

Original Research Article

Effects of Tomato Extract (*Lycopersicon Esculentum*) on Carbimazole-Induced Alterations in Kidney of Albino Rats

Uchendu Ikenna Kingsley

Division of Clinical Chemistry, Department of Medical Laboratory Science,
University of Nigeria, Enugu Campus, Nigeria

ABSTRACT

Acute Kidney Injury has a very high mortality and morbidity despite advances in clinical treatment. The health benefits of tomato extract on kidney injury has not been extensively studied. This research investigated the effects of tomato extracts on carbimazole-induced alterations in the kidney of albino rats. A total of 24 albino rats weighing (100±25g) were randomly divided into four groups (A-D) with six rats per group. Group A served as normal control and received no treatment. Group B received only Carbimazole (60mg/kg, oral) daily for 3 weeks and served as negative control. Group C received Vitamin C (200mg/kg, oral) in the presence of carbimazole challenge and served as the positive control and Group D which served as the test group received Tomato extract (30mg/kg, oral) in the presence of Carbimazole challenge. The Carbimazole administration resulted in Kidney injury with Serum Creatinine, Urea and Potassium levels: 1.57±0.12mg/dl; 47.00±1.00 mg/dl and 6.43±0.54mmol/l respectively. The administration of Tomato extracts resulted in the amelioration of the Carbimazole-induced kidney damage with Serum Creatinine, Urea and Potassium levels: 0.83±0.20mg/dl (P<0.05); 25.67±3.38mg/dl (P<0.01) and 4.77±0.12mmol/l (P<0.05) respectively. Histopathological results also revealed no significant kidney degeneration in the Tomato group; hence the kidneys were protected by the Tomato extract. The phytochemicals present in the Tomato extracts may have protected the kidneys from carbimazole-induced kidney injury. Intake of Tomato extract protects the kidneys from acute Kidney Injury.

Key words: ethnopharmacology, renoprotection, nephrototoxicity, *Lycopersicon esculentum*, carbimazole.

INTRODUCTION

Despite treatment advances, the mortality rate for Acute Kidney Injury (AKI) remains high-about 40% in critically ill patients. [1] Acute Kidney Injury (AKI) is the new consensus term for Acute Renal Failure. [2] It is one of the conditions that affect kidney structure and function. AKI is defined as an abrupt decrease in kidney function that includes, but is not limited to, Acute Renal Failure (ARF). It is a broad clinical syndrome encompassing various aetiologies, including specific kidney

diseases, non-specific conditions and extra-renal pathology. [3] Drug-induced Acute Kidney Injury (AKI) has also been on the increase with the increase in number of drugs and easy availability of over-the-counter medication. [4] Intake of drugs such as Carbimazole has been shown to cause some biochemical damage to the renal tubules of laboratory animals. [5]

Carbimazole is an antithyroid drug for treatment of hyperthyroidism which is also known as thyrotoxicosis. It is a 3-carbethoxy-methimazole derivative,

metabolized to methimazole in the liver. Serum thyroxine, thyroid-stimulating hormone and thyrotropin-binding inhibitory immunoglobulins are decreased after some weeks of treatment with carbimazole. [5] The use of carbimazole is associated with various adverse effects such as: Mild necrosis of renal tubules in rats and necrotizing glomerulonephritis. Intake of some dietary antioxidant fruits. Such as tomato fruit amongst others, has a lot of health benefits.

The edible Cherry fruit, commonly known as tomato (*Lycopersicon esculentum*) belongs to the nightshade family, Solanaceae. It contains phytochemicals which possess antioxidant, antibacterial, antifungal, antiviral and anti-carcinogenic properties. [6] Some studies have shown that antioxidant-rich foods or food products have potential bioactive substances that exhibit protective properties. [2,7-12] The antioxidants in tomato may as well help to scavenge harmful free-radicals, thereby offering health benefit.

Certain biochemical markers play an important role in accurate diagnosis, assessing risk and adopting therapy that improves clinical outcome. Over decade's research and utilization of biomarkers of kidney function has evolved substantially.

The test for electrolytes includes the measurement of sodium, potassium, chloride, and bicarbonate for both diagnosis and management of renal, endocrine, acid-base, water balance, and many other conditions, with Potassium used as a most convincing electrolyte marker of renal failure. The combination of decreased filtration and decreased secretion of potassium in distal tubule during renal failure cause increased plasma potassium. Hyperkalaemia is the most significant and life-threatening complication of renal failure. [13]

Millions of people worldwide die of kidney disease each year because they do not have access to affordable treatment. The effects of tomato extract on kidney function parameters have not been adequately

investigated. There is no available study that has tried to show that renal function could be affected by consumption of tomato extract. The increase in Acute Kidney Injury (AKI) might also be due to the poor feeding habits of individuals and a side effect of the treatment with nephrotoxic drugs. This study therefore hypothesizes that tomato extract could improve cases of drug-induced Acute kidney injury (AKI), thereby making their treatment affordable. This study aimed to investigate the effects of tomato extracts on carbimazole-induced alterations in kidney of albino rats.

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 processed 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 $4\pm 2^{\circ}\text{C}$ 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 [14] were employed for the analysis.

Reagents and solutions

Preparation of Vitamin C solution:

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

Preparation of carbimazole solution:

Twenty-five (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 Nephrotoxicity

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 (100±25g) 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 for acclimatization, before the onset of the experiment. 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. [15] In addition, proper care was taken as per the ethical rules and regulations of the concerned committee of the University of Nigeria Nsukka, Enugu State, Nigeria.

Ethical Approval

An 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 three weeks period together with the stipulated feed and water.

- Group A (Normal Control): No treatment was given to this group.
- Group B (Negative Control): was administered with Carbimazole (60mg/kg, oral) for twenty-one days.
- Group C (Positive Control): was administered with Carbimazole (60mg/kg, oral) and the standard drug Vitamin C (200mg/kg, oral), for twenty-one days.
- Group D: received Carbimazole (60mg/kg, oral) and tomato extract (30mg/kg, oral) for twenty-one 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 and subsequently the kidneys were excised for histopathological studies. The kidneys were isolated immediately after sacrificing the animal and washed with saline and then processed.

Biochemical analysis

The levels of Serum Electrolyte, Urea and Creatinine were estimated using the following methods:

Determination of serum electrolytes

Serum electrolytes were determined using Perlong Medical PL1000A Electrolyte Analyser. The electrolyte analyser applies the principle of advanced ion-selective electrode, which gives the instrument a stable and reliable measurement. It measures the ion concentrations of K⁺, Na⁺, Cl⁻, Ca⁺⁺, HCO₃, and pH values in the whole blood, serum and urine sample.

Determination of Serum Urea

Serum urea concentration was determined using the diacetylmonoxime method with protein precipitation according to Natelson *et al.* [16]

Determination of serum creatinine concentration

Serum creatinine concentration was determined using the Jaffe Reaction according to Fabing and Ertingshausen. [17]

Histopathological analysis

The excised kidneys were fixed in 10% formal saline for 24 hr and further processed using the conventional paraffin wax embedding technique for light microscopic examination. The paraffin-embedded Kidney tissues were sectioned at 5 microns and stained using the Haematoxylin and Eosin [H and E] Staining procedure. [18] The histological sections were examined using an Olympus™ light microscope.

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 ANOVA.

RESULTS

Phytochemical 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	-

Key: +++ = More intensely present; ++ = Present; + = Present (in trace amount); - = Absent

Biochemical results

Serum sodium Na, potassium K, creatinine, urea, calcium Ca, and bicarbonate HCO_3^- levels in all groups are shown in Figure 1. Carbimazole administration resulted in Kidney injury with Serum Creatinine, Urea and Potassium levels: $1.57 \pm 0.12 \text{ mg/dl}$; $47.00 \pm 1.00 \text{ mg/dl}$ and $6.43 \pm 0.54 \text{ mmol/l}$ respectively. However, the administration of Tomato extracts resulted in the amelioration of the

Carbimazole-induced kidney damage with Serum Creatinine, Urea and Potassium levels: $0.83 \pm 0.20 \text{ mg/dl}$ ($P < 0.05$); $25.67 \pm 3.38 \text{ mg/dl}$ ($P < 0.01$) and $4.77 \pm 0.12 \text{ mmol/l}$ ($P < 0.05$) respectively.

Note-worthy, the levels of Na was significantly decreased in the affected group (carbimazole alone); However, the administration of tomato extract (30mg/kg) and vitamin C (200mg/kg) separately in the presence of carbimazole challenge significantly elevated the decreased Na ($p < 0.05$) when compared to the affected group.

Furthermore, there were no significant differences or changes in HCO_3^- (renal/acid-base parameter), and Ca among the groups ($p > 0.05$).

The Histograms show Serum Na, K, creatinine, urea, Ca, and HCO_3^- levels following experimental treatments. The preliminary data shows that tomato extract significantly ameliorated the nephrotoxic effect of carbimazole. The preliminary data shows that there were no significant differences or changes in HCO_3^- (renal/acid-base parameter) and Ca levels among the groups. The data are presented as mean ±SEM of Serum Na, K, creatinine, urea, Ca, and HCO_3^- levels for individual treatment. Statistical analyses were performed using ANOVA. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

Histopathological result

Microscopic examination of the kidneys isolated from the rats at sacrifice revealed no histopathological alteration in the control rats (Figure 2A). Presence of significant necrosis of the renal tubules and severe interstitial degeneration were observed in the kidney of rats treated with oral administration of carbimazole (Figure 2B); however non-significant degenerations were observed in rats with co-administration of vitamin C and tomato extract separately (Figure 2C and D respectively). The kidneys of rats in group C and D showed no significant histological alterations when compared with the control group.

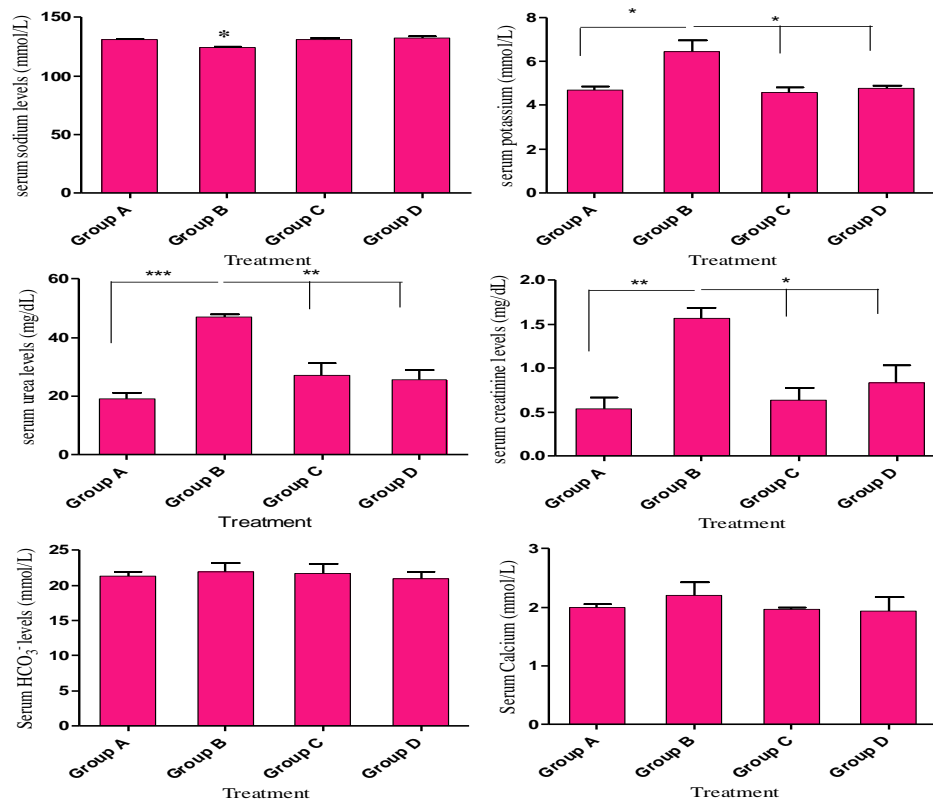


Figure 1: Comparison of renal biochemical concentrations in different experimental groups.

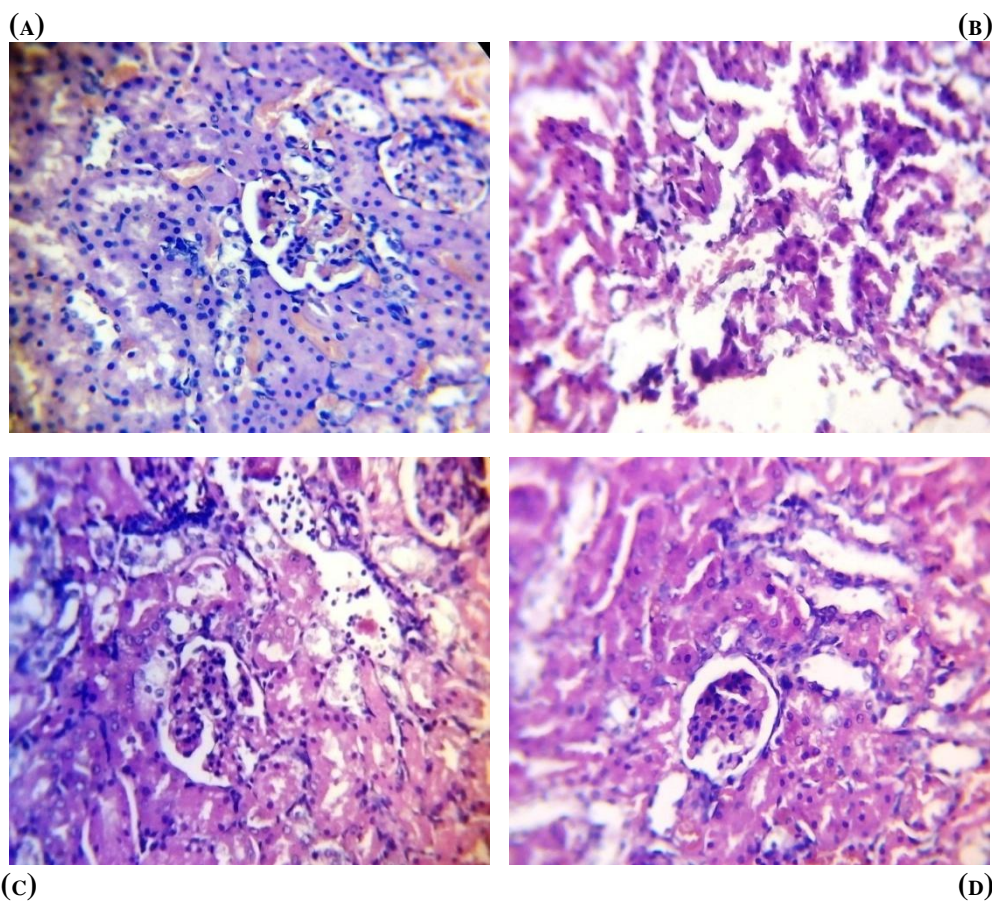


Figure 2(A-D): Histopathology and photomicrograph of kidney from (A) control, (B) Carbimazole-treated (C) vitamin C-treated, and (D) tomato extract-treated rats [Stain: H and E; ×40]

DISCUSSION

Studies have shown that minute changes in kidney function, has important implications in prognosis of diseases and AKI in critically ill patients is exacerbated by metabolic disorders due to loss of homeostatic function. [19] Oxidative stress by drugs has been suggested to be the source of many diseases; that is why this study sought to evaluate the effects of carbimazole-induced biochemical and histological alterations in kidney of albino rats.

Biochemical markers of kidney function: Serum creatinine, Urea and Electrolytes – Sodium Na^+ , Potassium K^+ , Calcium Ca^{++} , and Bicarbonate HCO_3^- levels were assayed for in the various groups and the results show that the levels of K^+ , Creatinine and Urea were significantly elevated in the negative control group (Carbimazole only). The increased Serum Urea and Creatinine observed were due to kidney injury and it is in tandem with the work of Higgins, [20] which states that blood and urinary concentration of both Urea and creatinine increases as kidney function declines.

Serum K^+ , Urea and Creatinine levels were seen to be significantly reduced in the positive control group (carbimazole and vitamin C), when compared with the negative control group (carbimazole only). The observation may be probably due to the antioxidant and anti-inflammatory properties of Vitamin C as stated by Kumar *et al.* [21]

The serum levels of sodium Na^+ was significantly reduced in the negative control group (Carbimazole only); however, with the administration of tomato extract (30mg/kg) and vitamin C (200mg/kg) separately, in the presence of carbimazole challenge, the levels of Na^+ were significantly increased ($p < 0.5$) when compared with the negative control, which is in tandem with the work of Giordina *et al.*, [22] which states that when Kidney function is affected, maintenance of

electrolyte balance is impaired leading to hypo-osmolar states in the body.

The group that received tomato extract (30mg/kg) in the presence of carbimazole challenge significantly showed a reduction in serum K^+ , Urea and Creatinine levels. This shows a protective effect from the intake of tomato extracts on the Kidneys, which agrees with the claim by Omodamiro and Amechi, [23] and Uchendu *et al.* [12] who reported that tomatoes have pharmacological properties which if properly harnessed, can be used in the management of diseases. The protective effects observed maybe as a result of the singular or combined effects of the phytochemicals present in tomatoes.

Few studies have been shown to support that “foods rich in tomatoes and tomato products are associated with reduced risk of disease”. [12,23] Preliminary phytochemical analysis of tomato fruits revealed abundance of: Alkaloids, Flavonoids, Reducing sugars and Carbohydrates amongst others.

An Alkaloid is a cyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms. It has been found to occur in about 25% of higher plants especially in some plant families amongst which is the solanaceae family. [24] In a study conducted in Malaysia, Tiong *et al.*, [25] discovered that alkaloids showed good anti-oxidant properties by alleviating Hydrogen peroxide-induced oxidative damage in β -TC6 cells of mice used in the experiment.

Flavonoids are a distinct group of phytonutrient found in almost all plants. It is a large family of more than 5,000 hydroxylated polyphenolic compounds that carry out important functions in plants, including fighting environmental stress and regulating cell growth. [26] The biological activities and bioavailability of flavonoids in humans are believed to be as result of their chemical nature. Chun *et al.* [27] reported that there has been a growing interest in dietary flavonoids due to their

most likely input to the health benefits of fruits and vegetable rich diets. They have also been found to have the ability to inhibit the oxidation of low-density lipoproteins (LDLs) which demonstrates their potentials as chain-breaking antioxidants. [28]

CONCLUSION

The present study showed that carbimazole induced Acute Kidney Injury (AKI) and that the administration of tomato extract ameliorated the effects in the test group. Thus, this data suggests that the intake of tomato extract could be of health benefit to patients suffering from drug-induced Acute Kidney Injury.

ACKNOWLEDGEMENT

The author expresses deep sense of gratitude to Mr. Chris Ireoba, The head of department of the Laboratory Division at Eastern Nigeria Medical Centre, Enugu, and all the technical staff for their kind cooperation.

REFERENCES

1. Dirkes S M, McCullough P A, Shaw A D, Haase M. et al. Acute kidney injury causes, phases and early detection. *American NurseToday* 2015; 10(7). Accessed on April 4, 2017
2. Uchendu I K, Orji O O, Agu C E. Attenuation of glycerol-induced acute renal failure in albino rats by soy beans (*Glycine max*). *International Journal of ChemTech Research* 2017; 10(12), 165-172
3. Mabrouk I I, Lamiaa A M. Early Prediction Biomarkers of Contrast Induced Acute Kidney Injury-A single Centre experience Egypt ISSN 2320-5407 *International Journal of Advanced Research* 2016; 4(3): 458-466
4. Kim S Y, Moon, A. Drug-Induced Nephrotoxicity and Its Biomarkers. *Biomolecules & Therapeutics* 2012; 20(3), 268-272.
5. Saber A S, Hoda A M, Amany E N. Effect of selenium on Carbimazole induced Histopathological and Histochemical Alterations in Poststate of Albino rats. *American journal of medicine and medical sciences* 2012; 2 (1): 5-11.
6. Oyetayo F L, Ibitoye M F. Phytochemical and Nutrient / Antinutrient Interactions in Cherry Tomato (*Lycopersicon esculentum*) Fruits. *International Journal of Advanced Biological Research* 2012; 2(4): 681-684.
7. Ikenna K U, Okechukwu S O, Chidozie E A, Oliver C O, Blessing E C Tochi F N. Hypolipidaemic and renoprotective effects of glycine max (soy bean) against lipid profile and renal biochemical alterations in hypercholesterolemic rat. *Int J Biomed Res* 2016; 7(12), 822-828.
8. Orji O C, Agu C E, Uchendu I K, Nsonwu A C, Offor J S. Anti-diabetic and renal protective effect of the fruit juice of *Citrus X Paradisi* on alloxan induced diabetic male albino wistar rats. *Der Pharmacia Lettre* 2016; 8 (19): 32-38
9. Kingsley, U I, Steven O O, Agu C E, Orji O C, Chekwube B E, Nwosu T F. Anti-hyperlipidemic effect of crude methanolic extracts of *Glycine max* (soy bean) on high cholesterol diet-fed albino rats. *J Med Allied Sci* 2017; 7 (1): 34-40
10. Ikenna K U, Chidozie E A, Oliver C O, Eluke B C, Ikechukwu J C, Nnedu EB, Tochi F N, Oluwanifemi P A. Effect of Soy (*Glycine max*) Against Alcohol-Induced Biochemical Alteration in Liver of Male Albino Rat. *Der Pharma Chemica* 2017; 9 (16):115-119
11. Anioke I, Okwuosa C, Uchendu I, Chijioke O, Dozie-Nwakile O, Ikegwuonu I, Kalu P, Okafor M. Investigation into Hypoglycemic, Antihyperlipidemic, and Renoprotective Potentials of *Dennettia tripetala* (Pepper Fruit) Seed in a Rat Model of Diabetes. *Hindawi BioMed Research International* 2017; Article ID6923629, 11pages
12. Uchendu I K, Agu C E, Orji O C, Nnedu E B, Arinze C, Uchenna A C, Okongwu U C. Effect of Tomato (*Lycopersicon Esculentum*) Extract on Acetaminophen - Induced Acute Hepatotoxicity in Albino Wistar Rat. *Bioequivalence and Bioavailability International Journal* 2018; 2(1): 000119.
13. Gowda S, Desai P, Kulkarni S, Hull V, Math A, Vernekar S. Markers of Renal function tests, *North American Journal of Medical Science* 2010; 2, 170-173.
14. Trease G E, Evans W C. Pharmacognosy. 11thedn. Brailliar Tridel; 1989
15. American Physiological Society. Guiding principles for research involving animals and human beings *Am. J. Physiol. Regul. Integr. Comp. Physiol* 2002; 283: R281-R83.

16. Natelson S, Scott M L, Beffa C. A rapid method for the estimation of urea in biologic fluids. *Am J Clin Pathol* 1951; 21(3): 275-281.
17. Fabing D L, Ertingshausen G. Automated reaction-rate method for determination of creatinine with the centrifichem. *J. Clin. Chem.* 1971; (17), 696-700.
18. Baker FJ, Silverton R E, Pallister C J. *Baker and Silverton's Introduction to Laboratory Technology*. 7th Editon, Butterworth-Heinemann, Wobrun, MA, USA, ISBN-13: 978075621908, 1998; page 448.
19. Himmelfarb J, Ikizer T A. Acute kidney injury: changinglexicography, definitions and epidemiology. *2001 International Journal of Nephrology* 2007; 71, 971-976.
20. Higgins, C. "Urea and creatinine concentration, the urea: creatinine ratio." www.acutecaretesting.org 2016; Accessed on July 7, 2017.
21. Kumar S, Bhowmik K P, Shravan P, Shweta S. Tomato: A Natural Medicine and Its Health Benefits, *Journal of Pharmacognosy and Phytochemistry* 2012; ISSN 2278- 4136
22. Giorgina B P, Marco C, Lorenzo O, Maria C, Deagostini V C, Giulio R. Acute renal failure, severe sodium and potassium imbalance and sudden tetraplegia, *NDT Plus* 2010; 3(3): 247–252
23. Omodamiro O D, Amechi U. The phytochemical content, antioxidant, antimicrobial and anti-inflammatory activities of *Lycopersicon esculentum* (Tomato). *Asian Journal of Plant Science and Research* 2013; 3(5):70-81.
24. Tadeusz A. *Alkaloids- Secrets of Life: Alkaloid Chemisty, Biological Significance, Applications and Ecological Role*, Elsevier publications Netherlands, 2007; ISBN: 13. 978-0-444-52736-3
25. Tiong, S.H; Looi, C.Y; Hazni, H.; Anya, A; Paydar, M; Wong, WF; Cheah S.C; Mustafa, M.R; Awang, K. Antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus* (L.) G. Don. *Molecules* 2013; 18(8): 9770-9784
26. Kumar S, Pandey A K. Chemistry and biological activities of flavonoids: an overview. *Scientific World Journal* 2013; 2013:162750.
27. Chun O K, Kim D O, Lee, C Y. Superoxide radical scavenging activity of the major polyphenols in fresh plums, *J Agric Food Chem* 2003; 51(27): 8067-8072.
28. Balasundram N, Kalyana S, Samir S. "Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses." *Food chemistry* 2006; 99(1): 191-203.

How to cite this article: Kingsley UI. Effects of tomato extract (*lycopersicon esculentum*) on carbimazole-induced alterations in kidney of albino rats. *International Journal of Research and Review*. 2018; 5(1):72-79.
