Original Research Article

CHANGES IN ANTIOXIDANT/PRO-OXIDANT RATIO IN RESPONSE TO *Cocos nucifera* TREATMENT BRAIN TISSUE HOMOGENATES

ABSTRACT

Objective: This study revealed further effect of *Cocos nucifera* juice on antioxidant/pro-oxidant ratio in brain tissue homogenates of wistar rats.

Materials and methods: 40 male wistar rats were collected and randomly selected into 4 groups. Treatment protocols were; *i*-normal saline, *ii*-3ml *C. nucifera* juice, *iii*-6ml *C.nucifera juice*, *iv*-9ml *C. nucifera juice*. The study period lasted for 42 days. Brain tissue homogenate was prepared and biochemical analysis for antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx); pro-oxidant biomarkers hydrogen peroxide (H_2O_2), thiobarbituric acid reactive substances (TBARS) and nitric oxide (NO) were assayed for.

Results: The result showed that there was an increase in antioxidants concentration and a simultaneous decrease in pro-oxidant species generation as the dose of treatment was increased. The ratio of antioxidants to pro-oxidants showed a direct proportionality to the treatment dose.

Conclusion: *C. nucifera* juice has the ability to improve the level of production or synthesis of the assayed antioxidant enzymes and also has the efficacy to suppress pro-oxidant specie generation.

Keywords; *C. nucifera*; Antioxidants; Pro-oxidants; Thiobarbituric Acid Reactive Substances; Brain Tissue Homogenates

INTRODUCTION

Oftentimes, excessive free radical generation occurs in the body and the oxidant system cannotcope with such phenomenon [1]. Under normal conditions, there is a balance between the levelof pro-oxidants and antioxidants in living systems[2]. A loss in this balance towards pro-oxidants is a condition called oxidative stress. Oxidative stress occurs when the generation of free radicals and active intermediates in a system exceeds the system's ability to neutralize and eliminate them[1]. To cope with the oxidative stress elicited by aerobic metabolism, animal and human cells have developed ubiquitous antioxidant defense system, which consists of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR)

together with a number of low molecular-weight (LMW) antioxidants such as ascorbate, α-tocopherol, glutathione, cysteine, thioredoxin and vitamins[1][3]. However, these antioxidant defense systems may be overwhelmed by various pathological or environmental factors so that a fraction of reactive oxygen species may escape destruction and form the far more reactive hydroxyl radicals. Oxidative stress has been implicated in several neurodegenerative diseases like Parkinsonism[1][4] and Alzheimer's disease[1][5]. Recent studies also suggest that free radical generation is positively correlated with learning, memory and motor impairment[1]. Coconut, botanically known as *Cocos nucifera* [6], belongs to the family of *Arecaceae* (*Palmae*), an important member of monocotyledons [7].Indonesia, Philippines, India and Brazil are the world's largest coconut producers,accounting for over 25% of world production[6][8]. *C.nucifera* (Coconut) juice acts as a natural energy or sports drink,as it is rich in mineral content especially in potassiumlevels.*C. nucifera* juice has a high demand among consumers for its zero fat content and low contents ofcarbohydrates and sodium. *C. nucifera* juice serves as a potential healthy drink for adults and oldpersons as it has promising health utilities.*C. nucifera* is considered to be sterile unless the fruit is damaged from an external source[8][9].Coconut water is rich in mineral content with high potassium and anti-oxidantcontents which has various medical utilities. *C. nucifera*also contains cytokinin [10] which is one of themany beneficial components it is composed of.Around the world, *C. nucifera* juice has been used in popular medicine for the management of various diseases, such as arthritis, diarrhea, liver and kidney diseases[11].It is believed to be antigingivitic, febrifugal, antibronchitis and antiblenorrhagic. This study revealed further effect of *C.nucifera* juice on antioxidant/pro-oxidant ratio in brain tissue homogenate.

MATERIALS AND METHODS

Plant material

C. nucifera juice was collected daily from fresh fruits into sterilized bottles. The fruits were purchased during the dry season dated between 16st November and 31st December, 2018.

Animal collection

40 male wistar rats aged 80 days with weight between 150 to 170 grams were collected within the matrices of Experimental Animals Unit, Department of Human Physiology, Madonna University. The animals were confirmed to be healthy by a veterinarian in same institution.

Ethical approval

Standard protocols regarding the use of animals in research were carefully observed. Every effort was aimed at reducing suffering and utilizing well refined methods to prevent pain, stress and restraint.

Study design

This study included 4 groups sampled randomly from a population of 40wistar rats. The treatments were; *i*- normal saline and served as control, *ii*-3ml *C. nucifera* juice, *iii*-6ml *C. nucifera*juice, *iv*-9ml *C. nucifera*juice. The animals were exposed to an almost constant temperature of 25±3 ℃in normal day/night rhythm and pelleted feed was provided *ad libitum*. The duration of study was 42 days; 14 days for acclimatization, 28 days for treatment protocols.

Sacrifice and sample collection

Sacrifice and brain sample collection was done on day 42 using methods previously described(Ilochi, et al., 2018) [1].

Biochemical analysis

Brain tissue homogenate was prepared following methods previously described. Antioxidants assayed for include the enzymatic antioxidants; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and pro-oxidants; hydrogen peroxide, thiobarbituric acid reactive substances (TBARS) and the nitrosative agent, nitric oxide. Biochemical assay for each marker was performed following standard protocols(Ilochi, et al., 2018) [1].

Statistical analysis

IBM®SPSS version 20.0 was the statistical tool used for this study. Data was expressed as Mean ± standard error of mean (SEM). One way analyses of variance (ANOVA) was calculated using recommended bio-statistical guidelines [11].

RESULTS

The following results were obtained from this study;

Table 1; effect of *C. nucifera* treatment on antioxidants in brain tissue homogenates

Treatments	SOD (µg/l)	CAT (µg/l)	GPx (µg/l)
Normal saline	221.1±0.4	102.0±0.1	98.2±0.4
3ml	221.3±0.3	227.2±2.1*	111.4±0.2*
6ml	322.1±0.3*	233.2±1.3*	117.0±1.3*
9ml	446.4±1.2*	241.1±0.1*	242.0±0.3*

Key; *=values statistically significant at 95% confidence interval compared to control.

Effect of *C. nucifera* treatment on antioxidants

C. nucifera juice treatment caused an increase in antioxidant enzymes and this increase was directly proportional to the dose administered. As the dose of treatment was increased, there was a significant increase in the brain tissue level of SOD,CAT and GPx compared to control. From this result, the juice has a dose-dependent positive effect on brain tissue antioxidant enzyme markers.

Table 2; effect of *C. nucifera* treatment on pro-oxidants in brain tissue homogenates

Treatments	H_2O_2(µg/l)	TBARS (µg/l)	NO (µg/l)
Normal saline	43.4±0.2	37.3±1.0	22.1±0.2
3ml	42.2±0.3	22.1±0.4*	21.3±1.7
6ml	20.3±1.4*	17.2±1.1*	24.0±0.1*
9ml	17.2±1.3*	12.4±0.2*	24.3±1.1*

Key; *=values statistically significant at 95% confidence interval compared to control.

Table 3; effect of *C. nucifera* on antioxidant/pro-oxidant ratio in brain tissue homogenate

Treatment	Antioxidant-Pro-oxidant Ratio					
Normal saline	SOD[2]	CAT[1]	GPx[1]	H_2O_2[2]	TBARS[2]	NO[1]
3ml	CAT[2]	SOD[2]	GPx[1]	H_2O_2[2]	TBARS[1]	NO[1]
6ml	SOD[2]	CAT[2]	GPx[1]	NO[1]	H_2O_2[1]	TBARS[1]
9ml	SOD[2]	GPx[1]	CAT[1]	NO[2]	H_2O_2[1]	TBARS[1]

Effect of *C. nucifera* treatment on pro-oxidants

C. nucifera juice treatment caused a decrease in level of brain tissue pro-oxidants,H_2O_2, TBARS and NO. This decrease was dose-dependent or negatively correlated with the dose of treatment. The juice may have enhanced the level of antioxidant thereby indirectly reducing the level of pro-oxidants in brain tissue.

Effect of *C. nucifera* on antioxidant/pro-oxidant ratio

The ratio of antioxidant to pro-oxidant revealed CAT to be a predominant antioxidant enzyme against the pro-oxidant $H_2O_2$when treatment dose of 3ml was administered. SOD showed highest level of prevalence against NO at treatment doses 6ml and 9ml.

DISCUSSION

Oxidative stress is a serious burden since it has been implicated in several diseases like diabetes, hypertension and neurodegenerative disorders[1]. Biomedical studies have been conducted extensively to determine possible prevention and management of oxidative burden[1] [12]. This study revealed further effect of *C. nucifera* juice on antioxidant enzymes and pro-oxidant generation in brain tissue homogenate of animal model. The phytochemical composition of *C. nucifera* juice has already been evaluated [11] [12]. From the outcome of this study, the juice significantly increased the level of antioxidants in all sample and this increase was directly proportional to the dose administered. As the dose of treatment was increased, there was a corresponding increase in all antioxidant enzymes. There was a corresponding dose-dependent progressive decrease in pro-oxidants in the brain tissue homogenates. This decrease in pro-oxidant occurred simultaneously with the increase in level of antioxidants. It can be deduced from this study that as the dose of *C. nucifera* juice treatment is increased, there will be a progressive increase in antioxidant/pro-oxidant ratio. The reason behind these physiologic manifestations may be because of some phytochemicals like flavonoids and other phenolic compounds which have the capacity to boost antioxidant level and suppress pro-oxidant generation in a system [11]. An increase in endogenous antioxidant generation may cause a progressive or proportionate decrease in pro-oxidant species generation in a living system [13]. This can be applied in the management of certain diseases with an oxidative causality [14]. The effect of the juice on antioxidant and pro-oxidant level was predominant when the highest dose in this study was administered, this may infer that at the dose of treatment, the concentration of the active agents in the juice were either just enough or more than enough to cause marked significant changes in the tested biomarkers.

CONCLUSION

From the outcome of this study, *C. nucifera* juice has the ability to enhance antioxidant/pro-oxidant ratio in neural tissues and may have the therapeutic potential in prevention and management of oxidative stress related changes.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we

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