



Scholars Research Library

Archives of Applied Science Research, 2013, 5 (5):198-201
(<http://scholarsresearchlibrary.com/archive.html>)



Toxic Effect of n-hexane Oil Extract of Two Spices on Rat Liver and Kidney Functions

Ogunka-Nnoka, C. U. and Bravil M.

Department of Chemistry (Biochemistry Unit), Rivers State University of Science and Technology, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.

ABSTRACT

Njasang (Ricinodendron heudelotii) and Ehuru (Monodora myristica) are used in some parts of Nigeria both as spices and in the treatment of various ailments. This study was conducted to investigate the toxic effect of n-hexane oil extract of these spices on rat liver and kidney functions. Twenty five albino rats were divided into five groups of five rats each. The mixed oil from the spices were administered alongside with olive oil in the ratio of 1:3ml, 1:2ml and 1:1ml (olive oil: oil extract) to rats in groups 1, 2 and 3 respectively. Group 4 rats received only 2ml olive oil and group 5 (control) received 0.9% normal saline daily for 28 days. The blood samples were collected via cardiac puncture and were assayed for serum enzyme activities (ALT, AST and ALP), Electrolytes (Na^+ , K^+ and Cl^-) and other biochemical (Urea, Creatinine, Total protein and Total Bilirubin). The result showed significant ($P < 0.05$) increase for ALT and AST at the various dose levels of the test samples compared with the control, while ALP increased only at a higher dose level (1:3ml). Results for serum electrolytes showed a dose-dependent increase in Na^+ and Cl^- concentrations, while K^+ decreased in the entire tested group. The effect of the various doses on serum Urea, Creatinine and Total Bilirubin increased significantly ($P < 0.05$) while Total Protein showed a non significant ($P > 0.05$) decrease at various dose levels. Olive oil treated group had a decreased effect in the concentrations of all the parameters studied except for Total Protein.

Keywords: Kidney, Liver, Njasang, Ehuru, Albino rat.

INTRODUCTION

Lipids which consist of fats and oils play important roles in the body, apart from imparting desirable taste and flavors to food, they are energy rich substances as well as carriers of fat soluble vitamins (1). Scientific evidence revealed that a diet rich in essential fatty acids help in the development of healthy brain, heart and immune system. However, fats and oils which contain more unsaturated fatty acids are particularly susceptible to oxidation. Intake of food containing oxidized lipid increases the concentration of secondary peroxidation products in the liver (2)

The liver and kidney are two major organs in the body. While the kidney regulates the amount of water and salts in the body, the liver is the centre of all metabolic activities in the body. Essential functions of these organs tend to be lost in the development of diseases (3). The damage of hepatocytes may lead to release of intercellular constituents into the plasma or circulation (4).

Plants are the major source of material used to combat different kinds of ailments. A number of components present in these plants are being explored either for their nutritional value or therapeutic effect. Njasang and Ehuru are widely used in Nigeria and other parts of Africa for these purposes.

Njasang (*Ricinodendron heudelotii*) is a dioecic plant having fruits which contain two cells in which the seeds lie (5). They serve as flavoring agents in dishes as well as thickeners. The proximate composition and nutritive value as well as the lipid profile had been studied (6, 7).

Ehuru (*Monodora myristica*) is a seed oil from the Annonaceae family. Widely used as spice and as a pain relief (8, 9). Literature has so far revealed the proximate composition, preliminary screening/Antibacterial activity and lipid profile (10, 11). The use of any plant for medicinal purposes requires safety of such plants and hence needs to be properly screened for their toxicity levels. The aim of this study therefore, is to investigate the toxic effect of n-hexane oil extract of njasang and ehuru on rat liver and kidney functions.

MATERIALS AND METHODS

Animal

A total of twenty-five wistar albino rats weighing between 120 to 160g was used for the study. The rats were obtained from the Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria. Animals were housed in cages of five groups with each cage containing five rats. They were fed with standard pellet diet and water *ad libitum*. The experiment was carried out in compliance with National Institute of Health (NIH) guidelines on care and use of laboratory animals.

Plant

Dry seeds of njasang (*Ricinodendron hendelotii*) and ehuru (*Monodora myristica*) were bought from mile 3 market in Port Harcourt, Rivers State, Nigeria. The seeds were cleaned, sundried, ground and sieved into fine powder. The sieved samples were stored in an air tight container until required for analysis.

Preparation of oil extract

Oil from the spices was obtained by soxhlet extraction (12). The oil sample recovered using rotary evaporator was stored in a sample bottle until required for use.

Experimental design

The rats which were divided into five groups of five rats each were administered with different concentration of the oil extract of njasang and ehuru mixed in equal proportions alongside with olive oil. Groups 1 – 3 received orally olive oil and oil extract mixture in the ratio of 1:3ml, 1:2ml and 1:1ml respectively; while group 4 received only 2ml olive oil and group 5 (control) received 0.9% normal saline. The various doses were administered orally for 28 days. Blood samples were collected from the animals into lithium heparin bottles under chloroform anesthesia via cardiac puncture. Blood samples were centrifuged and the sera were separated with sterilized sample bottle and kept in the refrigerator for biochemical assays.

Biochemical assay

Biochemical parameters studied include electrolytes (Na^+ , K^+ and Cl^-), serum enzymes such as Alanineaminotransferase (ALT), Aspartateaminotransferase (AST) and Alkaline-phosphatase (ALP), Urea, Creatinine Total protein, Total bilirubin. Na^+ and K^+ were determined by flame photometry using Jenway P7 Flame photometer. Chloride ion, activities of serum enzymes were determined using diagnostic kits (Quinica Clinica Applicada S.A Spain) following Reitman and Frankel (13) method. Bilirubin, Creatine and Total protein were determined using Randox kit.

Statistical analysis

The results are expressed as mean \pm Standard deviation (SD). Analysis of variance was used to test for differences in the groups. Differences were considered to be statistically significant at $P < 0.05$ (14)

RESULTS

Table I: Serum Enzymes (μL)

Extract treatment (ml) (O.O: OEM)	Enzymes		
	ALT	AST	ALP
Group 1(1:3)	15.48 \pm 1.23 ^a	83.30 \pm 2.40 ^a	151.52 \pm 6.01 ^a
Group 2 (1:2)	14.08 \pm 0.81 ^a	82.12 \pm 3.00 ^a	143.21 \pm 2.32 ^c
Group 3 (1:1)	12.40 \pm 1.42 ^b	78.44 \pm 1.71 ^b	141.50 \pm 2.04 ^c
Group 4 (olive oil)	9.50 \pm 0.47 ^c	73.30 \pm 1.42 ^c	138.90 \pm 2.02 ^d
Group 5 (control)	11.03 \pm 1.02 ^b	76.50 \pm 2.80 ^b	145.71 \pm 2.61 ^b

Results are mean \pm SD from four determinations. Means in the same column not followed by the same superscript differ significantly ($P < 0.05$). O.O = olive oil, OEM = oil extract mixture

Table II: Serum Electrolytes

Extract treatment (ml) (O.O: OEM)	Electrolytes (mmol/L)		
	Na ⁺	K ⁺	Cl ⁻
Group 1(1:3)	132.00 ± 4.21 ^a	9.80 ± 0.0 ^a	116.21 ± 2.07 ^a
Group 2 (1:2)	122.60 ± 2.34 ^b	9.60 ± 2.10 ^a	109.50 ± 3.41 ^b
Group 3 (1:1)	120.51 ± 4.01 ^b	9.30 ± 1.04 ^a	108.44 ± 1.18 ^b
Group 4 (olive oil)	112.06 ± 1.41 ^c	7.00 ± 1.14 ^{ab}	105.00 ± 2.60 ^c
Group 5 (control)	113.50 ± 2.20 ^c	10.70 ± 2.41 ^a	108.02 ± 3.10 ^b

Results are mean ± SD from four determinations. Means in the column not followed by the same superscript differ significantly ($P < 0.05$)

Table III: Liver and Kidney functions

Extract treatment (ml) (0.0: OEM)	Parameter			
	Total Protein (μ L)	Total Bilirubin (mmol/L)	Creatinine (mmol/L)	Urea (mmol/L)
Group 1(1:3)	61.92 ± 3.40 ^a	10.50 ± 0.00 ^a	92.75 ± 4.0 ^a	5.50 ± 0.14 ^a
Group 2 (1:2)	62.10 ± 2.14 ^a	10.25 ± 0.14 ^a	83.50 ± 2.71 ^b	3.95 ± 0.00 ^b
Group 3 (1:3)	62.85 ± 2.04 ^a	9.31 ± 1.21 ^a	81.00 ± 2.12 ^c	3.43 ± 0.21 ^b
Group 4 (olive oil)	63.45 ± 3.13 ^a	8.00 ± 1.07 ^{ab}	79.60 ± 4.21 ^d	2.42 ± 0.92 ^b
Group 5 (control)	63.02 ± 1.62 ^a	9.25 ± 0.89 ^a	79.84 ± 3.14 ^d	2.53 ± 0.15 ^b

Results are mean ± SD from four determinations. Means in the same column not followed by the same superscript differ significantly ($P < 0.05$)

Table I shows the serum enzyme activities. ALT and AST increased with extract treatments. There was significant difference ($P < 0.05$) when compared with the control especially with groups 1 and 2 rats. Serum ALP decreased significantly with extract treatment for groups 2 and 3; while group 1 rats treated with 1:3 dose level showed significant ($P < 0.05$) increase in serum ALP level compared with the control. All the serum enzymes (ALT, AST and ALP) reduced significantly ($P < 0.05$) when rats were treated with olive oil.

Table II shows the serum electrolytes of the treated and control rats. The concentration of sodium ion (Na⁺) and chloride ion (Cl⁻) in the serum samples at various doses levels of the extract increased. The increase in both Na⁺ and Cl⁻ appears to be dose-dependent. The potassium ion (K⁺) concentration decreased in all the tested groups. The decrease was not significant ($P > 0.05$) compared with the control. However the group administered with olive oil had reduced values of these electrolytes compared with the control.

The effect of the various doses on serum total bilirubin, creatinine and urea increased significantly ($P < 0.05$) compared with the control as shown in Table III above. The increase appears to be dose-dependent. Total protein decreased at different dose levels, compared with the control. Olive oil group however showed non significant ($P > 0.05$) increase in total protein concentration when compared with the tested group and control.

DISCUSSION

The increase observed in serum enzyme activities especially ALT and AST is an obvious sign of hepatic injury. Such injury is associated with the leakage of cellular enzymes located in the cytosol into the blood stream as a result of the disturbances caused in the transport function of the hepatocytes. (15, 16) A number of factors may have contributed to the unusual increase in serum enzyme levels observed in this study. For instance, substances that affect the integrity of the membrane like saponin has been reported to lyse red blood cells by destroying the erythrocytes (17, 18). Also, some free radicals generated from the solvent used for extraction or environment may induce lipid peroxidation causing liver damage (2, 19). The results on serum enzyme activities of this present study agree with the reports of other authors who administered ethanol extract of sorghum bicolor leaf sheath, three sources of vegetable oils and root bark of extract of *vitex doniana* to rats in different studies (1,17,18).

Electrolytes are important component of the heart, nerve, muscles and also used to maintain voltages across their cell membranes as well as in the maintenance of pH (22). Both Na⁺ and Cl⁻ ions increased with increase in extract administration. These ions are interrelated as they diffuse easily across plasma membrane. The movements of the ions help to regulate osmotic pressure. Both Na⁺ and Cl⁻ are regulated by the kidney. Sodium ion is easily filtered in the glomerula portion of the kidney. The rate of excretion is directly affected by the rate of filtration of sodium ion in the glomerulus. Therefore, the increase in Na⁺ and Cl⁻ could be related to malfunction of the kidney or alteration in the activity of Na⁺ - K⁺ ATPase which is required in the movement of these electrolytes across the membrane and sometimes over activity of parathyroid gland can lead to increase in chloride ion concentration (23, 24). Potassium ion however, is the major cation of intracellular fluid. The decrease in K⁺ observed in this study may be related to the increase in sodium load in the tubules which enhances the excretion of potassium ion. The present study corroborates our previous study (20) as well as the reports of Abdulrahman *et. al.*, (21).

The kidney and liver status were further assessed by the determination of the levels of serum creatinine, urea, total protein and total bilirubin. The extract administration increased serum urea and creatinine. Urea is a waste product formed from the breakdown of proteins which should be excreted through urine, while creatinine is a breakdown product of creatinine which is an important part of muscle that should be excreted through the kidney also. A high serum level of urea and creatinine indicates that the kidneys are impaired or dehydrated. Although creatinine is a better maker of kidney functions than urea and other kidney functions such as glomerula filtrate rate and dissolved salts. Increased Na⁺ and Cl⁻ ions further supported the diseased state of the kidney. Other researchers (20, 21) also observed increased creatinine and urea concentration when rats were administered with the extract of *sorghum bicolor* and *vitex doniana*. Total protein and Total bilirubin further explains the liver status, Protein concentration decreased, while total bilirubin increased. Ahmed *et. al.*, (25) reported that any change in serum protein concentration indicates impairment in the normal function of the liver. In this case, there may be possible alteration in protein synthesis. Increase in total bilirubin level is only noticed in a serious liver cirrhosis (24). However, olive oil used as the reference oil did not show any adverse effect on all the parameters studied.

CONCLUSION

This study showed that n-hexane oil extract from mixture of njasang and ehuru under the condition of this study negatively affected the parameters used to assess the kidney and liver status thus, indicating a toxic effect on these organs, in spite of the hypolipidaemic effect observed in our previous study as reported above.

REFERENCES

- [1] Imafidon, K. E. and Okunrobo, L. O. *Nigerian Journal of Basic and Applied Science*, **2012**, 19(2): 105 – 110
- [2] Slater, T. F. Pion Ltd London. **1972**, 21
- [3] Bolarin, D. M. and Bolarin, A. T. Revision Notes on Chemical Pathology, 1st edn. Lantern Books, Lagos Nigeria, **2005**, 210 – 247.
- [4] Ajayi, O. B. Fajemilehin, A. S. Dada, C. A and Awolusi, O. M, *J Nat. Prod. Plant Resour*, **2011**, 1(4): 47 – 55.
- [5] <http://en.wikipedia.org/wiki/Njangsa>
- [6] Besong, S. Fosung, C. Jones, A and Fondong, V. *Int. J. Poultry Science*, **2011**, 10(4): 257 – 265.
- [7] Ogunka-Nnoka, C. U. and Igwe, F. U., *Global Journal of Science Frontier Research*, **2013**, 13, Issue 6, version 1: 11-15
- [8] Keny, R. W. Trees of Nigeria. Clarendum Press. Oxford Science Publisher, **1989**, 30
- [9] Irrine, F. R. Woody plants of Ghana. Oxford University Press, London, **1967**, 40
- [10] Adekunle, S. R, J. *Plant Medica*, **1983**, 19: 97
- [11] Agomuo, E. N., Onyeike, E. N. Anosike, E. O. *Int. Sci. Res. Journal*, **2011**, 85 – 87
- [12] AOAC. Official Methods of Analysis. Association of Official Analytical Chemists, 14th edn. Washington DC, **1984**.
- [13] Reitman, S. and Frankel, S. *American Journal of Clinical Pathology*, **1957**, 28:56
- [14] Wahua, T. A. T. Applied Statistics for Scientific Studies. African Press, Aba, Nigeria, **1999**, 168 – 180
- [15] Meganathan, M. MadhanaGopal, K, Sasikala, P. Mohan, J., Gowdhaman, N. Balamurugan, K., Nirmala, P., Santhakuma, S., Samuel, V. *Global Journal of Pharmacology*, **2011**, 5(1): 50 – 53
- [16] Obi, E. Orisakwe, O. E., Asomugha, I. A. and Udemezue, O. O. *Indian Journal of Pharmaceutical Sciences*, **2004**, 36(5): 303 – 305
- [17] Sadipo, O. A.; Akinniyi, J. A. and Ogunbamaru. *Global J. Pure. Applied. Sci*, **2000**, 6: 83-87
- [18] Abii, T. A. and Elegalam, A. *J. Food Technol*, **2007**, 3: 229 – 232.
- [19] Javanoic, R., Savic, S., Vaseline, P., Kolgajic, V. and Desanka, K. *Krunva J*, **1991**, 2: 25– 31
- [20] Ogunka-Nnoka, C. U., Uwakwe, A. A. and Nnabuikwe, C. J. *Journal of Natural Sciences Research*, **2012**, 2(4): 66 – 70.
- [21] Abdulrahman, P. A., Onyeyili, A., Sanni, S. and Ogugbuaja V. O. *International Journal of Biological Chemistry*, **2007**, 1: 184 – 195
- [22] <http://www.hep.or.an/documents/factsheets/liverfunction2010>
- [23] Akpanabiata, I. M., Umoh, B., Udosen, G. O., Udoh, A. E. and Edet, E. E. *Indian Journal of Clinical Biochemistry*, **2005**, 20(2): 29 – 34
- [24] <http://www.medicinet.com>
- [25] Ahmed, M., Saeed, M. A., Alam H. and Ashga, Z. *Journal of Islamic Academy of Science*, **1992**, 5: 51 – 56.