Cocos nucifera (Coconut) water of different maturity stages affects hematological and coagulation parameters in rabbits: A positive lead

Sadia Saleem Rao* and Rahila Ikram
Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan

Abstract: *Cocos nucifera* (coconut) water is a traditionally used beverage and sportsdrink around the world. The recommendations on the use of plants for different conditions often lack proper scientific research. This study is first of its kind to explore, compare and document scientific evidence of the effect of different stages *cocos nucifera* (coconut) water on hematological and coagulation parameters in rabbits. For this study, we selected healthy rabbits and used their blood samples for our target analysis. 4ml/100g of young and mature *cocos nucifera* (coconut) water was administered orally once a day to respective groups for 60 days. Control group was administered distilled water orally. Results showed positive effect of coconut water on key hematological parameters including red cells and hemoglobin. Coagulation profile revealed significant increase in platelets and fibrinogen level with decrease in bleeding time and increase in clotting time by coconut water. This study revealed slightly dominating profile of young coconut water over mature coconut water which may be attributed to the unique composition differences between young and mature coconut water including micronutrients, carbohydrates and phenolic components. Outcomes of this research lead to the speculation about possible effect of *cocos nucifera* (coconut) water in anemia and bleeding disorders.

Keywords: Anemia, coconut water, coagulation

INTRODUCTION

Plants have been an important source of medicine for thousands of years now. The World Health Organization (WHO) also estimated that up to 80% of people still rely on herbal remedies for their health care (Rao and Najam, 2016). Recommendations for the use of dietary supplements are often based on small clinical trials, case reports, or predictions derived from known pharmacology and expert opinions. Scientific evidence therefore becomes essential because natural products are often widely adopted by the public before adequate data is available that support their safety and efficacy (Wang *et al.,* 2015). This calls for the need to explore alternative, effective and safer methods to manage different problems including hematological and coagulation disorders.

Coconut (*cocos nucifera*) tree is an important tree found in the tropical regions. It belongs to Arecaceae family. The coconut fruits have great nutritional importance (Yong *et al.,* 2009). It is considered as the most versatile natural beverage around the globe. Increasing scientific evidence also supports its role in healthcare. It has been used traditionally for a long time as sports drink and also has been used as intravenous fluid for multiple years in the past (Reddy and Lakshmi, 2014). It was therefore drawn from such uses that it may have some effect on hematopoietic system as a whole. Therefore, the objective of this study became the exploration of *cocos nucifera* (coconut) water of different maturity stages on blood components and blood coagulation.

**Corresponding author:* e-mail: dr.sadiasrao@yahoo.com

MATERIALS AND METHODS

Botanical description of material used

- Scientific name: *Cocos nucifera* linn
- Family: Arecaceae – Palm family
- Sub-family: Cocoideae
- Common Name: Coconut (Lima *et al*., 2015)
- Part used: *Cocos nucifera* water

Coconut water collection

Cocos nucifera fruits were collected locally from Karachi city, Pakistan region. The fruits were identified as young and mature stage by the Department of Pharmacognosy, University of Karachi with reference number CNSA-02-14/16. After the collection, the fruits were carefully cut from the top and the respective coconut water was saved in separate bottles and kept in refrigerator for daily use (Khan *et al.,* 2003).

Selection of animal

30 healthy rabbits were selected for this study. Their weights ranged from 1100-1300g. Selected rabbits were locally bred in the Department of Pharmacology, University of Karachi. Animals were kept under controlled temperature and 12/12 hours light/dark cycle. Selected animals were kept on their standard diet and were handled according to the Helsinki Resolution 1964. This study was approved by University Board of Advanced Studies and Research with number 02181/Pharm.

Experimental protocol

Three groups with 10 rabbits in each group were selected for the study. The dosing continued for the period of 60

Groups	Treatments	Dose
Group 1	Control (distilled water) orally	1ml once daily
Group 2	Young coconut water (YCW) orally	4ml/100g once daily*
Group 3	Mature coconut water (MCW) orally	4ml/100g once daily*

*(Prathapan and Rajamohan, 2011; Sandhya and Rajamohan, 2006)

days. Red blood cells, hemoglobin, hematocrit, mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet, white blood cells, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen were assessed after 30 days and 60 days of dosing. Bleeding time and clotting time was observed after 60 days of dosing.

Collection of sample and analysis

Bio-Vac EDTA-K_3 and Bio-Vac 3.2 percent sodium-citrate tubes were employed for the estimation of complete blood count and coagulation profile respectively. Huma-count by HUMAN (Germany) was used for hematological estimation including red blood cells, hemoglobin, mean cell volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, hematocrit and white blood cells. Fibrinogen was estimated by automated evaluation. Its method was based on Clauss method. For PT and aPTT, tests were performed on Humaclot duo coagulometer. The principle of turbidimetric clot detection was employed. These parameters were determined using standard kits supplied by HUMAN (Riaz *et al*., 2014).

Bleeding time and clotting time

For the estimation of bleeding time, Duke's method was employed. Ear of rabbit was cut with the help of lancet and the time of bleeding was noted from the time the bleeding started till the time the bleeding stopped. After every 10 seconds, spots of bleeding ear were made on the blotting paper till the bleeding ceased completely. Time was calculated accordingly (Prasad *et al*., 2012).

For the estimation of clotting time, capillary tube method was used. Rabbit's ear was pricked and blood was drawn into the capillary tube. Time of the appearance of blood drop on rabbit's ear was noted. The tube was held between the palms to provide body temperature. The tube was taken out of the palms after 30 seconds and was broken from the tip. It was continued after every 10 seconds interval until a thread (clotted blood) was observed between the broken pieces of capillary tube. The time of appearance of thread was noted accordingly (Prasad *et al*., 2012).

STATISTICAL ANALYSIS

Collected data is presented as mean $\pm$ standard deviation and analyzed by SPSS version 20.0. One way ANOVA with post hoc Tukeys test was performed. p-value of <0.001 was considered highly significant.

RESULTS

Table 1 shows the effect of young and mature coconut water on hematological parameters after 30 days of administration. Only mature coconut water showed significant ($p<0.05$) increase in red blood cells and MCHC after 30 days. Young and mature coconut water both showed highly significant ($p<0.001$) increase in hemoglobin and hematocrit level after 30 days of young and mature coconut water administration.

Table 2 shows the effect of young and mature coconut water on hematological parameters at day 60. After 60 days only young coconut water showed highly significant ($p<0.001$) increase in red blood cells, hemoglobin and hematocrit. MCH level was highly significantly ($p<0.001$) decreased after 60 days of young coconut water administration only. MCV was not changed after young and mature coconut water administration throughout the study.

Table 3 shows the effect of young and mature coconut water on white blood cells. Only young coconut water showed very significant ($p<0.01$) increase in white blood cells but only after chronic dosing.

Table 4 and table 5 shows the effect of young and mature coconut water on coagulation parameters. Only young coconut water showed highly significant ($p<0.001$) increase in platelet count and fibrinogen level after 30 days of dosing. However, after 60 days of dosing platelet count and fibrinogen level was highly significantly ($p<0.001$) increased by both young and mature coconut water. No significant change in PT and aPTT was observed after young and mature coconut water administration throughout the study period.

Table 6 shows the effect of young and mature coconut water on bleeding time and clotting time. Bleeding time was decreased while clotting time was increased after the administration of young and mature coconut water.

DISCUSSION

Coconut water was evaluated for its effect on hematological and coagulation system. Young coconut water overall increased hemoglobin, red blood cells and hematocrit level that show stimulating effect of young coconut water on bone marrow and red blood cells production Overall results from mature coconut water administration also showed positive effect on hematopoietic system (Riaz *et al.,* 2013). These findings

Table 1: Effect of young and mature coconut water on hematological parameters after 30 days

Groups	RBC (million/µl)	Hb (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	5.00±0.49	8.32±0.69	28.03±2.19	66.26±1.65	20.08±2.55	29.37±2.50
YCW	5.14±0.56	10.47±1.13***	34.87±3.60***	66.54±1.24	20.40±3.21	30.01±4.41
MCW	5.67±0.79*	11.50±1.37***	36.95±4.16***	65.35±1.84	20.30±4.54	31.10±3.90*

Table 2: Effect of young and mature coconut water on hematological parameters after 60 days

Groups	RBC (million/µl)	Hb (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	5.09±0.22	8.49±0.77	29.08±3.65	65.94±1.28	20.13±3.31	29.12±3.66
YCW	$^{***+++}$5.88±0.19	$^{***+}$10.66±0.51	$^{***+++}$37.90±4.21	64.43±1.51	$^{***+++}$18.16±4.11	$^{+++}$28.16±2.41
MCW	5.10±0.36	9.50±0.84	30.60±2.31	64.75±1.78	20.10±2.36	30.99±4.30

Table 3: Effect of Young and Mature Coconut Water on WBC

Groups	WBC (x10^9/L) (after 30 days)	WBC (x10^9/L) (after 60 days)
Control	4.31±0.97	4.37±1.26
YCW	4.94±0.89	6.73±1.50^{**+++}
MCW	3.55±0.59	3.10±1.15

Table 4: Effect of young and mature coconut water on coagulation parameters after 30 days

Groups	Platelets (x10^9/L)	Fibrinogen (mg/dl)	PT (seconds)	aPTT (seconds)
Control	285.80±26.01	270.7±41.64	10.05±1.20	180.5±1.51
YCW	440.20±36.98^{***+++}	407.9±33.10^{***+++}	9.74±1.02	181.0±0.99
MCW	321.50±74.30	290.2±25.22	10.0±1.05	180.7±1.15

Table 5: Effect of young and mature coconut water on coagulation parameters after 60 days

Groups	Platelets (x10^9/L)	Fibrinogen (mg/dl)	PT (seconds)	aPTT (seconds)
Control	289.30±41.10	271.4±31.92	10.15±1.80	181.9±1.33
YCW	681.10±31.89***	376.6±49.74***	10.66±1.69	180.59±1.63
MCW	653.30±27.84***	359.0±37.15***	11.00±1.05	182±1.85

n=10; data presented as mean ± standard deviation. Statistical significance analyzed by one-way ANOVA followed by post hoc Tukeys test; ***p<0.001, **p<0.01, *p<0.05 is considered highly significant, very significant, significant in comparison to control; +++p<0.001, +p<0.01 is considered highly significant, significant when young Cocos nucifera water compared with mature Cocos nucifera water; YCW=Young coconut water; MCW=Mature coconut water

Table 6: Effect of young and mature coconut water on bleeding time and clotting time

Groups	Bleeding Time		Clotting Time	
	Baseline	After Treatment	Baseline	After Treatment
YCW	Normal	Decreased	Normal	Increased
MCW	Normal	Decreased	Normal	Increased

n=10; YCW= Young coconut water; MCW= Mature coconut water

may play a beneficial role in the cases of anemia (Lee *et al*., 2014). According to Gupta and Flora (2005) carbohydrates have stimulatory effect on the hematopoietic system. Both young and mature coconut water contains glucose and fructose (Yong *et al*., 2009). Therefore, it may be assumed that this carbohydrate component in coconut water is playing its role in the stimulation of hematopoietic system. Yong *et al* (2009) reported that young coconut water contains more carbohydrate content than mature coconut water. Therefore, this change in concentration of carbohydrate could be attributed to the better hematopoietic potential of young coconut water over mature coconut water which is observed in this study. Only young coconut water showed increased level of white blood cells after chronic dosing. This is suggestive of positive role of young coconut water against immunosuppression (Riaz *et al*., 2013).

Young and mature coconut water both significantly increased the level of platelets. This increase is however fairly closer to the normal range of platelets in rabbits and hence may not impose risk of thromboembolic conditions. On the other hand, this finding can be employed for the patients with thrombocytopenia (Riaz *et al*., 2013). No significant change in PT and aPTT was noted after coconut water use therefore, it is postulated that these stages coconut water have no effect on extrinsic and intrinsic pathways (Chan *et al*., 2007).

Young and mature coconut water also showed increased in fibrinogen level and clotting time. This shows that fibrinogen is not being used. Researchers have reported that long term treatment with common anticoagulant warfarin also decreases the activation of coagulation system in cardiovascular disease patients. They have reported increased level of fibrinogen in such patients (Eritsland *et al*., 1992). The overall increase in fibrinogen level by both maturity stage coconut waters is assumed as a reduction in the activation of coagulation system. However, this could be confirmed further.

Mahayothee *et al* (2015) mentioned that coconut water contains p-coumaric acid which is one of the important phenolic components. P-coumaric acid is slightly more in concentration in young coconut water than in mature coconut water. According to Luceri *et al* (2007) p-coumaric acid reduces ADP-induced platelet aggregation without placing any effect on blood coagulation. This is in parallel to the level of platelets as it was also increased after young and mature water administration. More pronounced effect of young coconut water on platelets and fibrinogen may be attributed to the slight increase in the concentration of p-coumaric acid in young coconut water (Mahayothee *et al*., 2015).

CONCLUSION

Young and mature *cocos nucifera* (coconut) water showed significant effects on hematological and coagulation profile. The outcomes of this research lead to the speculation about possible effect of coconut water in anemia and bleeding disorders. Since young *cocos nucifera* (coconut) water showed overall dominating hematological and coagulation profile than mature *cocos nucifera* (coconut) water, young coconut water could be employed for more enhanced effects while mature coconut water may be reserved for milder effects.

REFERENCES

Chan KC, Yin MC and Chao WJ (2007). Effect of diallyltrisulfide-rich garlic oil on blood coagulation and plasma activity of anticoagulation factors in rats. *Food Chem. Toxicol*., **45**(3): 502-507.

Eritsland J, Seljeflot I, Arn esen H, Smith P, Westvik AB (1992). Effects of long-term treatment with warfarin on fibrinogen, FPA, TAT and D-dimer in patients with coronary artery disease. *Thromb Res*., **66**(1): 55-60.

Gupta R and Flora SJ (2005). Protective value of *Aloe vera* against some toxic effects of arsenic in rats. *Phytother. Res.,* **19**(1): 23-28.

Khan MN, Rehman MU and Khan KW (2003). A study of chemical composition of *Cocos nucifera* L. (coconut) water and its usefulness as rehydration fluid. *Pak. J. Bot*., **35**(5): 925-930.

Lee HW, Kim H, Ryuk JA, Kil KJ and Ko BS (2014). Hemopoietic effect of extracts from constituent herbal medicines of Samul-tang on phenylhydrazine-induced hemolytic anemia in rats. *Int. J. Clin. Exp. Path.,* **7**(9): 6179-6185.

Lima EBC, Sous CNS, Meneses LN, Ximenes NC, Junior S, Vasconcelos GS, and Vasconcelos SMM (2015). *Cocos nucifera* (L.) (Arecaceae): A phytochemical and pharmacological review. *Braz. J. Med. Biol. Res.,* **48**(11): 953-964.

Luceri C, Giannini L, Lodovici M, Antonucci E, Abbate R, Masini E, Dolara P (2007). p-Coumaric acid, a common dietary phenol, inhibits platelet activity *in vitro* and *in vivo*. *Br. J. Nutr*., **97**(03): 458-463.

Mahayothee B, Koomyart I, Khuwijitjar P, Siriwongwilaichat P, Nagle M, Müller J (2015). Phenolic compounds, antioxidant activity, and medium chain fatty acids profiles of coconut water and meat at different maturity stages. *Int. J. Food Prop*., **9**: 2041-2051.

Prasad SS, Kumar S, Patel K, Dumater C, Vajpeyee SK, Bhavsar VH (2012). To investigate the action of ginger-juice *Zingiber officinale* roscoe (Zingiberaceae) on blood coagulation process. *Int. J. Pharma. Sci. Res*., **3**(7): 407-415.

Prathapan A and Rajamohan T (2011). Antioxidant and antithrombotic activity of tender coconut water in experimental myocardial infarction. *J. Food. Biochem*., **35**(5): 1501-1507.

Rao SS and Najam R (2016). Efficacy of combination herbal product (*Curcuma longa* and *Eugenia jambolana*) used for diabetes mellitus. *Pak. J. Pharm. Sci*., **29**(1): 201-204.

Reddy EP and Lakshmi TM (2014). Coconut water - properties, uses, nutritional benefits in health and wealth and in health and disease: A review. *J. Curr. Trends Clin. Med. Lab. Biochem*., **2**(2): 6-18.

Riaz A, Khan RA, Mirza T, Mustansir T, Ahmed M (2014). *In vitro/in vivo* effect of *Citrus limon* (L. Burm. f.) juice on blood parameters, coagulation and anticoagulation factors in rabbits. *Pak. J. Pharm. Sci*., **27**(4): 907-915.

Riaz B, Najam R, Azhar I and Khan SS (2013). Hematopoietic effects of the brown algae Iyengariastellata in albino rabbits. *J. Pharm. Res*., **7**(3): 215-218.

Sandhya VG and Rajamohan T (2006). Beneficial effects of coconut water feeding on lipid metabolism in cholesterol-fed rats. *J. Med. food.,* **9**(3): 400-407.

Wang C, Moss J and Yuan CS (2015). Commonly used dietary supplements on coagulation function during surgery. *Medicines*., **2**(3): 157-185.

Yong JW, Ge L, Ng YF and Tan SN (2009). The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. *Molecules,* **14**(12): 5144-5164.