PharmacologyOnLine
Archives • 2021 • vol.2 • 707-728

EVALUATION OF EFFECT OF THERAPEUTIC DOSES OF ACETAMINOPHEN AND AMELIORATIVE ROLES OF ANTIOXIDANT VITAMINS IN WISTAR ALBINO RATS

[1]*Omeoga, Chukwudi Humphrey; [1]Egba Simeon Ikechukwu and [1]Uhuo Emmanuel Nnaemeka
[1]Department of Biochemistry, Michael Okpara University of Agriculture, Umudike Abia State

*omeoga.humphrey@gmail.com

Abstract

To evaluate the effect of therapeutic dose of acetaminophen and ameliorative effects of some antioxidant vitamins in Wistar albino rats. The animals were grouped to receive different doses of acetaminophen only, acetaminophen + vitamins C or E, and normal control. Administrations were done for weeks 1, 3, 5 and 7 while only feed and water administered on weeks 2, 4, and 6. Laboratory analysis were done using standard methods. In 7th week, groups administered only paracetamol, had significant increase in activities of AST, ALT, and ALP, as well as urea concentration, while at weeks 5 and 7, a significant increase was observed in MDA concentration. Furthermore, activities of SOD, CAT, GPx and GSH indicated significant increase in the groups treated with the antioxidant vitamins. Haematological parameters of the untreated animals showed significant decrease at the 5th and 7th week, while a significant increase was observed in the treated groups, and histopathology seemingly corroborated the serum chemistry. Therapeutic dose of acetaminophen consumed chronically could have deleterious effect on proper body functioning, and co-administration of antioxidant vitamins could ameliorate these effects.

Keywords: Acetaminophen, antioxidant, vitamin C, vitamin E

Introduction

Paracetamol, a common analgesic and anti-pyretic drug, considered safe at therapeutic doses, but possesses the toxicity potential when consumed in overdose usually to the liver and nephron.[1] It is a potent inducer of the cytochrome-P450 class of enzyme, whose effect leads to generation of very reactive metabolite (N-acetyl-p-benzoquinone imine, NAPQI) which has the toxic effect by its high reactivity with the sulfhydryl group of proteins, leading to oxidative damage of cellular proteins.[2] Paracetamol toxicity also causes depletion of both mitochondrial and cytosolic pools of reduced glutathione.[3,4] Kidney is the second target organ of paracetamol toxicity, even though nephrotoxicity may exist in the absence of hepatotoxicity following paracetamol overdose[5] and this has been widely ignored in studies aimed at treatment of paracetamol toxicity.[6]

Vitamin C, discovered by Szent-Gyorgyi[7] consists of dual inter-convertible compounds known as the L-ascorbic acid and L dehydroascorbic acid, and readily available in fruits such as oranges, lemon, grapefruits, and other citrus, as well as and most green vegetables while animal sources include the liver and kidney.[8] The administration of this vitamin can be through the mouth or intravenously[9] and well absorbed efficiently in the small bowel *via* a saturable active transport mechanism making it to be sufficiently delivered in virtually every tissue present in the human body, with significant concentrations present in the adrenal glands, pituitary and retina. Vitamin C is very soluble in water and is considered a very significant antioxidant in bodily fluids, extinguishing reactive oxygen species especially peroxide from damaging the biological membranes. Ascorbic acid is highly effective in scavenging oxygen radicals ($½O_2$, O_3^-, OH^-), and hypochlorous acid.[10] Research have shown that its effectiveness in free radical scavenging is due to it being an important distributor of electrons which is donated to quench the activities of these free radicals.[11]

Vitamin E refers to a group of fat-soluble compounds with notable antioxidant properties needed for essentially good health.[12] They are usually found in lipid-containing foods,[13] making it a compound that can be accumulated within the adipocytes. As an effective chain-breaking antioxidant, it serves to inhibit the generation of free radicals when lipids are oxidized or in the process of reactions involving free radicals.[14] Research have indicated that a combination of tocopherols possesses greater anti-lipid peroxidation effects induced in human erythrocytes compared to lone alpha-tocopherol (another form of vitamin E) alone.[15]

Acetaminophen is used frequently as an over-the-counter analgesic and antipyretic for minor aches and pains. When consumed in overdose, it possesses variety of consequences on the hepatocytes and renal functions. Thus, this research aims to study the chronic dose-dependent toxicity of acetaminophen and potential restoring effects of ascorbic acid and tocopherol.

Methods

Animal handling

Fifty-six (56) male Wistar aged 10-12 weeks and weighing 95 – 105 g procured from the Veterinary Department, University of Nigeria, Nsukka Enugu State were used for this study. The animals were transported in standard animal cages to the Department of Biochemistry animal house, Michael Okpara University of Agriculture, Umudike. They were acclimatized for a period of 7 days and fed Vital Growers Mash and water *ad libitum.*

Experimental design

The animals were randomly grouped into seven groups with eight animals in each group thus:

Group 1: Feed + water *ad libitum* only
Group 2: Paracetamol only (1500 mg/70kg body weight)
Group 3: Paracetamol only (1000 mg/70kg body weight)
Group 4: Paracetamol (1500 mg/70kg body weight) + vitamin C (100 mg/kg body weight)
Group 5: Paracetamol (1000 mg/70kg body weight) + vitamin C (100 mg/kg body weight)
Group 6: Paracetamol (1500 mg/70kg body weight) + vitamin E (1000 iμ/70kg body weight)
Group 7: Paracetamol (1000 mg/70kg body weight) + vitamin E (1000 iμ/70kg body weight)

The administration was done for weeks 1, 3, 5 and 7; twice daily, while vitamin E was administered once daily using gavage. Nothing, but water and feed were administered on the 2nd, 4th and 6th week. At the end of the experiment, the animals were anesthetized and sacrificed at the end of each administrative week. Blood samples were collected using 2 ml syringes and stored in EDTA, as well as non-EDTA sample bottles and used for laboratory analysis while the organs (kidney and liver) were harvest histopathological studies.

Tissue preparation for analyses

The blood samples for biochemical analysis were allowed to clot after which they were centrifuged and the sera collected for the analysis while the harvested organs were carefully dissected, made to be blood-free and fixed in 10% formalin.

Biochemical analyses

The concentrations of some renal function parameters (urea and total protein), activities of liver marker enzymes (aspartate amino transferase, alanine amino transferase and alkaline phosphatase), endogenous antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase and glutathione), lipid peroxidation marker (malondialdehyde) and hematological parameters were determined using standard methods of analysis. Analytical grade reagent kits were obtained from Biosystems Laboratories (S. A. Costa Brava, Barcelona, Spain) and Randox Laboratories (United Kingdom) were utilized.

Results

Results were statistically analyzed using one-way ANOVA (SPSS vs 22.0) and presented as mean ± S.E.M. Significance was accepted at $p<0.05$

Effect of acetaminophen on AST activity in Wistar rats

From the result in fig. 1, the enzyme activity in weeks 1 and 3 shows a non-significant ($p>0.05$) increase in the groups treated only with acetaminophen when compared against the control group. Also, the group administered 1500 mg/70 kg (55.02 ± 3.00) and 1000 mg/70 kg (54.67 ± 3.33) acetaminophen only had a significant ($p<0.05$) increase when compared with other groups in weeks 5 and 7 while the groups co-administered the various antioxidant vitamins and acetaminophen showed no significant ($p>0.05$) increase when compared with the control group in weeks 5 and 7, respectively.

Effect of acetaminophen on ALT activity in Wistar rats

In fig. 2, there was a significant ($p<0.05$) increase in the ALT activity when the group administered 1500 mg/70 kg acetaminophen (89.50 ± 1.83) is compared with the control (81.01 ± 4.50) in weeks 5 and 7 and non-significantly ($p>0.05$) increased when compared with the other groups. In week 7, there was a significant ($p<0.05$) increase when compared with the control group and other groups while the group administered 1000 mg/70 kg acetaminophen (90.20 ±1.12) showed a non-significant ($p>0.05$) increase when compared with the other treated

groups and significantly ($p<0.05$) increased when compared with the control group.

Effect of acetaminophen on ALP activity in Wistar rats

Fig. 3 shows that the result of week 5 shows that the enzyme activity was significantly ($p<0.05$) increased when the groups administered 1500 mg/70 kg (3.51 ± 0.04) and 1000 mg/70 kg (3.31 ± 0.22) acetaminophen are compared with the control (2.98 ± 0.14). In week 7, there was marked significant ($p<0.05$) increase when the acetaminophen-only treated groups were compared with the control and other groups.

Effect of acetaminophen on serum urea concentration in Wistar rats

The result of fig. 4 shows a significant ($p<0.05$) week-dependent increase when the group administered 1500 mg/70 kg acetaminophen is compared with the control group (37.64 ± 2.63). Also, the group administered 1000 mg/70 kg acetaminophen showed a marked significant ($p<0.05$) increase in the 7th week when compared with the control and other groups.

Effect of acetaminophen on serum total protein concentration in Wistar rats

The result for total protein (fig. 5) shows a significant ($p<0.05$) decrease in the group administered acetaminophen-only at doses of 1500 mg/70 kg and 1000 mg/70 kg at weeks 5 and 7 while the groups with acetaminophen and co-administration of antioxidant vitamins shows a significant ($p<0.05$) increase when compared with the acetaminophen-only and the control groups at week 7.

Effect of acetaminophen on serum malondialdehyde concentration in Wistar rats

Fig. 6 shows that MDA was non-significantly ($p>0.05$) increased in the acetaminophen-only groups at doses of 1500 mg/70 kg and 1000 mg/70 kg when compared with the control and other groups at weeks 1, 3 and 5 while there was a significant ($p<0.05$) increase at week 7.

Effect of acetaminophen on serum superoxide dismutase activity in Wistar rats

In fig. 7, it was observed that results of week 1, 3, 5 and 7 show a significant ($p<0.05$) decrease in superoxide dismutase activity of the groups administered only acetaminophen at doses of 1500 and 1000 mg/70 kg when compared with the control and other groups while the groups administered acetaminophen with co-administration of antioxidant vitamins showed no significant ($p>0.05$) increase when compared with the control group.

Effect of acetaminophen on serum catalase activity in Wistar rats

The result of fig. 8 above shows a non-significant ($p>0.05$) increase for all the duration of the study when the acetaminophen-only treated groups were compared with the control group.

Effect of acetaminophen on serum glutathione peroxidase activity in Wistar rats

From the chart (fig. 9), there is a non-significant ($p>0.05$) decrease in glutathione peroxidise activity in the group administered only acetaminophen from weeks 1 through the 3rd week. In the 7th week, there was a significant decrease in groups administered only acetaminophen when compared with the control group and other treated groups.

Effect of acetaminophen on serum glutathione activity in Wistar rats

From fig.10, there was a non-significant decrease in the groups administered only acetaminophen when compared with the control group and the other treated groups in the first week. From weeks 3 through 7, there was a significant ($p<0.05$) decrease when the acetaminophen-only groups are compared with the control and other treated groups.

Discussion

In this research, the effect of chronic therapeutic dose of acetaminophen and ameliorative potentials of vitamins C and E on Wistar albino rats were evaluated. Acetaminophen, a widely used analgesic and antipyretic has been regarded as the promoter of liver failure[16] making its ease of access a cause for concern with respect to the

negative effects. Although liver toxicity caused by paracetamol is dose-dependent usually occurring with an overdose, it may likely occur at therapeutic doses when taken under some predisposing health conditions such as in frequent consumption of alcohol use or in the setting of an underlying liver disease[17].

Enzymes of the cytoplasm such as ALP, ALT and AST are utilized in assessing hepatic disorders and as such, an alteration in their activities reflects the liver's integrity and an increased value is a indication of inflammation in the hepatocytes.[18] From this study, it was observed that there is a significant ($p<0.05$) increase in these cytoplasmic liver enzymes in the 5th and 7th weeks when the group treated with paracetamol only at therapeutic and modified therapeutic doses are compared with the control and antioxidant-coadministered groups. A study by Watkins et al.[19] in a study involving randomized placebo-controlled trial of 94 patients with no prior history of liver dysfunction reported that some patients had increased ALT levels of about 3 times the normal value. Considering their cytoplasmic nature, an injury to the liver causes a distortion of the membrane integrity and leakage of these enzymes into the circulatory system. Acetaminophen is considered safe at therapeutic doses[20] and to some people, a little above is seen as an ideal dose. Paracetamol metabolism normally undergoes glucuronidation and sulfation to the corresponding conjugates but when consumed in excess, there is usually an overwhelming metabolic condition whereby the glucuronidation and sulfation pathways are saturated and the cytochrome P450 pathway becomes heavily important. When this condition persists over time, it results to decreased hepatic glutathione which in most cases is not regenerated soon enough; this leads to accumulation of NAPQI, a very reactive and toxic metabolite. In the absence of maximum concentration of intracellular antioxidants such as glutathione, NAPQI reacts with the nucleophilic groups present on cellular macromolecules such as proteins or lipids to generate toxic free radicals leading to hepatotoxicity.[21]

Additionally, the activities of ALT, AST and ALP indicated a significant ($p<0.05$) decrease in the Wistar rats administered paracetamol with concomitant Vitamin C and Vitamin E when compared against those administered only paracetamol. This is an indicator that as a free radical suppressor, vitamin C possibly restricted reaction chain involved in generation of free radicals through chemical agents or mopped up reactive species before they got to their target hepatic cells.

Research on animal[22] and human[23] studies have depicted ascorbic acid as an effective free radical scavenger, mediating its effect by the termination of free reactive oxygen species (ROS). Therefore, it could be suggested from this study that the protective effects of ascorbic acid is likely as a result of its toxicity ameliorating effects, and quenching/scavenging of generation of free radicals and their activities.[24] The possible mechanisms of action of antioxidants were first established when it was observed that substances with anti-oxidative potentials are possibly those that are readily self-oxidized. Studies into the activities of vitamin E in the prevention of lipid peroxidation primed the realization of antioxidants as reducing agents that neutralize oxidative reactions, usually by the removal of reactive oxygen species prior to damaging biological cells. These observations agree with previous studies [25-28] which presented vitamin C as an efficient antioxidant compound in numerous biological systems.

Also in this study, serum protein showed a significant ($p<0.05$) decrease in the 7th week of the groups administered paracetamol only when compared with the control group while those with concomitant administration of the antioxidant vitamins showed significant ($p<0.05$) increase when compared against the control and the paracetamol-only groups.

Proteins, known as the basic components of every living cell, include various substances *viz:* enzymes, hormones, as well as antibodies responsible for adequate and effective organisms.[29] Plasma protein has numerous roles which include transportation, biological defence, clotting and inflammation defence. Its concentration and laboratory assays are imperative in estimating nutritional status, infection and various disorders[30], and as an indicator in the assessment of blood plasma or serum protein. Various research has explained that a significant decrease is usually attributed to the presence or possibility of chronic disorder of the hepatocytes.[31]

Through research, it has become understandably known of the boundless and awesome roles biological signifiers such as malondialdehyde, superoxide dismutase, and glutathione as antioxidants play in protecting the biological systems from various unpleasant effects and damages because of oxidative stress. This work shows changes in these endogenous biological antioxidants and marker of lipid peroxidation marker after administration of paracetamol. The significant ($p<0.05$) decrease in these enzymes at the 7th week in the group administered only paracetamol is a possible indication of the efficacy of the compound in generating free radicals and are concurrently extinguished by the biological antioxidants. This is in accordance with the increase in concentration of MDA at same positions. These observations (markers for oxidative stress) were seen to be reversed when the antioxidant vitamins were administered alongside acetaminophen.

The increased lipid peroxidation was associated by a significant decrease concentration of GSH. Glutathione is a tripeptide, present in numerous tissues and a very necessary scavenger of free radicals and NAPQI - the reactive intermediate in acetaminophen metabolism.[32] Glutathione plays a crucial part in the antioxidant resistance system; eliminating reactive oxygen species, and maintaining membrane protein thiols.[33] The significant ($p<0.05$) decrease in SOD and GSH levels as seen in the 7th week and the implicate increase in MDA concentration are in agreement with previous studies on acetaminophen-induced nephrotoxicity.[34-36]

Through the administration of antioxidant vitamins, there was a decrease in concentration of serum MDA, while GSH levels and SOD activity increased, showing the antioxidant potential of the vitamins. These suggest that antioxidant vitamins could bring about a reduction in oxidative stress. From this work, it was seen that glutathione peroxidase (GPx) was non-significantly ($p>0.05$) increased in the groups administered paracetamol and the vitamins when compared with the control group. Glutathione peroxidase is a significant intracellular compound mostly dependent on selenium as the cofactor. Its responsibility is in breaking down hydrogen peroxides (H_2O_2) and converting same to water; as well as and lipid peroxides to their respective alcohols chiefly in the cell powerhouse (mitochondria) and in other instances, the cytosol matrix.[37] Therefore, GPx executes an important and effective role in inhibition of the processes involved in lipid peroxidation which helps in shielding the cells from damages caused by free radicals.[38]

Further in this research, it was seen that administration of the vitamins concomitantly with paracetamol showed a significant increase in the serum antioxidants erythrocyte activity and decrease in MDA. Vitamin C modulates immune responses in several ways which include modulation of leukocyte function and lymphocyte proliferation[39] and reports of Hermsdorff *et al.* have lend credence to this by indicating an important improvement in activities of SOD and GPx after vitamin C supplementation.[40] Also, Bernardo et al.[41] reported that administration of vitamin C in small-bowel mucosal biopsy organ culture system averts toxicity due to

free radicals and boosts biological scavengers which include SOD, GPx, and CAT; while the second line of defence against oxidative stress is given by the scavengers with low molecular weight scavengers; and these include the thiols, particularly GSH, vitamin E, vitamin C, etc.

Paracetamol toxic overdose has been associated with many metabolic disorders such as serum electrolyte and urea derangements[42] which was supported by Ghosh et al.[34] who reported that, exposure of rats with a nephrotoxic dose of paracetamol altered some number of biomarkers. These changes were reported to ensue due to the inactivation of the mitochondrial pathway during acetaminophen-induced cell death. In this present study, administration of the varying doses of paracetamol shows no significant ($p>0.05$) effect on the serum electrolytes (chloride and sodium) so analysed.

The present study showed that at the 7th week, the tissue sections obtained from the liver of the rats administered 1500 mg/70kg body weight showed some biological alterations when compared with the control group and others. These include a mild widespread hepatocellular swelling with partial occlusion of adjacent sinusoids whereby the affected cells appear swollen and contain numerous minute intracytoplasmic clear vacuoles. Multifocal areas of leucocytic aggregations were also observed with random distribution in relation to the hepatic sinusoids. These are likely due to the presence of free radicals and the very reactive metabolite NAPQI. Also, sections of the kidney harvested from the animals in this group indicated normo-renal histo-architecture when compared with other groups.

The results for haematological parameters in the present study shows non-significant ($p\geq0.05$) decrease in haemoglobin (Hb), red blood cell count (RBC), packed cell volume (PCV) as well as white blood cell (WBC) and platelets count when groups 2 and 3 are compared with the control in the 1st and 3rd weeks. In the 5th and 7th weeks, a significant ($p\leq0.05$) decrease is seen when both paracetamol-only groups are compared with the control. This significant decrease was ameliorated by the antioxidant vitamins C and E as seen when groups 4 through 7 are compared with the control group. The observed acetaminophen toxicity to haematological parameters agrees with the report of Godwin *et al.*[43] who reported a significant ($p\leq0.05$) decrease in haematological parameters of the animals administered the toxic dose of acetaminophen. Platelets and white blood cells count of the untreated group decreased significantly. This agrees with a human study report of Arun,[44] where patients who suffered from a progressive deterioration of their renal function experienced decline in their immune status, following a decrease in white blood cell count. Although the precise mechanism of action behind this reduction has not been fully understood.

A strong correlation has been established between anaemia and the degree of renal dysfunction, with the major cause being the failure of the kidneys to produce erythropoietin.[45] Renal impairment has been linked directly to some haematological distortions. Therefore, the reduced red blood cell count could be due to a compromised erythropoietin production resulting from damage caused by acetaminophen toxicity. Erythropoietin is also implicated in the humoral regulation of the platelet mass.[46] Vitamins C and E are synthetic antioxidant drugs that prevents oxidative stress induced damage in tissues of living organisms.

Conclusion

This present chronic study has shown that adulteration of the approved therapeutic dose of paracetamol by individuals or even consumption of the recommended therapeutic dose is deleterious to the biological system; causing accumulated

metabolites to significantly induce adverse damages majorly on the hepatic cells. The corrective action as displayed by the antioxidant vitamins goes further to indicate the benefit of co-administration of these vitamins with paracetamol, especially in a situation where an individual finds it difficult to manage some health disturbances without consuming the compound, paracetamol.

The findings of this study have revealed the necessity of alerting the public on the adverse health consequences of over-consumption of paracetamol while at same time, showing the benefits of antioxidants vitamins for perceived addicts of this drug. Owing to these, drug regulatory body – the National Agency for Food and Drug Administration and Control (NAFDAC) should ensure to see that the medical history of patients is being monitored and if possible, patients should be made to purchase these vitamins alongside paracetamol whenever they want to consume this compound. This is imperative because we are in an environment where one can easily get this drug across the counter – anytime, any day.

Acknowledgement

I wish to acknowledge the staff of Department of Biochemistry, Michael Okpara University of Agriculture, Umudike for giving me assess to the laboratory and animal house without reserve.

Conflict of interest

The authors wish to state that there are no conflicts of interest.

References

1. Mazer, M. & Perrone, J. (2008) Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. Journal of Medicine & Toxicology, 4: 2-6.
2. *Pathan, M.M., Khan, M.A., Moregaonkar, S.D., Somkuwar, A., & Gaikwad, N. (2013).* Amelioration of Paracetamol Induced Nephrotoxicity by *Maytenus emarginata* in Male Wistar Rats. International Journal of Pharmacy and Pharmaceutical Sciences, 5: 471-474.
3. Sandhu, N.S., Chopra, D. & Kaur, S. (2010). Amelioration of Paracetamol-induced hepatotoxicity by a protein isolated from the leaves of the herbs *Cajanus acutifolus* Linn. International Journal of Pharmacy and Pharmaceutical Sciences, 2: 75-80.
4. Dasgupta, B., Kalita, J.C, Chowdhury, A. & Kotoky, J. (2012). Hepatoprotective activity of *Nelsonia canescens* (Lam.) Spreng on acute hepatotoxicity induced by Paracetamol. International Journal of Pharmacy and Pharmaceutical Sciences, 4: 107-112.
5. Gulnaz, H., Tahir, M., Muni, B. & Sami, W. (2010). Protective effects of garlic oil on acetaminophen induced nephrotoxicity in male Wistar rats. Biomedical, 26: 9-15.
6. Patel, P. B., Patel, T. K., Shah, P., Baxi, S. N., Sharma, H. O., & Tripathi, C. B. (2010). Protective Effect of Ethanol Extract of Gymnosporia montana (Roth) Bemth. in Paracetamol-induced Hepatotoxicity in Rats. Indian Journal of Pharmaceutical Sciences, 72(3),392–396.
7. Chatterjee, I.B. (2009). "The History of Vitamin C Research in India," Journal of Biosciences,34:185-194.
8. Stangeland, T., Remberg, S.F. & Lye, K.A. (2008). "Total Antioxidant Activity in 35 Ugandan Fruits and Vegetables," Elsevier, 113: 85-91.
9. Padayatty, S.J., Sun, H., Wang, Y. & Riordan, H.D. (2004). "Vitamin C

Pharmacokinetics: Implication for Oral and Intravenous Use," Annals of Internal Medicine, 140: 533-537.

10. Sminorff, N. & Wheeler, G.L. (2000). "Ascorbic Acid in Plants Biosynthesis and Function," Critical Reviews in Biochemistry and Molecular Biology, 19: 267-290.

11. Adikwu, E. & Deo O. (2013). Hepatoprotective Effect of Vitamin C (Ascorbic Acid). Pharmacology and Pharmacy, 4: 84-92

12. Niki, E., & Traber, M. G. (2012). A history of vitamin E. Annals of nutrition & metabolism, 61(3), 207–212.

13. Zingg, J.M. (2007). Vitamin E: an overview of major research directions. Molecular Aspects in Medicine, 28: 400-422.

14. *Rizvi, S., Raza, S.T., Ahmed, F., Ahmad, A., Abbas, S., & Mahdi, F. (2014). The role of vitamin e in human health and some diseases. Sultan Qaboos University medical journal, 14 2, e157-65 .*

15. Howard, A.C. Anna, K., McNeil, A.K. & McNeil, P.L. (2011). Promotion of plasma membrane repair by vitamin E. Nature Communications, 20: 597.

16. Gregory, B., Larson A.M., Reisch, J. & Lee, W. M. (2010). "Acetaminophen Dose Does Not Predict Outcome in Acetaminophen-Induced Acute Liver Failure." Journal of investigative medicine, 58(5), 707–710.

17. Cesar, Y. & Antoine, A. (2017). Acetaminophen Toxicity at Therapeutic Doses. Internal Medicine Review, 3:1-13

18. Makni, M.Y., Chtourou, H., Fetoui, E.M., Garoui, T. & Zeghal, N. (2011). Evaluation of the antioxidant, anti-inflammatory and hepatoprotective properties of vanillin in carbon tetrachloride-treated rats. European Journal of Pharmacology, 668, 133-139.

19. Watkins, P.B., Kaplowitz, N. & Slattery, T. (2006). Aminotransferase elevations in healthy adults receiving 4 grams of paracetamol daily: a randomized controlled trial. *JAMA,* 296: 87–93.

20. Ebenyi, L.N., Ibiam, U.A. & Aja, P.M. (2012). Effects of Sativum extracts on paracetamol induced hepatotoxicity in albino rats. International Research Journal of Biochemistry and Bioinformatics, 2: 93- 97.

21. Chen, Y.L., Chen, L.J., Bair, M.J., Yao M.L., Peng H.C., Yang S.S. & Yang, S.C. (2011). Antioxidative status of patients with alcoholic liver disease in South-eastern Taiwan. World Journal of Gastroenterology, 17: 1063–1070. [PubMed]

22. Odigie, I.P., Okpoko, F.B. & Ojobor, P.D. (2007). Antioxidant Effects of Vitamin C and E on Phenylhydrazine-Induced Haemolysis in Sprague Dawley Rats: Evidence for A better Protection by Vitamin E. The Nigerian Postgraduate Medical Journal, 14: 1-7.

23. Idogun, E.S. & Ajala, M.O. (2005). Ascorbic Acid and Alpha Tocopherol Antioxidant Status of Type 2 Diabetes Mellitus Patients seen in Lagos. The Nigerian Postgraduate Medical Journal, 12: 155-157.

24. Uboh, F.E., Ebongi, P.E., Akpan, H.D. & Usoh, I.F. (2012). Hepatoprotective

effect of vitamins C and E against gasoline vapor-induced liver injury in male rats. Turkey Journal of Biology, 36: 217-223.

25. Ayo, J.O., Minka, N.S. & Mamman, M.M. (2006). Excitability scores of goats administered ascorbic acid and transported during hot-dry conditions. Journal of Veterinary Science, 7: 127-131.

26. Chen, K., Suh, J., Carr, A. C., Morrow, J. D., Zeind, J., & Frei, B. (2000). Vitamin C suppresses oxidative lipid damage in vivo, even in the presence of iron overload. American journal of physiology. Endocrinology and metabolism, 279(6), E1406–E1412. https://doi.org/10.1152/ajpendo.2000.279.6.E1406

27. Frei, B. (2004). Efficacy of dietary antioxidants to prevent oxidative damage and inhibit chronic disease. Journal of Nutrition, 134: 3196-3198.

28. Ambali, S., Akanbi, D. & Igbokwe, N. (2007). Evaluation of subchronic chlorpyrifos poisoning on haematological and serum biochemical changes in mice and protective effect of vitamin C. The Journal of Toxicological Sciences, 32: 111-120.

29. Amin, K.A., Hameid, H.A. & Elsttar, A.H. (2010). Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food and Chemical Toxicology,48: 2994-2999.

30. Fatimah, A.A.H. & Omaima, M.M.H. (2016). Histopathological and biochemical studies in male Wistar albino rats injected with diethylnitrosamine and treated with Camel's milk and *Curcuma longa*. Egyptian Journal of Chemistry, Environment and Health; 2: 548-565.

31. Murray, N., Schiodt, F.V. & Ostapowicz, G. (2006). Alpha-fetoprotein and prognosis in acute liver failure. Liver Transplantation, 12: 1776–1781.[PubMed]

32. Yayla, M., Halici, Z., Unal, B., Bayir Y., Akpinar, E. & Gocer, F. (2014). Protective effect of et-1 receptor antagonist bosentan on paracetamol induced acute liver toxicity in rats. European Journal of Pharmacology, 726: 87–95.

33. Traber, M.G. & Jan, F.S. (2011). "Vitamins C and E: Beneficial Effects from a Mechanistic Perspective." Free radical biology & medicine 2011; 51: 1000–1013.

34. Ghosh, A. & Sil, P.C. (2007). Anti-oxidative effect of a protein from *Cajanus indicus* l against acetaminophen-induced hepato-nephro toxicity. Journal of Biochemistry and Molecular Biology, 40: 1039–1049.

35. Yousef, M.I., Omar, S.A., El-Guendi, M.I. & Abdelmegid, L.A. (2010). Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematoxicity in rat. Food Chemistry and Toxicology, 48: 3246–3261.

36. Abdul, H.Z., Budin, S.B., Wen J.N., Hamid, A., Husain, K. & Mohamed, J. (2012). Nephroprotective effects of

Zingiber zerumbet Smith ethyl acetate extract against paracetamol-induced nephrotoxicity and oxidative stress in rats. Journal of Zhejiang University of Science, 13:176–185.

37. Goth, L., Rass, P. & Pay, A. (2004). Catalase enzyme mutations and their association with diseases. Molecular Diagnosis, 8: 141–149.10.2165/00066982-200408030-00001

38. Gill, S.S. & T. Narendra, (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants, Plant Physiology and Biochemistry, 48,909-30 DOI. 10.1016/j.plaphy.08.016

39. Mahmoudabadi, M.M.S., Mohammad, M.S. & Ali, R.R. (2014). "Effect of EPA and Vitamin C on Superoxide Dismutase, Glutathione Peroxidase, Total Antioxidant Capacity and Malondialdehyde in Type 2 Diabetic Patients." Oman Medical Journal, 29: 39–45.

40. Hermsdorff, H.H., Barbosa, K.B., Volp, A.C., Puchau B., Bressan J., Zulet M.Á. and Martínez J.A (2012). Vitamin C and fibre consumption from fruits and vegetables improves oxidative stress markers in healthy young adults. British Journal of Nutrition, 107: 1119-1127.

41. Bernardo D, Martínez-Abad B, Vallejo-Diez S., Montalvillo E, Benito V, Anta B. (2012). Ascorbate-dependent decrease of the mucosal immune inflammatory response to gliadin in coeliac disease patients. *Allergologia et Immunopathologia* (Madr), 40: 3-8.

42. Palani, S., Raja, S., Kumar, R.P. (2009). Therapeutic efficacy of Pimpinella tirupatiensis (Apiaceae) on acetaminophen induced nephrotoxicity and oxidative stress in male albino rats. International Journal of Pharmtech Research,1: 925-934.

43. Godwin, E.E., Item, J.A., Ochuole, D.O. & Sunday, A.B. (2017). The Effect of *Vernonia calvoana* extract on some Haematological Indices in Acetaminophen Treated Male Wistar Rats. Global Journal of Pure and Applied Sciences, 2: 255-261

44. Arun, S., Prabhu, M.V., Chowta, N.K. & Bengrre, M.L. (2012). The hameatological pattern of the patients with chronic kidney disease in a tertiary care setup in South India. Journal of Clinical and Diagnostic Research, 6: 1003-1006.

45. Alpers, C.E. (2004). The kidney. In: Vinay Kumar, Abul, K. A. and Nelson Fasto. Robbins Pathologic basis of disease, 7th edition. Elsevier Incorporated, 20: 960 - 965.

46. Suresh, M., Mallikarjuna, R.N., Sharan, S.M. Hari, K. B., Sharavya, K. G. & Chandrasekhar, M. (2012). Hematological changes in chronic renal failure. International Journal of Scientific and Research Publications, 2: 78 – 85.

	WEEK 1					WEEK 3				
GROUPS	**RBC**	**PLATELETS**	**WBC**	**HB**	**PCV**	**RBC**	**PLATELETS**	**WBC**	**HB**	**PCV**
Group 1	520.50 ±0.50[a]	225.00 ±25.00[a]	9800.00 ±200.00[a]	13.20[a] ±1.00	39.00[a] ±1.00	520.50 ±0.50[a]	225.00 ±25.00[a]	9800.00 ±200.00[a]	13.20[a] ±1.00	39.00[a] ±1.00
Group 2	498.00 ±0.55[a]	220.50 ±10.00[a]	9750.00 ±120.00[a]	10.55 ±0.25[b]	41.00 ±1.00[a]	496.50 ±0.55[a]	242.00 ±12.00[b]	9700.00 ±99.00[a]	9.23 ±0.10[b]	35.00 ±0.20[a]
Group 3	500.50 ±0.50[a]	225.00 ±10.00[a]	9800.00 ±89.00[a]	11.15 ±0.05[a]	38.50 ±0.50[a]	515.00 ±0.50[a]	230.00 ±8.10[a]	9800.00 ±95.00[a]	11.05 ±0.15[a]	35.00 ±0.15[a]
Group 4	515.50 ±2.50[a]	224.00 ±0.50[a]	9800.00 ±120.00[a]	15.25 ±1.05[a]	38.50 ±2.50[a]	500.50 ±2.50[a]	231.00 ±11.50[a]	9830.00 ±110.00[a]	11.15 ±1.00[a]	36.25 ±1.12[a]
Group 5	512.50 ±10.00[a]	225.50 ±7.50[a]	9850.00 ±250.00[a]	11.30 ±0.90[a]	36.50 ±2.50[a]	515.50 ±10.00[a]	231.50 ±5.20[a]	9800.00 ±150.00[a]	13.00 ±0.00[a]	35.00 ±1.50[a]
Group 6	500.00 ±0.59[a]	223.50 ±2.50[a]	9850.00 ±150.00[a]	12.35 ±0.15[a]	34.50 ±0.50[a]	500.00 ±0.59[a]	228.50 ±3.15[a]	9850.00 ±225.00[a]	12.30 ±0.10[a]	36.00 ±0.10[a]
Group 7	507.50 ±2.50[a]	224.50 ±7.50[a]	9850.00 ±125.00[a]	13.50 ±0.30[a]	35.50 ±0.50[a]	518.50 ±2.50[a]	232.50 ±5.15[a]	9800.00 ±105.00[a]	13.00 ±0.00[a]	35.00 ±0.00[a]

Table 1: Effect of acetaminophen on haematological parameters of Wistar at weeks 1 and 3

[ab]Groups with different subscripts are significant ($p<0.05$) when compared with the control

Key: Group 1: Control

Group 2: Acetaminophen only (1500 mg/70 kg body weight)

Group 3: Acetaminophen only (1000 mg/70 kg body weight)

Group 4: Acetaminophen (1500 mg/70 kg body weight) + vitamin C

Group 5: Acetaminophen (1000 mg/70 kg body weight) + vitamin C

Group 6: Acetaminophen (1500 mg/70 kg body weight) + vitamin E

Group 7: Acetaminophen (1000 mg/70 kg body weight) + vitamin E

ISSN: 1827-8620

Table 2: Effect of acetaminophen on haematological parameters of Wistar at weeks 5 and 7
[ab]Groups with different subscripts are significant ($p<0.05$) when compared with the control

	WEEK 5					WEEK 7				
GROUPS	**RBC**	**PLATELETS**	**WBC**	**HB**	**PCV**	**RBC**	**PLATELETS**	**WBC**	**HB**	**PCV**
Group 1	520.50 ±0.50[a]	225.00 ±25.00[a]	9800.00 ±200.00[a]	13.20[a] ±1.00	39.00[a] ±1.00	520.50 ±0.50[a]	225.00 ±25.00[a]	9800.00 ±200.00[a]	13.20[a] ±1.00	39.00[a] ±1.00
Group 2	450.00 ±1.55[b]	185.20 ±5.00[b]	9050.00 ±80.00[b]	8.33 ±0.00[b]	30.00 ±1.00[b]	450.00 ±1.55[b]	185.20 ±5.00[b]	9050.00 ±80.00[b]	8.33 ±0.00[b]	30.00 ±1.00[b]
Group 3	465.00 ±0.50[b]	195.00 ±4.10[a]	9000.00 ±60.15[b]	9.55 ±0.05[b]	32.00 ±1.25[b]	465.00 ±0.50[b]	195.00 ±4.10[a]	9000.00 ±60.15[b]	9.55 ±0.05[b]	32.00 ±1.25[b]
Group 4	505.25 ±1.00[a]	201.00 ±2.05[a]	9500.00 ±80.00[b]	10.00 ±1.05[a]	33.00 ±1.00[b]	505.25 ±1.00[a]	201.00 ±2.05[a]	9500.00 ±80.00[b]	10.00 ±1.05[a]	33.00 ±1.00[b]
Group 5	518.00 ±5.10[a]	210.00 ±4.00[a]	9800.00 ±70.10[a]	11.05 ±0.00[a]	37.00 ±0.01[a]	518.00 ±5.10[a]	210.00 ±4.00[a]	9800.00 ±70.10[a]	11.05 ±0.00[a]	37.00 ±0.01[a]
Group 6	500.00 ±1.15[a]	228.15 ±3.15[a]	9550.00 ±115.00[a]	12.00 ±0.00[a]	35.00 ±1.50[a]	500.00 ±1.15[a]	228.15 ±3.15[a]	9550.00 ±115.00[a]	12.00 ±0.00[a]	35.00 ±1.50[a]
Group 7	515.00 ±1.00[a]	222.10 ±3.05[a]	9850.00 ±100.00[a]	14.05 ±0.02[a]	37.00 ±1.00[a]	515.00 ±1.00[a]	222.10 ±3.05[a]	9850.00 ±100.00[a]	14.05 ±0.02[a]	37.00 ±1.00[a]

Key
Group 1: Control
Group 2: Acetaminophen only (1500 mg/70 kg body weight)
Group 3: Acetaminophen only (1000 mg/70 kg body weight)
Group 4: Acetaminophen (1500 mg/70 kg body weight) + vitamin C
Group 5: Acetaminophen (1000 mg/70 kg body weight) + vitamin C
Group 6: Acetaminophen (1500 mg/70 kg body weight) + vitamin E Group 7: Acetaminophen (1000mg/70 kg body weight) + vitamin E

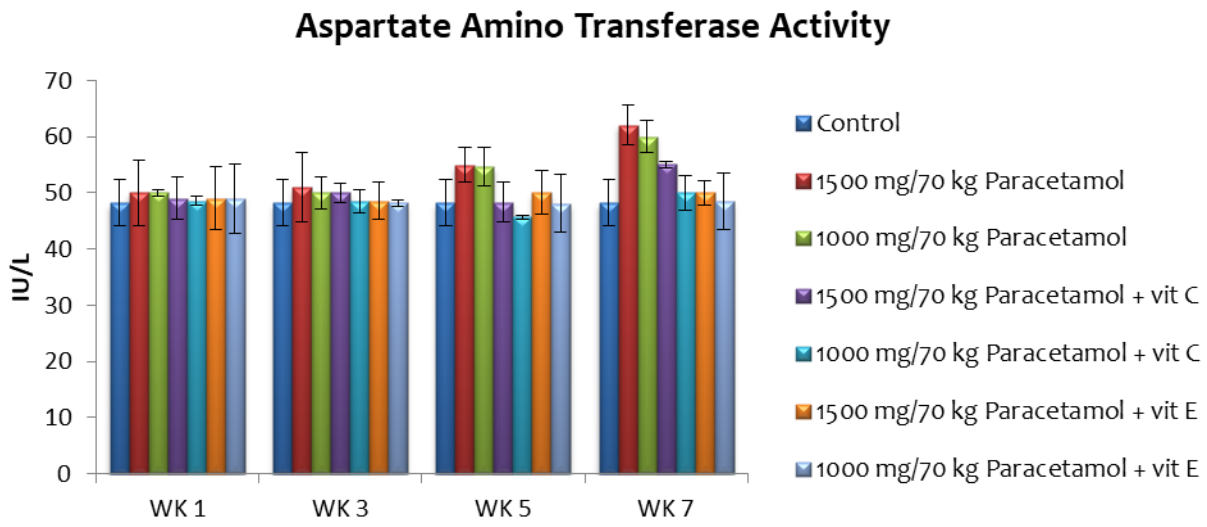


Fig. 1: Effect of acetaminophen on AST activity in Wistar rats

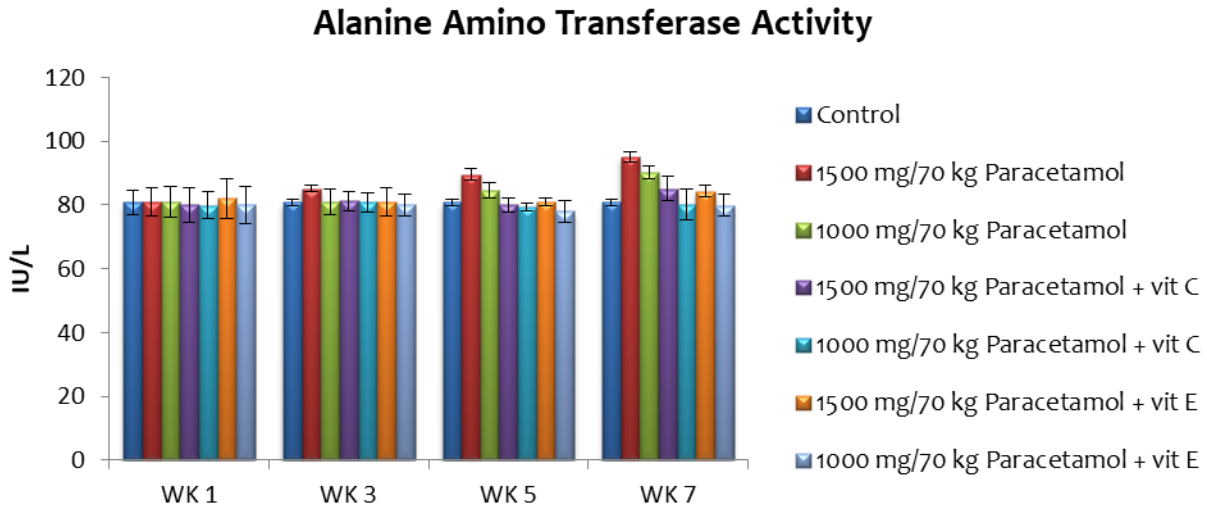


Fig. 2: Effect of acetaminophen on ALT activity in Wistar rats

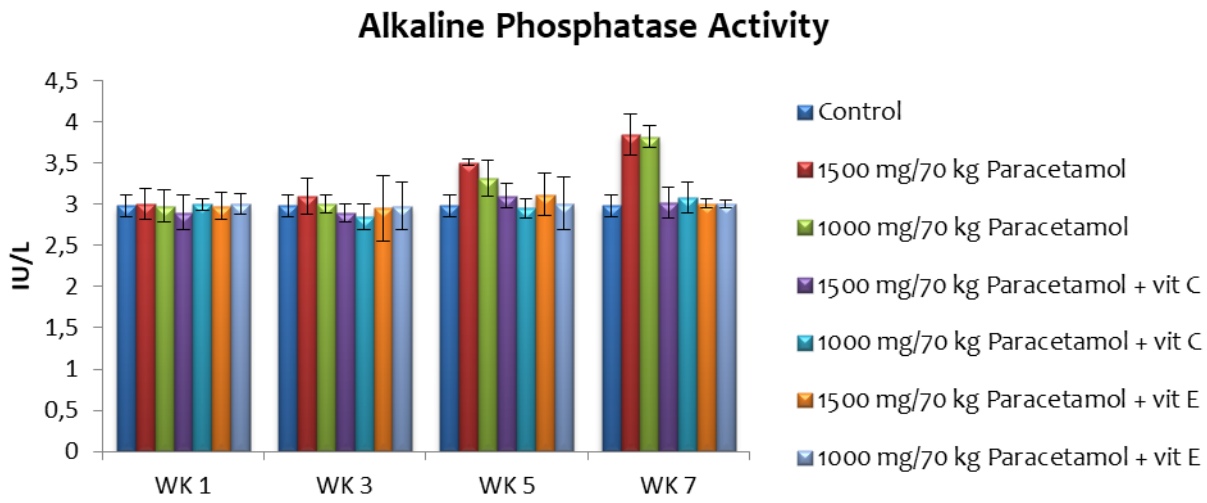


Fig. 3: Effect of acetaminophen on ALP activity in Wistar rats

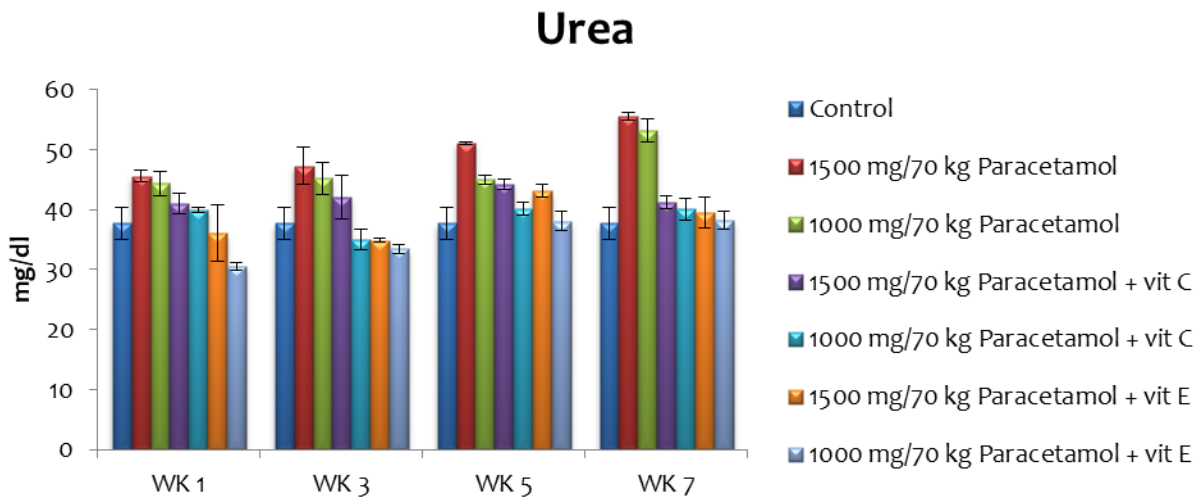


Fig. 4: Effect of acetaminophen on urea concentration in Wistar rats

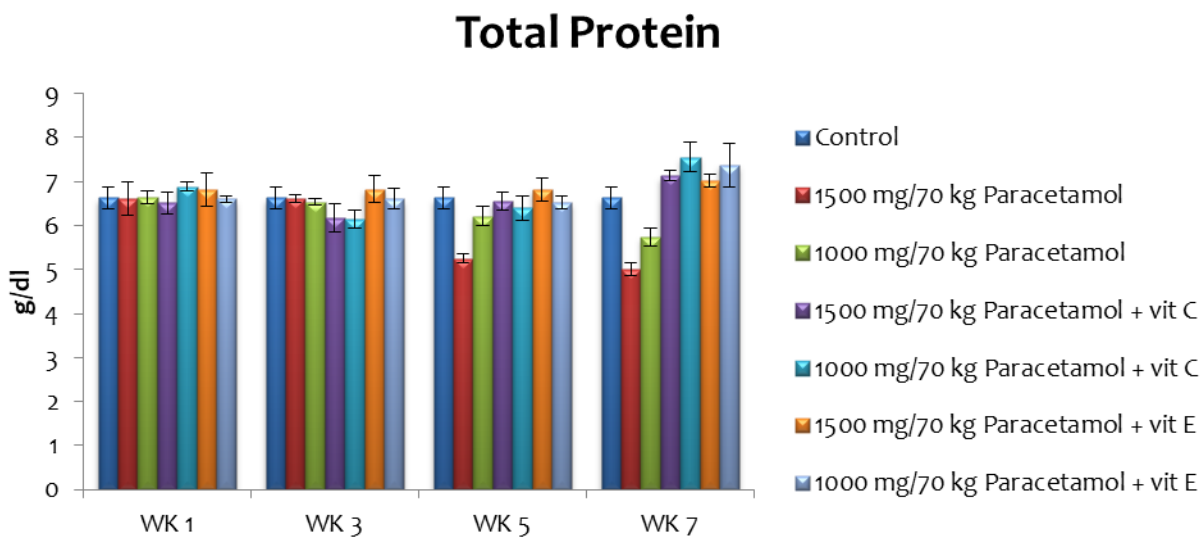


Fig. 5: Effect of acetaminophen on total protein concentration in Wistar rats

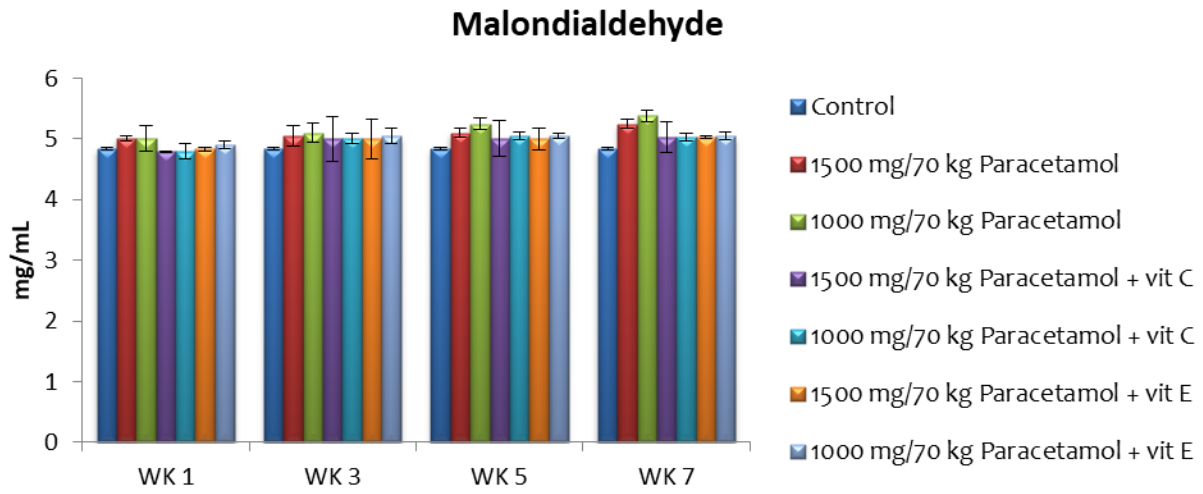


Fig. 6: Effect of acetaminophen on MDA concentration in Wistar rats

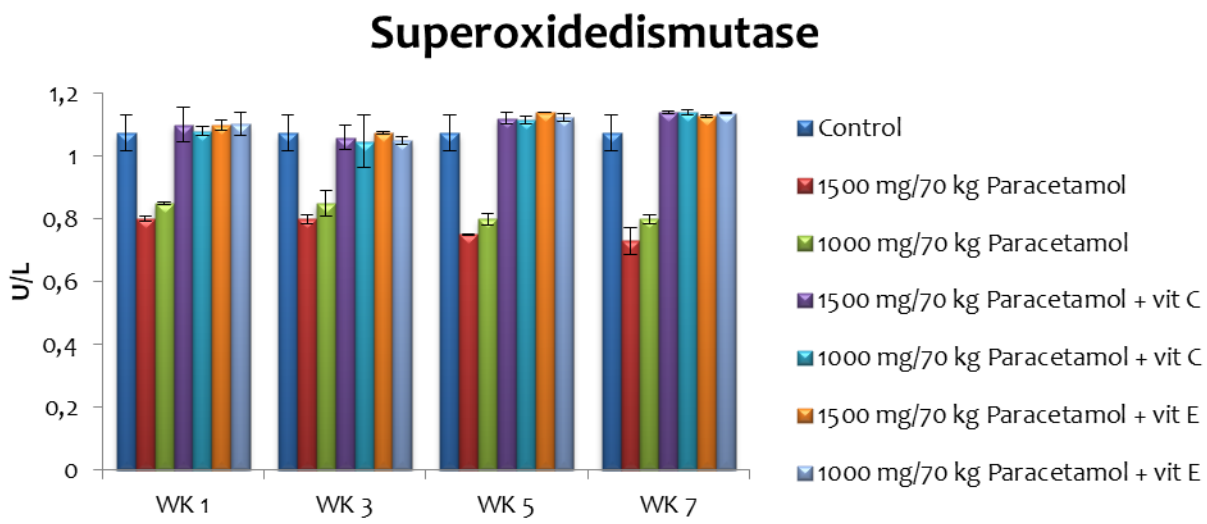


Fig. 7: Effect of acetaminophen on SOD activity in Wistar rats

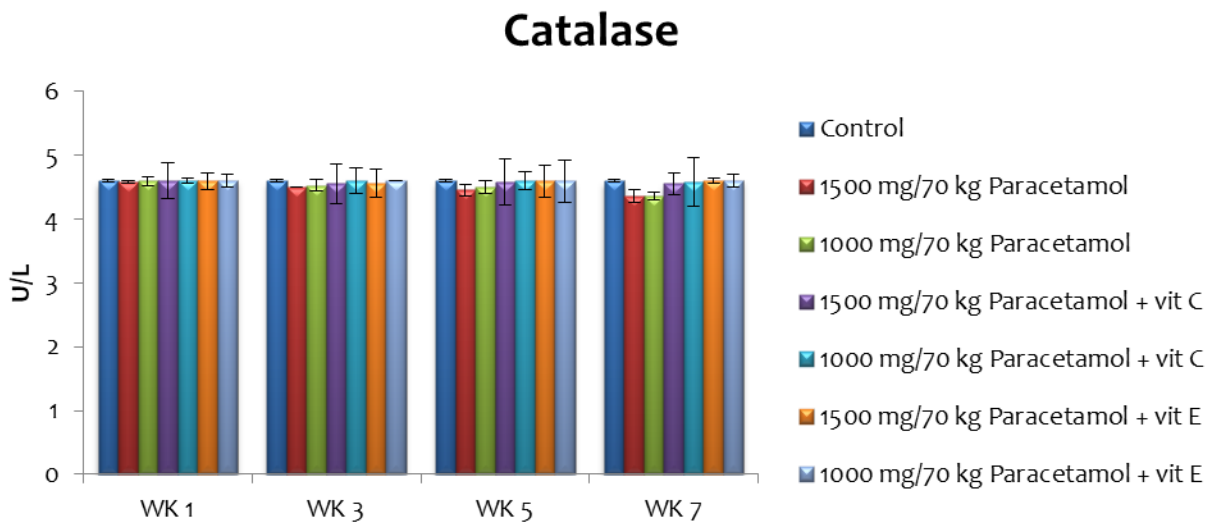


Fig. 8: Effect of acetaminophen on CAT activity in Wistar rats

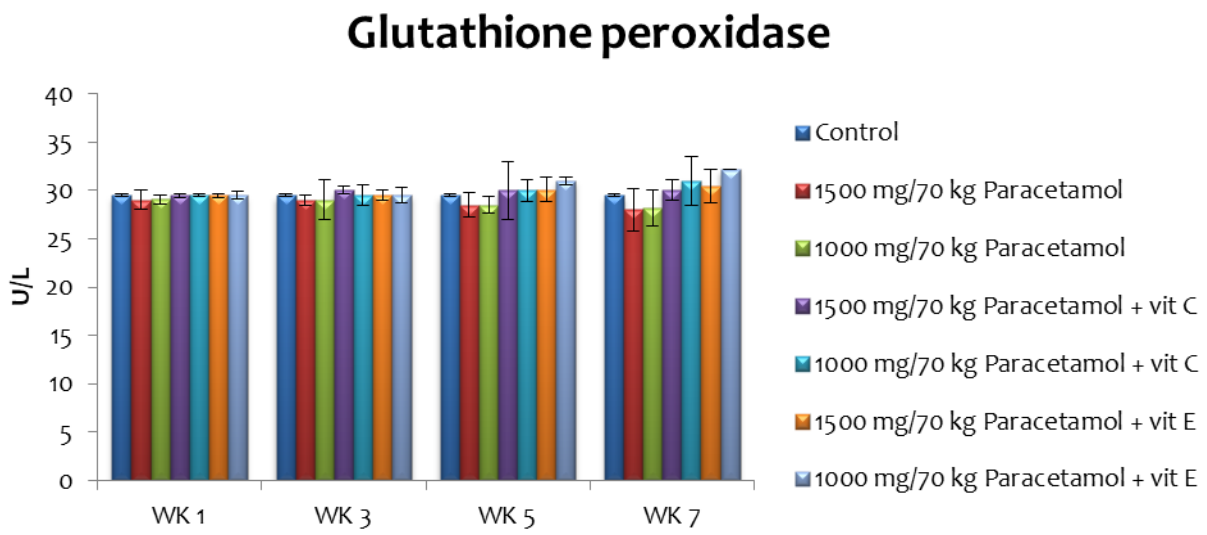


Fig. 9: Effect of acetaminophen on GPx activity in Wistar rats

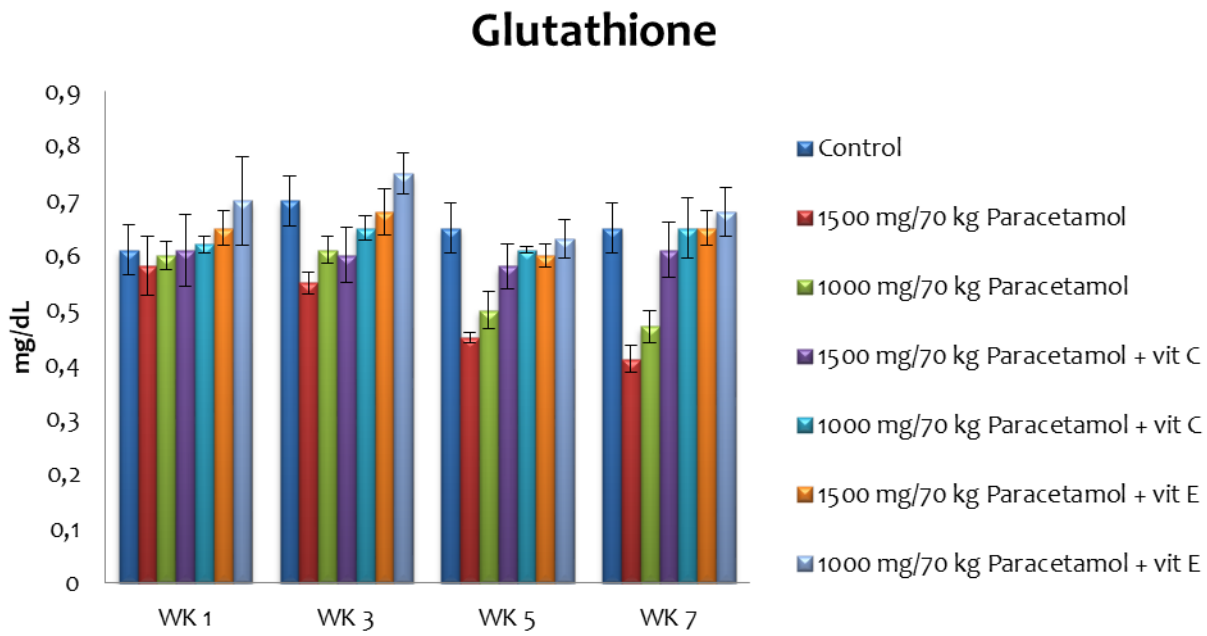
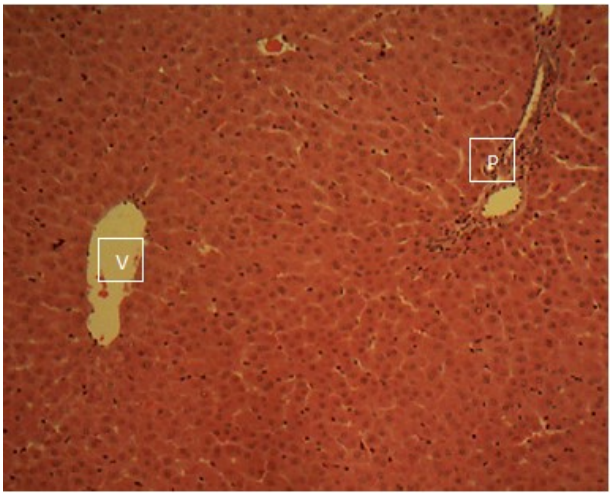


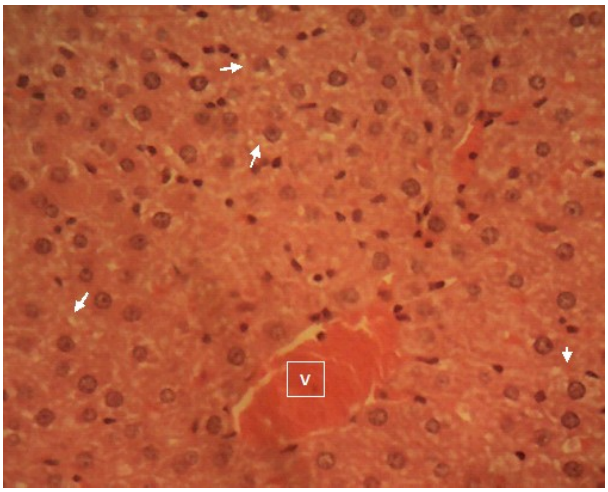
Fig. 10: Effect of acetaminophen on GSH activity in Wistar rats

Histopathology
The liver at 7th week of administration

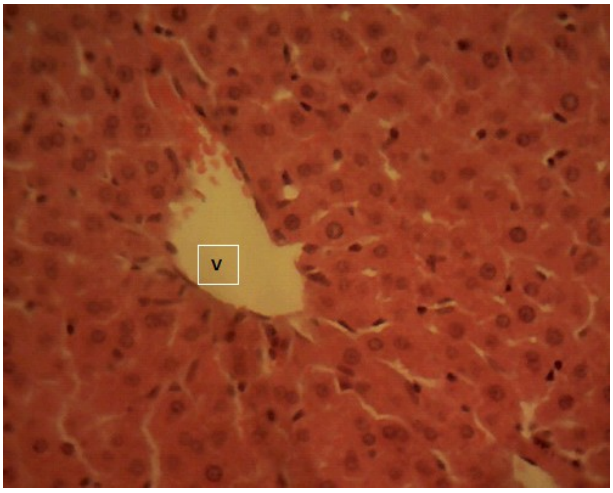
A



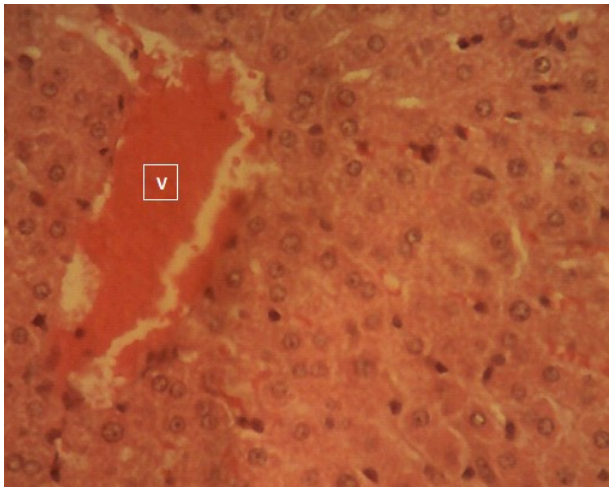
B



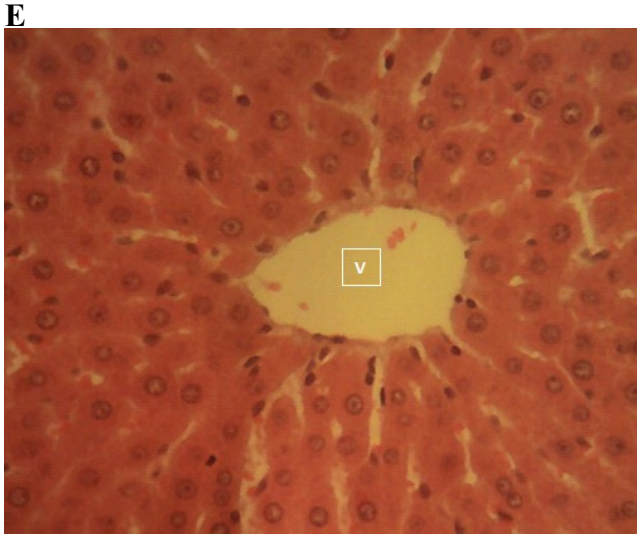
C



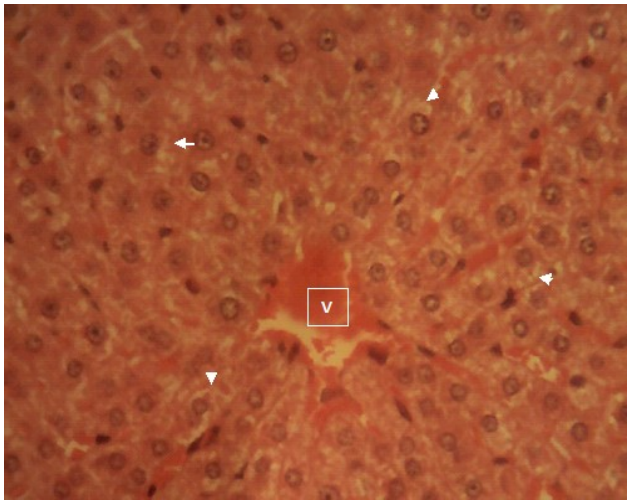
D



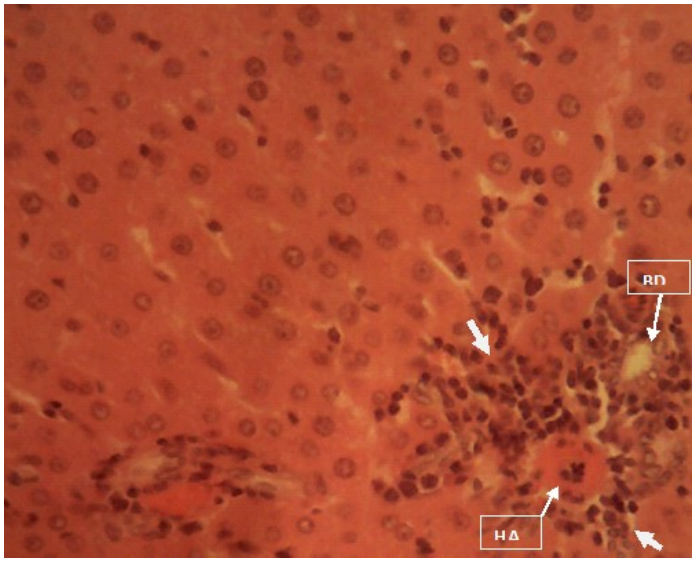
E



F

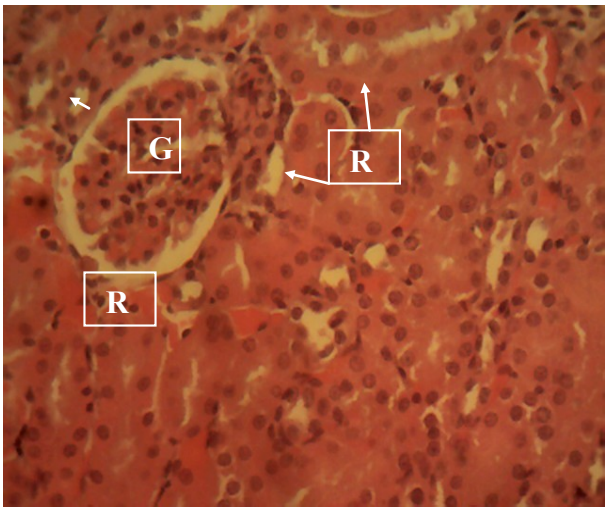


G

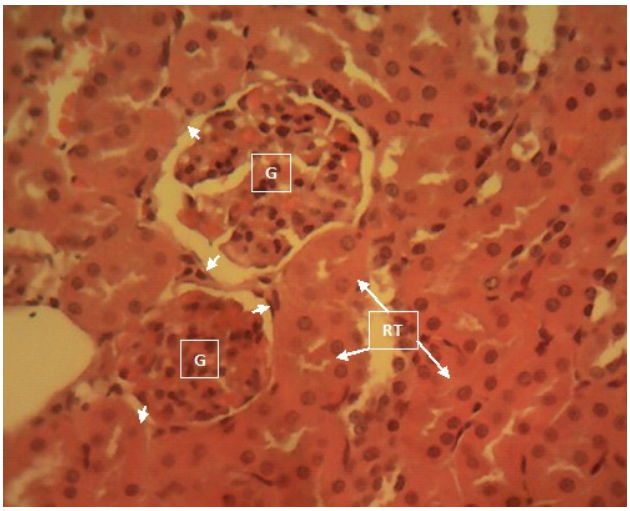


A: Sections of the liver collected from the control group showed the normal features of the hepatic histomorphology/histo-architecture for laboratory rodents. Normal hepatic lobules, with normal hepatocytes arranged in interconnecting cords around the central veins (V) were observed. The hepatic cords are separated from each other by the hepatic sinusoids as they radiate towards the periphery of the hepatic lobules where they meet with the components of the portal areas (P)/portal triad (Hepatic artery, Hepatic vein and Bile duct) suspended in loose connective tissue matrix. H&E x400. **B:** Sections of the liver collected from the animals administered 1500mg/70kg Acetaminophen only showed a mild widespread hepatocellular swelling with partial occlusion of adjacent sinusoids. The affected cells appear swollen and contain numerous minute intracytoplasmic clear vacuoles (arrow). Central vein (V). H&E x400. **C:** Sections of the liver collected from the animals administered 1000mg/70kg acetaminophen showed the normal hepatic histo-architecture for laboratory rodents. Central vein (V). H&E x400. **D:** Sections of the liver collected from the animals administered 1500mg/70kg Acetaminophen + Vitamin C showed a very mild widespread hepatocellular swelling with partial occlusion of adjacent sinusoids. The affected cells appear swollen and contain numerous minute intracytoplasmic clear vacuoles (arrow). Central vein (V). H&E x400. **E:** Sections of the liver collected from the animals administered 1000mg/70kg Acetaminophen + Vitamin C showed the normal hepatic histo-architecture for laboratory rodents (See control group for detailed histopathological descriptions). Central vein (V). H&E x400. **F:** Sections of the liver collected from the animals administered 1500mg/70kg Acetaminophen + Vitamin E showed a mild widespread, centrilobular hepatocellular swelling with partial occlusion of adjacent sinusoids. The affected cells appear swollen and contain numerous minute intracytoplasmic clear vacuoles (arrow). Central vein (V). H&E x400. **G:** Sections of the liver collected from the animals administered 1000mg/kg Acetaminophen + Vitamin E showed a mild periportal infiltration of inflammatory cells. The inflammatory cells composed primarily of mononuclear leucocytes aggregate around the components of the portal triads. A few random aggregates in the hepatic lobules were also observed. Hepatic artery (HA); Bile duct (BD). H&Ex400.

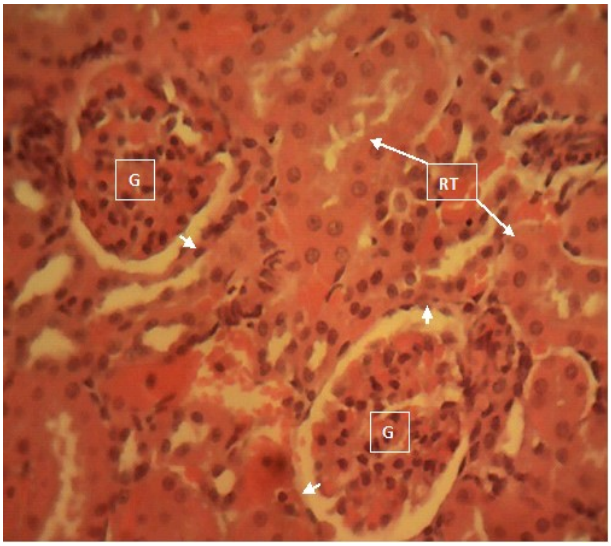
The kidney at 7th week of administration



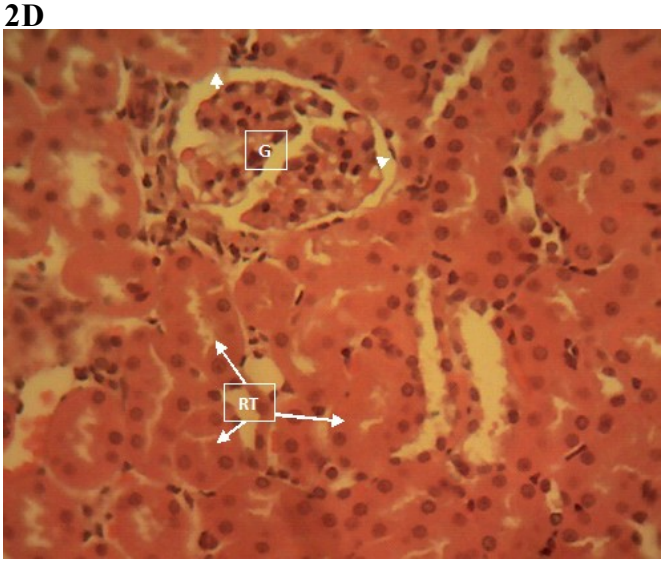
2B



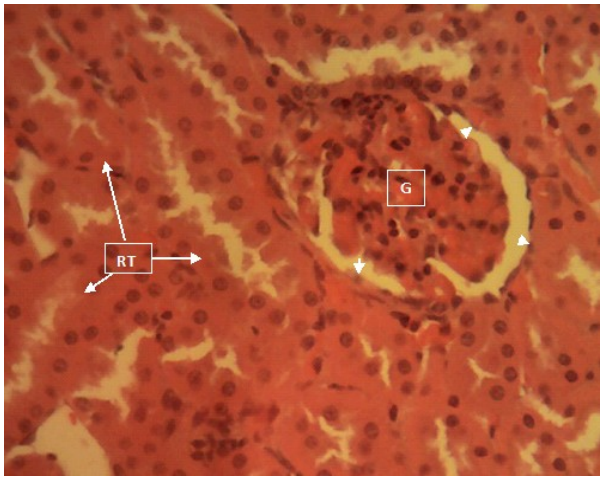
2C



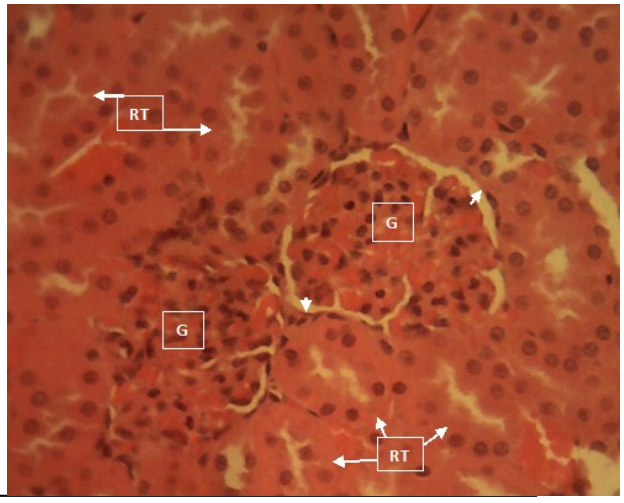
2D



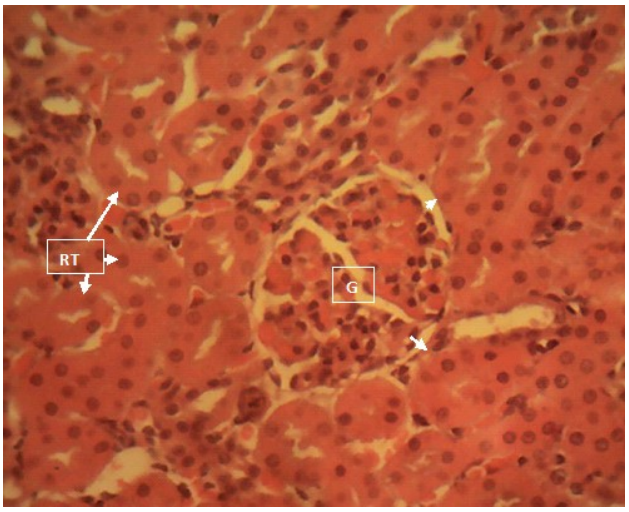
2E



2F



2G



2A: Sections of the kidney collected from the animals in the control group showed the normal features of the renal histomorphology/histo-architecture for laboratory rodents. The sections showed normal Glomeruli (G) in their thin Bowman's capsules (arrow) surrounded by a sea of renal tubules (RT) (proximal convoluted tubules, pars recta, distal convoluted tubules and collecting ducts) in the cortex and outer medulla. The inner medulla also showed normal renal tubules. The sections also showed normal renal interstitium composed of loose connective tissue with rich capillary network. H&E x160; x400. **2B:** Sections of the kidney collected from the animals administered 1500mg/70kg Acetaminophen showed the normal renal histo-architecture (see control group for detailed histological description). Glomeruli (G); Bowman's capsule (Arrow); Renal tubules (RT); Blood vessel (BV). H&E x400. **2C:** Sections of the kidney collected from the animals in this group showed the normal renal histo-architecture (see control group for detailed histological description). Glomeruli (G); Bowman's capsule (Arrow); Renal tubules (RT). H&E x400. **2D:** Sections of the kidney collected from the animals administered 1500mg/70kg Acetaminophen + Vitamin C showed the normal renal histo-architecture (see control group for detailed histological description). Glomeruli (G); Bowman's capsule (Arrow); Renal tubules (RT); Blood vessel (BV). H&E x400. **2E:** Sections of the kidney collected from the animals administered 1000mg/kg Acetaminophen + Vitamin C showed the normal renal histo-architecture (see control group for detailed histological description). Glomeruli (G); Bowman's capsule (Arrow); Renal tubules (RT). H&E x400. **2F:** Sections of the kidney collected from the animals administered 1500mg/70kg Acetaminophen + Vitamin E showed the normal renal histo-architecture (see control group for detailed histological description). Glomeruli (G); Bowman's capsule (Arrow); Renal tubules (RT)Blood vessel (BV). H&E x400. **2G:** Sections of the kidney collected from the animals administered 1000mg/kg Acetaminophen + Vitamin E showed the normal renal histo-architecture (see control group for detailed histological description). Glomeruli (G); Bowman's capsule (Arrow); Renal tubules (RT). H&E x400.