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Original article

The effects of tomato (*Lycopersicon esculentum*) extract on carbimazole-induced biochemical alterations in pancreas of albino rats

Ikenna Kingsley Uchendu[1], Chidozie Elochukwu Agu[2], Oliver Chukwuma Orji[1]

[1]Division of Clinical Chemistry, Department of Medical Laboratory Science, University of Nigeria, Enugu Campus, Enugu-400241, Enugu State, Nigeria.
[2]Prime Health Response Initiative - Global Fund HIV/AIDS Project, Ilorin, Kwara State, Nigeria.

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Corresponding author

Chidozie Elochukwu Agu
Division of Clinical Chemistry, Department of Medical Laboratory Science, University of Nigeria, Enugu Campus, Enugu-400241, Enugu State, Nigeria.
Phone: +234-8030984682
Email: chidozieagu@gmail.com

Abstract

Acute pancreatitis is a relatively common disease with an estimated annual mortality rate of about 7%. Drug-use is a major etiological factor in the pathogenesis and pathophysiology of the condition. The aim of this study was to evaluate the effects of tomato extract on carbimazole-induced biochemical alterations in the pancreas of albino rats. phytochemical analysis of the tomato extract was done. A total of 24 male albino wistar rats weighing (225±25g) were randomly divided into 4 groups (A−D) with six rats per group. Group A served as the normal control group and received no treatment. Group B received only carbimazole (60mg/kg, oral) and served as the negative control group. Group C received vitamin C (200mg/kg, oral) and served as the positive control and Group D which served as test group received tomato extract (30mg/kg, oral) in the presence of carbimazole challenge for 4 weeks. Carbimazole administration to rats resulted in pancreatic damage with glucose, amylase, triglycerides and total protein levels: 126.33±6.64 mg/dl; 484.00±18.33 IU/L; 0.75±0.09 mmol/l and 4.83±0.12 g/dl respectively. The daily administration of tomato extract resulted in the mitigation of the carbimazole-induced pancreatic damage with glucose, amylase, triglyceride and total protein levels: 106.33±296 mg/dl ($p<0.01$); 333.33±44.10 IU/L ($p<0.01$); 2.80±0.31 mmol/l ($p>0.05$) and 6.33±0.44 g/dl ($p>0.05$) respectively. Tomato (*Lycopersicon esculentum*) possesses pancreas-protective property against carbimazole- induced pancreatic damage**.**

Key words: Acute pancreatitis, Albino rats, Carbimazole, Pancreatic protection, Tomato

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Acute pancreatitis is a relatively common disease that affects about 300,000 patients per annum in America with a mortality of about 7%[1]. It is an inflammatory disease of the pancreas whose aetiology and pathogenesis have been intensively investigated for centuries, worldwide[1]. It can be initiated by several factors such as gallstone, alcoholism, trauma, infections, hereditary factors and drugs[2]. About 75% of acute pancreatitis is caused by gallstone or alcoholism while drug-induced pancreatitis is only considered to be a rare disease[2]. The total incidences of acute pancreatitis

in developed countries continue to rise as does the exposition of the general population to medication.

For a proper understanding of drug-induced pancreatitis, we must regard it not simply as one of many other types of acute pancreatitis but primarily as an acute drug reaction (ADR). A recent definition describes ADR as an appreciably harmful or unpleasant reaction; resulting from an intervention related to the use of a medicinal product, which predicts hazard from future and warrants prevention or specific treatment, or alteration of the dosage regimen or withdrawal of the product[3]. Drug use accounts for about 2% of all the causes of acute pancreatitis; the overall incidence in different studies vary from 0.1% to 2% with a tendency to increase over time[4]. The incidence of drug-induced pancreatitis may be somewhat under-estimated. Pathogenesis of acute pancreatitis is probably very uniform, differing only by initial injury mechanism. It consists of 3 steps: premature activation of trypsin in acinar cells, intra pancreatic inflammation and, extra pancreatic inflammation[5]. Several drugs have been implicated in drug-induced pancreatitis, and an example is carbimazole.

Carbimazole is a thionamide drug used in the treatment of hyperthyroidism and reduce thyroid function before surgery[6]. Many authors have reported that its use was accompanied by deleterious effects. It was reported that within 15 days following the start of carbimazole therapy, both minor (e.g. pruritus, rash, urticaria, fever and arthralgia) and potentially life-threatening (agranulocytocysis, hepatotoxicity with severe cholestatic jaundice) were developed[6]. The mechanism through which carbimazole causes acute pancreatitis is by increasing oxidant stress within the pancreatic cells; causing free radicals and superoxide to be more available, which eventually leads to the inflammation of the pancreas[7]. Some studies have shown that antioxidant-rich foods or food products have potential bioactive substances that exhibit protective properties against toxicant xenobiotics[8-16]. It is suggested that the curative effect of fruits or food products against damage induced by carbimazole may be due to their antioxidant properties. Common antioxidants include vitamin A, vitamin C, vitamin E, selenium and lycopene. These components are commonly found in fruits and vegetables such as tomato.

One of the most well known benefits of eating tomato is its lycopene content. Lycopene is a vital antioxidant that helps in the fight against cancerous cell formation as well as other kinds of health implications and diseases[17]. Antioxidants are molecules that protect the body cells from harmful effects of free radicals, molecules that form in the body through contact with oxygen. Tomato extracts contain carotenoids, beta carotene and vitamin E (a well known anti oxidant). Thus, the beneficial role of tomato fruit against drug-induced pancreatitis or oxidant stress cannot be over-emphasized.

The medicinal importance of tomato or tomato products has not been adequately investigated. This research was aimed to scientifically assess the medical benefits of tomato extracts; a natural fruit, and its role in combating pancreatic dysfunction induced by the toxicant drug, carbimazole.

Materials and methods

Collection of Tomato fruits

Fresh samples of tomato fruit (*Lycopersicon esculentum*) were purchased from Akwatta-Ogbette main market in Enugu, Nigeria.

Processing of tomato fruits

The tomato fruits were processes by washing thoroughly in clean water. After washing they were put in an electric blender (Saisho, China) and blended at maximum speed for five minutes. The extract was filtered through a clean muslin cloth and resultant filtrate preserved in the refrigerator at 4 ± 2^0C until when needed.

Phytochemical analysis of tomato fruit

Preliminary phytochemical screening of tomato fruits (*Lycopersicon esculentum*) for the presence of glycosides, flavonoids, saponins, steroids, tannins, carbohydrates, proteins and terpenoids was carried out at Department of Pharmacognosy, Faculty of Pharmaceutical Science, University of Nigeria Nsukka. Procedures outlined by Trease and Evans[18] were employed for the analyses.

Reagents and solutions

Preparation of Vitamin C solution:- The Vitamin C was purchased from EMZOR® pharmaceuticals. One hundred (100) tablets of 100 mg each (i.e. 10,000 mg) was grinded to powder, dissolved in distilled water and made up to 200 ml in a measuring cylinder to give a stock concentration of 50 mg/ml.

Preparation of carbimazole solution:- 25 tablets of 5 mg (i.e. 125 mg) carbimazole obtained from Hovid® Inc., Malaysia, were grinded to powder, dissolved in distilled water and made up to 250 ml in a measuring cylinder to give a stock concentration of 0.5 mg/ml.

Induction of acute pancreatitis:- Each experimental rat was treated with oral administration of 60mg/kg body weight carbimazole for three weeks.

Experimental animals and maintenance

Twenty-four (24) adult male albino wistar rats, with an 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 animals were housed in metallic cages in the animal house under ambient temperature (25±3°C) and 12-hour light and dark periodicity. They were adequately fed with commercial rat pellets (Neimeth Livestock Feeds Ltd., Ikeja) and water *ad libitum*. The animals were kept under observation for about 14 days before the onset of the experiment for acclimatization. All the animals used in this study were handled according to Institutional guidelines describing the use of rats and in accordance with the American Physiological Society guiding principles for research involving animals and human beings[19]. In addition, proper care was taken as per the ethical rule and regulation of the concerned committee of the University of Nigeria, Nsukka, Enugu State, Nigeria.

Ethical approval

Ethical approval for the use of animals for experimental research was applied for and obtained from the Institutional Ethics Committee at Department of Animal Science, University of Nigeria, Nsukka, Enugu State, Nigeria.

Experimental design

The rats were randomly allocated to four (4) groups (A−D) of five (5) animals per group in well ventilated cages. The experimental animals received the following treatments on a daily basis for four weeks period together with the stipulated feed and water.

- Group A (Normal control): No treatment was given to this group
- Group B (Negative control): Administered with carbimazole 60mg/kg, oral, for twenty-eight days
- Group C (Positive control): Carbimazole (60mg/kg, oral) and the standard drug Vitamin C (200mg/kg, oral) were administered for twenty-eight days
- Group D: Carbimazole (60mg/kg, oral) body weight and tomato extract (30mg/kg, oral) for twenty-eight days.

Sacrificing of animal and sample collection

Blood samples for biochemical analysis were taken by cardiac puncture of the left ventricle of heart under chloroform anesthesia.

Biochemical analysis

The serum amylase, triglyceride, total protein and plasma glucose levels were estimated using the following methods.

Measurement of serum amylase:- This was determined using enzymatic method with 4-Nitrophenyl-a-oligosaccharides as Substrate as described by Lorentz[20].

Measurement of plasma glucose:- This was determined using glucose oxidase-peroxidase method as described by Trinder[21].

Measurement of serum triglyceride:- This was determined using glycerol phosphate oxidase (GPO) method for Triglyceride as described by Fossati and Prencipe[22].

Measurement of Total Protein:- This was determined using Biuret method.

Statistical analysis

The statistical analysis was done using Graph pad prism 6.0. The results were reported as mean ±SEM (standard error of mean). Statistical significance $p<0.05$ (*), $p<0.01$ (**), or $p<0.001$ (***) was determined by using the independent two-sample students t-test or ANOVA.

Results

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

Table 1: Preliminary phytochemical analysis

Constituents	Indication
Carbohydrate	+
Reducing Sugar	+++
Alkaloids	+++
Glycosides	−
`Saponins	−
Tannins	−
Flavonoids	++
Resins	+
Proteins	−
Oils	−
Acidic Compounds	−
Terpenoids	−
Steroids	−

+++ = More intensely present, ++ = Present, + = Present (in trace amount); − = Absent

Table 2: Statistical analysis of pancreatic biochemical concentrations in different experimental animal groups

Treatment group	Amylase (IU/L)	Glucose (mg/dl)	Triglyceride (mmol/L)	Total protein (g/dl)
Normal control	209.30±3.84**	103.33±1.45*	2.25±0.26**	6.83±0.20**
Carbimazole alone (60mg/kg)	484.00±18.33	126.33±6.64	0.75±0.09	4.83±0.12
Vitamin C (200mg/kg)	286.00±41.59**	106.30±3.48*	2.83±0.23**	6.47±0.41*
Tomato extract (30mg/kg)	333.33±44.10	106.33±2.96*	2.80±0.31**	6.33±0.44*

Values are given as Mean ± SEM. **P<0.01 or *P<0.05 is significantly different when normal control, vitamin C (positive control) or tomato extract is compared with negative control (carbimazole alone).

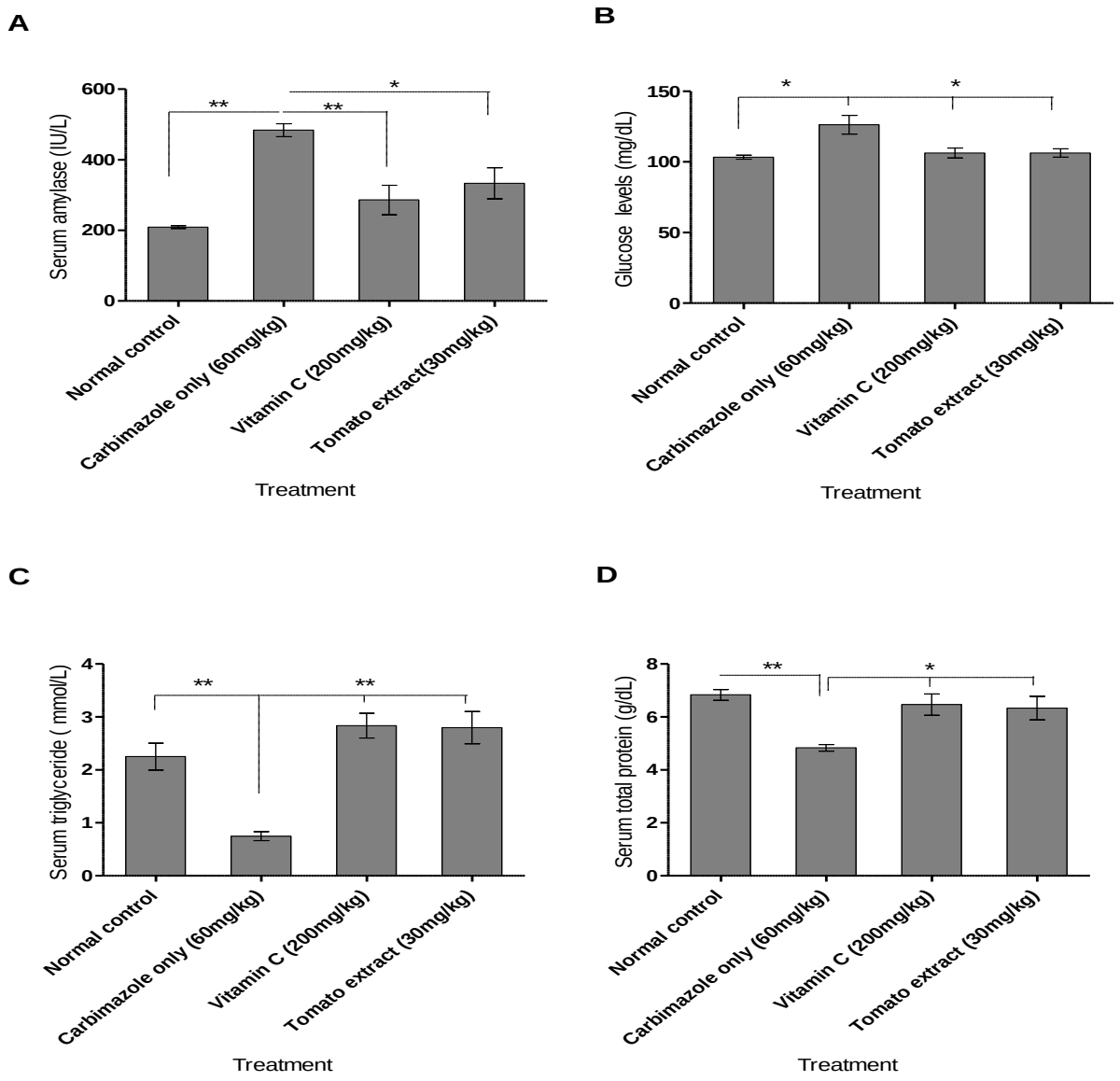

Fig 1. Comparison of pancreatic biochemical concentrations in different experimental groups

Histogram showing serum amylase, triglyceride, total protein and glucose levels following treatment with (Vitamin C + carbimazole), carbimazole alone or (tomato extract + carbimazole). The preliminary data shows that tomato extract or Vitamin C significantly induced lower amylase and glucose levels; and higher triglyceride and total protein levels when compared to carbimazole alone (negative control). Albino whister rats (n = 5) were daily administered with tomato extract or Vitamin C for 4 weeks and carbimazole (60mg/kg, oral). The data are presented as mean±SEM of serum amylase, triglyceride, total protein and glucose levels for individual treatment. See Materials and Methods for experimental details. Statistical analyses were performed using the student's t-test or ANOVA. (**p<0.01; *p<0.05).

Serum amylase, triglyceride, total protein and glucose levels in all groups are shown in table 2. The levels of amylase and glucose were highly elevated significantly in the affected group (carbimazole alone) in comparison with normal control group. However, the oral administration of tomato extract (30mg/kg) and vitamin C (200mg/kg) separately under carbimazole (60mg/kg) challenge significantly lowered the elevated levels of amylase (p<0.01) and glucose (p<0.05) when compared to the affected group. Furthermore and note-worthy, the levels of triglyceride and total protein were significantly decreased in the affected group (carbimazole alone) when compared to the normal control; however the oral administration of tomato extract (30mg/kg) and vitamin C (200mg/kg) separately under carbimazole (60mg/kg) challenge significantly elevated the decreased levels of triglyceride (p<0.01) and total protein (p<0.01) when compared to the affected group (Fig 1).

Discussion

Acute pancreatitis is an acute inflammatory condition of the pancreas with varying degree of involvement of surrounding tissues and/or distant organs[2]. This condition is viewed as a relatively common clinical condition and it is estimated to affect about 300,000 patients per annum in America with a mortality rate of about 7%[2]. The pathological process by which this inflammatory condition develops may be limited to the pancreatic gland only with edema or necrosis, or it may sometimes involve the surrounding tissues and/or distant organs, therefore the clinical manifestations range from mild abdominal pain to very serious presentations[23]. It is estimated that drug use accounts for about 2% of all the causes of acute pancreatitis[4]. The overall incidence varies in different studies from 0.1% -2% with a tendency to increase over time[4]. This therefore justifies any scientific enquiry into drug use, its debilitating effects on the pancreatic cells and how this can be salvaged using extracts from natural fruits or vegetables. The aim of this research was to evaluate the effects of tomato extract on carbimazole-induced biochemical alterations in pancreas of albino rats.

In this study, results showed clearly that carbimazole; a thionamide drug used in the treatment of hyperthyroidism and reduces thyroid function, induced severe pancreatic damage on the pancreatic cells in albino rats (Table 1) and this is in line with the position of Mennecier *et al.*[7], that such drugs could induce pancreatic dysfunction. Biochemical results showed that after the administration of the carbimazole (60mg/kg, oral) for 4 weeks, biochemical alterations in the pancreas were observed to show highly elevated blood glucose levels in the carbimazole only group when compared to the normal group. This is likely due to damage in the pancreatic beta acinus leading to insulinopenia (also known as hypoinsulinism) and resultant hyperglycemia. There was also increased amylase level in the blood. Furthermore, the levels of triglyceride and total protein were significantly decreased when compared to rats in normal group. All these alterations are indicative of pancreatic anomaly or dysfunction. This biochemical alteration believed to have been induced by the drug, carbimazole, were to a large extent reversed in rats in the positive control group (i.e. Carbimazole + Vitamin C group) and test group (Carbimazole + tomato extract group). The oral administration of tomato extract (30mg/kg, oral) and Vitamin C (200mg/kg, oral) separately under carbimazole (60mg/kg, oral) challenge significantly lowered the elevated levels of amylase and glucose. This also elevated the decreased levels of triglyceride and total protein when compared to the affected group. The possible recovery of the pancreatic cells was observed in the normalization of the altered levels of the pancreatic biochemicals.

The mechanism of action of the pancreatic protection by the tomato extract is not well understood or documented yet but it can be inferred that these restorative changes were similar to the changes observed in the positive group (i.e.Vitamin C administered group). It has been suggested that the mechanism through which carbimazole causes acute pancreatitis is by increasing oxidant stress within the pancreatic cells; causing free radicals and superoxide to be more available, which eventually leads to the inflammation of the pancreas[7].

Vitamin C (ascorbic acid), being an effective anti-oxidant actively slows down the formation of these dangerous oxygen molecules[12]. It is also known that common anti-oxidants such as Vitamin C, Vitamin E, Vitamin A and Selenium produce their restorative effects by slowing down the formation of these reactive oxygen species (ROS) and providing alternative route for their harmless clearance from the cell.

Tomatoes, which are actually fruits and not vegetables, are enriched with biomolecules whichhave health benefits for the body. A major health benefit of tomato is its antioxidant properties. Antioxidants are substances that protect the cells and tissues in the body from harmful effects of free radicals; molecules that for m in the body through contact with oxygen[12, 13].

Phytochemical analysis of Tomato *(Lycopersicon esculentum*) showed the presence of flavonoids, alkaloids, carbohydrates, and resins. This is in concordance with findings of Toor and Savage[24], who posited that the skin and seed fractions of tomato fruit contain abundant flavonoids, alongside other antioxidants. The antioxidant properties of flavonoids and/or alkaloids are well documented in literature[25-28]. Flavanoids are a class of secondary plant phenolics with significant antioxidant and chelating properties[26]. They are found ubiquitously among the plant kingdom as basic portions of our daily diet in vegetables and fruits as posited by Sandhar *et al*[31]. The best- described antioxidant property of flavoniods derives from its ability to directly scavenge the ROS, chelating free radicals immediately by donating a hydrogen atom or by single- electron transfer[32]. The presence of these vital biomolecules in tomatoes is mainly responsible for the antioxidant capacity of raw tomatoes and processed tomato products. The antioxidant effects of flavonoids and alkaloids; and their abundant presence in tomato extract strongly show that tomato fruit consumption can be effectively employed in combating drug- induced acute pancreatitis.

Conclusion

Oral administration of tomato extracts, under carbimazole challenge, significantly protected the pancreas of albino rats from severe pancreatic damage. The attenuating or protective action of tomato extract against pancreatitis by carbimazole was evident in the extract's ability to prevent further biochemical changes which are indicators of severe pancreatic damage. Tomato fruits consumption may possibly be the safest treatment or management against any pancreatitis by drugs with similar mechanism of action as carbimazole. Therefore, natural antioxidants in tomato possess the capacity of reducing the toxic effects of drug on pancreatic cells.

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Conflict of interest: The authors declare no competing interests.

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