

## EFFECT OF CAFFEINE -COCONUT PRODUCTS INTERACTIONS ON INDUCTION OF MICROSOMAL DRUG-METABOLIZING ENZYMES IN WISTAR ALBINO RATS

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**Summary:** Effect of caffeine-coconut products interactions on induction of drug-metabolizing enzyme in wistar albino rats was studied. Twenty rats were randomly divided into four groups: The control group (1) received via oral route a placebo (4.0ml of distilled water). Groups 2 to 4 were treated for a 14-day period with 50mg/kg body weight of caffeine, 50mg/kg body weight of caffeine and 50mg/kg body weight of coconut water, and 50mg/kg body weight of caffeine and 50mg/kg body weight of coconut milk in 4.0ml of the vehicle via gastric intubation respectively. One day after the final exposure, the animals were anaesthetized by inhalation of an overdose of chloroform. The blood of each rat was collected by cardiac puncture while the liver of each rat was harvested and processed to examine several biochemical parameters, ie, total protein and RNA levels, protein/RNA ratios, and activities of alanine and aspartate amino transferase (ALT and AST, respectively). The results showed that while ingestion of coconut milk and coconut water increased the values of protein and protein/RNA ratios, it decreased alanine and aspartate amino transferase (ALT and AST) activities. These effects, in turn, enhanced the induction of the metabolizing enzymes and a resultant faster clearance and elimination of the caffeine from the body, there by reducing the toxic effect on the liver.

**Key Words:** *Coconut water, coconut milk, caffeine, protein, RNA, aminotransferases, liver and enzymes.*

### Introduction

Humans are widely exposed to various foreign chemicals such as drugs, food additives and pollutants. These substances interfere with the absorption, metabolism, distribution and excretion of nutrients, and are mostly insoluble in the body fluids, thus, potentiating their toxic effects in tissues such as the liver and brain (Eteng *et al*, 1997; Ekam, 2001; Obochi, 2006). Genetics, environmental and physiological factors are involved in the regulation of drug biotransformation reactions and are thought to be responsible for prolonged pharmacological effects in the tissues (Bahtnager and Misra, 1988; Coon, 1996; Raisfield *et al*, 2000; Obochi, 2006). Drugs metabolizing activities within the cells take place primarily in the endoplasmic reticulum and involve microsomal enzymes or mixed function oxidases (Goldberg and Mitsugi, 1967; Craft *et al*, 1979; Bahtnager and Misra, 1988; Coon, 1996; Singh *et al*, 1998). These enzymes act to increase microsomal membrane phospholipids and proteins, resulting in the proliferation of the smooth endoplasmic reticulum, an adaptive

measure, to increase the volume (hypertrophy) of the smooth endoplasmic reticulum, and modification of these enzymes due to demethylation process (Suresh and hedge, 1971; Bahtnager and Misra, 1988; Singh *et al*, 1998). The significance of these enzymes modification is to increase the solubility or hydrophilic properties of these foreign chemicals thereby facilitating their excretion (Suresh and Hedge, 1971; Effiong, 2003; Obochi, 2006).

Caffeine, 1,3,7-trimethylxanthine, is consumed through processed cocoa, coffee, tea or kola nut based foods and beverages, stimulants, drugs and cosmetics (Eteng *et al*, 1997; Obochi, 2006). The metabolism and toxicity of caffeine are viewed to be dose dependent, resulting in non-linear accumulation of the methylxanthines, hence greater risk of cardiovascular diseases such as heart failure, high blood pressure and neuronal disorder such as schizophrenia (Cucinnel *et al*, 1965; Shaffner and Popper, 1969; Leon and Hedge, 1970). Metabolic derangement and subsequent toxicity of caffeine leads to weight loss, poor growth, low protein efficiency ratio and poor nitrogen

retention, leading to death (Leon and Hedge, 1970; Eten *et al*, 1997, Ekam, 2001). Thus, the toxicity of caffeine is viewed as a disposition demonstrating that caffeine is rapidly absorbed but slowly excreted (Leon and Hedge, 1970; Eteng *et al*, 1997; Ekam, 2001; Obochi, 2006).

Caffeine also acts to increase alertness, anxiety and hallucination due to its blocking of adenosine receptors which normally inhibits glutamate release (Ekam, 2001; Obochi, 2006). Glutamate supplies the amino group for the biosynthesis of amino acids, and is a substrate for glutamine and glutathione synthesis (Danbolt, 2001). Inhibition of glutamate release results in low protein synthesis and efficiency (Bertolini *et al*, 1980; Bahtnager and Misra 1988). Adenosine receptors are linked with an interplay of release, reuptake, metabolism and excretion of neurotransmitters (Obochi, 2006). Thus, blockage of adenosine receptors by caffeine results in alteration in behavioural pattern by delaying neuronal tube closure (Eteng *et al*, 1997; Obochi, 2006). Caffeine also activates phosphorylase and lipase, thus, enhancing glucogenolysis and lipolysis, resulting in loss of weight. Caffeine also inhibits androgen binding protein, resulting in decreased cauda epididymis sperm reserve, seminiferous tubular fluid volume, resulting in low sperm production and infertility (Eteng *et al*, 1997; Obochi, 2006).

Mechanisms of action of caffeine involve interaction with hormones, peptides and receptors on the surface of the plasma membrane to generate signals since these molecules cannot cross the plasma membrane (blood-brain-barrier). The amplification and subsequent transmission of such signals to the cell interiors require the participation of second messenger, usually a cyclic nucleotide, cAMP, while the hormones, peptides or receptors serve as the first messenger (Eteng *et al*, 1997; Ekam, 2001; Obochi, 2006).

Coconut water is essentially composed of water, amino acids, (Arginine, glutamic acid, Aspartic acid, alanine) vitamins (ascorbic acid, folic acid and pyridoxine), minerals (potassium, nitrogen, calcium, phosphorus, iron, sodium, chloride, bicarbonate), sugars, fats and nitrogenous substances (Baptist, 1956; Pandalai, 1958; Suresh and Hedge, 1971; Prosavior and Rubico, 1979; Davis, 1982; Pehowich *et al* 2000; Effiong, 2003). The mineral composition of coconut water potentiates it as an oral fluid for rehydration therapy, and as an antacid, a therapy for peptic ulcer, caused by high secretion of gastric acid (Church, 1972; Wright and Prescolt, 1973; Prosaviour et al, 1979; Anzaldo *et al*, 1980; Davis, 1982; Pehowich *et al*, 2000; Effiong, 2003).

Coconut water has been found to contain hydrolytic enzymes such as cellulase, which breaks down the wall of kernel to liberate oil, upon which lipase acts to release free fatty acids and glycerol. Other enzymes present include proteinases which give rise to various amino acids and nitrogen by hydrolysis of protein in the kernel (Baptist, 1956; Church, 1972; Anzaldo *et al*, 1980; Pehowich *et al*, 2000; Effiong, 2003).

Coconut milk is essentially composed of high amount of protein, amino acids (glutamine, Arginine, lysine, leucine, proline), water, sugars (lactose), fats, vitamins (Ascorbic acid, Nicotinic acid, Biotin pantothenic acid), minerals (nitrogen, calcium, iron, phosphorus) (Anzaldo *et al*, 1980; Davis, 1982; Pehowich *et al*, 2000; Effiong, 2003).

This current study focused on the assessment of hepatocellular drug-metabolizing enzyme induction or inhibition in animals exposed daily to caffeine, caffeine and coconut water, caffeine and coconut milk, or a combination of the two. Enzyme activities were examined in whole homogenate (WH) and post-mitochondrial supernatants (PMS) prepared from the liver of rats within each treatment regimen.

## Materials and Methods

### *Experimental Animals:*

Twenty (20) wistar albino rats weighing between 150-300g obtained from the disease free stock of the animal house, Department of Biochemistry, College of Medical Sciences, University of Calabar, Nigeria, were used for the study. The animals were randomly assigned on the basis of average body weight and litter origin into four (4) study groups of five (5) animals per group. Each rat in a study group was individually housed in a stainless cage with plastic bottom grid and a wire screen top. The animals room was adequately ventilated, and kept at room temperature and relative humidity of  $29 \pm 2^{\circ}\text{C}$  and 40 – 70% respectively with a 12 hour natural light – dark cycle.

### *Treatment Regimen:*

The animals were fed *ad libitum* with water and rat chow (livestock feeds Ltd, Calabar, Nigeria). Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily. The control group (1) received via oral route a placebo (4.0ml of distilled water). Groups 2 to 4 were treated for a 14-day period with 50mg/kg body weight of caffeine, 50mg/kg body weight of caffeine and 50mg/kg body weight of coconut water, and 50mg/kg body weight of caffeine and 50mg/kg body weight of coconut

milk in 4.0ml of the vehicle via gastric intubation (i.e, orally using orogastric tubes and syringes) respectively. The experiments were conducted between the hours of 9.00am and 10.00am daily.

#### *Sample Preparation:*

One day after the final exposure, the animals were anesthetized by inhalation of an over dose of chloroform, the blood of each rat was collected by cardiac puncture and centrifuged at 4000 x g for 30 minutes into serum. The liver of each rat was harvested, ground using mortar and pistle, and buffered with TRIS buffer, pH 7.4. A whole homogenate (WH) was prepared by centrifugation using a centrifuge at 4000xg for 30 minutes. Subsequently, aliquots of the whole homogenate (WH) obtained from each rat liver was subfractionated as follows an aliquot of 10ml of each whole homogenate was placed in a pyrex graduated test tube and was quantitatively transferred into centrifuge tubes. The tubes were balanced in a centrifuge and subfractionated by spinning for 10 minutes at 6000xg (1,900rpm). The resultant 6000xg post nuclear supernatant (PNS) was centrifuged at 10,000xg for 15 minutes to obtain the post mitochondrial supernatant (PMS), which was made up to 100ml mark with the TRIS buffer, pH 7.4, in a volumetric flask. The serum, whole homogenate and the post mitochondrial supernatant were stored at -70<sup>0</sup>c freezer and used for the various assays.

#### *Biochemical Assays:*

The whole homogenate (WH) and post mitochondrial supernatant (PMS) were used for the analysis of the protein, RNA, and Protein/RNA ratio; the serum that was collected was used for the estimation of alanine and aspartate amino transferase (ALT and AST, respectively) activities. Liver protein was determined using the Biuret method described by Lowry et al (1951). Liver RNA was determined with modifications of the method of Burton (1956). Protein/RNA ratio was calculated. Alanine and aspartate amino transferase (ALT and AST) were determined using the standard methods described by Mathieu et al (1982).

#### *Preparation of Caffeine:*

Synthetic caffeine was obtained from May and Baker (M&B) limited, Enfield, Middle Sex, United Kingdom (UK), and used for the study. A stock solution of caffeine was prepared by dissolving 20g of powdered caffeine in 500ml of hot distilled water. The solution was allowed to cool to room temperature. Out of the

stock solution, 50mg/kg body weight of caffeine was administered to all the test groups (i.e, groups 2 to 4) in 4.0ml of the vehicle via gastric incubation respectively.

#### *Preparation of Coconut Milk and Coconut Water:*

A matured coconut fruit was obtained from a coconut plantation in Calabar, Nigeria, and used for the study. The fruit was shelled, the nut removed and the water collected. The nut was then grated using a stainless grater. A stock solution of coconut milk was obtained by dissolving 50g of the grated mass of coconut in 500ml of distilled water, heated to 65<sup>0</sup>c and the residues removed using a sieve. The solution was boiled for 30 minutes, and oil scooped. The solution was again boiled with constant stirring for another 30 minutes, and the crude milk was allowed to cool to room temperature. 50mg/kg body weight of coconut water and 50mg/kg body weight of coconut milk obtained were administered to the animals in groups 3 and 4 respectively in 4.0ml of the vehicle via gastric incubation.

#### *Statistical Analysis:*

Data collected were expressed as mean  $\pm$  standard deviation (SD) and the student 't' test were used for analysis. Values of p<0.05 were regarded as significant.

#### **Results**

The results of the administration of caffeine, caffeine and coconut water and caffeine and coconut milk on levels of hepatic protein, RNA, protein/RNA ratios, ALT and AST are presented in Tables 1,2,3, and 4 respectively. The results for caffeine alone (i.e, groups 2) showed that there was a significant increase (p<0.05) in the values of hepatic protein contents, as well as in serum ALT and AST activities, in the caffeine-treated groups when compared to values observed with control rat samples. This finding was attributable to the increases in protein levels alone, as there were no significant differences (p<0.05) in values of the hepatic RNA levels and protein/RNA ratios as compared to those in the controls. However, the results for the caffeine and coconut water treated groups (ie, groups 3) showed that there was a significant increase (p<0.05) in the values of hepatic protein and protein/RNA ratios when compared to those of the controls while there was a significant decrease (p<0.05) in the values of ALT and AST activities. Also, the results for the caffeine and coconut milk treated groups (i.e., groups 4) showed that there was a significant increase (p<0.05) in the values of hepatic protein contents and protein/RNA ratios

when compared to those of the controls while there was a significant decrease ( $p < 0.05$ ) in the values of ALT and AST activities.

Comparing the values of the hepatic protein levels and protein/RNA ratios obtained in the caffeine and coconut water treated groups to those in the caffeine and coconut milk treated groups, it was observed that the values of the hepatic protein levels and protein/RNA ratios in

the caffeine and coconut milk treated groups were higher than those of the caffeine and coconut water treated groups and vice versa for the values of ALT and AST activities.

However, a study on the interactions of the three substrates (ie, caffeine, coconut waster and coconut milk) may be rewarding to diagnosis and or treatment.

Table 1. Effect of treatment on liver protein content in Wistar albino rats.

	Group (N)	WH (mg/ml)	PMS (mg/ml)
1.	Control	7.12 ± 0.57	5.64 ± 0.51
2.	Caffeine	10.43 ± 0.48*	8.36 ± 0.43*
3.	Caffeine + Coconut Water	18.66 ± 0.83*	16.59 ± 0.52*
4.	Caffeine + Coconut Milk	22.68 ± 0.76*	20.68 ± 0.67*

*N* = Number of rats per group = 5. Values are expressed as mean ± SD. WH = Whole homogenate and PMS = Post mitochondrial supernatant. \* Significantly different from control,  $P < 0.05$ , using student 't' test.

Table 2: Effect of treatment on RNA content in Wistar albino rats

	Group (N)	WH (mg/ml)	PMS (mg/ml)
1	Control	6.18 ± 0.48	5.13 ± 0.23
2	Caffeine	6.29 ± 0.57*	5.48 ± 0.34*
3	Caffeine + Coconut Water	6.67 ± 0.58*	5.63 ± 0.48*
4	Caffeine + Coconut Milk	6.85 ± 0.62*	5.74 ± 0.52

*N* = Number of rats per group = 5. Values are expressed as mean ± SD. WH = Whole homogenate and PMS = Post mitochondrial supernatant. \* Significantly different from control,  $P < 0.05$ , using student 't' test.

Table 3: Effect of treatment on Protein/RNA ratio in Wistar albino rats

	Group (N)	WH	PMS
1	Control	1.15 ± 1.19	1.10 ± 2.22
2	Caffeine	1.67 ± 0.84*	1.53 ± 1.26*
3	Caffeine + Coconut Water	2.80 ± 1.43*	2.95 ± 1.08*
4	Caffeine + Coconut Milk	3.31 ± 1.23*	3.60 ± 1.29*

*N* = Number of rats per group = 5. Values are expressed as mean ± SD. WH = Whole homogenate and PMS = Post mitochondrial supernatant. \* Significantly different from control,  $P < 0.05$ , using student 't' test.

Table 4: Effect of treatment on enzymes activities in wistar albino rats

Group (N)	ALT (u/l)	AST (u/l)
1 Control	22.63 ± 0.39	37.53 ± 0.61
2 Caffeine	37.24 ± 0.37*	45.41 ± 0.53*
3 Caffeine + Coconut Water	16.78 ± 0.41*	30.84 ± 0.67*
4 Caffeine + Coconut Milk	10.94 ± 0.38*	20.27 ± 0.46*

*N* = Number of rats per group = 5. Values are expressed as mean ± SD. WH = Whole homogenate and PMS = Post mitochondrial supernatant. \* Significantly different from control,  $P < 0.05$ , using student 't' test.

### Discussion

In this study, caffeine independently produced an increase in the activity of alanine and aspartate amino transferases (ALT and AST), resulting in hepatotoxicity due to inflammation of the cytoplasm, and a resultant leakage of cytoplasmic enzymes into the blood stream. These enzymes acted to block the transcription and translation steps of the genetic code, resulting in decreased processes of protein biosynthesis. High concentrations of tannic acid inhibited caffeine uptake, resulting in accumulation of methylxanthines, alanine, aspartate and nitrogen levels, leading to increased permeability of the liver cells. These effects, in turn, had led to inhibition of microsomal drug-metabolizing enzymes. However, an apparent caffeine – coconut water and caffeine-coconut milk interactions decreased the activities of ALT and AST, resulting in increase in Protein biosynthesis, hepatic protein levels and protein/RNA ratios. While the increase in the hepatic protein levels and protein/RNA ratios could be attributed to increased protein biosynthesis (since the RNA content is negligably affected), the decrease in ALT and AST activities could be attributed to the decreased permeability of the liver cells arising from increased glucuronidation (addition of highly polar groups such as sulfates and glucuronic acid). These effects, in turn, decreased the tannic acid, alanine, aspartate and nitrogen concentrations in the liver cells, which increased the rate of gastrointestinal absorption of caffeine metabolites. Thus, it seems that the decrease in ALT and AST activities enhanced the induction of microsomal drug-metabolizing enzymes, and these regulatory controls may act to conserve nutrients and energy for the cells. These results are in consonance with the studies of Orrhenuis *et al* (1965), Marshall (1978), Remmer and Merker (1993), and Singh *et al* (1998). The reports of these workers showed that increased protein synthesis due to increased glucuronidation of the endoplasmic reticulum and decreased permeability of the liver cells

resulted in induction of the microsomal drug-metabolizing enzymes. Also, it is likely that the toxic effects of caffeine may be modified by the rapid production of caffeine metabolites in the present of coconut water and cocoanut milk which could generate reactive electrophiles that could activate microsomal drug-metabolizing enzymes such as monooxygenase, cytochrome P<sub>450</sub>, glucuronyl transferase and nitroreductase (Nerbert and Gelboin, 1970, Matsunura and Omura, 1973, Bertolli *et al*, 1980, Salem *et al*, 1981, Singh *et al*, 1998). Also, the vitamins and amino acids present in the coconut water and coconut milk may likely have a role to play in providing cofactors for the activation of the drug-metabolizing enzymes and maintenance of liver cell integrity, even in the presence of hepatotoxic agents like caffeine (Ekam, 2001).

ALT catalyzes a reversible amino group transfer reaction in the Krebs (tricarboxylic acid) cycle necessary for tissue energy production while AST catalyzes transfer of the nitrogenous portion of an amino acid to an amino acid residue. ALT is released from the hepatocellular cytoplasm into the blood stream when there is acute hepatocellular damage; AST is found in the cytoplasm and mitochondria of many cells such as liver, heart and is released into serum in proportion to cellular congestion due to heart failure (Rodwell, 1996).

The Protein/RNA ratios are suggestive of enzyme induction and serum endoplasmic reticulum proliferation (Marshall, 1978; Hunter and chasseur, 1978; Bertolini *et al*, 1980; Salem *et al*, 1981; Bahtnager and Misra, 1988; Singh *et al*, 1998). This is because microsomal enzyme induction could most likely depends on de novo protein biosynthesis (Bertolini *et al*, 1980; Rodwell, 1996; Coon, 1996; Singh *et al*, 1998). In explaining the mechanism of de novo synthesis, the inducing agent acts to relieve repression of enzyme synthesis (Rodwell, 1996). The increased synthesis of enzyme protein could also be as a result of an increase in the translation process due to a reduced rate of turnover of messenger and other types of RNA

(Lessard and Peska, 1971; Rodwell, 1996; Singh *et al*, 1998).

The mechanisms of action of induction of the microsomal drug-metabolizing enzymes therefore, may involve activation of the transcription process. Transcription is mediated and regulated by regulatory protein- DNA interactions. The regulatory proteins are DNA-binding, proteins that recognize specific DNA sequences. Biosynthesis of these regulatory proteins enhanced glucuronidation of the endoplasmic reticulum, leading to increased cytoplasmic volume, and increased activities of the microsomal drug-metabolizing enzymes. Coconut products (ie coconut water and coconut milk) however, acted synergistically with caffeine to increase the induction of the microsomal drug-metablizing enzymes (since inducible enzymes are those whose rate of synthesis, and hence their amount, increases with the introduction of a specific substrate), leading to a faster clearance and elimination of caffeine from the body.

The endoplasmic reticulum (ER) is one of the major sites for protein synthesis in the cell (Craft *et al*, 1979; Remmer and Merker, 1993; Balogun and Malomo, 1998; Ebong *et al*, 1998). The majority of membrane and secretory proteins are synthesized on membrane – bound ribosomes and are translocated in the ER where folding, glycosylation, and disulfide bond formation take place to give the unique configuration and function of the proteins (Remmer and Merker, 1993; Coon, 1996; Raisfield *et al*, 2000). This may suggest that synthesis of new enzymes are dependent upon the formation of new functional membranes, and increased glucuronidation of the endoplasmic reticulum. The coconut products appeared to have enhanced formation of new functional membranes and increased glucuronidation of the endoplasmic reticulum, leading to induction of the microsomal drug-metabolizing enzymes.

In conclusion, the results have shown that coconut products-caffaine interactions increased hepatic protein levels and protein/RNA ratios and decreased ALT and AST activities. These effects in turn, enhanced induction of microsomal drug-metabolizing enzymes. Thus, the findings of this study may suggest that coconut water and coconut milk may be used as inducers of microsomal drug-metabolizing enzymes and or as antidotes to drugs such as alcohol, caffeine and antibiotics.

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Received: 22/5/2007

Accepted: 9/8/2007