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Research Article

Combined Effects of Vitamin C and Tomato Extract (*Lycopersicon esculentum*) on Function and Histological Structure of Liver in Male Albino Rats Treated with Carbimazole

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Abstract

Drugs have been implicated as the cause of most liver injuries. Carbimazole administration, in excess, is associated with various pathological conditions, which includes hepatocellular damage. Generation of oxidative stress is one of the plausible mechanisms of carbimazole-induced organ damage. The aim of the present study was to investigate the effects of carbimazole on liver function in male albino rats, as well as assess the ameliorative role of the combination of Vitamin C and tomato extracts treatments. Phytochemical tests were performed. A total of 25 male albino rats weighing 200 - 250g were randomly divided into five groups (A-E), with five rats in each group. Group A served as normal control and received no treatment. Group B served as the negative control and received only carbimazole (60 mg/kg, oral). Groups C, D and E served as the treatment groups. They all received carbimazole, and then Vitamin C only (200 mg/kg, oral), tomato extract only (30 mg/kg, oral), and vitamin C plus tomato extract respectively for 3 weeks. Carbimazole (60 mg/kg, oral) administration for 3 weeks resulted in liver injury in the rats with increase in AST, ALT and ALP levels: 67.33 ± 12.71 U/L, 56.68 ± 11.22 U/L and 316.00 ± 58.81 U/L respectively. However, with the administration of vitamin C and/or tomato fruit extracts, these values were reduced thus, respectively: vitamin C only: 24.33 ± 4.33 U/L, 20.67 ± 2.73 U/L, 131.70 ± 7.27 U/L (**ρ < 0.01); tomato fruit extract only: 24.67 ± 4.37 U/L, 21.00 ± 2.08 U/L, 156.30 ± 9.84 U/L (**ρ < 0.01); vitamin C plus tomato fruit extract: 24.00 ± 2.31 U/L, 20.00 ± 2.31 U/L and 125.00 ± 8.34 U/L (**ρ < 0.01). Histopathological results concomitantly revealed mild or no significant degeneration in the livers of vitamin C and/or tomato extract-treated rats when compared to the normal control rats. This showed that vitamin C and tomato fruit extract possess hepatoprotective properties against the hepatotoxic effect of carbimazole; their hepatoprotective effect is greatest when combined.

Keywords: Ethnopharmacology; Hepatoprotection; Liver Injury; Tomato Extracts; Vitamin C; Carbimazole

Introduction

Oxidative stress is defined as a disruption of the equilibrium between oxidant and antioxidant systems. Excessive oxidants in the system results to increased production of reactive oxygen species (ROS), which can cause oxidative damage in vulnerable targets such as unsaturated fatty acids in membranes, thiol groups in proteins, and nucleic acid bases in DNA. Oxidative damage, accumulating during the life cycle, plays a key role in the development of many diseases, liver disease being one of them [1,2]. Superoxide dismutase (SOD), malondialdehyde (MDA) and protein disulfide isomerase (PDI) have often been used to demonstrate oxidative damage in tissues [2].

The antioxidant compounds help scavenge excess free radicals which are unstable molecules linked to the development of a number of degenerative diseases and conditions and, thus, prevent abnormal oxidation changes in the human body [3]. The ascorbic acid (vitamin C) in the tomato fruit is widely reported as a free radical scavenger [4].

Vitamin C can be found in abundance in Fruits and vegetables. The vitamin C content of food may be reduced by prolonged storage and by cooking because ascorbic acid is a water-soluble acid and is destroyed by heat. Due to the functions of vitamin C and tomato constituents as antioxidants (free radical scavengers), they have been promoted as a means to help prevent and/or treat numerous health conditions which may include drug-induced liver damage [4].

Carbimazole (ethyl 3-methyl-2-sulfanylidene-imidazole-1-carboxylase) (C7H10N202S) is a drug used for the treatment of thyrotoxicosis. It is one of the two drugs that make up the anti-thyroid compound called the thioamides, the other being propylthiouracil. Carbimazole is a pro-drug as, after absorption; it is converted to methimazole [5]. Methimazole acts by multiple mechanisms. The major action is to prevent hormone synthesis by inhibiting the thyroid peroxidase-catalysed reactions and blocking iodine organification. In addition, it blocks coupling of the iodotyrosines, hence reducing the production of thyroid hormones T3 and T4. Such agents are called goitrogens [5]. Adverse reaction to methimazole

and its pro-drug (carbimazole) are nausea, gastrointestinal distress, an altered sense of taste or smell. But the most common adverse effect is a maculopopular pruritic rash, at times accompanied by systemic signs such as fever. Cholestatic jaundice, which is also associated with methimazole, may be fatal [6-9].

There are few literatures or researches on the protective effect of antioxidant-rich food and food products on the liver, and there is currently no literature on the effect of tomato extracts against hepatocellular damage induced by the toxicant xenobiotic, carbimazole. However, the tomato fruit has been reported to be a rich source of antioxidants such as vitamin C, vitamin E, carotenoids etc [4]. Owing to the deleterious and oxidant stress on delicate organs induced by carbimazole, as extensively reported by Saber., *et al*. [1,10]; we evaluated for the comparative effects of two antioxidants (tomato fruit and vitamin C), so as to know if there is synergy in their ability to protect the liver against oxidant injury by carbimazole.

Materials and Methods

Collection and processing of tomato fruits

Fresh samples of tomato fruits were purchased from Akwata, Ogbete Main Market, a local market in Enugu Metropolis, Enugu State, Nigeria. The tomato fruits were processes by washing thoroughly in clean water. After washing, they were ground in an electric blender (Saisho, China) at maximum speed for 5 minutes. The extracts obtained were passed through a 52 mm pore size sieve (Tungsten and Co., Germany), and were subsequently preserved in the refrigerator at a temperature between 4 - 6°C for 24 hours.

Phytochemical analysis

Preliminary phytochemical screening of tomato fruits was carried out at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. Procedures described by Trease and Evans [11], were used for the analyses.

Chemical reagents

Standard laboratory kits (Randox Laboratories Ltd, UK) for assay of liver biomarkers, Vitamin C (Emzor Pharmaceuticals, Nigeria), to serve as an antioxidant; and carbimazole (Hovid, Malaysia) for induction of hepatocellular damage were utilized for the experiment.

Experimental animals and maintenance

Twenty-five (25) adult male albino wistar rats, with average weight of (200 - 250g), were used in this study. They were obtained from the animal house of the College of Veterinary Medicine, University of Nigeria, Nsukka, Enugu state, Nigeria. The animal experimentation was carried out according to Institutional guidelines describing the use of rats, and approved by the institutional animal research ethical committee (UNTH/CSA. 985/VOL. 17).

Induction of hepatotoxicity

Each experimental rat was administered with high dose carbimazole (60 mg/kg, oral), daily for 21 days.

Experimental design

The 25 experimental animals were randomly allocated to five (5) groups (A-E) of five (5) animals per group.

- Group A (Normal Control): No treatment was given to this group.
- Group B (Negative Control) were administered with only carbimazole (60 mg/kg b. wt, oral) for 21 days.
- Group C were administered with carbimazole and the standard drug Vitamin C (200 mg/kg b. wt, oral) for 21 days.
- Group D were administered with carbimazole and tomato extract (30 mg/kg b. wt, oral) for 21 days.
- Group E received carbimazole, Vitamin C, and tomato extract for 21 days.

Sacrificing of animals and sample collection

Blood samples for the determination of liver biomarkers were taken by cardiac puncture of the left ventricle of the heart under chloroform anaesthesia and the liver harvested for histological analysis.

Biochemical analysis

Measurement of Bilirubin (Total and Direct)

Colorimetric method as described by Malloy and Evelyn [12].

Measurement of ALT and AST

Determination of ALT and AST were by colorimetric method as described by Reitman and Frankel [13].

Measurement of ALP

Determination of ALP was by colorimetric method as described by Kind and King [14].

Histopathological analysis

The excised livers were fixed in 10% formal saline for 24 hours and further processed using the conventional paraffin wax embedding technique for light microscopic examination. The paraffin-embedded liver tissues were sectioned at 5microns and stained using the Haematoxylin and Eosin [H and E] staining procedure by Baker., *et al* [15]. The histological sections were examined using an Olympus TM light microscope.

Statistical analysis

The statistical analysis was done using Graph pad prism 6.0. The results were reported as mean ± SEM. Statistical significance $\rho < 0.05$ (*), $\rho < 0.01$ (**) or $\rho < 0.001$ (***) was determined by using ANOVA.

Results

Phytochemical results

The result of the preliminary phytochemical analysis is represented in table 1.

Constituent	Indication
Carbohydrate	+
Reducing Sugar	+++
Alkaloids	+++
Glycosides	–
Saponins	–
Tannins	–
Flavonoids	++
Resins	+
Proteins	–
Oils	–
Acidic Compounds	–
Terpenoids	–
Steroids	–

Table 1: Preliminary phytochemical analysis of tomato fruit.

Biochemical results

Serum Total bilirubin, Conjugated bilirubin, AST, ALT and ALP levels in all groups are shown (Figures 1.1 and 1.2). High dose of Carbimazole (60mg/kg, oral) administration for 3 weeks resulted to liver injury in the rats with an increase in AST, ALT and ALP levels: 67.33 ± 12.71 U/L, 56.68 ± 11.22 U/L and 316.00 ± 58.81 U/L respectively. However, with the administration of vitamin C and/or tomato fruit extract, these values were reduced thus, respectively: vitamin C only: 24.33 ± 4.33 U/L, 20.67 ± 2.73 U/L, 131.70 ± 7.27 U/L (**ρ < 0.01); tomato fruit extract only: 24.67 ± 4.37 U/L, 21.00 ± 2.08 U/L, 156.30 ± 9.84 U/L (**ρ < 0.01); vitamin C plus tomato fruit extract: 24.00 ± 2.31 U/L, 20.00 ± 2.31 U/L and 125.00 ± 8.34 U/L (**ρ < 0.01). The levels of Serum Total bilirubin and Conjugated bilirubin, were highly elevated significantly in the affected group following the oral administration of carbimazole (60 mg/kg). However, co-treatment with vitamin C (200 mg/kg), tomato extract (3 0mg/kg) and vitamin C plus tomato extract separately significantly decreased the elevated Serum total bilirubin (ρ < 0.05) and Conjugated bilirubin (ρ < 0.05) when compared to the affected group. Note-worthy, the tomato fruit extract showed a strong anti-hepatotoxicity against carbimazole.

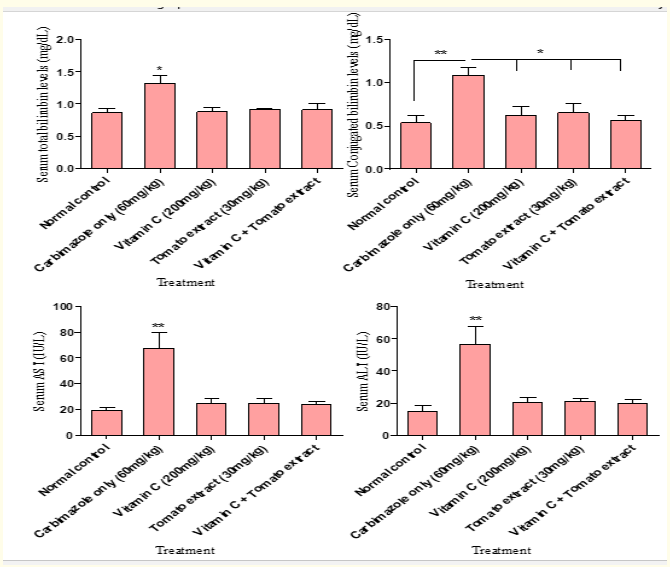


Figure 1.1: Comparison of liver biochemical concentrations in different experimental groups. The histograms show serum total bilirubin and conjugated bilirubin, AST and ALT levels following experimental treatments. The preliminary data show that tomato fruit extract significantly ameliorated the hepatotoxic effect by carbimazole. The data are presented as mean ± SEM of serum total bilirubin and conjugated bilirubin, AST and ALT levels for individual treatment. Statistical analyses were performed using ANOVA (*ρ < 0.05; **ρ < 0.01).

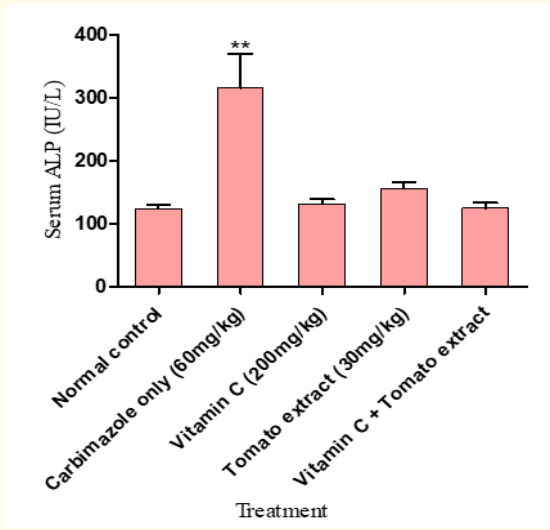
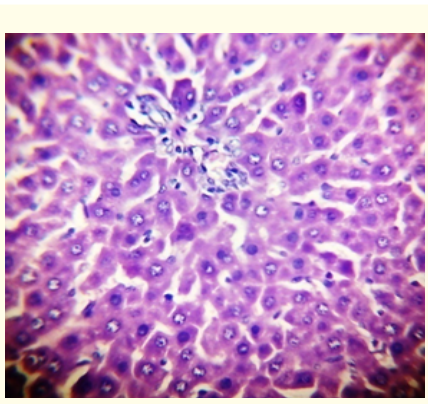
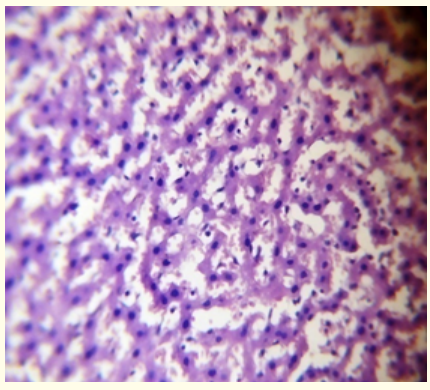


Figure 1.2: Comparison of chloride levels in different experimental groups. Histogram showing serum ALP levels following experimental treatments. The preliminary data show that tomato fruit extract and/or vitamin C significantly reduced the elevated ALP levels induced by the hepatotoxicant, carbimazole. The data are presented as mean ± SEM of ALP levels for individual treatment. See Materials and Methods for experimental details. Statistical analyses were performed using ANOVA (**ρ < 0.01).

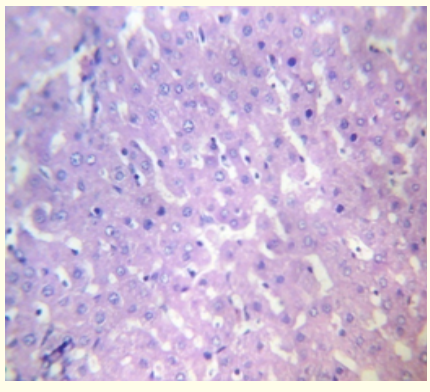
Histopathological results



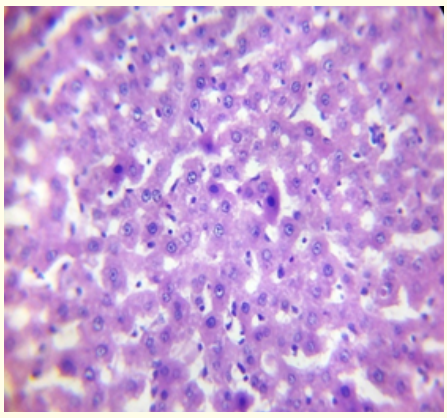
(A)



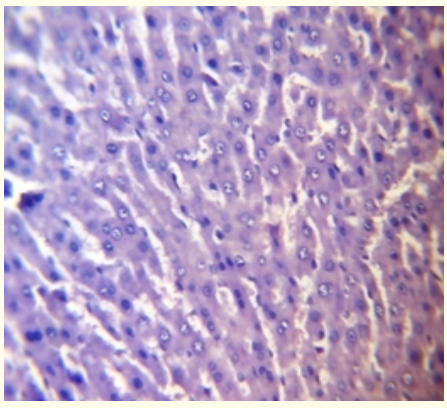
(B)



(C)



(D)



(E)

Figure 2 (A-E): Histopathology and photomicrograph of liver from (A) normal control, (B) carbimazole alone -treated, (C) vitamin C-treated and (D) tomato extract-treated rats and (E) rats co-treated with vitamin C and tomato fruit extract. Microscopical examination of the liver isolated from the rat at sacrifice revealed no histopathological alteration in the control rats (2A). The presence of severe cell necrosis and severe hepatocellular parenchyma degeneration were observed in the liver of rats treated with carbimazole only (2B); however mild or no significant degenerations were observed in rats with co-treatment with vitamin C, tomato fruit extract and vitamin C + tomato fruit extract separately (2C, 2D and 2E respectively). The livers of rats in group C, D and E showed no significant histological alterations when compared with the control group [Stain: H and E; ×40].

Discussion

Liver injury results from a number of factors and drugs are one of them. Most cases of acute liver injury that occur annually are caused by drugs [16]. Liver injury generally can be direct or indirect. Direct liver injury occurs with certain drugs such as acetaminophen or with ischaemia, whereas indirect liver injury is immunologically mediated injury that occurs with hepatitis viruses [17]. Drugs that cause liver injury are now known as hepatoxicants, and this includes carbimazole.

Carbimazole causes liver injury in a number of ways. But the most common of them is idiosyncratic, immune-mediated injury to hepatocytes, which is characterized by elevations in ALT, AST and ALP [17].

The aim of this study was to investigate the hepatotoxic effect of carbimazole and the combined hepatoprotective effects of vitamin C and tomato fruit extract. The result shows that 3 weeks of high dose carbimazole administration resulted to elevation of serum ALT, AST and ALP, which suggests a liver injury in the experimental rats. The mechanism by which the liver injury was caused is likely by an immunological reaction to a metabolic product of carbimazole metabolism [18].

Carbimazole is metabolized by cytochrome P450 (CYP450) enzymes and flavoprotein mixed-function oxidase (FMO) [19]. The two major metabolites of carbimazole metabolism through CYP450 enzymes are N-methylthiourea and glyoxal. N-methylthiourea is further oxidized by FMO, giving two acid species: sulfinic and sulfenic acids. Sulfenic acids are electrophilic species that form irreversible adduct with cellular nucleophilic sites and may have a role in carbimazole-reactive metabolite that is responsible for the hepatoxicity induced by this drug [19].

The active phytochemicals present in this tomato extract are the flavonoids, and alkaloids. Flavonoids constitute the largest group of naturally occurring phenolics in tomatoes [4]. They demonstrate antioxidant activities and have also been shown to possess many health promoting properties. Most of the known alkaloids are related to tissue protection.

Alkaloids are a class of naturally occurring organic nitrogen containing bases which have important physiological effect on both humans and other animals. Lycopene is an antioxidant found abundantly in tomatoes. It has the ability to destroy free radicals, thereby preventing their harmful effects on cells and the immune system [20]. Lycopene has also been reported to be very active against prostate cancer [20,21].

In the present research, the hepatotoxic effect induced by carbimazole was significantly reduced by vitamin C and tomato fruit extract. This may be because of their antioxidant properties. As antioxidants, they may have interacted with the free radicals released during carbimazole metabolism. The synergistic interaction of these antioxidants when in combination may be responsible for the reduction of the elevated ALT, AST and ALP induced by the hepatotoxicant, carbimazole. Some recent studies have shown that antioxidant-rich foods or food products have potential bioactive substances that exhibit protective properties against toxicant xenobiotics [22-32].

Vitamin C, an essential cofactor for α-ketoglutarate-dependent dioxygenases, has a very strong reducing power (high redox potential) and facile regeneration, which enables it to function physiologically as a water-soluble antioxidant [33]. It protects the cells from oxidative damage by scavenging reactive oxygen species, neutralization of lipid hydroxyperoxyl radicals which are dependent on vitamin E, and protecting proteins from alkylation by electrophilic lipid peroxidation products [3]. Vitamin E, on the other hand, is an essential α-tocopherol and lipid-soluble antioxidant that functions by scavenging hydroperoxyl radicals in lipid milieu [33].

Conclusion

Carbimazole induced liver injury in the rats and therefore could do the same in humans. This can be treated by the administration of vitamin C and tomato fruit extract as these substances contain antioxidants which can significantly reduce and reverse the damaging effect of the drug to the liver.

Acknowledgement

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Competing Interests Statement

The authors declare no competing interests.

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