, 6, 24, and 48 h after the injection of CCl_4.

Group 8 (aTP_{120}): This group was the post-treatment group with the dose of 120 in which the rats were administered with 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day and received 120 mg/kg TPE via gavage 2, 6, 24, and 48 h after the injection of CCl_4.

Group 9 (aSC_{100}): This group was the post-treatment group with silymarin in which the rats were administered with 1.5 mg/kg of CCl_4 and olive oil mixture on the 14th day and received 100 mg/kg silymarin via gavage 2, 6, 24, and 48 h after the injection of CCl_4.

50 h after the last injection, the rats were anesthetized with the injection of 200 µl (160 µl ketamine 10% + 40 µl xylazine) of anesthesia agent. Then their abdominal areas were opened and the blood samples were directly taken from their hearts. The samples were centrifuged at 3000 rpm for 10 min and after making aliquots in vials of 1.5 ml were stored in a freezer at −80 °C to perform biochemical experiments.

Immediately after blood collection, a piece of each rat's liver (right-distal lobe) was removed and kept in formalin 10% for histopathological examinations. A part of their livers was also washed with saline and kept in liquid nitrogen.

2.4. Measurement of serum factors

Serum levels of ALT, AST and ALP were measured using standard assay kits according to the manufacturer's instructions. The concentrations of glucose, cholesterol, and triglyceride were measured with GOD-POP, CHOD-POP, and GPO-POP methods, respectively. Direct measurement method was used to determine HDL-C and LDL-C concentrations while albumin was measured through bromocresol green method according to the instructions provided by Pars Azmoon kit (Tehran, Iran). The experiments were conducted according to the instructions provided in each laboratory kit using an Auto-Analyzer (Biochemistry Analyzer BT 1500, Italy).

2.5. Preparation of tissue lysate

200 mg of liver was chopped and poured in a tube and 2 ml of homogenization buffer (tris buffer) was added to it. Then, it was homogenized at 10,000 rpm for 2 min in a homogenizer set. The obtained suspension was centrifuged at 12,000 rpm for 20 min so that the unhomogenized cells deposit. The pure homogenous solution was used in the measurement of malondialdehyde, superoxide dismutase, and