In Vivo Trace Element Determination: Acute Toxicity Test and Effect of Unripe *Cocos nucifera* Fluid on Body Organs

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ABSTRACT

In vivo mineral elements effect of unripe coconut water, its acute toxicity test, and effect of unripe coconut water on body organs were investigated. it was observed that the liquid extract showed a significant increase in the levels of K^+, Cl^-, Zn^{2+} and Na^+ when compared to normal control. No death of the tested mice was observed in the acute toxicity test, showing that the liquid extract is not toxic. AST and ALT tests were used to evaluate the health of the liver. Creatinine, urea, uric acid and acid phosphate tests were used to evaluate the health of the kidney. Results showed that unripe coconut water is non-toxic to body organs.

(Keywords: albino mice, unripe coconut water, malaria parasite (Mp^+), trace elements, ALT, AST, creatinine, urea, uric acid, acid phosphate)

INTRODUCTION

A natural product is a chemical compound or a substance produced by a living organism that is found in nature. Within the field of organic chemistry, the definition of natural products is usually restricted to mean purified organic compounds isolated from natural sources (Bhagya et al., 2010). Natural products sometimes can be either toxic or have biological activity that can be of therapeutic benefit in treating diseases. As such, natural products are active components not only for most traditional medicines but also for many modern medicines (Antherden, 1999).

Unripe coconut water is one of the world's most versatile natural products with increasing scientific evidence that support its role in health and medicinal application (Sofowora, 1984; Ghasemzadeh, *et al.*. 2010). The aim of this work is to investigate the effect of unripe coconut water on the mineral element in the blood, its toxicity and also its effect on the body organs.

EXPERIMENTAL

Sample Collection

Samples of unripe coconut were collected from the department of biochemistry in the University of Nigeria, Nsukka campus, authenticated at the Department of Plant Science and Biotechnology by a Botanist. The mesocarp was carefully removed to get the endocarp which harbors the clear liquid of unripe coconut water. This clear liquid was extracted from the endocarp with the aid of a syringe into a clean container.

Inoculation of the Parasitaemia

Parasitaemia was maintained in the laboratory by the method of David et al., 2006 and Ochei, 2007. Ten drops of the parasitized blood obtained with the aid of a capillary tube through the ocular region of the mice, was diluted with normal saline (1 mL). Thereafter diluted parasitized blood (0.2 mL) was used to infect the three mice that served as the host from where other experimental animals were infected.

Group I (positive control): was inoculated with malaria parasite (Mp^+) and treated with 5 mL/kg body weight of normal saline.

Group II (normal control): was not inoculated with malaria parasite (Mp^+) and treated with 5 mL/kg body weight of normal saline.

Group III (standard control): was inoculated with malaria parasite (Mp^+) and treated with 5 mg/kg body weight of Artesunate (standard drug).

Group IV**:** was inoculated with malaria parasite (Mp^+) and treated with 200 mL/kg body weight of the unripe coconut water.

Group V: was inoculated with malaria parasite (Mp^+) and treated with 300 mL/kg body weight of theunripe coconut water.

Determination of Trace Elements

Serum Chloride Determination

Reagents: Chloride reagent (1.5 mL), distilled water (10 µL) and standard reagent (10 µL)

Procedure: Blood serum (10 µL) was pipetted into a test tube, distilled water (10 µL) was pipetted into a blank tube and standard reagent (10 µL) was also pipette into a standard tube. Chloride reagent (1.5 mL) was added to all the tubes. The mixture was left for 5 min before taking the measuring the absorbance at the wavelength of 520 nm in the spectrophotometer.

Serum Sodium Determination

Reagents: Sodium filtrate reagent (1000 µL), distilled water (50 µL) and standard reagent (50 µL), Sodium acid reagent (50 µL) and color reagent (50 µL)

Procedure: Blood serum (50 µL) was pipetted into a test tube, distilled water (50 µL) was pipetted into a blank tube and standard reagent (50 µL) was also pipette into a standard tube. Sodium filtrate reagent (1000 µL) was added to all the tubes and the mixture was centrifuged for 5 min. Sodium acid reagent (50 µL) and color reagent (50 µL) were added after which the absorbance was measured at the wavelength of 550 nm in the spectrophotometer.

Serum Potassium Determination

Reagents: Potassium filtrate reagent (1000 µL), distilled water (10 µL) and standard reagent (10 µL)

Procedure: Blood serum (10 µL) was pipetted into a test tube, distilled water (10 µL) was pipetted into a blank tube and standard reagent (10 µL) was also pipette into a standard tube. Potassium reagent (1000 µL), was added to all the tubes. The absorbance was measured at the wavelength of 520 nm in the spectrophotometer.

Serum Zinc Determination

Reagents: Ammonia-ammonium chloride buffer (10 mL), distilled water (25 mL), hydroxylamine (12%, 2 mL), 2 drops of Erichrome-black-T indicator and EDTA (0.01 M).

Procedure: Blood serum (10 mL) was pipetted into a test tube, distilled water (25 mL), Ammonia-ammonium chloride buffer (10 mL), hydroxylamine (12%, 2 mL) and 2drops of Erichrome-black-T indicator were added in the test tube. The mixture was titrated with EDTA (0.01 M) to blue color as reported in the literature as reported in literatures.

Acute Toxicity Screening Test

Wistar albino mice of either sex weighing 20–34 g were housed in separate cages, acclimatized for one week and then divided into five groups of five mice each. The route of administration was via oral route with the aid of an incubation tube.

Liver Function Test Activity

Determination of the Aspartate Aminotransferase (Ast) Activity

Reagents: $R_{1=}$[(Phosphate buffer (100 mmol/L), L-aspartate (100 mmol/L) and α-oxoglutarate (2.0 mmol/L)] pH 7.4, R_2 = 2,4-dinitrophenyl-hydrazine (2 mmol/L), NaOH (0.4 M) and distilled water.

Procedure: Blood serum sample (100 µL) was pipetted into a sample tube and distilled water (100 µL) was also pipetted into a blank tube. R_1 (500 µL) was added to the sample tube and the blank tube, the solutions were allowed to stand for 30 min before adding R_2 to both the sample tube and the blank tube.

The solutions were also allowed to stand for another 20 min before adding NaOH (0.4 M).The absorbance was read at the wavelength of 546nm in the spectrophotometer.

Determination of the Alanine Transaminase (ALT) Activity

Reagents: $R_{1=}$ [(Phosphate buffer (100 mmol/L), L-alanine (200 mmol/L) and α-oxoglutarate (2.0 mmol/L)] pH 7.4, R_2 = 2,4-dinitrophenyl hydrazine (2mmol/L), NaOH (0.4 M) and distilled water.

Procedure: Blood serum sample (100 µL) was pipetted into a sample tube and distilled water (100 µL) was also pipetted into a blank tube. R_1 (500 µL) was added to the sample tube and the blank tube, the solutions were allowed to stand for 30 min before adding R_2 to the sample tube and the blank tube. The solutions were also allowed to stand for another 20 min before adding NaOH (0.4 M).The absorbance was read at the wavelength of 546nm in the spectrophotometer.

The Kidney Function Test Activity

Determination of Urea

Reagents: R_1 = EDTA (116 mmol/L), sodium nitroprusside (6 mmol/L) and urease (1 g/L), R_2 = diluted phenol (120 mmol/L), R_3 = diluted sodium hypochlorite (27 mmol/L) and sodium hydroxide (0.14 M).

Procedure: Blood serum sample (10 µL) was pipetted into a test tube, standard reagent (10 µL) was pipetted into a standard tube and distilled water (10 µL) was also pipetted into the blank tube. R_1 (100 µL) was added to all the tubes, R_2 (2.5 mL) was added to all the test tube and R_3 was also added to all the test tubes. The mixtures were allowed to stay for 15 min before taking the absorbance at the wavelength of 546 nm in the spectrophotometer.

Determination of Uric Acid

Reagents: Distilled water, standard reagent and R_1 = Hepes buffer or 3,5-Dichloro-2-hydroxybenzenesulfonic acid (50 mmol/L) pH= 7.0

Procedure: Blood serum sample (20 µL) was pipetted into a test tube, standard reagent (20 µL) was pipetted into a standard tube and distilled water (20 µL) was also pipetted into the blank tube. R_1 (1000 µL) was added to all the tubes. The absorbance was taken at the wavelength of 546 nm in the spectrophotometer.

Determination of Acid Phosphatase (ACP)

Reagents:R_1 = Citrate buffer (7.5 mmol/L) pH=5.2, R_2 1-naphthyl phosphate (10 mmol/l) and R_3= sodium tartrate (135 mmol/L).

Procedure: Blood sample (100 µL) was pipetted into a test tube and R_2 (1000 µL) was added to the tube. The mixture was incubated for 5 min at the temperature of 37^{o}C. The absorbance was taken at the wavelength of 546 nm in the spectrophotometer as reported in literature.

Determination of Creatinine

Reagents:R_{1a}= Picric acid (35 mmol/L), R_{1b}= sodium hydroxide (0.32 mmol/L).

Procedure: Blood serum sample (100 µL) was pipetted into a test tube, standard reagent (100 µL) was pipetted into a standard tube and equal volume of R_{1a} and R_{1b} (1000 µL) was added to all the tubes after which the absorbance was read at the wavelength of 510 nm in the spectrophotometer.

RESULTS AND DISCUSSION

Table 1: Results of Serum Chloride Determination (mmol/L).

GRP 1	GRP 2	GRP 3	GRP 4	GRP 5
60.364	76.818	71.818	98.182	81.364
51.818	85.909	91.364	92.273	91.364
58.182	84.567	88.636	105.455	86.913
55.162	79.223	83.763	87.321	96.722
57.761	83.097	79.677	77.541	89.987
Av=56.657	Av=81.923	Av=83.052	Av=92.154	Av=89.270

Serum Zinc Determination (mmol/L)

GRP 1	GRP 2	GRP 3	GRP 4	GRP 5
0.095	0.159	0.115	0.164	0.299
0.017	0.104	0.202	0.231	0.278
0.014	0.124	0.142	0.254	0.172
0.020	0.108	0.152	0.198	0.144
0.016	0.119	0.164	0.175	0.161
Av=0.0324	Av=0.1228	Av=0.155	Av=0.2044	Av=0.2108

The tables above showed that the levels of trace elements in serum were significantly lower in **GRP1** when compared to **GRP2**. This decrease may be attributed to the damageable effect of malaria parasite on the quantity of trace elements. This is in agreement with the works of

authors Muller and kappes 2007. In their opinion, levels of trace elements in serum are inversely proportional to the level of malaria parasite in the blood. **GRPS, 4 and 5** showed a slight increase in the concentrations of K^+, Cl^-, Zn^{2+} and a significant increase in the concentration of Na^+ when compared to **GRP2.** This increase may be due to the low level of malaria parasite in the blood and the minerals present in the unripe coconut water.

Table 2: Acute Toxicity Test **(Ld_{50})** Result.

GRPS	DOSAGE	MICE 1	MICE 2	MICE 3	MICE 5
PHASE 1					
GRP 1	10 mL/Kg	ND & NST	ND & NST	ND & NST	ND & NST
GRP 2	100 mL/Kg	ND & NST	ND & NST	ND & NST	ND & NST
GRP 3	1000 mL/kg	ND & NST	ND & NST	ND & NST	ND & NST
PHASE 2					
GRP 1	1900 mL/Kg	ND & NST	ND & NST	ND & NST	ND & NST
GRP 2	2600 mL/kg	ND & NST	ND & NST	ND & NST	ND & NST
GRP 3	5000 mL/kg	ND & NST	ND & NST	ND & NST	ND & NST

ND: No Death
NST: No Sign of Toxicity.

Unripe coconut water is not toxic to the body system

Results Of Liver Function Test Activities

Table 3: Results of Aspartate Aminotransferase (AST) Activity (U/L).

GRP 1	GRP 2	GRP 3	GRP 4	GRP 5
21	11	15	18	17
25	15	14	19	11
20	14	15	17	19
21	12	13	12	11
36	11	12	11	15
Av=24.6	Av=12.6	Av=13.8	Av=15.4	Av=14.6

AST and ALT tests are used to evaluate the health of the liver. These tests are used to detect liver damage and liver injury. The amount of AST and ALT in the blood is directly proportional to extent of tissue damage (Johnston, 1999). **GRP1(UC)** showed high level of AST and ALT indicating high liver injury caused by the malaria parasite in the liver. **GRPS 4 and 5** showed a decrease in AST and ALT level similar to **GRP2**. These decrease show that unripe coconut water is not toxic to the liver.

Table 4: Results of Alanine Aminotransferase (ALT) Activity (U/L).

GRP 1	GRP 2	GRP 3	GRP 4	GRP 5
49	20	26	28	25
50	21	22	24	30
55	25	21	26	23
46	29	30	20	21
47	30	25	21	22
Av=49.4	Av=25.0	Av=24.8	Av=23.8	Av=24.2

Results of Kidney Function Test Activities

Table 5: Results of Determination of Creatinine (mmol/L)

GRP 1	GRP 2	GRP 3	GRP 4	GRP 5
3.047	1.00	1.O11	1.640	1.712
2.127	1.213	1.106	1.713	0.986
3.275	1.441	1.220	1.410	1.616
4.156	1.525	1.01	1.051	1.593
3.784	1.096	1.128	1.211	1.367
Av=3.278	Av=1.255	Av=1.095	Av=1.405	Av=1.455

Table 6: Results of Determination of Urea (mmol/L).

GRP 1	GRP 2	GRP 3	GRP 4	GRP 5
9.431	4.764	6.431	6.669	4.193
8.954	3.907	5.955	5.002	6.669
9.002	5.682	5.246	5.955	5.478
7.685	4.776	4.026	4.748	4.765
9.876	3.823	3.125	5.866	4.932
Av=8.990	Av=4.590	Av=4.957	Av=5.648	Av=5.207

Table 7: Results of Determination of Uric Acid (mmol/L).

GRP 1	GRP 2	GRP 3	GRP 4	GRP 5
6.431	2.764	3.431	2.669	3.193
5.954	2.907	2.955	3.002	2.669
5.002	2.568	3.246	3.955	3.478
7.554	3.426	2.547	2.566	2.579
6.742	2.812	2.362	2.687	2.699
Av=6.337	Av=2.895	Av=2.908	Av=2.976	Av=2.924

Table 8: Results of Determination of Acid Phosphate (mmol/L).

GRP 1	GRP 2	GRP 3	GRP 4	GRP 5
5.743	1.086	1.229	2.486	1.400
4.458	1.229	1.715	1.320	2.201
6.229	1.500	1.892	1.687	1.488
5.276	1.364	1.206	1.206	1.207
4.658	1.257	1.146	1.448	1.345
Av=5.273	Av=1.287	Av=1.438	Av=1.629	Av=1.528

Creatinine is a chemical waste that is generated from muscle metabolism. It is transported through the blood stream to the kidney. Uric acid is a chemical waste produced when the body breaks down food that contains organic compounds. They are dissolved in the blood, filtered through the kidney and expelled in the urine. Urea is formed when ammonia produced from the liver reacts with CO_2. The kidney filters out most of the Creatinine, urea and uric acid and disposes them in the urine. Elevated levels of **Creatinine, urea, uric acid and acid phosphate** signify impaired kidney function or kidney disease. **GRP1 (UC)** showed elevated level of creatinine, urea uric acid and acid phosphate compared to **GRP2 (NC).** This increase is due to the level of malaria parasites in the blood.

GRPS 4 and 5 showed **decreases in Creatinine, urea, uric acid and acid phosphate** level similar to **GRP2**. These decreases suggest that unripe coconut water is not toxic to the kidney.

CONCLUSION

AST and ALT tests were used to evaluate the health of the liver. Creatinine, Urea, Uric acid and Acid Phosphate test were used to evaluate the health of the kidney. Results showed that unripe coconut water is non-toxic to the body organs.

Unripe coconut is not toxic to the body system at large from the knowledge of acute toxicity test. Also this liquid is seen to have an appreciable increase in the level of trace elements in blood serum.

REFERENCES

1. Antherden, L.M. 1999. *Textbooks of Pharmaceutical Chemistry. 6th edition*. Oxford University Press: London. J 0-814.
2. Bhagya, D., L., Prema, and T. Rajamohan. 2010. "Beneficial Effect of Tender Coconut Water on Blood Pressure and Lipid Levels in Experimental Hypertension". *Journal of Cell and Tissue Research*. 10(1):2139-2144.
3. Bremen, J. 2001. "The Ears of the Hippopotamus: Manifestation, Determinants, and Estimates of the Malaria Burden". *Am. J. Trop. Hyg*. 64(1, 2 S):1-11.
4. Muller, S. and B. Kappe. 2007. "Vitamin and Co-Factor Biosynthesis Pathway in Plasmodium and other Apicomplexan Parasites Trends in Parasitology". 2(4):1-177.
5. Nussemblatt, V. and K. Semba. 2002. "Micro-Nutrient Malnutrition and Pathogenesis of Malarial Anemia". *Acta. Tropica*. 82:321-337.
6. Ochei, J. and A. Kolhatkar. 2007. "Care and Use of Experimental Animals". In: *Medical Laboratory Science Theory and Practice*. Tata McGraw-Hill: New Delhi, India. 1213-1232.
7. Sergeant, G.R., Y. Grandison, K. Mason, B. Sergeant, A. Sewell, and S. Vaidya. 1980. "Heamatological indices in Normal Negro Children: A Jamaican Cohort from Birth to Five Years". *Clin. Lab. Haematol*. 2:169-178.
8. Sofowora, A. 1984. *Medicinal Plants and Traditional Medicine in Africa*. John Wiley and Sons, Ltd.: London. 100–102.
9. Wander, K., B. Shell-Duncan, and T.W. McDade. 2009. "Evaluation of Health of the Liver and Kidney Deficiency as a Nutritional Adaptation to Infectious Disease: An Evolutionary Medicine Perspective". *Am. J. Hum. Biol.* 21(2):172.

SUGGESTED CITATION

Onyeyilim, E.L., B.E. Ezema, K.G. Akpomie, F.K. Ojo, and O.L. Alum. 2017. "*In Vivo* Trace Element Determination: Acute Toxicity Test and Effect of Unripe *Cocos nucifera* Fluid on Body Organs". *Pacific Journal of Science and Technology*. 18(2):330-334.