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Research Article

Effects of Coconut Water on Reproductive Functions of Female Wistar Rats: A Preliminary Investigation.

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Abstract

Coconut water (CW) has been reported to possess sex hormone-like activity. The effects of CW on oestrus cycles and reproductive organs of female Wistar rats were investigated. Five groups (n=6) of rats were treated as follows; 0.5 ml/100 g body weight distilled water (Control), 0.1, 0.25, 0.5 and 1.0 ml/100 g body weight CW respectively for 21 days. Body weights and oestrus cycles were monitored daily. Animals were sacrificed in proestrus at which time; the ovary, uterus, kidney, liver, heart and brain were assessed for changes in weight. Histological analysis of the ovary, uterus and liver was carried out. CW caused significant reductions ($P<0.05$) in body weight gain. Oestrus cycle lengths were significantly decreased ($P<0.05$), showed more regular rhythms with an increase in occurrence of the proestrus phase ($P<0.05$) and reduced frequency of the metestrus phase ($P<0.05$). CW had no significant effect on organ weights, also the ovary, uterus and liver did not show any structural modifications. The results suggest that CW 1) does not have any apparent toxic effect on the reproductive and other major organs of female Wistar rats; 2) may have fertility-enhancing properties in female rats via the regularisation of irregular oestrus cycles and promotion of onset of the proestrus phase of the oestrus cycle; and 3) has a body weight reducing effect. Further studies are on-going to reveal possible mechanisms for the fertility-enhancing effects of coconut water.

Keywords: Coconut water, oestrus cycle, toxicity, rat

INTRODUCTION

Coconut water is obtained from within the fruit of the coconut (*Cocos nucifera* L.) tree, a monospecie palm with tall and dwarf varieties. Coconut trees, which are found in large populations within the tropics (Gunn *et al*., 2011), have been described as the "tree of life" because every part of the plant is useful for purposes ranging from nutrition to art and science (Amarasiri and Dissanayake, 2006). Coconut water contains amino acids, sugars and several minerals which are essential for an adequate diet (Solangi and Iqbal, 2011). It is a non-toxic, pleasant tasting source of hydration both orally and parenterally (Campbell-Falck *et al*., 2000). Although the many virtues of coconut water on health (such as its hypolipidemic, antidiabetic and antioxidant properties) have been extolled (DebMandal and Mandal, 2011; Pinto *et al*., 2015), the possible link between coconut water consumption and fertility has been overlooked. It may not be a mere coincidence that the tropics with their abundant population of coconut trees host more than 40% of the world's inhabitants. Coconut extract has been reported to have oestrogenic activity (Jawad and Ali, 2010) and coconut oil has been reported to have testosterone improving properties (Dosumu *et al*., 2010). However, the effect of coconut water consumption on the female reproductive system is yet to be elucidated. It should be noted that coconut water is easier to access than other products of the coconut fruit such as its milk, extract or oil. This preliminary study was therefore carried out to determine the effects of oral coconut water administration on body weights, oestrus cycles and reproductive organs of female Wistar rats and the lowest effective doses to produce these effects.

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MATERIALS AND METHODS

Animals: Thirty female Wistar rats (100-150 g) obtained from and housed in the central animal house, College of Medicine, University of Ibadan, Oyo State, Nigeria were randomly assigned into five groups (n=6). All animals had access to standard laboratory rat pellets (Ladokun feeds, Ibadan, Nigeria) and drinking water *ad libitum*.

Plant Material and Water Extraction: Coconut fruits were obtained locally in Ibadan, Nigeria and authenticated in the Department of Botany, University of Ibadan, Oyo state, Nigeria. Coconut water was extracted fresh each day by puncturing one of the "eyes" of a dehusked coconut fruit and decanting it into a sterile container.

Experimental Design: A two-week pre-treatment phase of oestrus cycle monitoring was carried out to determine baseline cycle rhythms for each animal. Previous studies on the effects of coconut water on lipid profile and female reproductive endocrinology employed doses of coconut water ranging from between 2 ml/100 g body weight to 10 ml/100

g body weight (Radenahmad and Vongvatcharanon, 2006; Sandhya and Rajamohan, 2006; 2008; Bhagya *et al*., 2010). One of the aims of this study was to determine if lower doses could produce similar effects in order to avoid unnecessary overdosing of the experimental animals. To achieve this, four of the five groups were given 0.1, 0.25, 0.5, and 1.0 ml/100 g body weight coconut water respectively for three weeks (Bhagya *et al*, 2010), while the fifth group (control) received 0.5 ml/100 g body weight distilled water for three weeks. Body weights and oestrus cycles were monitored daily.

Oestrus Cycle Monitoring: The Marcondes technique (Marcondes *et al*., 2002) of vaginal smear monitoring was used in the daily observation of the oestrus cycles of each rat. After viewing, the slides were fixed using Papanicolaou's technique (Stockard and Papanicolaou, 1917).

Autopsy: At the end of the study, the rats were sacrificed in the proestrus phase of their oestrus cycle by cervical dislocation. Their ovaries, uteri, brains, adrenal glands, livers and kidneys were harvested, freed of adherent fat and weighed. Relative organ weights were calculated as the percentage of the ratio between the organ weight and body weight for each animal. The ovaries, uteri and livers were then fixed in 10% formalin for histological analysis.

Statistical Analysis: Data were expressed as means ± SEM. Statistical analyses were done using Student's *t*-test. P values <0.05 were considered to be statistically significant.

RESULTS

Effect of coconut water on body and organ weight: Coconut water administration at doses of 0.1, 0.25, 0.5 and 1.0 ml/100 g for three weeks produced significant reductions in body weight in a dose-dependent manner (figure 1). Coconut water administration did not produce any significant effect on organ weights (figures 2 and 3) neither did it result in any visible histologic alterations to the tissues of the reproductive organs (ovary and uterus) and livers of the rats (plates 1-3). There was also no significant change in mammary gland size (figure 4).

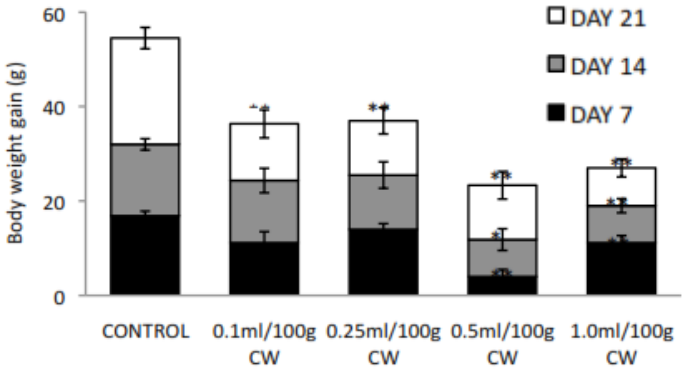

Fig. 1
Effect of coconut water (CW) on body weight of female Wistar rats. *P<0.05, **P<0.01 compared with control.

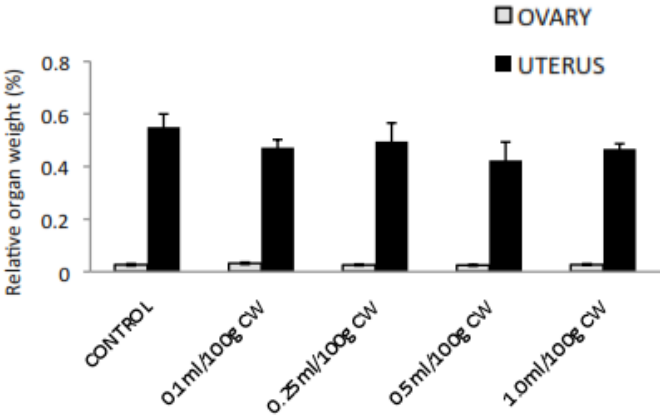

Fig. 2
Effect of coconut water (CW) on weight of ovary and uterus of Wistar rats.

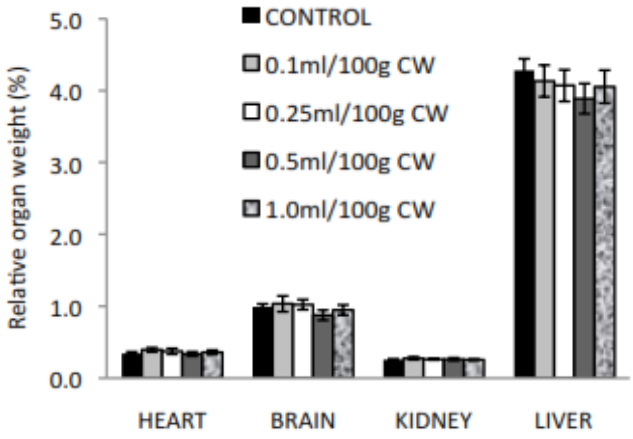

Fig. 3
Effect of coconut water (CW) on vital organ weights of female Wistar rats.

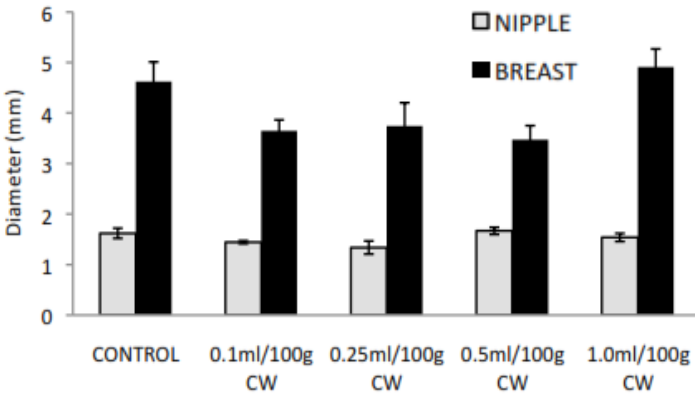

Fig. 4
Effect of coconut water (CW) on mammary gland size in female Wistar rats.

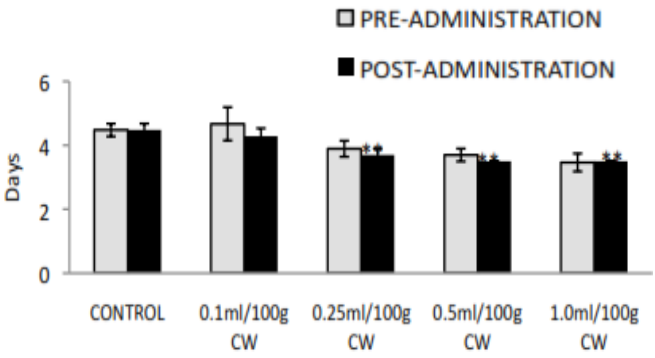

Fig. 5
Effect of coconut water (CW) on oestrus cycle length in Wistar rats. **p<0.01 compared with the control.

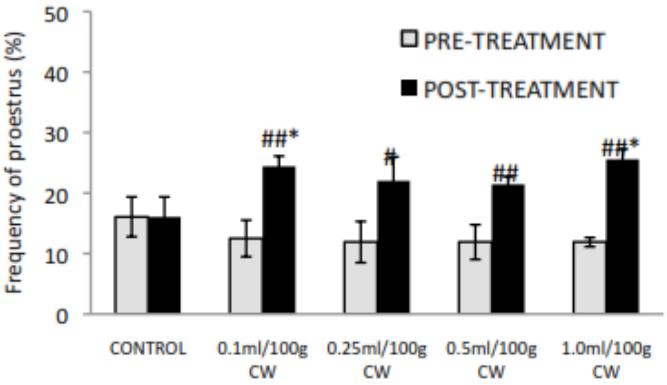

Fig. 6
Effect of coconut water (CW) on proestrus phase of oestrus cycle. *p<0.05 compared with control group. #p<0.05, ##p<0.01 compared with pretreatment period.

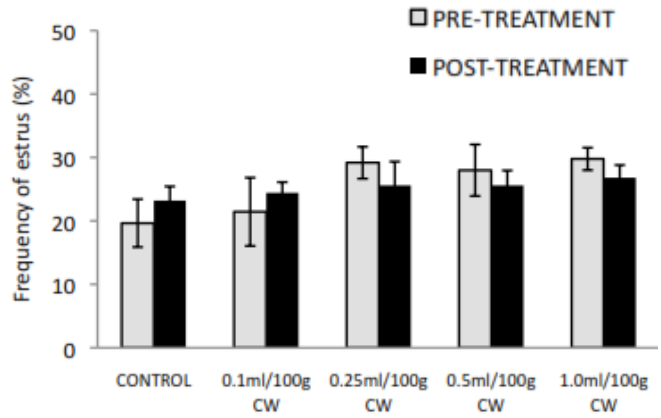

Fig. 7
Effect of coconut water (CW) on estrus phase of oestrus cycle of Wistar rats.

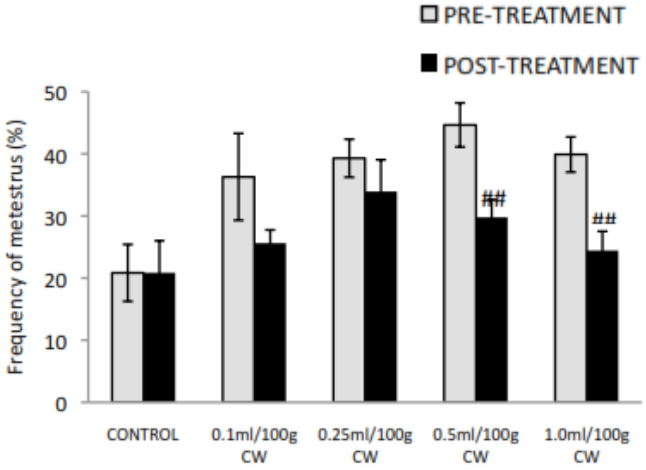

Fig. 8
Effect of coconut water (CW) on metestrus phase of oestrus cycle. ##p<0.01 compared with pretreatment period

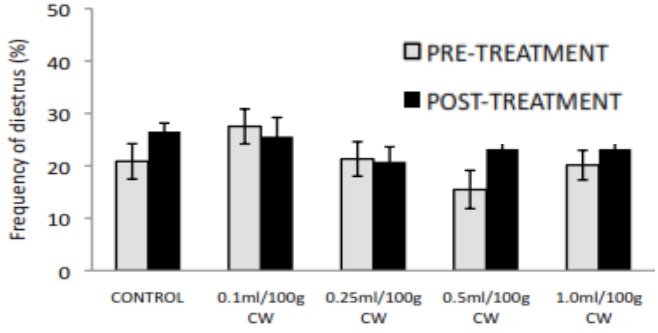

Fig. 9
Effect of coconut water (CW) on vital organ weights of female Wistar rats.

Effect of coconut water on oestrus cycle rhythm: There was a significant reduction (P <0.01) in oestrus cycle lengths from about 4½ days in the control group to about 3½ days in the groups treated with 0.25, 0.5 and 1.0 ml/100 g body weight coconut water (figure 5). Coconut water produced a significant increase (P <0.05) in frequency of the proestrus phase of the oestrus cycle in all four experimental groups (figure 6), and a significant reduction (P <0.05) in the metestrus phase in the groups which received 0.5 and 1.0 ml/100 g (figure 8).

DISCUSSION

Coconut water has been reported to have hypolipidemic properties (Sandhya and Rajamohan, 2008). This effect may be responsible for the significant loss of weight observed in this study. However, considering that the animals used in this study were of normal weights at the commencement of the study and that they consumed standard diets, the hypolipidemic effect of lower doses of coconut water are currently being explored using high fat diet(s). This will help to determine the effect of lower doses of coconut water on lipid profile, as well as steroidogenesis and female reproductive function in totality.

Coconut water did not affect the weight and/or structure of the female reproductive organs (ovary and uterus) and the vital organs (brain, heart, liver and kidney). This supports previous findings on the non-toxic nature of coconut water and justifies its reported use for *in vitro* organ storage in addition to oral and parenteral rehydration therapies (Kuberski *et al*., 1979; Campbell-Falck *et al*., 2000; ; Saat *et al*., 2002; Jawad and Ali, 2010; Schettino *et al*. 2015). Further studies are also being conducted in our laboratory to elucidate the effects of coconut water on the physiological actions of these organs.

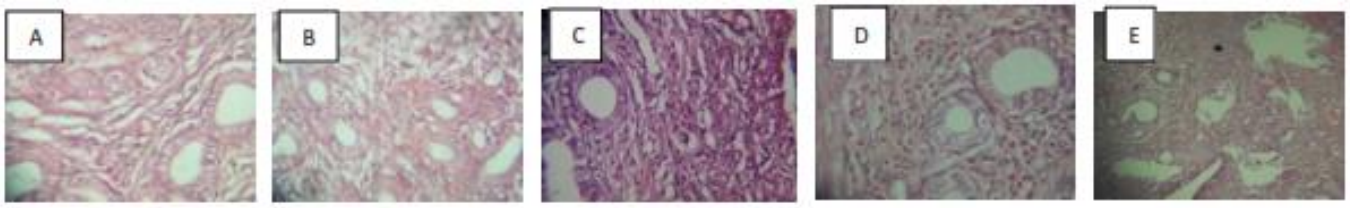

Plate 1:
Photomicrographs of uterus sections of rats treated with coconut water for three weeks (mag. X 40, H&E stain). Sections from (A) Control (B) 0.1 ml/100 g (C) 0.25 ml/100 g (D) 0.5 ml/100 g and (E) 1.0 ml/100 g show no visible signs of lesion.

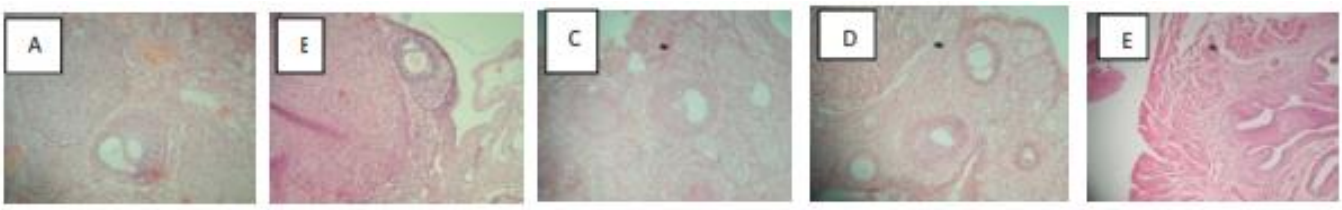

Plate 2:
Photomicrographs of ovarian sections of rats treated with coconut water for three weeks (mag. X 40, H&E stain). Sections from (A) Control (B) 0.1 ml/100 g (C) 0.25 ml/100 g (D) 0.5 ml/100 g and (E) 1.0 ml/100 g show no visible signs of lesion.

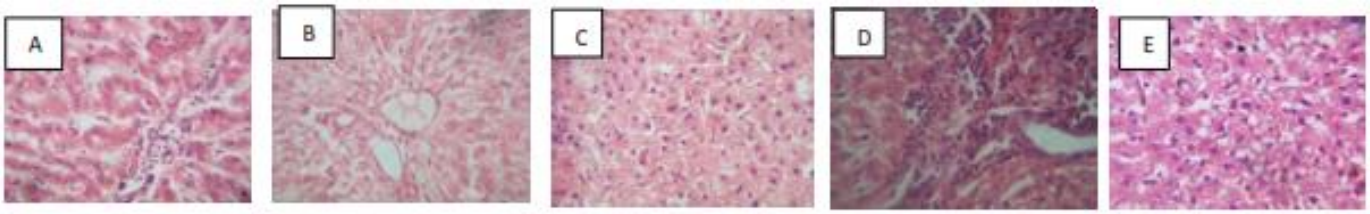

Plate 3:
Photomicrographs of the hepatocytes of rats treated with coconut water for three weeks (mag. X 400, H&E stain). Sections from (A) Control (B) 0.1 ml/100 g (C) 0.25 ml/100 g (D) 0.5 ml/100 g and (E) 1.0 ml/100 g show no visible signs of lesion

The reduction in length of oestrus cycles translated to more regular rhythms being observed in the coconut water treated groups. The female reproductive cycles are critical to their reproductive capabilities, thus, this increased cyclicity may be a pointer to the possible neuronal or endocrinological mechanisms whereby coconut water may exert its effects on the rat female reproductive system. Assuming that all the cycles are ovulatory, there will be a greater availability of ova for fertilization. This hypothesis is being explored as it may yield a phenomenal breakthrough for fertility treatments. Coconut water increased the occurrence of the proestrus phase of the oestrus cycle. The proestrus phase signals the beginning of an oestrus cycle and most of the female reproductive hormones are present in appreciable quantities in proestrus. Coconut water has been suggested to have oestrogen-like activity (Yusuh *et al*., 2010; Radenahmad *et al*., 2011) and even though the scope of this study did not involve the assay of reproductive or other hormones, the insignificant difference in size of the mammary gland observed in this study during the proestrus phase does not suggest an increase or otherwise in oestrogen activity. Also, the ovaries did not show any significant structural change to suggest a negative-feedback effect of coconut water administration. Further studies are being carried out in our laboratory to confirm the presence of any sex hormone-like action of coconut water as the reduced frequency of metestrus phase occurrence also supports a possible fertility-enhancing property of coconut water. This is because, circulating oestrogen, luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels are least in the metestrus phase suggesting declining fertility in this phase (Caligioni, 2009). Therefore assessing the reproductive hormone levels in coconut water treated female rats will help determine the mechanisms through which coconut water affects the female reproductive cycle.

From the results of this study, it was concluded that oral administration of coconut water at doses of 0.1, 0.25, 0.5 and 1.0 ml/100 g body weight for three weeks does not adversely affect the vital and reproductive organs in female Wistar rats; these doses of coconut water have the ability to normalize irregular oestrus cycles by promoting the appearance of the proestrus phase; and these doses resulted in significant reductions in body weight gain with 1.0 ml/100 g body weight coconut water producing the most significant reductions in body weight gain.

REFERENCES

Amarasiri, W. A. L D. and Dissanayake, A. S. 2006. "Coconut Fats." *Ceylon Medical Journal* 51(2): 47–51.

Bhagya, D., Prema, L. and Rajamohan , T. 2010. "Beneficial effects of Tender Coconut Water on Blood Pressure and Lipid Levels in Experimental Hypertension". *Journal of cell and tissue research* 10(1): 2139–2144.

Caligioni, C.S. 2009. "Assessing reproductive status/stages in mice". *Current protocols in neuroscience* Appendix 4I.

Campbell-Falck, D., Thomas, T., Falck, T. M., Tutuo, N. and Clem, K. 2000. "The Intravenous Use of Coconut Water." *The American journal of emergency medicine* 18(1): 108–111.

DebMandal, M. and Mandal, S. 2011. "Coconut (Cocos Nucifera L.: Arecaceae): In Health Promotion and Disease Prevention." *Asian Pacific journal of tropical medicine* 4(3): 241–247.

Dosumu, O., Duru, F., Osinubi, A., Oremosu, A. and Noronha, C. 2010. "Influence of Virgin Coconut Oil (VCNO) on Oxidative Stress, Serum Testosterone and Gonadotropic Hormones (FSH, LH) in Chronic Ethanol Ingestion." *Agriculture and Biology Journal of North America* 1(6): 1126–1132.

Gunn, B. F., Baudouin, L. and Olsen, K. M. 2011. "Independent Origins of Cultivated Coconut (*Cocos Nucifera* L.) in the Old World Tropics." *PLoS ONE* 6(6): 8.

Jawad, A. A. H. and Ali, M. H. 2010. "Toxicological and Some Reproductive Aspects of The Effects of Alcoholic Extract of Coconut on Male Albino Mice." *Bas. J. Vet. Res* 9(1): 71–77.

Kuberski, T., Roberts, A., Linehan, B., Bryden, R. N. and Teburae, M. 1979. "Coconut Water as a Rehydration Fluid." *The New Zealand medical journal* 90(641): 98–100.

Marcondes, F. K., Bianchi, F. J. and Tanno, A. P. 2002. "Determination of the Estrous Cycle Phases of Rats: Some Helpful Considerations." *Brazilian journal of biology (Revista brasleira de biologia)* 62(4A): 609–614.

Pinto, I. F. D., Silva, R. P., Filho, A. B. C., Dantas, L. S., Bispo, V. S., Matos, I. A., Otsuka, F. A. M., Santos, C. and Matos, H. R. 2015. "Study of Antiglycation, Hypoglycemic, and Nephroprotective Activities of the Green Dwarf Variety Coconut Water (Cocos Nucifera L.) in Alloxan-Induced Diabetic Rats." *Journal of Medicinal Food* Online ahead of print (February 4 2015). doi:10.1089/jmf.2014.0046.

Radenahmad, N. and Vongvatcharanon, U. 2006. "Serum levels of 17 β -estradiol in ovariectomized rats fed young-coconut-juice and its effect on wound healing". *Songklanakarin J. Sci. Technol.*, 28(5): 897–910.

Radenahmad, N., Saleh, F., Sawangjaroen, K., Vongvatcharanon, U., Subhadhirasakul, P., Rundorn, W., Withyachumnarnkul, B. and Connor, J. R. 2011. "Young Coconut Juice, a Potential Therapeutic Agent That Could Significantly Reduce Some Pathologies Associated with Alzheimer's Disease: Novel Findings." *The British journal of nutrition* 105(5): 738–746.

Saat, M., Singh, R., Sirisinghe, R. G. and Nawawi, M. 2002. "Rehydration after Exercise with Fresh Young Coconut Water , Carbohydrate-Electrolyte Beverage and Plain Water." *Journal of Physiological Anthropology and Applied Human Science*: 93–104.

Sandhya, V.G. and Rajamohan, T. 2006. "Beneficial effects of coconut water feeding on lipid metabolism in cholesterol-fed rats". *Journal Of Medicinal Food*, 9(3): 400–407.

Sandhya, V. G. and Rajamohan, T. 2008. "Comparative Evaluation of the Hypolipidemic Effects of Coconut Water and Lovastatin in Rats Fed Fat-Cholesterol Enriched Diet." *Food and chemical toxicology* 46(12): 3586–3592.

Schettino C. J . M., Petroianu, A., de Souza Vasconcelos, L., Cardoso, V. N., das Graças Mota, L., Barbosa, A. J. A., Vianna Soares, C. D. and Lima de Oliveira, A. 2015. "Coconut Water Solutions for the Preservation of Spleen, Ovary, and Skin Autotransplants in Rats." *Transplantation Proceedings* 47 (2): 536–544.

Solangi, A. H. and Iqbal, M. Z. 2011. "Chemical Composition of Meat (Kernel) and Nut Water of Major Coconut (Cocos Nucifera L .) Cultivars at Coastal Area of Pakistan." *Pak. J. Bot.* 43(1): 357–363.

Stockard, C. R. and Papanicolaou, G. N. 1917. "The Existence of a Typical Oestrous Cycle in the Guinea-Pig - with a Study of Its Histological and Physiological Changes." *American Journal of Anatomy* 22(2): 225–283.

Yusuh, M., Phochanukoon, N., Radenahmad, N., Eksomtramate, M., Ruangsri, P., Chantanasuksilpa, A. and Nitiruangjaras, A. 2010. "Changes of Condyle Cartilage in Orchidectomized Rats Fed with Young Coconut Juice: Novel Preliminary Findings." *Songklanakarin J. Sci. Technol.* 32(4): 333–339