Australian Journal of Basic and Applied Sciences, 5(9): 1116-1122, 2011
ISSN 1991-8178

Anti-Nociceptive and Anti-Inflammatory Potential of Coconut Water (*Cocos Nucifera* L.) In Rats and Mice.

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Abstract: Scientific evidence has established the role of coconut water in health and medicinal applications. The present study was aimed at investigating its anti-nociceptive and anti-inflammatory potential in laboratory animals. Hot plate, tail flick, formalin-induced paw licking and acetic acid-induced writhing tests were done to investigate the anti-nociceptive activity while carrageenan-induced paw edema was used to investigate the anti-inflammatory activity of coconut water after oral feeding for two, four and six weeks. Animals treated with normal saline and reference drugs served as negative and positive controls respectively. Coconut water significantly reduced nociception and inflammation in a duration-dependent manner. Latencies were increased in the coconut water treated over 0-90 min observation period in the hot plate test and 46-54^{0}C tail immersion test ($p<0.05$). In formalin test, coconut water showed a reduced paw-licking time both in early and late phase when compared with normal saline treated group ($p<0.001$). Also, it ameliorated acetic acid-induced writhing and carrageenan-induced paw edema by 55% and 50.2% respectively at the end of six weeks. Data were comparable with analgesic and anti-inflammatory activities of the standard drugs used in the study. The results demonstrate analgesic and anti-inflammatory activity of coconut water, which may be linked to its unique chemical composition.

Key words: Coconut water, Hot plate test, Tail immersion test, Acetic acid-induced writhes, Carrageenan-induced paw edema.

INTRODUCTION

Coconut water is a refreshing and nutritious beverage widely consumed in the tropics culturally or traditionally for its beneficial properties to health (George and Sherrington, 1984; Janick and Paull, 2008). The numerous medicinal properties have been documented (Yong, *et al*, 2009).

It is believed that coconut water could be used as an important alternative for oral rehydration and intravenous hydration of patients particularly in remote regions (Campbell-Falck, *et al*, 2000). It may also offer protection against myocardial infarction (Anurag and Rajamohan, 2003) and control of hypertension (Alleyne, *et al*., 2005). It is also effective in the treatment of kidney and urethral stones, urinary infections and mineral poisonings (Macalalag, and Macalalag, 1987).

Aside other significant and useful components in it, coconut water contain cytokinins, a class of phytohormones (Kende and Zeevaart, 1997) with significant anti-ageing, anti-carcinogenic, and anti-thrombotic effects (Rattan and Clark, 1994; Vermeulen, *et al*., 2002). Inorganic ions and vitamins in coconut water play a vital role in strengthening the human body antioxidant system (Evans and Halliwell, 2001). Other components which include sugars, alcohols, lipids, amino acids, nitrogenous compounds, organic acids and enzymes all play different functional roles in plant and human systems due to their distinct chemical properties (Santoso, *et al*., 1996; Arditti, 2008).

Furthermore, coconut water has been shown to attenuate ulcer formation in rats (Nneli and Woyike, 2008), thereby hypothesizing modulation of chronic pain known to be associated with inflammatory disorders of the gastrointestinal mucosa. Previously, we had reported from our laboratory that not only protein malnutrition but micronutrients deficiencies cause overt hypersensitivity to acute and chronic pain in laboratory animals (Ibironke, *et al*., 2009). Since coconut water contains a blend of vitamins, minerals, amino acids, sugars, antioxidants, enzymes, health enhancing growth hormones, and other important nutrients which justified its wide application for health and nutritional benefits, the present study was aimed at investigating its anti-nociceptive and anti-inflammatory properties. This is imperative because of the paucity of documented information on possible analgesic potential of coconut water.

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MATERIALS AND METHODS

Collection and Preparation of Coconut Water:

Coconuts were harvested from coconut tree (*Cocos nucifera*) grown in Okada village in Ovia North-East LGA of Edo state. Fresh coconut of 10 months' maturity (mature stage) used for this study were dehusked and broken carefully, and the liquid endosperm was used for each day's oral feeding. Four milliliters of coconut water per 100 g body weight (Sandhya and Rajamohan, 2008) was given to the rats in the experimental groups for two, four and six weeks.

Experimental Animals and Design:

Male albino rats of Wistar strain (180-220 g) and Swiss mice (29-35 g) were used for this study. The animals were obtained from the Central Animal House of Igbinedion University, Okada and kept at standard environmental conditions, subjected to 12 h light-dark cycle, 25 ± 2^0C and relative humidity 50-60%. They were fed with rat and mouse cubes and water *ad libitum.*

The animals were divided into five groups for each experimentation viz: Group 1, normal saline treated; Group 2, 3 and 4, two, four and six weeks oral feeding with coconut water respectively; Group 5, standard drug. This is replicated for hot plate, tail immersion, formalin-induced paw licking, acetic acid-induced writhing and carrageenan-induced edema tests.

Nociceptive Evaluation Test:

Hot Plate Test In Rats:

Pain reflexes in response to a thermal stimulus were measured using a hot plate apparatus (Ugo Basile, Italy) as described by Eddy and Leimback (1953). The control group received normal saline (2 ml per 100 g body weight). The test groups received four milliliters of coconut water for two, four and six weeks while indomethacin (5 mg/kg) was used as a reference drug. Rats were habituated to the apparatus for 3 minutes before the start of the test. They were placed on the hot plate of 25.4 cm x 25.4 cm at 45 ± 1.0^0C which was surrounded by an opened top acrylic cage (19 cm tall), with the start/stop button on the timer. Response latencies were measured at 30 minutes interval and the average of the results were taken.

Tail Flick Test:

The tail flick latency was measured using a medication of D' Amour and Smiths, (1941) tail immersion method. Each animal was gently hand-held with its tail wholly immersed in water at 46^0C, 50^0C and 54^0C after pretreatment with coconut. The latency for flicking the tail out of the water was recorded with a stopwatch.

Formalin-Induced Paw Licking Test In Rats:

20µL of 2.5% formalin was injected into the plantar surface of the left hind-paw (Hunskaar and Hole, 1987). The test was carried out in a transparent plastic chamber (30 x 30 x 30) cm with a mirror placed at the base of the chamber to allow an unobstructed view of the rats. Each animal was allowed to explore the chamber five minutes before receiving an injection of formalin. The time mice spent licking the injected paws was measured as an index of pain or nociception. The test animals were pre-treated with coconut water for two, four and six weeks and aspirin (150 mg/kg) thirty minutes before the administration of formalin. The early phase (initial nociceptive response) was 5 minutes after formalin injection (0-5min). The final phase (second nociceptive response) was between 15 to 30 minutes, post injection.

Acetic Acid-Induced Abdominal Writhing In Mice:

The mice were injected, i.p. with 0.2ml of 3% acetic acid solution. This induced the characteristic writhing (Siegmund, *et al*, 1957; Koster, *et al*., 1959). The number of writhing and contraction of the abdominal muscle together with stretching of the hind limbs was observed between 5 and 15 minutes. The data were computed according to the following formula:

$$\%\ inhibition = \frac{\text{WRITHING (control)} - \text{WRITHING (test)}}{WRITHING\ (test)} \text{X } 100$$

Anti-Inflammatory Activity:

Carrageenan-Induced Paw Edema In Rats:

An injection of 0.1 ml 1% carrageenan was delivered into the right hind paw of each rat under the sub plantar aponeurosis which produced pedal inflammation in male Wistar rats according to Winter, *et al.,* (1962). The animals were pre-treated with the coconut water, and ibuprofen, a reference drug was given 30 minutes before carrageenan injection. Measurement of the paw volume was carried out by plethysmometrically. The inhibiting activity was calculated according to the formula:

$$\%\ inhibition = \frac{(Ct - Co)control - (Ct - Co)test}{(Ct - Co)control} \text{ X } 100$$

[Where C_o is the mean paw size in the control group and Ct is the mean paw size in the treated group].

Statistical Analysis:

Data were analyzed by SPSS (version 15) and Microsoft Excel's XL Toolbox statistical package (2.60 version) using descriptive statistics, ANOVA and student's t test at $p<0.05$ (Glantz, 2005).

Results:

The Anti-Nociceptive Effects Of Coconut Water On Hot Plate Latency Assay In Rats:

Coconut water showed a statistically different anti-nociceptive property when compared with the normal saline treated. Hot plate latency was increased over the 0-90 minutes' observation period. Also, duration-dependent anti-nociceptive effect was observed in the assay. While latencies of the animals treated with coconut water for six weeks were 1.50 ± 0.1 min, 1.82 ± 0.12 min, 2.10 ± 0.11 min and 2.80 ± 0.13 min, four weeks treated group exhibited 1.22 ± 0.06 min, 1.35 ± 0.15 min, 1.53 ± 0.1 min and 1.93 ± 0.15 min at 0, 30, 60 and 90 minutes respectively ($p<0.05$). Animals treated with indomethacin tolerated the pain till 3.54 ± 0.85 min and 4.30 ± 1.05 min at 30 and 60 minutes observation period respectively. The anti-nociceptive potential of coconut water on hot plate test is shown in figure 1.

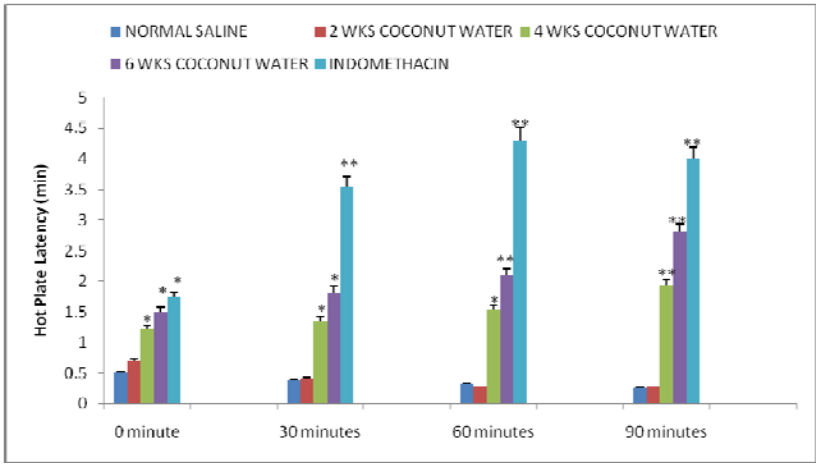

Fig. 1: The anti-nociceptive effect of coconut water on hot plate latency assay in rats. Each bar represents mean ± SEM (n=7 rats). *p<0.05, **p<0.001; significant difference from NS.

The Anti-Nociceptive Effects of Coconut Water on Tail Flick Assay in Rats:

The anti-nociceptive effect of coconut water was also exhibited in the tail immersion test (Figure 2). Four weeks and six weeks pretreatment of coconut water increased tail flick latencies (0.49 ± 0.01 min, 0.51 ± 0.16 min) in water temperatures 50 and 54^0C when compared with the normal saline group (0.24 ± 0.01, 0.19 ± 0.02) ($p<0.05$). Also, indomethacin reduced the pain sensitivity by putting the latency at 0.48 ± 0.06 min, 0.82 min ± 0.07 and 0.60 min ± 0.02, $p<0.001$.

The Anti-Nociceptive Effects of Coconut Water on Formalin-Induced Paw Licking in Rats:

Coconut water showed duration-dependent anti-nociceptive properties both at early and late phases of the paw licking in the rat (Figure 3). At the early phase, the licking time for four and six weeks group is 50.00 ± 2.5 seconds and 40.00 ± 3.5 seconds respectively compared with 65.00 ± 2.0 in the normal saline group ($p<0.05$). At the late phase, two, four and six weeks' groups all reduced the licking time with 145 ± 5.15, 100 ± 3.5 and 73 ± 4.0 seconds respectively versus 180 ± 7.5 seconds in normal saline group. Aspirin group, both at early and late phase, exhibited reduced time at 27 ± 1.45 and 48 ± 2.10 seconds respectively.

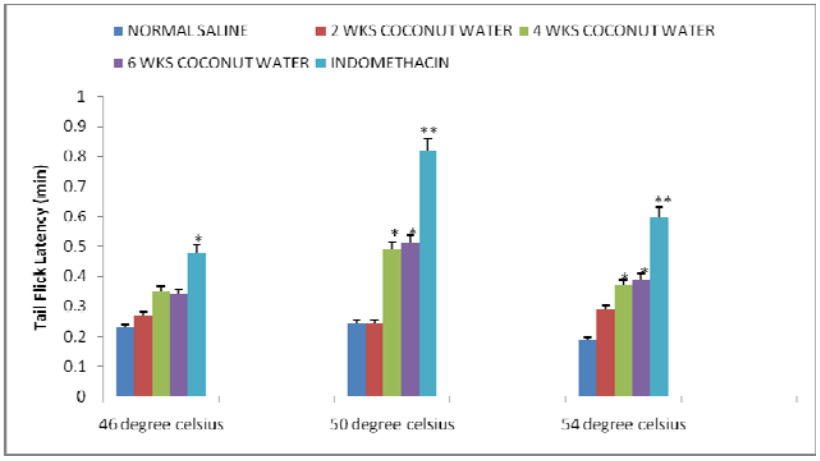

Fig. 2: The anti-nociceptive effect of coconut water on tail flick latency assay in rats. Each bar represents mean ± SEM (n=7 rats). *p<0.05, **p<0.001; significant difference from NS.

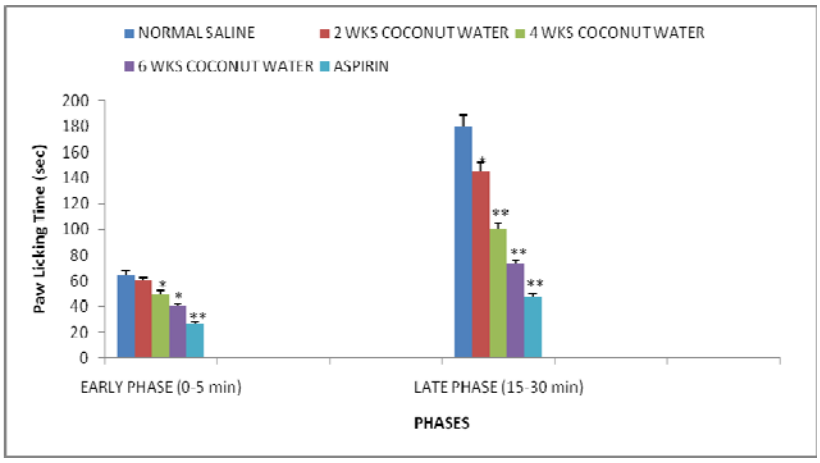

Fig. 3: The anti-nociceptive effect of coconut water on formalin-induced paw licking in rats. Each bar represents mean ± SEM (n=7 rats). *p<0.05, **p<0.001; significant different from NS.

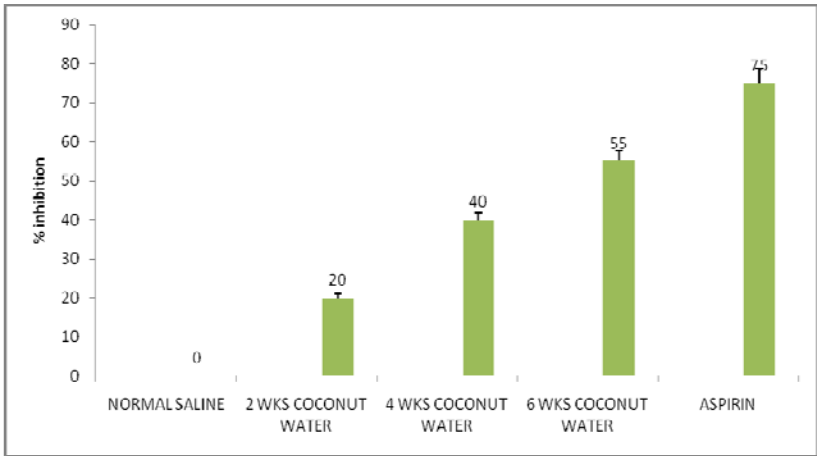

Fig. 4: The anti-nociceptive effect of coconut water on acetic acid-induced writhes in mice. Each bar represents the percentage inhibition of the mean number of writhing/10 minutes compare with control (n=7 rats).

The Anti-Nociceptive Effects of Coconut Water on Acetic Acid-Induced on Abdominal Writhing in Mice:

The anti-nociceptive effect of coconut water on mice using acetic acid-induced abdominal writhing is shown in figure 4. While aspirin afforded 75% ($p<0.001$), coconut water at two, four and six weeks caused 20%, 40% and 55% ($p<0.05$) inhibition respectively when compared with the normal saline treated.

The Anti-Inflammatory Effects of Coconut Water on Carrageenan-Induced Paw Edema in Rats:

The effect of coconut water on carrageenan-induced paw edema is shown in figure 5. This property is pronounced in four and six weeks' treatments of animals with coconut, exhibiting 10.6% and 20.6%, 12.6% and 30.4%, 20% and 40.8%, 23.7% and 50.2% over first, second, third and fourth hour respectively. Ibuprofen tolerated the edema by 90.3% at the third hour.

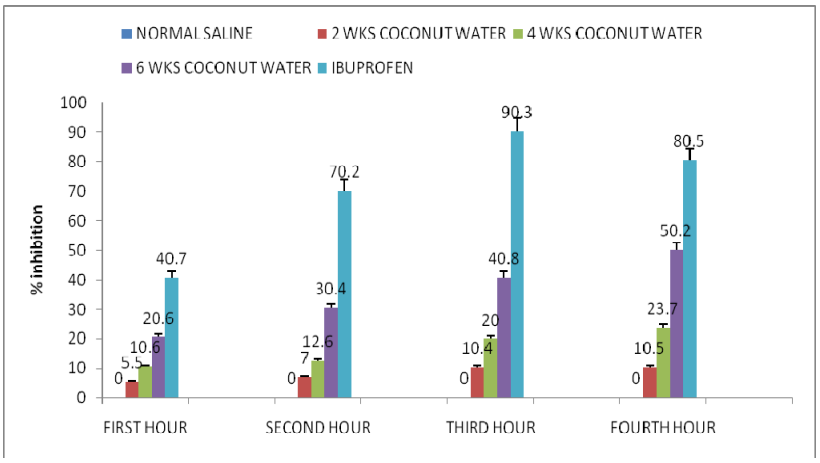

Fig. 5: The anti-inflammatory effect of coconut water on carrageenan-induced paw edema in rats. Each bar represents percentage inhibition of the mean paw volume compared with control (n=7 rats).

Discussion:

The results of the study show that coconut water possesses analgesic and anti-inflammatory properties in a duration-dependent manner. The analgesic property was demonstrated on the basis of thermal nociception in the test models of hot plate and tail immersion, and chemical nociception in formalin-induced paw licking and acetic acid induced writhing tests. Anti-inflammatory effect was evaluated using the widely accepted test model of carrageenan-induced paw edema.

The hot plate and tail flick tests are based on phasic stimulus of high intensity with central mechanisms (Tjolsen, *et al*., 1992; Ramabadran, *et al*, 1989). This study indicated that coconut water had latent periods in both hot plate and tail flick tests significantly increased. The hot plate is a specific central anti-nociceptive test in which opoid agents exert their analgesic effects *via* supra spinal and spinal receptors (Tjolsen, *et al,* 1992). The tail immersion test indicated that the pharmacological actions were mediated by μ opioid receptors rather than κ and δ receptors (Ramabadran, *et al*., 1989). The activity of coconut water on these models of pain suggests clearly that it might be centrally acting.

Formalin-induced paw licking represents a chemically-induced burning pain. In the test, the nociceptive response has two phases: the early acute phase (0-5 min) reflects direct effect of formalin on nociceptive C fibers whereas the late chronic phase (15-30 min), accompanied by well extended nociceptive response (Hunter and Singh, 1994) and functional changes in nociceptive C fibers (Tjolsen, *et al*, 1992) is manifested by behaviorally as flinching and licking of the affected paw. Experimental findings had also indicated that substance P and bradykinins participate in the early phase while histamine, serotonin and prostaglandins are involved in the late phase (Shibata, *et al*, 1989). Coconut water, as revealed in the study, reduced the paw licking time in these animal models of pain both at early phase late phase significantly even at values comparable to aspirin, a reference drug. It may also be suggested that coconut water possess peripheral anti-nociceptive effect since the biphasic model of pain is represented by direct effect on nociceptors and inflammatory nociceptive responses (Siegmund, *et al*, 1957).

In the acetic acid induced writhes model of pain, the activity consists of contraction of the abdominal muscles with a stretching of the hind limbs which is nociceptive (Hernandez-Perez and Rabanal, 2002). Acetic acid causes pain by inducing release of endogenous mediators, such as PGE2 and PGF2α in peritoneal fluids as well as lipooxygenase products, which stimulate the nociceptive neurons (Blane, 1967). Therefore, the results of

the acetic acid-induced writhing strongly suggest that a probable mechanism of coconut water may be linked partly to inhibition of lipooxygenase and/or cyclooxygenase in peripheral tissues and interfering with the mechanism of transduction in primary afferent nociceptor.

In the carrageenan-induced paw edema model of inflammation, the anti-inflammatory activity of coconut water was significantly high in the study. Carrageenan-induced inflammation in the rat paw is a standard model of edema formation and hyperalgesia. Several lines of evidence indicate that the COX-2-mediated increase in prostaglandin (PG) E2 production in the central nervous system (CNS) contributes to the severity of the inflammatory and pain responses in this model. COX-2 is rapidly induced in the spinal cord and other regions of the CNS following carrageenan injection in the paw (Guay, *et al*, 2004). Hence, this anti-inflammatory potential of coconut water may be linked descriptively with inhibiting prostaglandin production, thereby reducing inflammation and pain.

The anti-nociceptive and anti-inflammatory effects observed in this study, like other biological properties of coconut water (Yong, *et al*, 2009), may be due to its unique composition of sugars, vitamins, minerals, amino acid acids and cytokinins. This may be corroborating to earlier reports that lack or deficiency of micronutrients (vitamins and minerals) reduced pain threshold in both lactating and adult rats (Rocinholi, *et al*, 1997; Ibironke, *et al*., 2009).

Conclusively, these results show that coconut water possesses analgesic and anti-inflammatory properties.

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