Symbiosis

ISSN Online: 2376-4589
www.symbiosisonlinepublishing.com

Research Article | SOJ Biochemistry | Open Access

Effects of Alkaloids of *Cocos Nucifera* Husk fiber on Some Selected Enzymes in the Albino Mice

Oluwayemi Joshua Bamikole[1] , Godwin Okwori Adikwu[2]

[1]*Institute of Child Health, University of Ibadan. Nigeria*

[2]*Department of Biochemistry, University of Ilorin. Nigeria*

Received: 21 February, 2019; Accepted: 15 March, 2019; Published: 22 March, 2019

***Corresponding author:** Oluwayemi Joshua Bamikole, Institute of Child Health, University of Ibadan, Nigeria, Tel: +2347068505498, E-mail: bamikoleyemi@gmail.com*

Abstract

Cocos nucifera is a medicinal plant used in Nigeria for treatment of malaria. In this study, the effects of administration of alkaloids of *C. nucifera* husk fiber on the activities of some selected albino mice enzymes were investigated. The mice were administered the alkaloids orally at varying concentrations of 31.25, 62.50, 125, 250 and 500 mg/kg body weights while the controls were administered with distilled water. At the end of 7 days, the animals were sacrificed, and the assays for the activities of Alkaline Phosphatase (ALP), Gamma-glutamyl transferase (GGT), Aspartate Amino transferase (AST), Alanine Amino transferase (ALT), Glutamate Dehydrogenase (GLDH) in serum and kidney were carried out. From the results, there was significant decrease ($p<0.5$) in the activities of ALP and GGT in the kidney at doses of 125, 250 and 500 mg/kg body weights while there was significant increase ($p<0.05$) in their activities at 31.25, 62.50 and 125 mg/kg body weights in the serum. Generally, there was no significant change ($p>0.05$) in the activities of AST, ALP and GLDH in the serum and kidney. The findings in the study suggest that the alkaloids have adverse effect on the integrity of plasma membranes of kidney cells at higher doses which can cause a significant adverse effect on the heart.

Introduction

Alkaloids generally exert pharmacological activities particularly in mammals such as humans. Even today many of our most commonly used drugs are alkaloids from natural sources and new alkaloidal drugs are still being developed for clinical use. The decoctions of root stem barks, leaves and fruits of plants are resorted to and are used extensively as anti malarial remedies basically because of its cheaper costs without attention being paid to their possible toxicological consequence [2]. In this study, the effects of the alkaloids of *Cocos nucifera* husk fiber ethanolic extract (West African tall variety) on selected enzymes were evaluated in relationship to toxicity and body function (Kidney, Heart).

Cocos Nucifera

The coconut palm (*Cocos nucifera*) is in the tropics. It is particularly important in the low islands of the Pacific where, in the absence of land-based natural resources, it provides almost all the necessities of life. It has been nicknamed the 'tree of heaven' and 'tree of life'. Today, it remains an important economic and subsistence crop in many small Pacific island states [4].

Coconut is believed to have its origins in the Indo-Malayan region, from whence it spread throughout the tropics. Its natural habitat is the narrow sandy coast, but it is now found on soils ranging from pure sand to clays and from moderately acidic to alkaline. It thrives under warm and humid conditions but tolerates short periods of temperatures below 21°C (70°F) [4].

Common Uses of Coconut Husk

Coconut husks are used as mulch. They decompose slowly but are a good source of potassium. In low-rainfall areas, husks are buried in trenches to serve as water reservoirs during drought. Decomposed husk is placed in holes when planting coconut seedlings on sandy soils. Placed on the ground convex surface up, husks are commonly used as mulch around coconut seedlings and other plants to control weeds [4].

Materials and Methods

The extract was prepared according to the method of *Adebayo et, al* [1]. The samples were shade dried at room temperature and pulverized into powder. 500 grams (500 g) of the powder was percolated in 2.1 L of n-hexane for 72 hours in a tightly stoppered glass container. This was shaken at intervals. The resulting mixture was filtered with Whatmann filter paper (110mm). The filtrate was then concentrated under pressure using rotary evaporator at 40 0C, thereby generating a semisolid extract fraction of approximately 162ml, used for subsequent extraction.

The alkaloid was extracted using the method of Manske [8]. To 162ml of the semisolid extract was 22ml of 1M HCl added, after which it was basified by the addition of 6.5 ml of 5M NaOH yielding a white precipitate alongside with the solidification of the extract. The basified solution together with 18ml of 0.9% NaCl and 125ml of chloroform were separated with a separating

 ***Corresponding author email:** bamikoleyemi@gmail.com*

funnel thrice, at each interval producing an upper (aqueous) layer and a lower (organic) layer. A total of three organic layers containing the alkaloids were concentrated on a water bath at a temperature of 37^0C. The percentage yield was 0.335% alkaloid.

Animal Grouping and Administration of Alkaloids

The forty-eight mice were randomly assigned into five groups, of eight Mice each. Daily administration of 200µl of the alkaloid of ethanolic extract fraction of Cocos nucifera was done orally for seven days as follows:

Group A (Control): Administered appropriate volume of distilled water solution.

Group B: Administered 31.25 mg/Kg body weight of extract fraction.

Group C: Administered 62.5 mg/Kg body weight of extract fraction.

Group D: Administered 125 mg/Kg body weight of extract fraction.

Group E: Administered 250 mg/Kg body weight of extract fraction.

Group F: Administered 500 mg/Kg body weight of extract fraction.

The extract fraction was dissolved in warm distilled water solution to form a suspension before administration.

Toxicological Studies

At the end of the 7-day experimental period, the Mice were sacrificed by slight diethyl ether anaesthesia, the neck area was quickly cleared of fur and the jugular veins exposed, from which blood was collected into EDTA bottle to prevent clotting. The EDTA blood sample was centrifuged at 3000 rpm for 10 minutes and the serum pipetted out. This was stored frozen until needed for analysis.

Also, the mice were quickly dissected and the kidneys were isolated, cleaned of blood stains, weighed and suspended in an ice-cold 0.25M sucrose solution (an isotonic solution) to maintain the organs' integrity. Each organ was homogenized separately, using mortar and pestle, in ice-cold 0.25 M sucrose solution (1:6, w/v). The homogenates were frozen overnight to allow cell lysis. This ensures maximum release of enzymes.

The Kidney-body ratio, Total Protein Concentration, Total Protein concentration, Alkaline Phosphatase activity Aspartate Amino transferase activity, Alanine Amino transferase activity, AST activity, Gamma-glutamyl Transferase Activity, Glutamate Dehydrogenase Activity in serum and tissue homogenate, ALT activity in tissue supernatant and serum were determined.

Statistical Analysis

Experimental data are presented as Mean ± Standard Error of Mean (SEM). Statistical analysis was implemented using computer software SPSS 20.0 version statistical package program (SPSS, Chicago, IL). One-way analysis of variance was used to compare variables among the different groups. Level of significance (Post hoc comparisons) among the various treatments was determined by Duncan's Multiple Range Test. The values were considered statistically significant at $p<0.05$.

Results

Effect of alkaloids of Cocos nucifera Husk Fiber on kidney to Body Weight Ratio

The administration of alkaloid of Cocos nucifera husk fibre (WAT) at all doses investigated in this study did not cause any significant change at ($p > 0.05$) in the kidney-body weight ratio of mice compared to control Table 1.

Table 1: Effect of alkaloids of Cocos nucifera husk fibre on Kidney to Body Weight Ratio (%) of mice

Treatment (mg/Kg body weight)	Kidney/Body Weight (%)
CONTROL(distilled water)	1.096 ± 0.0329^{ab}
31.25	1.191 ± 0.0244^{a}
62.50	1.245 ± 0.0379^{ab}
125	1.061 ± 0.0705^{b}
250	1.204 ± 0.0374^{ab}
500	1.307 ± 0.125^{ab}

Values are expressed as Mean±SEM (n=8). Values in each column with different superscript are significantly different ($p < 0.05$).

Alkaline Phosphatase

The alkaloid fraction significantly increased ($p <0.05$) kidney ALP activity at the dose of 31.25 mg/Kg body weight, decreased it at doses of 125 mg/kg, 250mg/kg and 500 mg/kg body weights while it did not significantly alter ($p>0.05$) it at 62.50 mg/kg body weight dose compared to control. Serum ALP activity was significantly increased ($P<0.05$) at doses of 62.5, 125 mg/kg body weights, deceased at 500 mg/kg body weight of the alkaloids but was not significantly altered ($P>0.05$) at other doses compared to the control Table 2.

Gamma-glutamyl transferase

The activity of gamma-glutamyl transferase in albino mice kidney was significantly decreased ($p<0.05$) at doses of 62.50mg/kg and 125mg/kg body weights of alkaloids administered while it was not significantly changed ($p>0.05$) at other doses compared to the control. Serum GGT activity increased significantly ($p<0.05$) at doses of 31.25mg/kg and 62.50mg/kg body weights and decreased significantly ($p<0.05$) at other doses compared to the control Table 3.

Aspartate Amino Transferase

The extract fraction significantly increased ($P<0.05$) kidney and serum aspartate amino transferase activity at the dose of 31.25mg/kg and 62.50 body weights of alkaloids respectively while it was not significantly altered ($P>0.05$) at other doses of the alkaloids compared to control Table 4.

Table 2: Alkaline Phosphatase activities in the kidney and serum of mice administered alkaloids of Cocos nucifera husk fibre

GROUP	Specific Activity (nmol/min/mg protein)	
	Kidney	Serum
CONTROL(distilled water)	254.58±13.13^{b}	368.7943±8.5^{b}
31.25mg/kg b.wt	548.37±59.74^{c}	346.6312±27.8^{b}
62.50mg/kg b.wt	275.45±34.28^{b}	734.2199±29.2^{d}
125mg/kg b.wt	164.86±8.911^{a}	518.2624±38.1^{c}
250mg/kg b.wt	145.41±12.65^{a}	331.9149±27.1^{b}
500mg/kg b.wt	90.72±11.57^{a}	217.4941±24.4^{a}

Values are expressed as Mean±SEM (n=8). Values in each column with different superscript are significantly different ($p < 0.05$).

Table 3: Gamma-glutamyl transferase activities in kidney and serum of mice administered alkaloids of Cocos nucifera husk fibre

GROUP	Specific Activity (U/L)	
	Kidney	Serum
CONTROL(distilled water)	5.211±0.58^{c}	2817.80±568.60^{c}
31.25mg/kg b.wt	4.053±0.58^{bc}	10036.00±4034.90^{e}
62.50mg/kg b.wt	2.316±1.16^{ab}	6098.80±4888.50^{de}
125mg/kg b.wt	1.158±0.00^{a}	617.60±77.20^{a}
250mg/kg b.wt	4.632±0.00^{c}	1273.80±176.90^{b}
500mg/kg b.wt	4.632±0.00^{c}	231.60±66.80^{a}

Values are expressed as Mean±SEM (n=8). Values in each column with different superscript are significantly different ($p < 0.05$).

Table 4: Aspartate Aminotransferase activities in kidney and serum of mice administered alkaloids of Cocos nucifera husk fibre

GROUP	Specific Activity (nmol/ml/mg protein)	
	Kidney	Serum
CONTROL(distilled water)	0.29±0.05^{a}	0.007±0.002^{a}
31.25mg/kg b.wt	0.43±0.05^{b}	0.011±.002^{ab}
62.50mg/kg b.wt	0.29±0.05^{a}	0.017±0.002^{b}
125mg/kg b.wt	0.22±0.02^{a}	0.013±0.002^{ab}
250mg/kg b.wt	0.22±0.03^{a}	0.013±0.003^{ab}
500mg/kg b.wt	0.27±0.03^{a}	0.012±0.002^{ab}

Values are expressed as Mean±SEM (n=8). Values in each column with different superscript are significantly different ($p < 0.05$).

Alanine Amino transferase

TThe alkaloids of *Cocos nucifera* husk fibre at all doses administered did not cause any significant change in alanine amino transferase activities in the kidney and serum ($p>0.05$) at all doses when compared with control Table 5.

Glutamate Dehydrogenase

The alkaloids at a dose of 250mg/kg body weight caused a significant increase ($p<0.05$) in kidney's glutamate dehydrogenase activity but did not alter the activity at other doses compared to the control Table 6.

Table 5: Alanine Aminotransferase activities in the kidney and serum of mice administered alkaloids of Cocos nucifera husk fibre

GROUP	Specific Activity (nmol/ml/mg protein)	
	Kidney	Serum
CONTROL(distilled water)	0.27 ± 0.05^{ab}	0.03 ± 0.006^{a}
31.25mg/kg b.wt	0.15 ± 0.01^{a}	$0.04\pm.008^{a}$
62.50mg/kg b.wt	0.19 ± 0.04^{ab}	$0.05\pm.005^{a}$
125mg/kg b.wt	0.30 ± 0.02^{b}	0.04 ± 0.002^{a}
250mg/kg b.wt	0.27 ± 0.06^{ab}	$0.04\pm.006^{a}$
500mg/kg b.wt	0.18 ± 0.04^{ab}	$0.04\pm.007^{a}$

Values are expressed as Mean±SEM (n=8). Values in each column with different superscript are significantly different ($p>0.05$).

Table 6: Glutamate Dehydrogenase activity in kidney of mice administered alkaloids of Cocos nucifera husk fibre

Group	Specific Activity (µmol/min/mg protein)
	Kidney
CONTROL(distilled water)	0.145 ± 0.020^{a}
31.25mg/kg b.wt	0.171 ± 0.029^{a}
62.50mg/kg b.wt	0.330 ± 0.050^{a}
125mg/kg b.wt	0.375 ± 0.034^{a}
250mg/kg b.wt	0.726 ± 0.176^{b}
500mg/kg b.wt	0.304 ± 0.037^{a}

Values are expressed as Mean±SEM (n=8). Values in each column with different superscript are significantly different ($p < 0.05$).

Histological Observation of the Kidney

In the photomicrographs, the presence of few renal corpuscles confirms that the area of the kidney sectioned and stained is the renal cortex. The renal cortex is easily identified even at low magnification by the presence of renal corpuscles, which are absent in the renal medulla. However, the bowman capsule shown in B reveals the obliteration of the bowman space by the alkaloids at 31.25 mg/kg body weight, possibly due to inflammation.

Discussion

Organ-Body weight ratio

Organ-body weight ratios are normally investigated to determine whether the size of the organ has changed in relation to the weight of the whole animal [1]. Changes in the organ body weight ratio may be an indication of cell constriction or inflammation since the cells are the unit components of the organs. The constriction in the organ may occur as a result of loss of fluid from the organ due to damage, while increase in organ-body weight ratio may suggest inflammation (Moore and Dalley,

1999). Thus, since there was no significant change in the kidney-body weight ratio as a result of administration of alkaloids of *Cocos nucifera* husk fibre in this study, it may imply that the alkaloids do not cause inflammation or constriction in the kidney.

Enzyme Studies

The measurement of the activities of the marker enzymes in tissues and body fluid plays a significant and important role in diagnosis, disease investigation and in the assessment of drug or plant extract for safety/toxicity risk [7]. Tissue enzyme assay can also indicate tissue cellular damage long before structural damage can be picked by conventional histological techniques. Such measurement can also give an insight to the site of cellular tissue damage as a result of assault by the plant extract [2].

Membrane Bound Enzymes

Alkaline phosphatase (ALP) has been reported to be a marker enzyme for plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974), it is often used to access the integrity of the plasma membrane such that any alteration in the activity of the enzyme in the tissue and serum would indicate likely damage to the plasma membrane [14]. Any perturbation in the membrane caused by interaction with xenobiotics could lead to alteration in ALP activity [9]. Decrease in ALP activity caused by stressors probably indicates an altered transport of phosphate and an inhibitory effect on the cell growth and proliferation (Goldfischer, 1964) [5]. Inhibition of ALP activity reflects alteration in protein synthesis and uncoupling of oxidative phosphorylation [13].

Gamma-glutamyl transferase (GGT) is predominantly used as a diagonistic marker for liver disease. Elevated serum GGT serum activity can be found in diseases of the liver, biliary track and pancreas [3].

Alkaline Phosphatase

The decrease in activity of ALP in the kidney at higher doses of alkaloids administered when compared with the control might be due to the *in situ* inactivation of the enzyme molecule by the alkaloid or inhibition of the enzyme activity at the cellular level either at the transcriptional or translational levels or damage to the cell membrane resulting to leakage. The increased serum activity may be due to leakage from kidney tissue and the decrease observed at a higher dose may be as a result of increased clearance from the blood.

Gamma-Glutamyl Transferase

Decreased activity of serum and kidney's gamma-glutamyl transferase observed may be as a result of decreased GGT synthesis by inhibition at either transcriptional or translational levels or allosteric inhibition of the enzyme in situ. It could also be as a result of leakage from membrane damage. This can be observed in the increased serum activity at lower doses, though it became reduced at higher doses possibly due to increased clearance.

Amino Acid-Metabolizing Enzymes

It is well known that amino transferases play crucial role in amino acid metabolism and in providing intermediates for gluconeogenesis [7]. Amino transferases are active both in the cytoplasm and mitochondria of tissue cells where they form an important bridge between protein and carbohydrate metabolism [10]. Amino transferases respond to any stress or altered physiological condition [6]. Stress generally is known to elevate amino transferase activity [12]. Under stress conditions, animals need more energy resulting in higher demand for carbohydrate and their precursors to keep the glycolytic pathway and TCA cycle at sustained levels [11].

Aspartate Amino Transferase and Alanine Amino Transferase

The kidney is known as the secondary site of amino acid catabolism. Generally, AST and ALT activities were not significantly changed in the kidney and serum. This implies that the energy needs of the kidney remain fairly unchanged and the alkaloids did not adversely affect the inter conversion of the amino acids to their respective intermediates in carbohydrate metabolism.

Glutamate Dehydrogenase

Generally, GLDH activity was not affected by the alkaloid. The increased activity observed at 250 mg/kg body weight of alkaloids in the kidney may be as a result of increased synthesis of the enzyme or activation of the enzyme in situ. This may lead to increased catabolism of amino acids at this dose.

Conclusion

The present study evaluated the toxicity potentials of alkaloid fraction of Cocos nucifera husk fiber ethanolic extract. The results of the study suggest the following:

i. The alkaloids of Cocos nucifera husk fiber may adversely affect the plasma membrane of kidney at higher doses which might cause an adverse effect on the heart.

ii. They may have little or no adverse effect on amino acid metabolism in the kidney at all doses.

Thus, the consumption of the ethanolic extract fraction of Cocos nucifera husk fiber at higher doses may cause kidney dysfunction. Efforts should be directed at the isolation of the specific active principles (alkaloid) in order to reduce the risk of their toxicity.

Acknowledgement

We acknowledge Dr. Adebayo, Adewumi Muyiwa, and the Lab technicians of the Department of Biochemistry, University of Ilorin.Nigeria

Funding

None

References

1. Adebayo JO, Balogun EA, Malomo SO, Soladoye AO, Olatunji LA, Kolawole OM, et al. Antimalarial Activity of Cocos nucifera Husk Fibre: Further Studies. Evidence-Based Complementary and Alternative Medicine. 2013;2013:1-9.
2. Adebayo JO & Krettli AU. Potential anti malarials from Nigerian plants: a review. J Ethnopharmacol. 2011;133(2):289-302. doi:10.1016/j.jep.2010.11.024.
3. Betro MG, Oon RC, Edward JB. "Gamma-glutamyl transpeptidase in diseases of the liver and bone". Am. J. Clin. Pathol. 1973; 60 (5): 672-678. doi:10.1093/ajcp/60.5.672.
4. Chan E, and Elevitch C R. Cocos nucifera(coconut). Species Profiles for Pacific Island Agroforestry. Permanent Agriculture Resources (PAR). Hōlualoa, Hawai; 2006; 2(1): 1-27.
5. Engstrom L. Studies on bovine liver alkaline phosphatase, phosphate incorporation. Biochimicaet Biophysica Acta. 1964; 92: 71-74.
6. Knox WE. and Greengard O. An Introduction to enzyme physiology. In: Advanced Enzyme Regulation. Pergamon Press,New York, London. 1965; 257-248.
7. Malomo S O. Toxicological implication of ceftriaxone administration in rats. Nig. Journal Biochem Molecular Biol. Is 2000;15(1): 33-38. doi:10.1155/2013/803835.
8. Manske R H. The Alkaloids. Chemistry and physiology, New York Academic press. P.675. 1965; 8.
9. Molina R, MorenoI, Pichardo S. et.al. Acid and alkaline phosphatase activities and pathological changes induced in Tilapia fish (Oreochromis sp.) exposed subchronically to microcystins from toxic cyanobacterial blooms under laboratory conditions. Toxicon; 2005; 46(7):725–735. doi: 10.1016/j.toxicon.2005.07.012.
10. Rafelson JM E, Hayashi JA. and Beckoro-vainy A. Basic Biochemistry. Macmillan Publishing Co. Inc. New York 1980;121-122. doi:10.1016/0307-4412(80)90131-4.
11. Tiwari S. and Singh A. Piscicidal activity of alcoholic extract of Nerium indicum leaf and their biochemical stress response on fish metabolism. African Journal of Traditional Complementary and Alternative Medicines; 2004; 1: 15-29.doi: 10.4314/ajtcam.v1i1.31092
12. Velisek J, Inlason T, Gomulka P, Svoboda Z, et al. Effects of cypermethin on rainbow trout (Onchorhynchus mykiss). Veterinani Medicina; 2006; 51(10): 469-476.
13. Verma, S., Saxena, M. and Tonk, I. The influence of Ider 20 on the biochemical and enzymes in the liver of Clarias batrachus. Environmental Pollution; 1984; 33: 245–255.
14. Yakubu, M.T. Aphrodisiac and toxicological potentials of aqueous extratct of Fadogia agretis stem in male albino rats. Ph. D. Thesis, University of Ilorin, Nigeria. 2006; 7(4): 399-404.doi: 10.1111/j.1745-7262.2005.00052.x.