EUROPEAN ACADEMIC RESEARCH
Vol. IV, Issue 8/ November 2016

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Changes in Creatinine, Urea, Glutathione-S-Transferase and Uric Acid Levels in Acetaminophen Extra Overdosed Rabbits Treated with Watermelon Juice (Citrullus lanatus)

MATHEW FOLARANMI OLANIYAN[1]
Department of Medical Laboratory Science
Achievers University, Owo-Nigeria
BOSEDE OLUWATOSIN ODEJOBI
Department of Medical Laboratory Science
Achievers University, Owo-Nigeria
OKE S.A.
Ladoke Akintola University Teaching Hospital
Ogbomoso, Nigeria

Abstract:

Background: *Watermelon juice that contains antioxidants in addition to other vitamins and minerals is being used as beverage to alleviate thirst in Nigeria. Acetaminophen extra is most available to people and could be irrationally used to relieve pain.* ***Aim and Objective:*** *This work was designed to evaluate the effect of watermelon juice in rabbits overdosed with acetaminophen extra using Creatinine, Urea, Glutathione-S-transferase, and Uric acid.*

Materials and Methods: *Thirty rabbits classified into six experimental groups labelled A-F (and sub groups as C1, C2, E1 and E2) with A as control were investigated. An overdose of acetaminophen extra of 1500mg/kg was administered into some of the rabbits for 5 days to induce toxicity. Fifteen millilitre of watermelon juice was used to protect and treat acetaminophen extra toxicity. Plasma Creatinine*

[1] Principal and corresponding author: olaniyanmat@yahoo.com

Urea, Glutathione-S-transferase, and Uric acid were analysed biochemically by spectrophotometry.

Result: *The result showed a significantly increase plasma values of urea in rabbits simultaneously administered with 15ml/kg of watermelon juice and 1500mg/kg of acetaminophen extra than the normal control (<0.05). There was a significant increase in plasma value of GST in rabbits given watermelon juice only without acetaminophen extra and a significantly decrease difference in rabbits given 1500mg/kg of acetaminophen extra without watermelon juice and rabbits given both 15ml/kg of watermelon juice and 1500mg/kg of acetaminophen extra simultaneously than the normal control rabbits fed with normal meal and water (p<0.05). There was a significantly decrease urea level and a significantly increase plasma uric acid level in rabbits given 1500mg/kg of acetaminophen extra without watermelon juice than the normal control rabbits (p<0.05).*

Conclusion: *This study revealed a significant decrease and increase in plasma level of urea and uric acid respectively in the rabbits after the administration of 1500mg/kg acetaminophen extra. There was also a significant decrease in plasma Glutathione-S-transferase, in experimental rabbits following the administration of 1500mg/kg of acetaminophen extra for 5 days and also after the administration of 15ml/kg of watermelon juice compared with normal control rabbits. Further study should be carried out on the effect of watermelon juice on drug toxicity using another chemical substance.*

Key words: Creatinine, Urea, Glutathione-S-Transferase, Uric Acid, Acetaminophen, Overdosed, Rabbits, Watermelon Juice.

INTRODUCTION

Citrullus lanatus (water melon) is made up of about 93% water, hence the name "water" melon [(1)].Naturally, it is a rich source of lycopene(in the red/pink succulent part), a carotenoid that has a great antioxidant capacity and potential health benefits [(2)]. It also contains amino-acid citrulline, cucurbitacin,

triterpenes, sterols and alkaloids [3] [4] [5]. Every part of watermelon fruit is highly nutritive including the back and the seeds [6].

The fruit has some non-scientific traditional health benefits applied to treat some ailments in Nigeria, Ayurveda and Indian traditional system of medicine. In addition it contains phytochemicals like flavonoids, alkaloids, saponins, glycoside, tannins and phenols [7] [8]. The root can be used as purgative and emetic [8] while the seed is demulcent, pectoral and tonic [9]. It is sometimes been used to treat urinary tract infections [8] as well as bed wetting . The fruit is also diuretic [8] and very effective in the treatment of dropsy and renal stones [10]. It has antihypertensive effects and the fatty oil in the seed, as well as aqueous or alcoholic extracts, had been reported to paralyze tapeworms and roundworms [11][12]. The rind of the fruit has been recommended for alcohol poisoning and diabetes[9]. *Citrullus lanatus* in Northern Sudan is used for burns, swellings, rheumatism, gout and as laxative [13] [14]. Lycopene is characterized by its distinctive red color in fruits and vegetable [15]. The fruit is used as a purgative in Senegal; also used to treat diarrhoea and gonorrhoea in Nigeria [16] [17]. Plant medicine (phytomedicine) has been used in healthcare delivery in many parts of Africa and the rest of the world [17] [18]. Fruits and vegetables are natural sources of various bioactive compounds [19] which could be attributed to their phytochemical constituents especially flavonoids, anthocyanins, vitamins C and E, phenolic compounds, dietary fiber, and carotenoids present in fruits and vegetables [20]. An example of these medicinal plants is Citrullus lanatus. Although several of its uses in traditional medicine have been documented, many of these claims are yet to be validated by scientific researchers. Consequently, This work was designed to evaluate the effect of watermelon juice in rabbits overdosed with acetaminophen

extra using Creatinine, Urea, Glutathione-S-transferase, and Uric acid.

MATERIALS AND METHODS

Study area

This study was carried out at Achievers University animal house, Owo Local Government area of Ondo state in Nigeria.

Study population

Thirty rabbits were purchased in Owo through the Department of Biological Sciences, Achievers University, Owo – Nigeria and were divided into six groups.

Experimental Groups

Group A: 5 rabbits were fed with normal meal and water for 7 days (normal control)

Group B: Consist of 5 rabbits fed with normal meal and 15ml/Kg body weight of watermelon juice daily for 7 days

Group C: 5 rabbits were given 1500mg/kg of acetaminophen extra per oral for 5 days (C1) and thereafter were fed with normal meal and 15ml/Kg of watermelon juice daily for 7 days after 5 days of post acetaminophen extra administration(C2)

Group D: 5 rabbits given 1500mg/kg of acetaminophen extra per oral and fed with normal meal and water for 7 days

Group E: 5 rabbits fed with normal meal and 15ml/Kg of watermelon juice daily for 7 days after which each rabbit were given 1500mg/kg of acetaminophen extra per oral for 5 days with normal meal and water.

Group F: 5 rabbits given 1500mg/kg of acetaminophen extra per oral and were simultaneously fed with normal meal and 15ml/Kg of watermelon juice daily for 5 days .

Biological sample

Blood samples were collected from the vein lining the ears of each of the rabbits as control and from the experimental rabbits before and after the administration of 1,500mg/Kg BW and 15ml/Kg BW.

Preparation of watermelon juice

Water melon was purchased from Owo markets and was presented to the Department of biological sciences, Achievers University, Owo for confirmation and certification. The succulent red part was removed and kept in a sterile bowl. The seeds was aseptically removed. The remaining succulent red part of the watermelon was blended using electric blender and thereafter was filtered undiluted. Fifteen milliliter of the filtrate was administered to the rabbits as watermelon juice.

Preparation of acetaminophen extra powder

Acetaminophen extra tablets of GlaxoSmithKline was purchased and grinded into powder using Laboratory pestle and mortal.

Blood sample preparation

Whole blood samples collected from each of the rabbits was collected into Lithium heparinized tubes. The blood samples were spun using bench/macro centrifuge for the extraction of the plasma.

ESTIMATION OF UREA, URIC ACID, CREATININE AND GST

The UREA,URIC ACID, CREATININE were measured by using reagent kits purchased from Randox Laboratories Limited, Antrium (United Kingdom) according to the method described by Fawcett and Scott,(1960) and GST assay was

carried out using reagent from Sigma Aldrich (America) with biosino biochemical (China) according to the manufacturer's instruction.

ANALYTICAL METHODS

CREATININE

Method: Jaffe-slot alkaline picrate creatinine method

Principle: Creatinine reacts with picric acid in an alkaline medium. The absorbance of yellow-red colour produced is measured in a colorimeter using a blue-green filter 490nm (llford No.603) or in a spectrophotometer at 490nm wavelength[21].

UREA

Method: Berthelot reaction

Principle: Urea is split into ammonia and carbondioxide by the action of urease, an enzyme obtained from Jack Bean meal. The ammonia then reacts with alkaline hypochlorite and phenol in the presence of a catalyst-sodium nitroprusside. The resulting product is indophenol (a blue color) and the concentration of ammonia is directly proportional to the absorbance of indophenol [22].

$$\text{Urea} + H_20 \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{hypochlorite} + \text{phenol} \longrightarrow \text{indophenol (blue compound)}$$

GLUTATHIONE –S TRANSFERASE

Method: Enzymatic reaction

Principle: The principle is based on the fact that known glutathione-S-transferase demonstrate a relatively high activity with 1-chloro-2, 4,-dinitrobenzene as the second substrate, consequently, the conventional assay for glutathione-S-transferase activity utilizes 1-chloro2,4,- dinitrobenzene as

substrate. The absorption increase at the new wavelength of 340nm provides a direct measurement of enzymatic reaction [23].

URIC ACID

Method: Colorimetric method

Principle: Uric acid is converted by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase, oxidizes 3,5-Dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red-violet quinoneimine compound [24].

$$\text{Uric acid} + O_2 + 2H_2O \xrightarrow{\text{uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{3,5-dichloro-2-hydroxybenzenesulfonic acid} + \text{4-aminophenazone} \xrightarrow{\text{Peroxidase}} \text{N-(4-antipyryl) -3- chloro-5-sulfonate-p-benzo-quinoneimine}$$

STATISTICAL ANALYSIS OF DATA

A statistical package for social sciences (SPSS) 20.0 was used for the analysis of the data appropriately. Continuous variables were displayed as means and standard deviation (SD) and categorical variables were displayed as percentage. The level of significance was taken at 95% confidence interval and $P \leq 0.05$ was considered significant.

Results

The results obtained showed no significant difference in the plasma value of creatinine in normal control rabbits compared with: rabbits given 15ml/Kg of watermelon juice without acetaminophen extra; rabbits given 1500mg/Kg of acetaminophen extra for five days without water melon juice; rabbits simultaneously administered with 15ml/Kg of watermelon juice and 1500mg/Kg of acetaminophen extra for 5 days ($p>0.05$). No significant increase was obtained in the plasma value of creatinine in rabbits given 1500mg/Kg

acetaminophen extra for 5 days before and after they were given 15ml/Kg of watermelon juice for seven days before and after the inducement of toxicity by giving 1500mg/Kg of acetaminophen extra for 5 days ($p>0.05$). There was no significant increase difference in the plasma value of urea and no significant increase difference in the plasma uric acid in normal control rabbits compared with rabbits given 15ml/Kg of watermelon juice without acetaminophen extra ($p>0.05$). No significant decrease difference was obtained in the plasma value of urea and no significant increase difference in plasma uric acid in rabbits given 1500mg/Kg acetaminophen extra before and after they were given 15ml/Kg of water melon juice and also in rabbits given 15ml/Kg of watermelon juice for seven days before and after the inducement of toxicity by giving 1500mg/Kg of acetaminophen extra for 5 days ($p>0.05$).There was a significantly higher difference in plasma value of urea in rabbits simultaneously administered with 15ml/Kg of watermelon juice and 1500mg/Kg of acetaminophen extra for 5 days than the normal control ($p<0.05$). There was a significant increase in plasma value of GST in rabbits given watermelon juice only without acetaminophen extra and a significantly decrease level in rabbits given 1500mg/Kg of acetaminophen extra without watermelon juice and rabbits given both 15ml/Kg of watermelon juice and 1500mg/Kg of acetaminophen extra simultaneously than the normal control rabbits fed with normal meal and water ($p<0.05$). There was a significantly lower urea level and a significantly higher plasma uric acid level in rabbits given 1500mg/Kg of acetaminophen extra without watermelon juice than the normal control rabbits($p<0.05$).

Table 1: The mean and standard deviation of CREATININE, UREA, GST and GST obtained in the rabbits

	GROUP A	GROUP B	GROUP C1	GROUP C2	GROUP D	GROUP E1	GROUP E2	GROUPF
Cr	0.3± 0.1	0.1± 0.1	0.2± 0.1	0.2± 0.1	0.2± 0.1	0.1± 0.1	0.2± 0.1	0.3± 0.1
Urea	5.8±0.9	4.8±1.0	4.3±1.9	3.2±0.7	4.1±0.9	4.8±1.0	4.2±0.9	7.7±1.6
GST	9.8±5.2	14.1±1.9	5.5±2.5	5.9±1.7	1.8±0.5	14.1±1.9	3.7±0.6	4.8±1.2
UA	3.6±1.8	5.0±2.9	5.3±1.3	6.0±1.3	8.7±4.1	5.3±1.3	5.4±2.8	3.6±1.8

Table 2: The comparative analysis of mean and standard deviation of CREATININE, UREA, GST and URIC ACID obtained in the rabbits

		A Vs B	A Vs D	C1 Vs C2	E1 Vs E2	A Vs F
Creatinine (mg/dl)	**"t"**	2.12	2.12	0.45	0.0	0.0
	"P"	0.10	0.10	0.35	0.5	0.5
	Comment	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
Urea (Mmol/L)	**"t"**	1.83	3.20	1.23	0.84	-2.64
	"P"	0. 10	0.01	0.14	0.21	0.06
	Comment	p>0.05	P<0.05*	p>0.05	p>0.05	P<0.05*
GST(Mmol/dl)	**"t"**	-4.88	6.02	-0.10	10.90	2.13
	"P"	0.00	0.00	0.47	0.00	0.04
	Comment	P<0.05*	P<0.05*	p>0.05	P<0.05*	P<0.05*
Uric acid (mg/dl)	**"t"**	-0.52	-2.01	0.10	-0.20	0.00
	"P"	0.31	0.05	0.50	0.43	0.50
	Comment	p>0.05	P<0.05*	p>0.05	p>0.05	p>0.05

Figure 1: Showing creatinine mean result

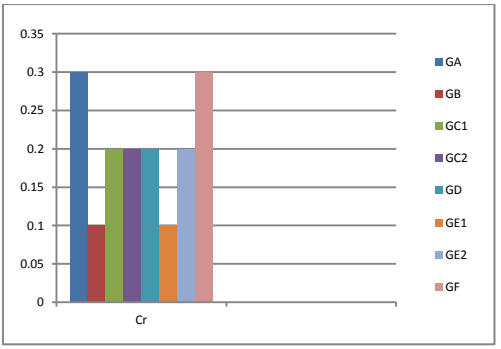


Figure 2: Showing the mean result of urea and uric acid

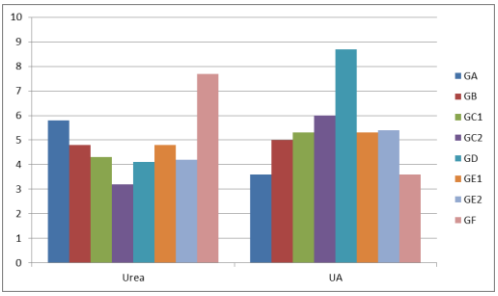
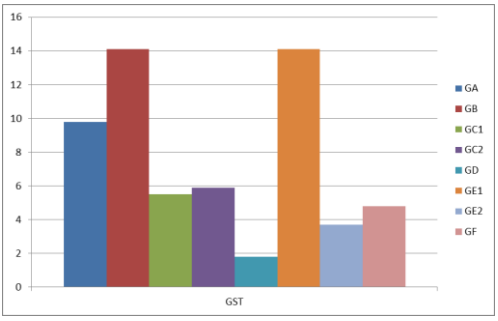


Figure 3: Showing the mean result of GST



DISCUSSION

he result showed a significantly increase plasma value of glutathione S-transferases in rabbits given watermelon juice only without acetaminophen extra; decrease GST in rabbits given 1500mg/Kg of acetaminophen extra without watermelon juice and rabbits given both 15ml/Kg of watermelon juice and 1500mg/Kg of acetaminophen extra simultaneously than the normal control rabbits fed with normal meal and water this could be as a result of over utilization of GST in detoxifying possible toxicity caused by the administration of 1500mg/Kg of acetaminophen extra because glutathione S-transferases are enzymes with many functions including a key role in cellular detoxification [25]. It protect cells against toxicants by conjugating them to glutathione, thereby neutralizing their

electrophilic sites, and renders the products more water-soluble [25]. The glutathione conjugates are metabolized further to mercapturic acid and then excreted. GSTs are implicated in a variety of diseases by virtue of their involvement with GSH [25]. There was a significantly decrease urea level and a significantly increase plasma uric acid level in rabbits given 1500mg/Kg of acetaminophen extra without watermelon juice than the normal control rabbits.

In this work decrease plasma urea level obtained in the rabbits after the admi nistration of 1500mg/Kg acetaminophen extra than in the control rabbits is consistent with the report of Waring *et al.*[26] that low serum urea concentration is not an independent risk factor for hepatotoxicity after paracetamol overdose. This could also be attributed to the fact that glutathione depletion increases the incidence of toxicity after paracetamol overdose. Risk factors for toxicity, including chronic ethanol excess and malnutrition, are associated with low serum urea concentrations. Therefore, they hypothesized that low serum urea concentration might itself be predictive of hepatotoxicity in patients that present to hospital after paracetamol overdose [26].

Raised uric acid found in the rabbits in this study after the administration of 1500mg/Kg of acetaminophen extra than in normal control rabbits agrees with the report of Wilding amd Heath [27] who reported on the effect of paracetamol on uric acid determination that apparently raised values of serum uric acid may be reported in patients receiving therapeutic levels of paracetamol and levels of serum uric acid were determined over a five-hour period in three patients who had received therapeutic doses of paracetamol.

CONCLUSION

This study revealed a significant decrease in plasma level of urea and a significant increase in plasma uric acid in the rabbits after the administration of 1500mg/Kg acetaminophen extra. There was also a significant decrease in plasma GST in experimental rabbits following the administration of 1500mg/Kg of acetaminophen extra and a significant increase after the administration of 15ml/Kg of watermelon juice compared with normal control rabbits.

RECOMMENDATION

Further study should be carried out on the effect of watermelon juice on drug toxicity using another chemical substance.

REFERENCES

1. Baker, T.P., Corwsin, B. and Jett, L.W. (2002).Watermelon Bacterial Fruit Blotch. University of Missouri Extension. Accessed 18
2. Rhodes, B. and Zhang, X.P. (1999). Hybrid seed production in watermelon. Hybrid seed production in vegetables: rationale and methods in selected crops. Food Products Press, New York. Page 69
3. Yuan, G., Wahlqvist, M.L., He, G., Yang, M. Li, D. (2006). Natural products and anti-inflammatory activity. *Asia Pac. J. Clin. Nutr*; **15**: 143
4. Wada, M. (1930). Über Citrullin, eine neue Aminosäure im Presssaft der Wassermelone, Citrullus vulgaris Schrad. *Biochem* Zeit;**224***:* 420
5. Mandel, H., Levy, N., Izkovitch, S. and Korman, S.H. (2005).Elevated plasma citrulline and arginine due to

consumption of Citrullus vulgaris (watermelon). *Berichte der deutschen chemischen Gesellschaft* ;**28 (4):** 467–472

6. Maynard D. and Maynard N. (2012). Cucumbers, melons and watermelons. The Cambridge World History of Food, Part 2. Cambridge University Press.
7. Mabberley, D.J. (1987). Mabberley's Plant-Book: a portable dictionary of plants, their classification and uses. Cambridge University Press.
8. Grieve, M. and Leyel, C.F.(1984). A modern herbal: Penguin Harmondsworth.
9. Duke, J. A. & Ayensu, E. S., 1985. Medicinal Plants of China. Strichzeichnungen. Reference Publ., Inc. Algonac. Michigan,. Vols **2**:20-4 ISBN 0-917266-.
10. Chiej, R. (1984). Encyclopaedia of medicinal plants. *MacDonald* ISBN 0-356-10541-5
11. USA (2012). Watermelon extract supplementation reduces an. [Am J Hypertens. NCBI". Ncbi.nlm.nih.gov. Retrieved 2012-08-13
12. Chopra, R.N. (1958). Indigenous drugs of India: *Academic Publishers*;10541-105415
13. Schippers, R.R. and Budd, L. (1997). African indigenous vegetables. Rome: IPGRI, and England: Nat. Res. Institute.
14. Fenko, A., Schifferstein, H.N., Huang, T.C. and Hekkert, P.(2009). What makes productsfresh: The smell or the colour?*Food Qual Prefer*; **20**:372–379
15. Mutanen, M. and Pajari, A.M. (2011). Diet and cancer. . Dordrecht Springer;Vegetables, whole grains, and their derivatives in cancer prevention. Vol. 2.
16. Pinner, R.S., Teutsch, L., Simonsen, L., Klug, J., Graber, M. and Berkelman, R. (1996). Trends in infectious diseases mortality in the United States. *Journal of American Medical Association*; 275 Page 189-193

17. Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. 2nd Edition. Spectrum Books Limited Ibadan, Nigeria, Page 289
18. Elujoba, A.A., Odeleye, O.M. and Ogunyemi, C.M.(2005).Traditional Medical Development for medical and dental y system in Africa. *African Journal Traditional, Complementary and Alternative Medicine*;**2(1)**: 46-61
19. Pennington, J.A.T. and Fisher, R.A (2010). Food component profiles for fruit and vegetable subgroups. *Journal of Food Composition and Analysis*; vol. 23, Page 411
20. Gonzalez-Aguilar, G., Robles-Sanchez, R.M., Martınez-T´ellez, M.A., Olivas, G.I.Alvarez-Parrilla, E. and De La Rosa, L.A.(2008). Bioactive compounds in fruits: health benefits and effect of storage conditions, Stewart Postharvest Review, vol. 4, no. 3, article 8, Page 1–10
21. Cheesbrough M. (2006).District Laboratory Practice in tropical countries, Part 1. Second Edition. Cambridge University Press, South Africa. Page 350
22. Olaniyan, M.F. (2015). Basic Analytical Methods in Clinical biochemistry. Published by IISTE, ISBN-13:978-1519140975, United States. Page 77
23. Habig,W.H., Pabst, W.B. and Jakoby, J. (1974). Glutathione-S- transferases. The first enzymatic step in mecapturic acid formation. *Journal of Biological Chemistry*;11-25
24. Fossati, P., Prencipe, L. and Berti, G. (1980) **Uric Acid Estimation**. *Clinical chemistry*;**26(2)**:227-231
25. Sheehan D., Meade G., Foley V.M. and Dowd CA 2001. Structure, funetion and evolution of glutathione transferases: implications for elassification of non-mammalian members of an ancient enzyme superfamily. *Biochem. J.* **360**:1-6

26. Waring W.S., Stephen A.F., Robinson O.D., Dow M.A., and Pettie J.M. Serum urea concentration and the risk of hepatotoxicity after paracetamol overdose Q J Med 2008; 101:359–363
27. Wilding, P., Heath, R. 1975. Annals of Clinical Biochemistry 12:142. Cited by Wooton and Freemon (1982), pp. 79.