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Diallyl disulfide, an organo-sulfur compound in garlic and onion attenuates trichloromethane-induced hepatic oxidative stress, activation of NFkB and apoptosis in rats

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ABSTRACT

Trichloromethane (TCM) serves as an ingredient in pesticide formulations and fire extinguishers. It is a reported hepato- and renal-toxin. We therefore investigated the chemo-preventive effect of diallyl disulfide (DADS) on TCM-induced hepatotoxicity. Twenty five rats, divided into five groups of five animals each were used. TCM at the dose of 200 mg/kg was orally administered, and concomitantly treated with DADS (50 mg/kg), 5 days per week for 3 weeks. Compared with control, there was a significant increase in hepatic expressions of nuclear factor kappa B (NFkB), TUNEL positive cells (apoptosis), and concentrations of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and nitric oxide (NO). Also, a significant decrease in expressions of p53, and activities of catalase (CAT) and glutathione peroxidase (GPx), as well as level of reduced glutathione (GSH) was recorded following TCM administration. Following treatment, DADS intervention significantly reduced the hepatic NFkB expressions, apoptotic positive cells as well as levels of MDA, H_2O_2, and NO, and also significantly increased the level of GSH, activities of CAT and GPx compared with TCM group, while its effect on expressions of p53 was insignificant. Hepato-protection by DADS against TCM-induced hepatotoxicity may therefore be via suppressions of NFkB activation, apoptosis, and oxidative stress in rats.

1. Introduction

Trichloromethane (TCM) is the most predominant by-product of water disinfection with chlorine-based chemicals [1], also formed in large quantities as a consequence of chlorination of cooling water in power plants and in the process of bleaching paper [2]. TCM has a widespread use in industry as solubilizer, diluent, and dispersant [3]. It is one of the major environmental toxicants [4], classified as a group 2B carcinogen [5]. TCM produces cancer via a non-genotoxic-cytotoxic mode of action, thus it is of interest in mechanistic studies [6]. TCM has similar acute toxic effects in animals and humans, affecting the liver, kidneys and the central nervous system [7]. TCM is metabolized by oxidative or reductive cytochrome P_{450}-dependent pathways with the former being predominant [8]. In the oxidative pathway, TCM is metabolized to phosgene [9], a highly reactive electrophilic metabolite, binding covalently to cell constituents possessing nucleophilic groups, including lipids, proteins, and glutathione [9–12], causing cytotoxicity [13,14]. TCM-induced reactive oxygen species generation is one of the direct causes of hepatic injury [6,15] The generation of these species and their overwhelming effects on cells' ability to mop them cause oxidative stress and then tissue injury [16,17]. TCM decreases GSH levels, resulting in oxidative stress in both liver and kidney of rodents [18,19].

Plant-derived natural products are gaining wide attention in chemo-prevention because of little or no side effects as against the chemically-derived therapeutics. A lot of naturally occurring phytochemicals of health beneficial importance have been identified. One of such is diallyl disulfide (DADS), found in garlic and onions, and a major component of the secondary metabolites. Increasing interest is being shown for DADS due to its numerous biological activities [20,21] that has been well documented including the ability to prevent urotoxicity, genotoxicity, nephrotoxicity, hepatotoxicity and cancer [22–27]. Specifically, DADS could account for the noticeable protective effects of garlic on cancer development.

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Several studies have suggested a protective effect of consumption of cooked or raw garlic on gastric cancer. Experimental studies in rodents have shown that organosulfurs such as diallyl sulfide (DAS) and DADS, as well as garlic powder, inhibit chemical-induced carcinogenesis in liver, lung, mammary gland, oesophagus, and colon, when administered during the initiation or the promotion stages [28].

DADS has been shown to reduce the hepatic DNA breaks induced by N-nitrosodimethylamine or aflatoxin B1, as well as the mutagenicity of N-nitrosopiperidine and benzo[a]pyrene. These effects could be attributed to the modulation of drug metabolizing enzymes, which play a key role in chemical activation and detoxication [29]. DADS promotes the activities of protein and mRNA levels of microsomal P_{450} 1A2 and P_{450} 2B1/2. It stimulates the activities of various phase II enzymes such as UDP-glucuronyl transferase, epoxide hydrolase and glutathione S-transferase.

Till date, one proposed explanation for DADS anti-promoting effects has been its ability to slow the growth of established tumor cell lines, and has been shown both *in vivo* and *in vitro* [30].

Evaluation of biochemical and molecular mechanism by which TCM causes hepatotoxicity in rats and the search for alternatives that ameliorate this toxicity may provide substantial information with clinical implications. It is on these premises that the present study investigated the chemo-therapeutic effects of DADS on TCM-induced hepatotoxicity in male wistar rats.

2. Materials and methods

2.1. Chemicals and test substances

TCM (98% purity), is a product of AD Chemicals Limited, Great Britain. DADS (98% purity) was purchased from Sigma Chemical Co., Saint Louis, MO, USA. Rat NFkB and p53 monoclonal primary antibodies were purchased from ABCAM UK; rat monoclonal secondary antibodies were purchase from Dako (Agilent Technologies, USA). Promega DeadEnd™ Colorimetric TUNEL System, for detection of apoptosis in tissue sections was purchased from Promega Corporation (Madison, WI, USA). Pure (100%) cholesterol free Mazola corn oil is a product of ACH Food Companies, Inc., Memphis, TN, USA. All other chemicals and reagents were of analytical grade, products of Sigma Chemical Co., Saint Louis, MO, USA or BDH Chemical Ltd, Poole, England.

2.2. Experimental animals and study design

Twenty five (25) male wistar albino rats of an average weight of 150 g used for this study were obtained from the animal house of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. They were housed in steel metal cages in the animal house of our department and were served food and water *ad libitum*. Permission to use the animals was approved by the Institution's Animal Ethical Committee. After 3 weeks of acclimatization, the rats were divided randomly into five groups of five animals each. Group I rats served as normal control and were fed food and water only, group II rats served as vehicle control and were administered corn oil, group III was administered 200 mg/kg TCM only [31], group IV was administered 200 mg/kg TCM and concomitantly treated with 50 mg/kg DADS [27], while group V was administered 50 mg/kg DADS only. All administrations were done orally.

2.3. Sample collections and preparations

Administrations lasted for fifteen (15) days (5 days/week for 3 weeks), and 24 h after, animals were sacrificed. They were handled and used in accordance with the international guide for the care and use of laboratory animals [32]. Liver was harvested, washed in ice-cold saline (0.9% w/v) solution, blotted dry, and weighed. A section of the liver was cut and fixed in 10% phosphate buffered formalin for terminal deoxynucleotidyl transferase dUTP nick end labeling (*TUNEL*) assay, immunohistochemistry, and histopathology. The rest was suspended in ice-cold 0.1 M phosphate buffer (pH 7.4) for homogenization. Homogenization was followed by centrifugation at 5000 rpm for 10 min. The resulting supernatant was aliquoted into Eppendorf tubes and used for other biochemical analyses.

2.4. Liver immunohistochemistry

This was performed as described by Ajayi et al. [33]. Poly-L-lysine charged slides were rehydrated in xylene as well as decreasing concentration of ethanol (100–50%). Heat-induced epitope retrieval was done in citrate buffer (pH 6.0) for 20 min followed by immersion in cold water for 10 min. The sections were marked with paraffin pen (PAP) pen and the endogenous peroxidase activities in the tissues were blocked with 5% hydrogen peroxide for 5 min in a dark cupboard. The sections were subsequently incubated overnight at 4 °C with anti-NFkB or anti-p53 primary monoclonal antibodies. The slides were thereafter washed with Tris buffer saline and further incubated with Horseradish peroxidase labeled anti-rabbit monoclonal secondary antibodies (Dako, Agilent Technologies, US). Immune complexes were visualized using 0.05% 3, 3-diaminobenzidene (DAB), countered stained with hematoxylin and the slides were visualized under light microscope. The percentage of tissue stained positive cells was scored.

2.5. TUNEL assay

This was carried out based on the manufacturer's protocol (Promega DeadEnd™ Colorimetric TUNEL System). Briefly, paraffin embedded tissue sections were washed in xylene for 5 min, followed by immersion in 100% ethanol for 5 min, rehydrated in decreasing concentrations of ethanol (100–50%) for 3 min, and washed by immersion in 0.85% sodium chloride and phosphate buffer saline (PBS) for 5 min each. Apoptosis was detected by fixing slides in 4% paraformaldehyde in PBS for 15 min, addition of proteinase K solution and incubation at room temperature for 10–30 min. Equilibration buffer was added to equilibrate at room temperature for 5–10 min, followed by addition of recombinant terminal deoxynucleotidyl transferase (rTdT) reaction mixture to the tissue sections on the slides and then incubated for 60 min at 37 °C. Reaction was stopped by immersion in 2× saline-sodium citrate (2× SSC) buffer for 15 min, blocked by immersion of slides in 0.3% hydrogen peroxide for 3–5 min. Streptavidin HRP was added to slides, incubated for 30 min at room temperature, stained with diaminobenzidine (DAB) and developed until a light brown background appeared. Visualization of stained slides was done using Nikon E100 light microscope (Novel Optics, NanJing, China) for apoptotic cells.

2.6. Estimation of MDA concentration

MDA concentration, a marker of lipid peroxidation (LPO) was determined by the method of Buege and Aust [34]. In this procedure, 0.1 mL of the supernatant was added to 2 mL of trichloroacetic acid–thiobarbituric acid–hydrochloric acid (TCA/TBA/HCl) (1:1:1 ratio) reagent, boiled at 100 °C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The absorbance of the supernatant was read