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Therapeutic potential of Vanillylacetone against CCl_4 induced hepatotoxicity by suppressing the serum marker, oxidative stress, inflammatory cytokines and apoptosis in Swiss albino mice

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ABSTRACT

The aim of this research was to investigate the therapeutic potential of Vanillylacetone against carbon tetrachloride (CCl_4) induced hepatotoxicity in mice through understanding the serum marker, oxidative stress mechanism and cytokine networks. Carbon tetrachloride is highly hepatotoxic used as research based on animal model. The mice were classified into five groups and each had eight mice. Group-I was controlled and the vehicle was given orally. Group-II was toxic and carbon tetrachloride (1.5 ml/kg) twice a week for 15 days was administered by intra-peritoneal injections. Group- III and IV were pre-treated with Vanillylacetone 50 & 100 mg kg^{-1} body weight given every day p.o. while, Group-V received only Vanillylacetone (100 mg kg^{-1} body weight) for 15 days orally. The finding indicates that the administration of CCl_4 causes significant elevation of enzyme markers, oxidative stress, inflammatory cytokine and apoptotic markers in Group-II as compared to Group-I. The administration of Vanillylacetone (50 and100 mg kg^{-1}) significantly suppresses the elevated serum enzymes, oxidative stress (TBARS), an inflammatory cytokine (IL2 and TNFα) and apoptotic markers (Caspase-3 and 9) in Group-III and IV as compared to Group-II. It was also noticed that the higher dose of Vanillylacetone (100 mg) is more effective than lower dose of Vanillylacetone (50 mg). There were no significant changes observed with higher dose of Vanillylacetone (100 mg kg^{-1}) in Group-V as compared to Group-I. Histopathological analysis also supported the above findings. Overall, this results shows that Vanillylacetone has a good antioxidant and therapeutic properties which can help in preventing the chemically (CCl_4) induced hepatotoxicity.

1. Introduction

Several drugs, toxins and herbs have been reported to cause liver injury and hepatotoxicity. The management of liver disorder is still challenging in the modern medicine. The literature review indicates that many plants are tested for antioxidant, anti-inflammatory and hepatoprotective effects in rats and mice. It is also a fact that the discovery of novel compound has originated from the plant kingdom. Vanillylacetone is one of the active compounds isolated from Ginger (*Zingiber officinale*), which is also called "Zingerone" that have high therapeutic importance, including good antioxidant, anti-inflammatory and anti-apoptotic properties (Kim et al., 2010; Mohammed, 2018). The chemical structure of Vanillylacetone is given in Fig. 1. The purpose of this research was to evaluate the therapeutic effects of Vanillylacetone against CCl_4 induced liver toxicity at a cellular and molecular level in mice.

Carbon tetrachloride (CCl_4) has been used in the past in manufacturing industry and nowadays its uses have been discontinued due to environmental contamination by accumulation in the atmosphere as well as in the ground water (Nordic Council of Ministers, 2003). Carbon tetrachloride is one of the most potent hepatotoxic agents due to this reason it has been used as a model to induce hepatotoxicity in animals which is widely accepted in scientific research (Seifert et al., 1994). Exposure to high concentration of CCl_4 can affect the CNS, Liver, Kidney, which leads to coma or death (Liu et al., 1993). CCl_4 produces reactive free radicals such as trichloromethyl radical (CCL3•) inside the liver, which causes the hepatotoxicity (Sahar and Dalia, 2014). A single dose of CCl_4 administration to rats produces cell necrosis and fatty changes, which is directly associated with the leakage of hepatic enzyme into the serum (Shen et al., 2009; Janbaz et al., 2002). The toxicological evaluation of hepatic injury was carried out interms of serum markers, oxidative stress (tissue markers), inflammatory cytokines

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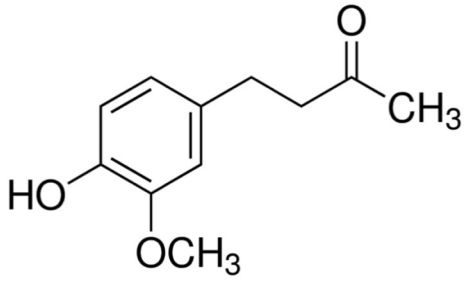

Fig. 1. Chemical structure of Vanillylacetone.

(immune markers) and apoptotic markers etc. Oxidative stress generally reflects an inequity between the reactive oxygen species (ROS) and antioxidant defense system that leads to tissue damage (Alam et al., 2018; Mohammed et al., 2016). The inflammatory cytokine is a type of signaling molecule that is excreted from immune cells like helper T Cell and macrophages, that promotes inflammation (Zhang, 2007; Noosheen and David, 2007). Apoptosis is a another biochemical event or programmed cell death that occurs in multicellular organisms that lead to morphological changes and death in the cell to replace the older cell with newer cells. The liver is one of the important vital metabolic organs of the body and their toxicity or disorder affects several important homeostatic mechanism resulting in cirrhosis, jaundice, tumors, metabolic degenerative and cell necrosis etc. (Williams and Burk, 1990). Liver diseases are leading to significant burden on society due to its different treatment challenges. In many Western countries, alcohol abuse is one of the main cause of liver diseases, but in other countries also experience lifestyle related liver disease and drug induced liver injury. Overall, there is always demand to search a safer molecule to combat the various types of liver disorder and fulfil the need. Therefore, we have focused on active compound Vanillylacetone from natural sources (ginger) to treat the carbon tetrachloride induced hepatotoxicity in mice and to understand the molecular mechanism.

2. Material and methods

2.1. Drug, chemicals and kits

Vanillylacetone (≥98%), Oxidative stress chemicals (oxidized glutathione, reduced glutathione,glutathione reductase, nicotinamide adenine dinucleotide phosphate, 1-chloro-2,4-dinitrobenzene, ethylene diamine tetraacetic acid, 5–5'dithio-bis-2-nitrobenzoic acid, thiobarbituric acid, (−)-epinephrine, sodium azide, hydrogen peroxide, sulfosalicylic acid and trichloro acetic acid were purchased from Sigma-Aldrich, Co. Germany through Byoni trading company of Saudi Arabia. The liver marker assay kits were purchased from Human Gesellschaft fur Biochemical and Diagnostic mbH, Germany. Interleukin (IL2-ab100706 and TNFα ab100747) and apoptosis kits (Caspase3-ab39401 and Caspase9-ab65608) were purchased from the Abcam Cambridge MA USA.

2.2. Animal adaptation

Male mice (35 ± 5 g) were procured from the central animal house, Medical Research Centre, Jazan University and mice were kept for acclimatization under standard condition (light/dark cycle 12 h, Temperature 23 ± 2 °C) before start the experiment in the College of Pharmacy, Jazan University. All animals were given laboratory diet and water. Animal care and handling were practiced according to the International standard and Institutional Animal Care and Use committee guidelines (IACUC) (National Research Council, 1996).

2.3. Experimental design

Mice were separated into 5 groups, each group had 8 mice. Group-I was controlled and the vehicle was given orally. Group-II was toxic and carbon tetrachloride CCl_4 (1:1 v/v) solution in olive oil containing 1.5 ml/kg intra-peritoneal injections twice a week for 15 days (Mohammed, 2018). Group-III and Group-IV were given CCl_4 and treated with Vanillylacetone (50 & 100 mg kg^{-1} body weight) every day orally. Group-V was given a higher dose of Vanillylacetone (100 mg kg^{-1} body weight) for 15 days P.O. On day 16, mice were sacrificed and blood was collected immediately. The collected blood sample was kept at room temperature for 30 min and then centrifuged at 3000 RPM for 10 min. The serum was isolated and preserved at -20 °C in a deep freezer for further biochemical analysis. The liver of each mouse was dissected out for biochemical analysis. A 10% homogenate of the liver was prepared in 0.1 M phosphate buffer (pH 7.4) and further centrifuged at 3000 RPM for 5 min at 4 °C to remove the debris and the supernatant (S1) was taken for the study of lipid peroxidation (LPO). While the balance homogenate was again centrifuged at 10,500 × *g* for 30 min at 4 °C to separate the post mitochondria supernatant (PMS) for the antioxidant glutathione (GSH) and other antioxidant enzyme analysis.

2.4. Biochemical assay of serum marker for hepatotoxicity

The important biochemical parameter such as Alanine aminotransferase (ALT), Aspertate aminotransferase (AST), Alkaline Phosphatase (ALP) and bilirubin were estimated in the serum. The biochemical parameters were estimated by spectroscopically (Shimadzu, Japan) using commercially available test kits from Humans for biochemical and Diagnostic, Germany.

2.5. Estimation of TBARS content in liver tissue

The lipid peroxidation test was done by the Utley et al. (1967) method to calculate the TBARS. The supernatant (S1) was taken and incubated in metabolic shaker and on the other hand same amount of supernatant was kept at 4 °C for 1 h incubation. Further TCA (10% chilled) and TBA (0.65%) was used for each test. The mixture was again centrifuged at 3000 RPM for 10 min. Thereafter S1 was further heated in boiling water bath for 10 min. After the cooling of the test tube sample was observed for the pink color and reading was taken at 535 nm.

2.6. Estimation of glutathione GSH in liver tissue

Glutathione was analyzed by the method of Jollow (1974) with minor changes. First PMS was mixed with 4% sulfosalicylic acid in a ratio of 1:1 and then samples were incubated at 4 °C for 1 h. The reaction mixture was centrifuged at 3000 rpm for 15 min at 4 °C. The developed color was read at 412 nm.

2.7. Estimation of antioxidant enzyme in liver tissue

The activity of antioxidant enzyme such as Catalase, Superoxide dismutase, Glutathione peroxidase, Glutathione reductase and Glutathione-s-transferease (CAT, SOD, GPx, GR and GST respectively) was estimated by the Claiborne (1985); Stevens et al. (2000); Mohandas et al. (1984); Carlberg and Mannervik (1975) and Habig et al. (1974), respectively.

2.8. Estimation of inflammatory cytokines (IL2 and TNFα) in liver tissue

The Abcam's cytokines assay kit was used for the measurement of IL2 and TNFα as per its standard guideline and for the each test standard curve was plotted. The standard and sample was analyzed by