International Journal of Pharmaceutical Research and Innovation, Vol. 5: 1-6, 2012

Effect of aqueous stem bark extract of *Ziziphus mucronata* on rat kidney functional status after ten days daily treatment

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ABSTRACT

The side effects commonly encountered with the use of peripheral analgesics/anti-inflammatory agents like non steroidal anti-inflammatory drugs currently available have been that of gastrointestinal tract, kidney and liver damage. Quite often, herbal remedies are indicated by herbalists for the management of pains associated with acute medical illnesses and these also exhibit similar side effects. Here the effect of the aqueous stem bark extract of *Ziziphus mucronata* (plant with demonstrated analgesic activity) on kidney function is investigated to ascertain its safety. The LD_{50} was determined and based on that three test doses (1000mg /kg, 500mg/kg and 250mg/kg) of the extract were administered orally to three groups of rats (six rats per group) daily for ten days to carry out kidney function test. The control group was administered with distilled water which served as vehicle in preparing the extract. On the 10^{th} day, blood samples were collected and serum separated for the test. Flame photometric method was used for sodium and potassium determinations, jaffe reaction, titration, diacetyl monoxime and titration method for creatinine, bicarbonate, urea and chloride determination respectively.

Results obtained revealed LD_{50} of the extract to be greater than 5000mg/kg. Also the administration of all test doses showed no significant difference ($p > 0.05$) between the test and control groups for serum sodium, potassium, chloride, bicarbonate, urea and creatinine concentrations. This study has therefore shown to some extent that aqueous stem bark extract of *Ziziphus mucronata* may not have significant effect on the rat kidney function after ten days daily treatment. However histological studies are needed to authenticate this claim.

Key words: *Ziziphus mucronata,* rat kidney function, sodium, potassium, chloride, bicarbonate, urea and creatinine.

INTRODUCTION

Pain is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage;or described in terms of such damage"[1]. Pain affects patients' quality of life and is considered a reliable index of injury. Every one has had experience with acute pain. Acute pain usually stops when tissue heals, however when pain persists, its connection to actual tissue injury becomes more tenuous and psychological factors may begin to predominate[2]. Acute pain can be defined as pain that is caused by noxious stimulation due to injury, a disease process, or the abnormal function of muscle or viscera. The most common forms of acute pain include post traumatic, post operative, and obstetric pain as well as pain associated with acute medical illnesses. Most forms of acute pain are self limited or resolve with treatment in a few days or weeks[3].

Quite often, herbal remedies are indicated by herbalists for the management of pains associated with acute medical illnesses. Recently there has been intensive researches into medicinal plants with possible analgesic/anti-inflammatory effects in quest for alternative source. Significant number of such plants have been identified without much work done on their nephrotoxic effects[4,5,6,7]. The side effects commonly encountered with the use of peripheral analgesics/anti-inflammatory agents like non steroidal anti-inflammatory drugs currently available have been that of gastrointestinal tract, kidney and liver damage[8,9,10,11]. This has constituted a drawback to their clinical use. Now that there is clamour for

either co-recognition of herbal and orthodox medical practices as done in India or co-integration as practiced by China, there is need to ascertain the safety profile of these herbal medicines(ethnopharmacovigillance).

Ziziphus mucronata willd,(Rhamnaceae),is a semi-deciduous plant which has a strong spines, paired with one straight and one curved. It grows in Northern Nigeria where it is known as "*magarya kura*"(Hausa) and part of Southern Nigeria where it is called "*Eka nase adie*"(Yoruba).The plant is used ethno medicinally for the treatment of septic swelling of the skin, for lumbago, and pains especially toothache[12]. Major constituents of the plant have been investigated and reported by many authors[13].The stem bark yielded 4% tannins[14].

In our previous works our team of researchers has investigated and demonstrated anti-nociceptive and anti-inflammatory activities of this plant[4,5].Here the effect of the aqueous stem bark extract of *Ziziphus mucronata* on kidney function is investigated to ascertain in part its safety as an alternative analgesic.

MATERIALS AND METHODS

Plant Materials

Ziziphus mucronata stem bark was collected from Zaria, Kaduna state, Nigeria.The plant was authenthicated by a taxonomist, Mall. U.S. Gallah of the herbarial section, Department of Biological Sciences,Faculty of Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen (No. 1622) was kept in the Department's Herbarium for future reference.

Preparation of Extract

The stem was carefully washed, removed, cut into pieces, air-dried and powdered using a pestle and mortar. The powdered material(100g) was cold macerated in distilled water(200ml) with occasional shaking for 24 hours and then filtered and evaporated on a water bath at 40^0C to dryness.

Animal

Wistar rats (180-210g) of either sex were used. The animals were obtained from Animal House of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. They were housed and fed with standard diet and water ad libitum. The ethical matters as they relate to the use of experimental animals in accordance with "Principle of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985) were followed throughout the various studies conducted. The experimentation protocol followed was approved by the Department of Pharmacology and Therapeutics Animal Ethical Committee, Ahmadu Bello University Zaria, Nigeria.

Acute toxicity studies

The extract was administered to rats orally and the LD_{50} was estimated in rats using method of Lork[15]. Three groups of 3 rats were treated with the aqueous extract of the plant at doses 10,100,1000mg/kg body weight orally and observed for 24 hours. In the second phase, 3 groups of one rat each were administered orally with the extract at specific doses of 1600,2900 and 5000mg/kg. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the rat survived.

Ten days toxicity studies

Three test doses of the extract within 20% range of the LD_{50} were administered orally to three groups of rats (six rats per group).Group 1,2 and 3 received 1000mg /kg, 500mg/kg and 250mg/kg of the extract respectively, daily for ten days. The control group was administered distilled water which served as vehicle in preparing the extract. On the 10^{th} day, 4.0ml of blood samples were collected into plain containers by cardiac puncture under ether anesthesia from each

rat. The blood samples were allowed to clot for two hours, centrifuged at 4000 revolution per minute (rpm) for 10minutes and serum separated for kidney function tests.

Kidney function tests

Serum sodium and potassium levels were determined by flame photometry method, chloride, bicarbonate, urea and creatinine levels were determined according to methods used by Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria [16,17].

Determination of sodium and potassium

0.1ml of the serum was added into universal bottle and 9.9ml of distilled water added. 5ml of working standard and 5ml of distilled water added to separate universal bottles respectively. The test bottles were capped with Para film and mixed by inversion.

To determine sodium the following procedure was followed. Sodium filter was adjusted to 590nm and the galvanometer was switched on. The gas supply was fully turned on and the flame ignited. The air pressure was regulated to 10 lb/sq inch. The gas was adjusted smoothly to obtain discrete cones of flame. The galvanometer reading was then set at zero using the working standard and reset at zero. The test was read, checking the standard after 2-3 test readings. The amount of sodium in mmol/L = galvanometer reading x 2.0.

In the determination of potassium, the potassium light filter was adjusted to 770nm.The galvanometer set with potassium working standard to 70 and the procedure continued as for sodium. The amount of potassium mm/L = galvanometer reading x 0.1.

Determination of chloride

Titration method was used. 0.2ml of serum was added to 1.8ml of distilled water. 3drops of di-phenylcarbazone indicator was added and mixed. This was titrated with mercuric nitrate to violet-blue coloured end point. The percentage chloride was calculated by comparing the volume of test sample required to volume required of standard sample.

Determination of Bicarbonate

Titration method was used also. 200ul of the serum sample was added to 2.0ml of de-ionized water, followed by addition of 2.0ml of 0.01N hydrochloric acid and one drop of neutral red indicator. Then 0.01N sodium hydroxide solution was titrated against this solution with mixing till the pink colour changed to light orange(end point). The titre was subtracted from the volume of hydrochloric acid added to get the volume of acid used by the bicarbonate. A bicarbonate standard containing 25mmol/L was treated in the same manner. The concentration of bicarbonate in the samples was calculated using the formula, T/S x concentration of standard. “T” being titre of the test while “S”is the titre of the standard.

Determination of Urea

The test sample was prepared by adding 0.1ml of serum to 10ml of distilled water. 2ml of mixed colour reagent (0.02g/ml diacetylmonoxime and 0.005g/ml thio-semicarbazide) and of mixed acid(0.02g/ml ferric chloride in 85% phosphoric acid and 0.43%sulphuric acid) each was added. This was thoroughly mixed and incubated at 100^0C for 20 minutes, cooled and the resulting red mixture was read at 520nm.The urea level in mmol/L was calculated by comparing test sample to standard sample.

Determination of Creatinine

The test sample was prepared by adding 1ml of serum to 3ml of distilled water. 1ml of 10% sodium tungstate and 1ml 2/3N sulphuric acid were added mixed well centrifuged for 10 minutes. 3ml of the supernatant was added to 0.75 N sodium hydroxide, 1ml of picric acid was added mixed and allowed to stand for 15minutes(Jaffe’s reaction). The resulting red colour was read at 520nm. The creatinine level in µmol/L was calculated by comparing test sample to standard sample.

Statistical Analysis

All data were expressed as Mean±SEM. Stastical analysis was carried out using the student’s t-test and the differences considered significant when $p<0.05$.

RESULTS

Preparation of Extract

The powdered plant material cold macerated in distilled water gave a yield of 9.41% dry weight of the extract.

Acute toxicity studies

The LD_{50} of the extract was calculated to be greater than 5000mg/kg body weight. No physical symptoms of toxicity was observed during the acute toxicity studies.

Ten days toxicity studies

The administration of the extract to the rats at different dose levels for ten days showed no significant difference ($p> 0.05$) between the test and control groups for sodium, potassium, chloride, bicarbonte, urea and creatinine serum levels as shown in table 1.

DISCUSSION

The oral LD_{50} of the aqueous stem bark of Z*iziphus mucronata* was found to be greater than 5000mg/kg suggesting safety of the plant in accordance with the recommendation of Organisation for Economic Co-operation and Development(OECD). In addition, no physical symptoms of toxicity and death were noticed. The chemical labeling and classification of acute systemic toxicity based on oral LD_{50} values recommended by the Organisation for Economic Co-operation and Development(OECD)[18] are as follow : very toxic ≤ 5mg/kg, toxic > 5 ≤ 50mg/kg, harmful >50 ≤500mg/kg and no label >500 ≤ 2000mg/kg. However LD_{50} has not been regarded as a biological constant because it is affected by many variables such as animal species and strain, age, gender, diet, bedding., ambient temperature and time of the day. LD_{50} therefore serves to give an idea about the safety of drugs.

From the result of the kidney function tests, all the parameters analysed; sodium, potassium, chloride, bicarbonate, urea and creatinine, showed no significant change (*p>0.05*) in serum levels of the treated rats at the doses tested compared to the controls. The urea and creatinine usually determine the general function of the kidney while the electrolytes are determinants of the tubular function. These parameters: urea and creatinine which were analysed in these rats were not statistically different from the control groups, suggesting that the aqueous stem bark extract of *Ziziphus mucronata* is not nephrotoxic in rats within the period of the study. Creatinine is derived from creatine and phosphocreatine, a major contituent of muscle mass. Once creatinine is released into plasma, it is excreted by the kidney almost exclusively by glomerular filtration. A decrease in glomerular filteration rate as in renal dysfunction would result in an increase serum creatinine concentration. Therefore serum creatinine is a reliable index of renal dysfunction. The serum urea level follows similar pattern, but may be affected by some factors, such as increase protein catabolism and hydration status. Changes in serum sodium, potassium, chloride and bicarbonate concentrations may indicate renal tubular dysfunction. In this study all serum levels of sodium, potassium, creatinine, bicarbonate, urea and chloride in the treated groups do not differ significantly from the control groups.

In contrast, the peripheral analgesics currently in use may act in various ways to bring about renal damage.The damage could be due to principal pharmacological action ie inhibition of prostaglandin biosynthesis. This action may result in acute ischaemic renal failure, sodium and water retention(leading to or exacerbating hypertension and/or heart failure).There could be hyporeninaemia and hypoaldosteronism which could lead to hyperkalaemia which may worsen the cardiac status. On the other hand the effect on the kidney may not be related to principal pharmacological action as in the case of renal failure and proteinuria but through other mechanisms .The action may be unknown whether

Table 1. Kidney Function Test Results obtained from Rats Serum treated daily with Aqueous Stem Bark Extract of *Ziziphus mucronata* at Different Doses for ten days.

Dose (mg/kg)	Sodium level (mmol/L)	Potassium level (mmol/L)	Chloride level (mmol/L)	Bicarbonate Level (mmol/L)	Urea Level (mmol/L)	Creatinine Level (µmol/L)
Control	144.00 ±9.39	6.03±0.54	94.33±5.42	35.33 ±2.11	4.57±1.96	89.83 ±10.40
250	154.00 ±3.61	7.67 ±0.42	102.67±2.23	35.67 ±1.97	5.57 ±2.60	89.83 ±10.40
500	148.00 ±3.63	6.63±0.30	96.00 ±3.42	38.67±1.33	5.57 ±1.55	68.83 ±7.25
1000	139.66± 4.36	6.38± 0.48	93.5± 5.55	38.67± 1.33	5.75± 4.45	93.83± 8.18

Values are Mean ± SEM, n = 6

or not related to principal pharmacological action as in papillary necrosis and chronic renal failure[19]. In addition to the above mechanism of renal damage, analgesics may accumulate in the renal cortex. These drugs are ultra filtered in the glomeruli and then undergo back diffusion in the proximal tubules and recycling via the blood back to the glomeruli. This recycling event may lead to kidney damage[20]. Analgesics may covalently modify biomolecules in the kidney[21].Studies on the aqueous stem bark extract of *Ziziphus mucronata* we earlier conducted, produced good analgesia and anti-inflammatory effect ,that were postulated to be through prostaglandin inhibition. However in this study, the extract may not be having inhibitory effect on kidney prostaglandins or the plant may be containing kidney protecting substances.

Researches into medicinal plants could be viewed from two perspectives. First, medicinal plants have been useful in the development of new drugs and continue to play an invaluable role in drug discovery processes[22,23]. Secondly researches into medicinal plants may be designed to establish scientific bases for their use in herbal medicine in order to either co-integrate herbal medicine with orthodox medicine or co-recognize the two practices. These herbs/plants are relatively cheap and available and their uses are dependent on ancestral experience[24]. The majority of the population in the developing countries remain dependent on them for healthcare[25]. Irrespective of the perspective, the safety profile of these plants will justify their ultimate use.

This study has revealed in part that the aqueous extract of stem bark of *Ziziphus mucronata* might be safe in terms of renal side effects (effects limiting the use of currently available peripheral analgesics),when used for short period of time. However histological studies, chronic toxicity studies and studies on other organs that are affected by peripheral analgesics such as gastrointestinal tract and liver would need to be conducted before clinical trials in human subjects is advocated.

CONCLUSION

This study has shown that the aqueous stem bark extract of *Ziziphus mucronata* has no effect on rat kidney function when used for short period of time at the doses tested. Studies on other organs such as gastrointestinal tract and liver in animals and evaluation in human subjects is required to authenticate this finding.

ACKNOWLEDGEMENT

This work was supported by Ahmadu Bello University, Zaria, Macarthur Post Doctoral Grant.

The authors acknowledge with thanks the assistant of Mal Ibrahim Adamu, Mr John Kono and Alh. Ya'u Dari of the technical section of Department of Pharmacology and Therapeutics, A. B. U. ,Zaria. We also gratefully acknowledge the assistance of Chief Medical Laboratory Scientist of Department of Chemical Pathology A.B.U.Teachihg Hospital,Zaria, Mr M.E.Ekanem.

REFERENCE

1.International Association for the study of pain subcommittee on Taxonomy of pain terms,1979 :A list with definition and notes on usage. Pain.6:249-252.

2.Sheik,T.L, Muktar, H.M., Odigie, V.I., 2007. An overview of the basis of pain. Book of Proceedings, Society for the Study of Pain, Nigeria, Seminar on pain management and rational use of opioids,1(1):3-5.

3.Nwasor, E.O.,Danbaba, M.A., Ogunride, G.O.,Onalo, R., Okoh, E.O. and Abubakar, U., 2007. Acute and chronic pain management.. Book of Proceedings, Society for the Study of Pain, Nigeria, Seminar on pain management and rational use of opioids,1(1):7-28.

4.Danjuma, N. M., Zezi A U., Abdulrahman, E. M. and Isah, H., 2006. Acute and chronic anti-inflammatory activity of the aqueous stem-bark extract of *Ziziphus mucronata* WILLD, Biological and Environmental Sciences Journal for the Tropics (BEST), 3(2) : 7-9.

5.Danjuma, N. M., Zezi, A. U., Abdulrahman, E. M., Maiha, B. B. Abdu-Nasir, S. and Jegede, O. M., 2007. Evaluation of the anti-nociceptive activity of the aqueous stem bark extract of *Ziziphus mucronata* Willd (Rhamnaceae), Nigerian Journal of Pharmaceutical Research, 6(1):6-8

6.Yaro, A. H., Anuka, A. J.,Salawu, O.A.and Zezi, A.U., 2008. Evaluation of analgesic and anti-inflammatory effects of methanolic extract of *Chrysanthellum indicum* Linn VATKE in rodents, Biological and Environmental Sciences Journal for the Tropics (BEST), 5(2) :56-61

7.Adeyemi, O.O.,Okpo S.O. and Onakade, A.A., 2005. Ant-inflammatory activity of the methanolic extract of *Acanthus montanus.* West Afr. J. of Pharmacology and Drug Research, 21(1&2) :13-17.

8.Zezi, A. U., Abdu-Aguye, I., Anuka, A. J., Danjuma N. M. and Yaro A. H., 2007.Comparative effect of paracetamol with paracetamol-caffeine combination on the functional integrity of the rat kidney during ten days daily treatment, Nigerian Journal of Pharmaceutical Research, 6(1) :101-106

9.Zezi, A. U. Danjuma, N. M. Abdu-Aguye, I., Maiha, B. B.and Yaro, H. A., 2007. Preliminary comparative effect of paracetamol with paracetamol-diclofenac combination on the functional integrity of the rat kidney during ten days daily treatment, Nigerian Journal of Pharmaceutical Sciences, 6(2) : 42-49

10.Zezi, A.U. Abdu-Aguye, I. Rafindadi, A. H. Ahmed, S.A. Anuka, J.A. and Danjuma, N.M., 2007.Comparative effect of paracetamol with paracetamol-diclofenac combination on the structural integrity of the rat kidney during 10 days daily drug treatment, Nigerian Journal of Pharmaceutical Sciences, 6(2):50-54

11.Sandler, D. P., Smith, J. C., Weinberg, C. R., Bucka Low, V., M. Jr.,Dennis, V. W., Blythe, W. B., and Burgess, W. P.,1989. Analgesic use and chronic renal disease. N. Engl. J. Med., 320:1238-1243.

12.Dalziel, J. M.,1937.The Useful Plants of West Tropical Africa. Crown overseas agents for the colonies, London pp:.300.

13.Prsonal Communication: Natural product alert (NAPRALERT) data base, the University of Illinois, Chicago U.S. A.

14.Oliver, B.,1960. Medicinal Plants in Nigeria. Published as a private edition by the Nigeria College of Arts, Science and Technology pp:44.

15.Lork, D.,1983. A new approach to practical acute toxicity testing. Archives of Toxicology, 54:275-287.

16. Kaplan, L. A. and Pesce, A. J., 1989. Clinical Chemistry, Theory, Analysis and Correlation, 2nd Edition, Mosby, pp 346 – 356.

17.Edmund, L. David, J.N. and Christopher, P.P., 2006. Kidney Function Tests. In : Carl, A.B.,Edward, R. A. and David, E.B.(Eds.),Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th Edition, Elsevier Saunders, St. L ouis, Missouri, pp 797-835.

18.Walum, E., 1998.Acute oral toxicity. Environ.Health Perspect.106:497-503.

19.Murray, M. D., and Brater, D. C., 1993. Renal toxicity of the nonsteroidal anti-inflammatory drugs. Ann. Rev. Pharmacol. Toxicol., 33: 435-465.

20. Beyer, K. H. and Gelarden, R. T. , 1984. Biochemical effects of salicylates: Factors influencing drug distribution and actions. In: Rainsford, K.D., Aspirin and the salicylates, Butterworths, London, pp. 229.

21.Nery, R., 1984. Biochemical effects of salicylates: Factors influencing drug distribution and actions. In: Rainsford, K. D., Aspirin and the salicylates, Butterworths, London, pp. 232

22.Fornsworth,N. R., 1994.Ethnopharmacology and drug development. In: Ethnobotany and the Search for New Drugs, Ciba Foundation Symposium 185, Prnce,G.T. and J. Marsh(Eds).John Wiey and Sons,Chichester,pp:42-59.

23.Cragg, G.M., Newman,D.J. and Snader,K.M., 1997. National products in drug discovery and development. J. Nat. Prod.,60:52-60

24.Marin-Bettulo, G. B.,1980. Present aspects of the use of medicinal plants in traditional medicine. J. Ethnopharmacology.,2:5-7.

25.Amos,S.E.,Kolawole,P. Akah,C. Wambebe and K. Gamaniel,2001. Behavourial effects of the aqueous extract of *Guiera senegalensis* in mice and rats. Phtomedicine,8:356-361.