



Effects of Methanolic Leaf Extracts of *Azadirachta indica* and *Spondias mombin* on the Kidneys Histology of Zidovudine Stress Induced Wistar Rats

C. O. Ubah^{1*}, O. R. Asuquo¹, G. E. Oko² and M. A. Eluwa¹

¹*Department of Anatomical Sciences, College of Medical Sciences, University of Calabar, P.M.B 1115, Calabar, Nigeria.*

²*Department of Biochemistry, College of Medical Sciences, University of Calabar, P.M.B 1115, Calabar, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors COU and ORA designed the study, performed the statistical analysis and wrote the protocol. Authors COU and GEO wrote the first draft of the manuscript. Authors MAE and COU managed the analyses of the study. Authors COU, ORA and GEO managed the literature searches. All authors read and approved the final manuscript

Article Information

DOI: 10.9734/JOCAMR/2019/46169

Editor(s):

(1) Dr. Sahdeo Prasad, Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Texas, USA.

(2) Dr. Francisco Cruz-Sosa, Metropolitan Autonomous University, Iztapalapa Campus Av. San Rafael Atlixco, Mexico.

Reviewers:

(1) Robert E. Smith, Park University, USA.

(2) T. Pullaiah, Sri Krishnadevaraya University, India.

Complete Peer review History: <http://www.sdiarticle3.com/review-history/46169>

Original Research Article

Received 21 October 2018

Accepted 07 January 2019

Published 24 January 2019

ABSTRACT

The kidneys play a role in the maintenance of homeostasis by ensuring the excretion of waste and toxic substances from the body. Oxidative stress could be defined as an imbalance between the production of reactive oxygen species and an inability of the body system to scavenge the presence of free radicals. Intake of certain drugs and toxic substances exposes the kidneys to oxidative stress effects and this may lead to impairment of homeostasis and malfunctioning of the kidney. This study was carried out to access the efficacy in administration of single herbal extracts of either *Azadirachta indica* or *Spondias mombin* when compared to the combination of both herbal extracts in ameliorating the effects of oxidative stress in wistar rats kidney. The study was carried out using 25 male adult wistar rats of weight 180-200 g, the animals were randomly selected and

*Corresponding author: E-mail: ubahchidiebere7@gmail.com;

were designated into groups A (Negative control group that received Rat chow and water, group B is the positive control group that received the administration of 450 mg/kg body weight of zidovudine drug, group C is the *A. indica* group that received 450 mg weight of zidovudine drug and 500 mg/kg body weight of methanolic leaf extract /kg body, group D is the *S. mombin* group that received 450 mg/kg body weight of zidovudine drug and 500mg/kg body weight of methanolic leaf extract and group E received a 450 mg/kg body weight of zidovudine and a combination of 500 mg/kg body weight of both methanolic leaf extracts of *A. indica* and *S. mombin* leaf. T administration was carried out once a day using orogastric tube for a period 21 days. At the end of the admnistration, the rats were sacrificed using chloroform inhalation technique and the kidney was fixed in 10% neutral buffered formal saline. Light mcroscopic evaluation of the kidney showed normal histological appearance of the kidney in group A as witnessed by the presence of glomerulus, proximal convoluted tubule (PCT), distal convoluted tubule (DCT), bowmans space (BS), while group B witnessed alterations in the histology of the liver as shown by the presence of haemorrhage in the glomerulus, shrinkage in the proximal and distal convoluted tubule and shrinkage of the bowmans space, group C and D witnesseed a restoration of the kidneys histology as evidenced by a reduction of haemorrhage in the glomerulus and shrinkage PCT and DCT. Group E showed an enlargement of the Bowmans space and shrinkage of the PCT and DCT. Hence the results proved the efficacy of single administration of herbal extracts in ameliorating the effects of oxidative stress when compared with the combination of the herbal extracts.

Keywords: *Azadirachta indica*; *Spondias mombin* leaf; kidneys; Zidovudine.

1. INTRODUCTION

Medicinal plants are considered as healthy sources for the prevention of various oxidative stress related diseases [1], this is because they are rich in certain phytochemical constituents having anti-oxidative activities such as phenolic compounds and carotenoids [2]. Medicinal plants derived anti-oxidants can protect renal damage through reduction of lipid peroxidation and an increase in the levels of anti-oxidants. [3]. Various sections and traditions make use of native substances as lone herbs, join of plants and union of herbs. Combination of herbs could lead to complications as numerous associations can happen within the person constituent. Complications may arise because of numerous constituent in the native extracts. [4]. However the impacts from plant-plant association are likely uncertain and complex [5], [6], [7], [8], [9], [10], [11], [13], [14]. Oxidative stress can be defined as a disproportion among the system display of active kind air and a functional body capacity to remove the active intermediate or to restore the outcome injury [15]. It is caused when the existence of liberal substance overwhelms the free scavenging mechanism of antioxidants [16]. Oxidative stress is also an important factor which can contribute to kidney damage by increasing the production of oxidants, especially insufficiency of antioxidants defense system [17]. Oxidative stress induced damage on the kidney is associated with an

increase in the production of reactive oxygen species [18].

The kidneys are paired bean shaped organs located on the posterior abdominal cavity [19]. It functions in the maintenance of homeostasis through the excretion of metabolic waste products, regulation of extracellular volume, as well as regulation of electrolyte composition and acid base balance [20]. Exposure of the kidney to several drugs, toxic xenobiotics, or chemicals can cause toxic damage to the kidney due to its high rate of blood flow [1]. *A. indica* (neem tree) is a native plant of South eastern Asia, and it is distributed in India and other neighboring countries [22]. It is called dogonyaro in Hausa, and Ogwuakuma in Igbo [23]. *A. indica* plays therapeutic role in the management of health due to the presence of rich source of various types of ingredients. Most important active chemical components of *A. indica* is azadirachtin, nimbolin, nimbin, nimbol, sodium nimbinate, gedunin, salannin and quercetin [24]. *A. indica* is rich in phytochemical constituents like azadirachtin, nimbolide and ascorbate which possess significant antioxidant properties, that enables it to scavenge free radicals present in the body [25].

S. mombin belongs to the family *Anarcadiaceae*, and it is one of the medicinal herbs in southern Nigeria [26]. It has several names; it is termed english in plum hog, Yoruba akika, tsardamaster in Hausa, Chabbuh in Fulani and nuskakara in

Efik [27]. *Spondias* also possess anthelmintic, antioxidant, antimicrobial and anti-inflammatory actions, sedative and anxiolytic potentials [25, 26,27,28,29,30]. Therefore, this study was carried to evaluate the effects of oxidative stress on the histology of the kidney of adult male Wistar rats to compare the impacts of single administration of herbal extracts with the combination of herbal extracts in ameliorating the effects of oxidative stress.

2. MATERIALS AND METHODS

The leaves *A. indica* and *S. mombin* were obtained from a local community in Ugep, Yakurr local Government Area of Cross River State, Nigeria. Taxonomical identification was conducted by a botanist in the Department of Botany University of Calabar, Calabar, Nigeria. Both leaves were grounded to powdered form and extracted by cold extraction method using methanol as the solvent for a period of 72 h with the aid of a Soxhlet apparatus. The extract obtained was filtered through Whatman paper 1 and the filtrate was evaporated to dryness on rotary evaporator at (50°C). The extracts were preserved in clean glass container for further use.

2.1 Animals

This study was approved by the Department Ethics Committee of the University of Calabar, Calabar. Twenty-five male adult Wistar rats with an average weight of 200 g were bred in the animal house of the department of Anatomical Sciences and were used for this study. The rats were fed with rat chow, water ad libitum.

2.2 Experimental Protocol

This study was carried out using twenty-five male adult Wistar rats of average weight 200 g and there were randomly distributed into five sections (A, D, E, B, C, n=5).

Group A the Negative normal group that distilled water and rat chur, Group B is the Positive control group that was induced with 450 mg/kg body weight of zidovudine drug for a period of three weeks. Group C is the Experimental group that was induced with 450 mg/kg body weight of zidovudine drug for a period of one week and received 500 mg/kg body weight of *A. indica* for a period of two weeks. Group D represents Experimental group that was induced with 450 mg/kg body weight of zidovudine drug for a

period of one week and received 500 mg/kg body weight of *S. mombin* for a period of two weeks. While Group E Experimental group received 450 mg/kg body weight of zidovudine drug for one week and 500 mg/kg body weight of *A. indica* and *S. mombin* for a period of two weeks. At the end of the administration, the animals were anaesthetized using chloroform inhalation technique.

2.3 Stress Induction

Oxidative stress was induced using Zidovudine obtained from the Plan President Emergency for Aids and liberation section, Teaching University of Calabar Hospital, Calabar town, Cross-River State, Nigeria.

The animals in all the experimental faction collected 450 mg/kg body weight of the zidovudine. The drug was dissolved in 150 ml of distilled water and administered once daily to group C, D, and E for a period of seven days, while group B received the drug for a period of three weeks.

2.4 Determination of Body Weights of Experimental Animals

The final weights of the animals were recorded a day after the last dose of administration.

2.6 Collection of Experimental Specimen

At the end of the administration, the animals were anaesthetized using chloroform inhalation technique. The abdomen was dissected out to access the kidney which was located on top of each Adrenal gland.

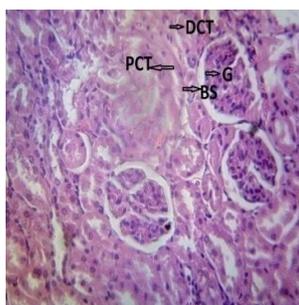
3. RESULTS

3.1 Histological Observation of the Kidney

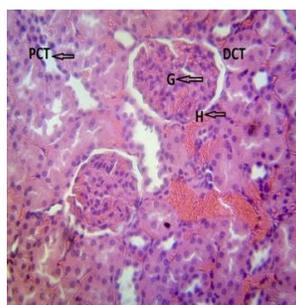
The photomicrograph below shows the histological observation of the kidney when exposed to zidovudine oxidative stress effects.

4. DISCUSSION

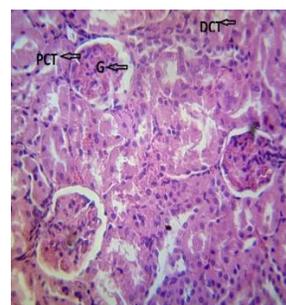
This study was carried out to assess the effect of zidovudine drug on the histology of the rat kidney in other to compare the efficacy of single administration of methanolic extracts of *A. indica* or *S. mombin* to a combination of both herbal extracts.



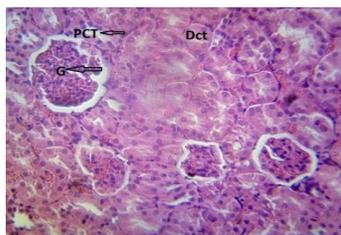
Photomicrograph of normal histology of negative control kidney group showing the presence of the glomerulus (G), distal convoluted tubule (DCT), proximal convoluted tubule (PCT), and bowmans space (BS).H and E ×400.



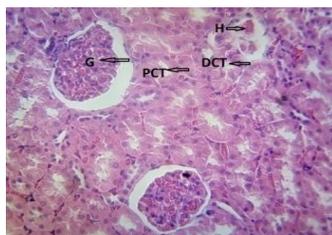
Photomicrograph of positive control group of Rat kidney showing the presence of haemorrhage, shrinkage of distal and proximal convoluted tubule and shrinkage of bowmans space (BS). H and E ×400.



Photomicrograph of *A. indica* group kidney group showing Presence of glomerulus, and a restoration of haemorrhage in the distal convoluted tubule (DCT), proximal convoluted tubule (PCT), with a normal bowmans space. H and E ×400.



Photomicrograph of *S. mombin* group showing Presence of glomerulus, and a restoration of haemorrhage in the distal convoluted tubule (DCT), proximal convoluted tubule (PCT), with a normal bowmans space. H and E ×400.



Photomicrograph of combined kidney group showing the presence of dilated bowmans space, with presence of shrinkage in the glomerulus, pct and dct of Rat kidney. (H&E ×400).

Exposure of the kidney to certain chemical agents or drugs could be manifested by the presence of vascular congestion (glomerulus), inflammatory cell infiltration with the presence of hyaline globule in the collecting tubule [31].

Light microscopic evaluation of the kidney showed normal histological appearance of the kidney in group A as witnessed by the presence of glomerulus, proximal convoluted tubule (PCT), distal convoluted tubule (DCT), and bowmans space (BS), while group B witnessed alterations in the histology of the liver as shown by the presence of haemorrhage in the glomerulus, shrinkage in the proximal and distal convoluted tubule and shrinkage of the bowmans space, group C and D witnessed a restoration of the Kidneys histology as evidenced by a reduction of haemorrhage in the glomerulus and shrinkage

PCT and DCT. Group E showed an enlargement of the Bowmans space and shrinkage of the PCT and DCT.

Results of group B and E is similar to the studies carried out by [32] which reported the presence of wider capsular space, congested glomerular tufts, and degeneration of the tubules when treated with cisplatin. Also results of group B,C,D and E is similar to works carried out by [33] on the ameliorative effect of pomegranate on the histopathology of the kidney of diabetic induced oxidative stress. The study revealed the presence of shrinkage and lesions in the bowmans capsule when exposed to oxidative stress, but intake of pomegranate herbal extract rich in anti-oxidants led to a reversal in histological changes of the kidney.

The restoration in the histology of group C, and D may be due to the presence of anti-oxidants present in the above herbal extracts, while the widening of the bowman space may be because of the drug on the kidney histology. Studies carried out by [34] showed that methanolic leaf extract of *A. indica* can ameliorate the effects of oxidative stress on the kidney. This may be due to its antinephrotoxic potential.

5. CONCLUSION

The results of the study proved that single administration *A. indica* and *S. mombin* ameliorated the effects of oxidative stress on the kidney histology of male Wistar rats when compared with the combination of both herbal extracts in ameliorating the effects of oxidative stress on the kidney. The effects of combined herbal therapy could not be compared with the single administration of the herbs, this may be due to interaction between the phytochemical components of both herbal extracts.

ETHICAL APPROVAL

This study was approved by the Department Ethics Committee of the University of Calabar, Calabar.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Rafien-kopaie M, Baradaran A. Plants anti-oxidants from laboratory to clinic. *Journal of Nephropathology*. 2013;152-153.
- Huang WY, Cai YZ, Corke H, Sun M. Survey of anti-oxidants capacity and nutritional quality of selected edible and medicinal plants in Hong kong. *Journal Food Campos Anal*. 2011;510-517.
- Khan MR, Siddiqui S, Parveen K, Javed S, Diwakar S, Siddiqui WA. Nephroprotective action of tocotrienol-rich fraction (TRF) from palm oil against potassium dichromate induced acute renal injury in Rats. *Chem Biology Interac*. 2011;186: 228-238.
- Wills RB, Bone K, Morgan M. Herbal products: Active constituents, modes of action and quality control. *Nutritional Research Review*. 2000;13:47-77.
- Chen XW, Sneed KB, Pan SY, Cao C, Kanwar JR, Chew H, Zhou SF. Herb-herb interactions and mechanistic and clinical considerations. *Current Drug Metabolism*. 2012;13:640-651.
- Colalto C. Herbal interactions on absorption of drug: Mechanism of action and clinical risk assessment. *Pharmacology Research*. 2010;62:207-227.
- Fasinu PS, Bowic PJ, Rosen KB. An overview of the evidence and mechanism of herb drug interactions. *Frontiers in Pharmacology*. 2012;3:69.
- Gurley BJ, Fifer EK, Gardner Z. Pharmacokinetic herb-drug interactions (part 2): Drug interactions involving popular botanical dietary supplements and their clinical. *Planta Medica*. 2012;78:1490-1541.
- Gurley BJ. Pharmacokinetic herb drug interactions (part 1): Origins, mechanisms, impact of botanical dietary supplements. *Planta Medica*. 2012;78:1478-1489.
- Hermann R, VonRichter O. Clinical evidence of herbal drugs as perpetrators of pharmacokinetic drug interactions. *Planta Medica*. 2012;78:1458-1477.
- Izzo AA. Interactions between herbs and conventional drugs: Overview of the clinical data. *Medical Principles and Practice*. 2012;21:404-425.
- Delima T, Vierira M, Huang AM. Botanical drug interactions: A scientific perspective. *Planta SMedica*. 2012;78:1400-1415.
- Zhou LM, Zuo Z, Chow MS, Danshen S. An overview of its chemistry, pharmacology, pharmacokinetic and clinical use. *Journal of Clinical Pharmacology*. 2005;45:1345-1359.
- Chandra K, Syed SA, Abid M, Sweetey R, Najam AK. Protection against FIA induced Oxidative stress induced DNA damage as a model of arthritis and invitro anti-arthritis potential of *Costus speciosus* Rhizome extract. *International Journal of Pharmacology & Phytochemical Reseach*. 2015;7(2):383-389.
- Halliwell B. Oxidative stress and neuro-degeneration where are we now. *Journal of Neurochemistry*. 2006;97:1634-1658.
- Grasseli E, Compalati AD, Voci A, Vecchione G, Ragazzoni M, Gallo G, Bomo P, Sumberaz A, Testino G, Vergani V. Altered oxidative stress/ antioxidant status in blood of alcoholic subjects is associated with alcohol Liver disease.

- Journal of Alcohol Dependence. 2014; 143:112-119.
17. Nasri H. World kidney day. Acute kidney injury; A public health awareness. Iran Journal of Public Health. 2013;42:338-340.
 18. Ghule AE, Jadhav SS, Bodhankar SL. Renoprotective effect of *Linum usitatissimum* seeds through haemodynamic changes and conservation of anti-oxidant enzymes in renal ischaemia-reperfusion injury in Rats. Arabian Journal of Urology. 2011;9:215-221.
 19. Cotran RSS, Kumar V, Fausto NR, Stanley L, Abbas AK. Robbins and cotran pathologic basis of disease. St Louis, Mo; Elsevier Saunders; 2005.
 20. William JB, Linda MB. Colour atlas of veterinary histology. 2nd Ed. Lippincott Williams and Wilkins; 2000.
 21. Pfaller W, Gstraunthaler G. Nephrotoxicity *In Vitro*. What we know and what we need to know. Environmental Health Perspective. 1998;2:559-569.
 22. Kumar VS, Navaratnam V. Neem (*Azadirachta indica*): Prehistory contemporary medicinal uses to human kind. Asia Pacific Journal of Biomedical Science. 2013;3:505-514.
 23. Ahmed S, Bamofrey M, Munsh A. Cultivation of neem (*Azadirachta indica*) in South Arabia. Economic Botany. 1989;45:35-38.
 24. Hossain MA, Shah MD, Sakari M. Gas chromatography-mass spectrometry analysis of various organic extracts of *Merremia borneensis* from sabah. Asian Pacific Journal of Tropical Medicine. 2011;4(8):637-641.
 25. Hossain MA, AL-toubi WAS, Weli AM, AL-Riyami OA, Al-sabahi JN. Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. Journal of Taibah University for Science. 2013;7(4):181-188.
 26. Aiyeloja AA, Bello OA. Ethnobotanical potentials of plants in Nigeria. A case study of Enugu State. Educational Research and Review Science International Journal. 2006;1(1):16-22.
 27. Gill S. Ethnomedicinal use of plants in Nigeria. Uniben Press Nigeria. 1992;222-223
 28. Urugulaga L, Laghton F. Plant polyphenol anti-oxidants and oxidative stress. Biological Research Journal. 2001;33:159-165.
 29. Ademola IO, Fagbemi BO, Idowu SO. Anthelmintic activity of extracts of *Spondias mombin* against gastrointestinal nematodes of sheep. 2005;235.
 30. Kramer A, Mosquera E, Ruiz J, Rodriguez E. Ethnobotany and biological activity of plants utilized during pregnancy and child birth in the Peruvian amazon. Emanations from the rainforest and the Carribean. 2002;4.
 31. Eroschenko VP. Atlas of histology with functional correlations (9th Ed.) Williams and Wilkins. Lippincott. 2000;12.
 32. Azu OO, Francis IOD, Abraham AO, Crescie CN, Stephen OE. Protective agent *Kigelia Africana* fruit extract against cisplatin-induced kidney oxidant injury in spraguep Dawley Rats. Asian Journal of Pharmaceutical Clinical Research. 2010;3:84-88.
 33. Aboonabi A, Rahmat A, Othman F. Effects of pomegranate on histopathology of liver and kidney on generated oxidative stress diabetic induced Rats. Journal Cytology & Histology. 2014;6:294.
 34. Dhar R, Dawar H, Garg S, Basir SF, Talwar GP. Effect of volatiles from neem and other natural products on gonotrophic cycle and oviposition of *Anopheles stephensi* and anclici facies (*Diptera Culicidae*). Journal of Medical Entymology. 1996;33(2):195-201.

© 2019 Ubah et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle3.com/review-history/46169>