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Short Communication

Bio-Modulation of Platelet Count Following the Administration of Tender Coconut Water in New Zealand Male Rabbits

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ABSTRACT

Coconut is a fruit of great health benefits. This study was aimed at investigating the bio-modulatory effect that oral ingestion of tender coconut water (TCW) exerts on the body during acute inflammatory processes in New Zealand male rabbits. A longitudinal study was adopted in this study using fifteen rabbits (n = 15) and having 4 treatments comprising them. Treatment 1 served as the basal stage without TCW administration. Treatment 2 includes 2 weeks post coconut water administration. Treatment 3 includes 4 weeks post TCW administration. Treatment 4 includes withdrawal of TCW administration. Oral doses of TCW, 5 mL/kg body weight were administered daily and maintained for six weeks. Blood samples were collected at weeks 2, 4 and 6 for the estimation of haematological parameters including white blood cell counts (WBC) and differentials, erythrocyte sedimentation rate (ESR), platelet counts and C-reactive protein concentration. Monocyte concentration (11.07 ±1.19%) in treatment 2 was significantly ($p < 0.05$) higher than those of treatment 1, 3 and 4%. Platelet concentration in group 1 was increased significantly ($p < 0.05$) compared to treatments 2 and 3 following the administration of TCW and increased again upon withdrawal of TCW [treatment 4 (280.27 ± 25.30 x 10^9/L)]. C-reactive protein was increased significantly in treatment 1 (2.93 ± 0.42) compared to treatments 2, 3 and 4. The data obtained from this study showed that the physiology of platelet during acute inflammatory conditions may be modulated by the administration of TCW.

Keywords: Tender Coconut water (TCW), Rabbits, Platelets, haematological parameters.

Introduction

The world is being awakened to the importance of 'phyto-pharmacy' and the lucid influence it has on the average health or wellbeing of the indulgent. Coconut water is the clear liquid inside young green coconuts (fruits of the coconut palm). Fresh coconuts are typically harvested from the tree while they are green. The volume of tender coconut water (TCW) that can be extracted from a mature coconut fruit is usually around 300 mL, depending on the species of coconut and stage of maturity. Suspended within the aqueous endosperm that constitutes 15 to 30% the weight of the coconut fruit, are B-vitamins such as nicotinic acid, pantothenic acid, and riboflavin.[1] Platelets or thrombocytes are the formed elements of blood. Platelets are small colorless, non-nucleated and moderately refractive bodies. Platelets are formed from bone marrow. Pluripotent stem cell gives rise to the colony forming unit-megakaryocyte (CFU-M). This develops into megakaryocyte.[2] Cytoplasm of megakaryocyte form pseudopodium. A portion of pseudopodium is detached to form platelet, which enters the circulation. Production of platelets is influenced by colony-stimulating factors and thrombopoietin.

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Normally, platelets are of several shapes, viz. spherical or rod-shaped and become oval or disc-shaped when inactivated. Platelets have three important properties: Adhesiveness, Aggregation, and Agglutination.[3]
Platelet P-selectin is implicated in recruiting leukocytes not only to the inflammation site but also in pure thrombosis. This confirms the central role of P-selectin in platelet interactions with leukocytes. Formation of platelet leukocyte rosettes *in vivo* depends on platelet activation. Under physiological conditions, platelets and leukocytes do not bind to each other. Such interaction becomes possible in pro-thrombotic or pro-inflammatory state with increased number of blood platelet leukocyte aggregates (PLA) observed in such diseases as diabetes mellitus, stroke and others.[4,5]
Binding of platelets to leukocytes can mediate recruitment of the latter to the vessel wall and render leukocytes more pro-thrombotic promoting synthesis of tissue factor by monocytes.[6,7]
Acute inflammatory conditions as seen by the obvious presence of insensible stressors which include inappropriate or variable temperature, humidity, ventilation, or illumination, inappropriate cage or enclosure size, noise, restraint, too infrequent change in bedding or removal of waste, unprofessional behaviours or practices, sample collection can induce stress in animals and alter the physiology of platelets. Stress is the effect of external or internal factors (stressors) which cause a change in biologic equilibrium. Stress initiates an adaptive response to restore baseline physiology and/or mental state. The adaptive response is seen as a change in physiology, psychologic state, or behavior.[8]
Some studies have been done on coconut water, no doubt, as it relates to its use frequently in cosmetics, in maintaining the electrolyte balance, in treating diverse ailments related to oxidative stress including liver function, its cardio protective properties, lowering of blood pressure etc. However, to the best of our knowledge there is paucity of information on the role of TCW on platelet count and acute phase reactants response(s) upon ingestion.[9]

Materials and Methods

Laboratory animals

New Zealand rabbits bought from a commercial vendor in Aduwawa, Benin City were used for this study. The rabbits were kept in the animal house of the department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. The animals were exposed to 12 hours light/dark cycle and maintained at room temperature (25 ± 1ºC) throughout the duration of the study. The animals were maintained according to the guidelines for the care of laboratory animals.

Ethical consideration

Ethical approval was obtained from the ministry of Agricultural and Natural resource, with reference number v.1041/15 Benin City, Edo state. This is because, while dealing with animals it is advised to get approval from the ministry of Agriculture for ethical reasons.

Inclusion Criteria

Four months old apparently healthy New Zealand white male rabbits that have acclimatized to the weather condition and environment of the study.

Exclusion Criteria

Other species of rabbit and rabbits that have been treated with drugs or immune compromised rabbits were excluded from this study.

Administration of TCW

Animals were feed only for 7 days before the commencement of experimental treatment in the laboratory animal house to acclimatize.

Collection of tender coconut water

Fresh coconut at tender stage (six months maturity) were harvested from the coconut tree (*Cocos nucifera*; dwarf variety) grown in Benin City, dehusked, carefully broken and liquid endosperm collected and used for each day experiment.

Coconut water was administered by oral intubation using Gavage's technique. 5 mL of TCW/kg body weight was given once daily throughout the duration of experiment. Coconut water aspirate into the syringe and the needle of the syringe was replaced by gavage's tube (oral gastric tube and administered to the animal).

Inducing Acute Inflammation

Here we explored monocyte and granulocytes concentrations in response to acute irritation and or inflammation by relying on data from possible injury and microbial infection responses. We used an inflammatory trigger, where a small nick was made with a scalpel through the vein of the marginal ear, from which blood was collected directly into a pipette. Care was taken however not to cut through the entire ear edge while making the nick in the vein. The hair directly over the vein was plucked and 70% alcohol was immediately applied to clean the area, but the surrounding hair was wet with water, making visualization of the vein easy. Petroleum jelly was then applied to the site. The vein was distended by occluding the venous return through digital pressure on the marginal ear vein at the base of the ear. Before sample collection even if the inciting agent may also be microbial. This simple procedure helped to induce some inflammatory process in the animal.

Blood Sample Collection

Marginal ear vein puncture was used to obtain venous blood samples. The hair directly over the vein was plucked or shaved and 70% alcohol was applied to clean the area and wet the surrounding hair, making visualization of the vein easy. Petroleum jelly was then applied to the site. To distend the vein, the venous return was occluded by digital pressure on the marginal ear vein at the base of the ear by applying a paper clip across it. Care was taken not to cut through the entire ear edge while making the nick in the vein.

White Blood Cell Count (WBC)

The Automated Haematologic Analyzer (Sysmex KX – 21N; Kobe, Japan) was used to analyse the total as well as the differential white blood cell count which were WBC (µL), LY (%), MO (%), and GR (%), based on standard methods and following the manufacturer's operational guidelines. All samples were analyzed within 30 minutes of sample collection.

Erythrocyte Sedimentation Rate

This was determined by using Westerngreen method. Briefly, Westerngreen tube was filled to the 100 mark with citrated blood. This was allowed to stand vertically at room temperature for I hour. Reading was taken and recorded in mm/hr.[10]

High Sensitivity C-Reactive Protein

This was determined by using Enzyme linked Immunosorbent Assay (ELISA).[11]

Principle: This is based on an immunoenzymometric assay. Monoclonal antibody is directed against a distinct antigenic determinant on the C-reactive protein molecule (CRP). This mouse monoclonal anti CRP is used for solid phase immobilization on the micro titer wells. A goat anti CRP antibody is in the antibody enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies resulting in the CRP molecules being sandwiched between the solid phase and the enzyme linked antibodies. TMB reagent is added and incubated for 20 minutes resulting in the development of blue colour which changes to yellow on addition of IN HC1. The concentration of the CRP is directly proportional to the colour intensity of the test sample.[12]

Statistical Analysis

Data collected were analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0 (IBM Inc., USA). The analysis of variance (ANOVA) was used to compare means and results were expressed in mean ± standard error of the mean. Inter relationship among the parameters were determined using correlation analysis. A p-value of less than 0.05 ($p < 0.05$) was considered significant.

Results and Discussion

Table 1 showed that the mean and standard error of mean of all the parameters in this longitudinal study (where the experimental animals served as their own control, in order to observe the progressive scientific changes that occur with time in the same animals) analysed for the control (basal animals) and the test animals, after two weeks and four weeks of administration of tender coconut water and after two weeks of withdrawal of administration of tender coconut water. The results obtained showed that granulocytes, lymphocytes and monocytes values were significantly increased across the four treatment groups ($p < 0.05$). However, the blood level of total WBC increased in groups 2, 3 and 4 after the commencement of CW but was not statistically significant.

Table 2 showed that the CRP was significantly raised and varied significantly across the groups with the highest value seen in treatment group 1 while the other Treatment groups were not statistically different ($p > 0.05$). Weight, and ESR declined within the time of administration of coconut water and slightly increased after withdrawal but was not statistically significant ($p > 0.05$).

Laboratory animals are sensitive in their responses to various degree of laboratory handling. Insensible stressors are generated during routine experimental procedures while the animals are still alive. Some of these laboratory handling include noise, restraint, too infrequent change in bedding or removal of wastes, unprofessional behaviours or practices, moderate to frequent sample collection, variable temperature, humidity, ventilation, illumination, inappropriate cage, enclosure size, personnel entering of the animal room, personnel handling of the animal, cage cleaning, cage change with transfer to a quiet room, cage change without transfer to a quiet room, handling and weighing of the laboratory animals etc.[13] The experimental result reveals that there was a significant increase in the mean monocyte concentration between the basal treatment group and the other treatment groups. Thus, there could be the introduction of minor infectious agents during the course of the experiment, which is an insensible stressor, and can affect platelet concentration because of the *in vivo* interaction between platelets and leucocytes. The mean concentration of platelet was observed to be significantly higher in the Basal treatment group than in the other groups where TCW was administered at different weeks. This could be due to a progressive increase of the level of insensible stressors over

Table 1: Platelet concentration, total white cell count and the differential leucocytes between the Basal treatment group and the test treatment groups.

Parameters	Group 1 (n=15)	Group 2 (n=15)	Group 3 (n=15)	Group 4 (n=15)	F-Value	P-Value	Level of Significance
White Blood Cells ($x10^9$/L)	7.08 ± 0.74	6.01 ± 0.89	6.48 ± 0.88	6.61 ± 1.04	0.202	0.894	NS
Granulocytes (%)	7.90 ± 0.53	49.13 ± 4.26	33.67 ± 3.99	31.47 ± 2.65	20.111	0.000	HS
Lymphocytes (%)	86.80 ± 1.31	40.33 ± 3.97	58.53 ± 3.25	60.13 ± 2.62	30.990	0.000	HS
Monocytes (%)	5.30 ± 0.91	11.07 ± 1.19	7.87 ± 1.03	7.73 ± 0.83	4.904	0.005	HS
Platelets ($x10^9$/L)	378.2 ± 38.93	263.67 ± 17.90	256.53 ± 21.24	280.27 ± 25.30	4.161	0.010	HS

Table 2: The weight and some inflammatory biomarkers between the Basal treatment group and the test treatment groups.

Parameters	Group 1 (n = 15)	Group 2 (n =15)	Group 3 (n=15)	Group 4 (n=15)	F-Value	P-Value	Level of Significance
Weight (g)	1.55 ± 0.07	1.49 ± 0.07	1.43 ± 0.07	1.40 ± 0.070	0.756	0.524	NS
Erythrocyte sedimentation rate (mm/h)	1.25 ± 0.67	0.67 ± 0.33	0.47 ± 0.34	0.27 ± 021	1.094	0.360	NS
C-reactive protein (ug/mL)	2.93 ± 0.42	0.93 ± 0.13	0.54 ± 0.05	0.52 ± 0.04	36.324	0.000	HS

time during the experiment. However, the concentration of platelets which was expected to increase over time was observed to have significantly decreased in treatment groups 2 and 3, because of the administration of TCW. But upon withdrawal of TCW in treatment group 4, the platelet concentration was significantly increased. That therefore showed that the administration of TCW had a bio-modulatory effect on the mean platelet concentration and as such their physiological activity.[14]

Furthermore, in this experiment CRP concentration was significantly raised and varied significantly across the groups with the highest value seen in Treatment group 1. It was observed that the concentration of CRP decreased progressively with the continuous administration of TCW, indicating the presence of acute inflammatory state in the animals and also corroborates the bio-modulatory effect of the administration of TCW on inflammatory processes because of the presence of insensible stressors.

Conclusion

The data obtained from this study revealed the bio-modulatory role of tender coconut water on platelet concentration and function during acute inflammatory conditions. It also showed that the mean concentration of some inflammatory indices could be modulated by the administration of TCW.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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