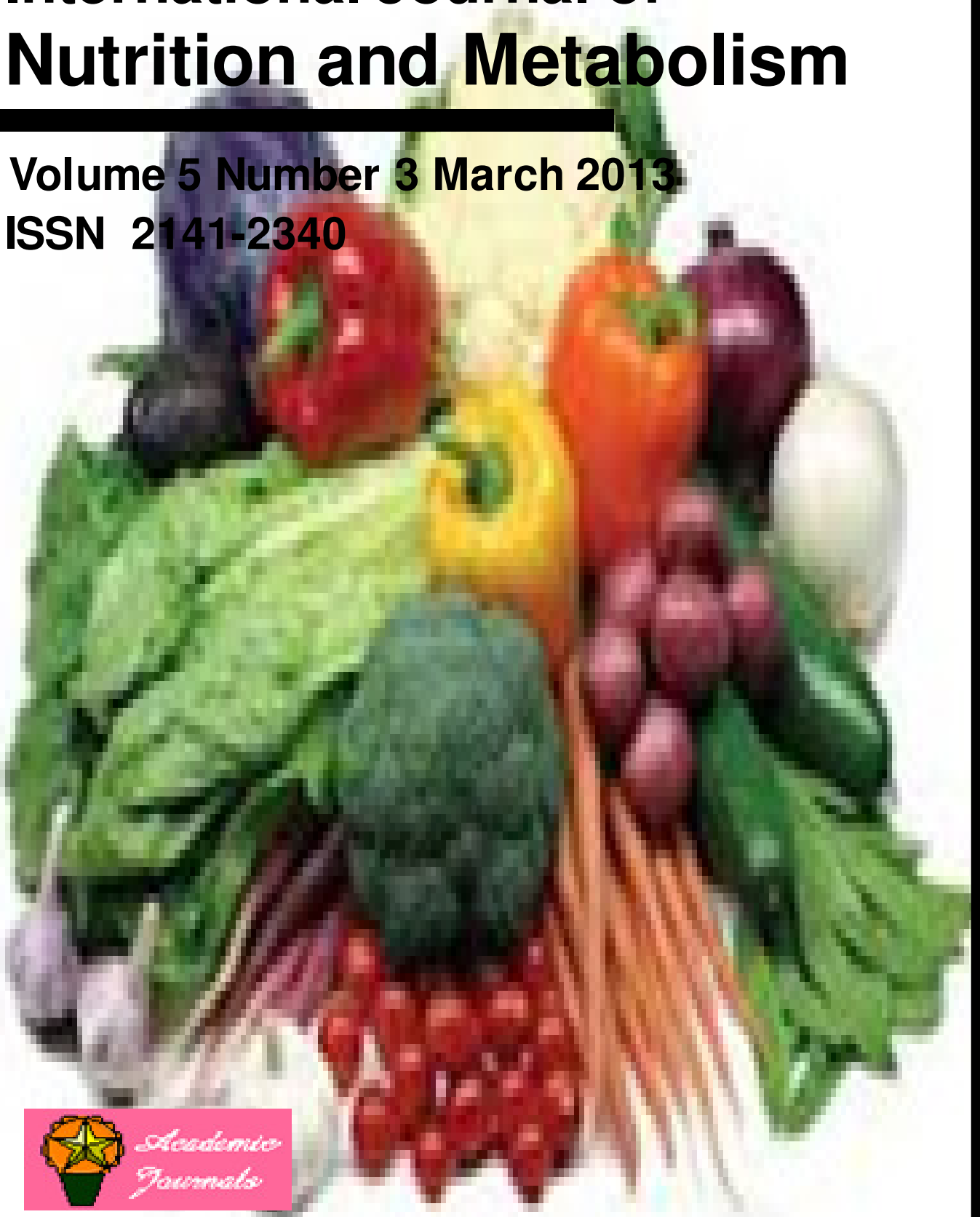


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# International Journal of Nutrition and Metabolism

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Full Length Research Paper

# Polyphenol-rich beverages promote a sustainable and renewable generation of energy and prevent neurotoxicity

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Accepted 21 March, 2013

**Polyphenol-rich beverages, formulations 1 and formulation 2 composed of herbal and berry extracts were developed. Their impact on adenosine triphosphate (ATP) responses was investigated *in vitro* and compared to that of well-known commercial drinks (Red Bull, Coca-Cola, Antioxia, Tetley green tea). Results show that mitochondrial activity, intracellular and extracellular ATP responses are impacted upon the exposure to polyphenol-rich beverages to promote a sustainable and renewable energy supply in human oral CAL27 mucosal cells. While herbal extracts are important for the renewable energetic activity, berry extracts play a role in regulating energy conservation. Prior exposure of polyphenol-rich beverages to CAL27 cells prevented subsequent neurotoxicity of SH-SY5Y dopaminergic cells undergoing oxidative stress. Formulation 1 was best at modulating ATP and neuroprotective responses. On the other hand, Red Bull caused energy depletion, and did not prevent neurotoxicity. In summary, polyphenol-rich drinks are potential energy drinks that promote a sustainable and renewable generation of energy. In particular, Formulation 1 may be a healthy caffeine-free alternative to energy drinks with side effects, such as energy crashing and neurotoxicity.**

**Key words:** Anthocyanins, adenosine triphosphate (ATP), conservation, dopamine, energy crashing, energy drink, mitochondria, neuroprotection, polyphenols, red bull.

## INTRODUCTION

People are increasingly concerned about the products they consume and prefer to choose food products with functional botanical natural ingredients over synthetic ones. For this reason, potential health benefits of various botanical ingredients have been widely studied. The beneficial effects of plants typically result from the combination of secondary products present in plants, such as polyphenols, a structural class of molecules characterized by the presence of large multiples of phenol units. Indeed, polyphenols do not only have properties associated with food color and aroma, but they may also play an important role in the prevention of

many chronic diseases in humans, such as cancer, diabetes, neurodegenerative and cardiovascular diseases (Sears and Rocordi, 2012; Korkina et al., 2012; Xie et al., 2011; Heim et al., 2002). In addition to provide protection from DNA cleavage, polyphenols can modulate various functions, including hormone regulation, enzyme inhibition, immune regulation, lipid peroxidation, capillary permeability, and membrane strengthening (Acquaviva et al., 2003; Lefevre et al., 2008; Rossi et al., 2003).

Energy drinks are popular among people who are looking for ways to boost their energy level for the optimal accomplishment of their everyday tasks. However, many

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health concerns, partly linked to their high levels of caffeine have been reported, including addiction, cardiovascular diseases, impaired cognition and even death. Thus, considerable efforts are undertaken by the food industry to develop healthy energy drinks that are low in caffeine and that are less damaging to consumers.

Novel natural health products that are polyphenol-rich, Formulation 1 (F1) and Formulation 2 (F2), were developed. They were especially designed to serve as adaptogens. In traditional Chinese medicine, the notion of 'adaptogen' has existed for thousands of years, which refers to the ability to regulate the various body functions and increase energy. It was proposed that polyphenols act as adaptogens (Stevenson, 2012). The role of our adaptogenic formulations in energy modulation was investigated to evaluate their potential as energy drinks.

In all forms of life, adenosine triphosphate (ATP), produced in mitochondria, the powerhouse of the cell and most complex cellular organelle is the useable form of chemical energy for the majority of basic metabolic processes (Boyer, 1998). ATP level is a crucial parameter of energy homeostasis, a balanced state ensuring the health and wellness. Levels of ATP are modulated by the diet, which directly affects mitochondrial activity. Most ATP produced by cells is generated from the oxidative phosphorylation process using simple and complex sugars or lipids as a source of energy. Smaller amounts of ATP are generated from the reaction of glycolysis in the cytosol of the cell. Cells stabilize their energetic potential by adjusting the rate of ATP synthesis to the state of energy demand (Fitz, 2007). Optimal metabolic functions can be achieved by consuming food ingredients having the ability to boost ATP responses.

While intracellular ATP (ATP<sub>i</sub>) is the main energy source required for most intracellular reactions, extracellular ATP (ATP<sub>e</sub>) released by cells can act as a signaling molecule and influence numerous biological processes, including platelet aggregation, vascular tone, neurotransmission (peripheral and central), cardiac function, muscle contraction, pain and immune responses, male reproduction, fertilization and embryonic development (Gordon, 1986; Volonte et al., 2003; Burnstock, 2006; Le Feuvre et al., 2002; Ostrom et al., 2000). ATP<sub>e</sub> has dramatic cytotoxic properties and may be involved in P2X7-mediated neurodegeneration (Le Feuvre et al., 2002). Furthermore, ATP<sub>e</sub> can excite gustatory primary afferent fibers and adjacent cells in taste buds (Huang et al., 2009) known to be innervated by gustatory afferent fibers projecting into the brain, especially the mesolimbic dopaminergic system (MLDS) involved in reinforcement, reward and motivation, in addition to its other functions in motor, mood, stress and addiction.

Dopamine (DA) is the principal neurotransmitter responsible for MLDS activity. Therefore, ATP<sub>e</sub> may

affect the dopaminergic system and should be explored to prevent addiction. In this study, the ability of various beverages, including F1 and F2, to modulate ATP responses in human oral mucosal cells to promote energy homeostasis was investigated. Results show that polyphenols-rich beverages, especially F1, cause a sustained and renewable energy lift in oral epithelial cells, and prevent neurotoxicity of dopaminergic cells.

## MATERIAL AND METHODS

### Reagents

F1 and F2 are natural health products sold under JustBio's trademarks that were developed in our laboratory. F1 helps relieve nervousness due to mental stress, and F2 helps maintain cardiovascular functions. Concentrated shots of F1 and F2 can be diluted into water to be consumed as functional beverages. Table 1 describes the ingredients of the beverages used in this study. Herbal extracts were generated from dried herbs purchased from La Clef des Champs (Val-David, Quebec). Berry concentrates used in F1 and F2 (blueberry: 65 Brix, cranberry: 50 Brix, and apple: 70 Brix) were purchased from Fruit d'Or (Villeroy, Quebec) and Vergers Paul Jodoin Inc. (Saint-Jean-Baptiste, Québec).

### Cell cultures

The human oral cancer cell line CAL27 was purchased from the American Type Culture Collection (ATCC CRL-2095, Massachusetts US). Cells were cultured in Dulbecco's modified eagle medium (DMEM) high-glucose 4.5 g/L culture medium supplemented with 2 mM of L-glutamine, 1 mM of sodium pyruvate, 10% (v/v) fetal bovine serum and 100 µg/ml Penicillin/streptomycin. All cultures reagents were purchased from Thermo scientific Hyclone, Ottawa, Ontario, Canada. CAL27 cells were plated 24 to 48 h prior to the assay in 96 well plates at a density of 20,000 to 30,000 cells per well. SH-SY5Y, human neuroblastoma exhibiting moderate levels of dopamine beta hydroxylase activity (ATCC CRL-2266) were grown in Eagle's minimal essential medium (EMEM)/F12 culture medium containing 10% (v/v) fetal bovine serum. To determine the impact of mucosal treatments on dopaminergic submucosal cells, CAL27 cells were first treated with various beverages for 24 h and their conditioned media was then transferred onto SH-SY5Y cells which were seeded in 96-well plates.

### Polyphenols and anthocyanins levels

Polyphenols levels present in the beverages studied were measured by spectrophotometry using an adaptation of a protocol already described by Grubestic et al. (2005). Extraction for the total amount of polyphenols was performed by adding 50% Folin (Sigma-Aldrich) reagent and 20% sodium carbonate solution to diluted samples for 1 h. Absorbance was measured at 760 nm using a Synergy Biotek HT reader. Gallic acid (GA) was used as positive control, and results were expressed as mg equivalent GA per 100 ml of samples. Anthocyanin levels were measured by a differential-pH spectrophotometric method. An aliquot of each sample were mixed with pH 1.0 buffer prepared by dissolving KCl into a 0.2 N HCl solution. At pH 1.0, anthocyanins exist in the

**Table 1.** Ready-to-drink beverages used and their ingredients.

Beverage	Ingredient
Formulation 1	Lemon balm ( <i>Melissa officinalis</i> L.), Skullcap ( <i>Scutellaria lateriflora</i> L.), maple syrup, blueberry concentrate, cranberry concentrate, and natural aroma
Formulation 2	Hawthorn ( <i>Crataegus oxyacantha</i> L.), Skullcap ( <i>Scutellaria lateriflora</i> L.), maple syrup, cranberry concentrate, apple concentrate, and natural aroma
Red Bull	Taurine, glucuronolactone, caffeine, niacin (niacinamide), pantothenic acid (calcium d-pantothenate), vitamine B6 (pyridoxine HCl), riboflavine, vitamine B12 (cyanocobalamine), sucrose, glucose, citric acid, inositol, and natural aroma caramel
Coca-Cola original	Glucose-fructose, Coca-Cola mix, caramel color, phosphoric acid, natural flavor, and caffeine
Antioxia	Fruit juice from concentrates (grape, apple, pomegranate, cranberry, blueberry, lemon, elderberry, blackberry), natural flavor, and ascorbic acid (Vitamin C)
Tetley green tea	Green tea (from real brewed green tea concentrate), raw sugar cane, pomegranate juice from concentrate, natural flavor, citric acid, ascorbic acid (vitamin C), and sodium citrate

colored oxonium or flavylum form. Absorbance at 510 nm was measured. Samples were mixed also with pH 4.5 buffer prepared by dissolving sodium acetate in deionized water and by adjusting the pH to 4.5 with HCl. At pH 4.5, anthocyanins are predominantly in the colorless carbinol form. Absorbance at 510 nm was also measured at pH 4.5. The difference in absorbance was proportional to the anthocyanin content. Anthocyanin analyses were carried out by TransBIOTech (Lévis, Canada).

#### Mitochondrial activity

CAL27 were seeded at a density of 20,000 to 30,000 cells per well in 96-well tissue culture plates. Colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was dissolved in Hyclone, balanced salt solution, with calcium and magnesium, without phenol red (HBSS) buffer to obtain a final concentration of 0.5 mg/ml per well. Cell plates were incubated for 2 h at 37°C. Conditioned medium was then removed, and acid-isopropanol (0.04 N HCl in isopropanol) was added to all wells. Plates were read at 530 nm/630 nm in a Synergy Biotek HT reader. The amount of dark blue crystals determined by spectrophotometry serves as an estimate for the number of mitochondria and hence the number of living cells in the test sample. This method is an adaptation of a previously described assay (Mosmann, 1983).

#### ATP responses

CAL27 were seeded at a density of 30,000 cells per well in 96-well tissue culture plates and were incubated in humidified incubator at 37°C for 2 days to allow cell attachment and stable cell growth. After two days of incubation, culture medium was aspirated and the cells were washed and incubated with Hank's buffered salt solution (HBSS) for 20 min. After HBSS incubation, cells were exposed to a first dose of beverages (diluted 1 in 24) for a period of 3 min. After 3 min, successive doses of beverages (diluted 1 in 6) were added for

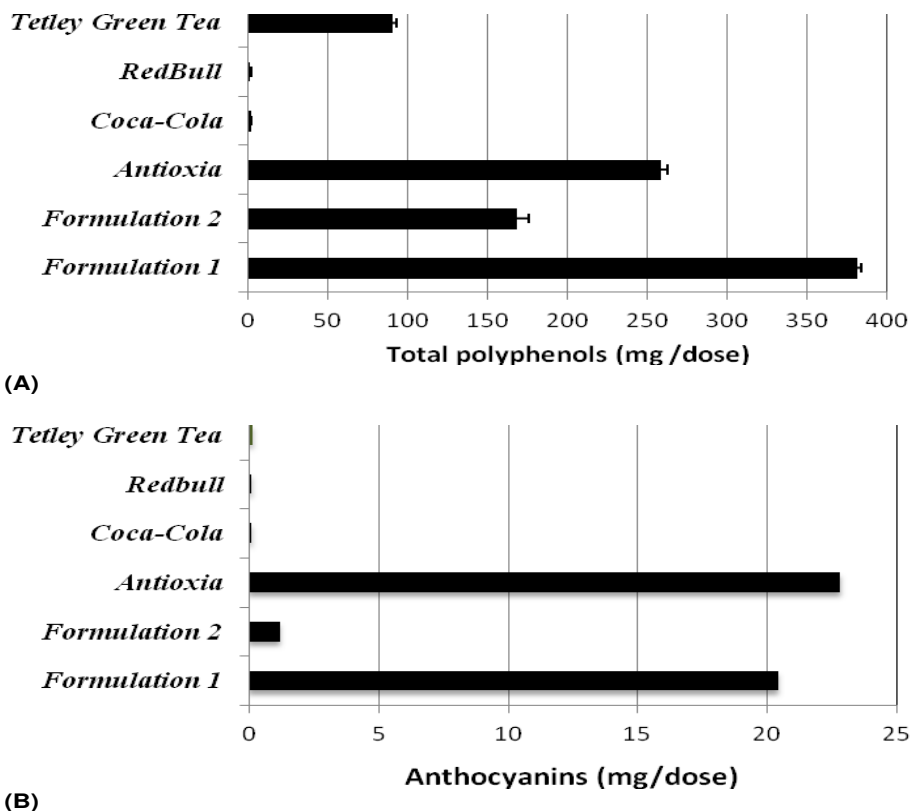
a total of 5 doses and a volume of 100 µl in each well. CAL27 cells were incubated for 25 min. After incubation, the supernatant was aspirated and placed in a second 96-well plate for measurement of ATPe. HBSS was added to each well of the first plate and was frozen at -80°C for cell lysis. Cell lysates were obtained through a freeze-thaw cycle. Levels of ATPi and ATPe were measured using the ATP determination kit from Molecular Probes purchased from Invitrogen (Life Technologies Inc., Burlington, Ontario). Levels were detected by luminescence at 560 nm with a Synergy HT Biotek reader.

#### ROS production

Cellular levels of reactive oxygen species (ROS) were obtained by measuring the oxidation of 5-(and6)-chloromethyl-20,70-dichlorodihydrofluoresceindiacetate (CM-H<sub>2</sub>DCFDA; Invitrogen), a cell-permeant indicator. SH-SY5Y were seeded in 96-well plates at a cell density of 20,000 cells per well. Cells were treated for 1 h with 5 µM CM-H<sub>2</sub>DCFDA dissolved in HBSS. After removing the CM-H<sub>2</sub>DCFDA solution, cells were treated with samples prepared with ROS buffer (HBSS containing 2% FBS). After 30 min of exposure with samples, a first reading was taken using the Synergy HT Biotek plate reader. Various concentrations of 2,2'-Azobisisobutyramidinium chloride (AAPH) (40, 16, and 6.4 mM) were added. Plates were read at 485 nm/530 nm every 30 min for 2 h. The fluorescence intensity is an indicator of H<sub>2</sub>O<sub>2</sub> intracellular level, so values were expressed in Relative fluorescence unit (RFU).

#### Salivary DA

Saliva samples were collected from 7 healthy individuals (men and women) who do not smoke, have no addictions, normal weight, exercise at least 2 to 3 times a week. Volunteers were given 20 ml-size samples to drink on separate days. Before swallowing,



**Figure 1.** Polyphenol and anthocyanin levels in beverages studied. A. Polyphenol levels in formulations studied. Values are expressed as mean mg/dose  $\pm$  SEM of  $n=6$ . Gallic acid was used to make the standard curve. B. Anthocyanin levels in formulations studied. Values are expressed as mean mg/dose.

samples were kept in the mouth for at least 15 to 20 s, after which saliva was collected over the next 5 min. Saliva samples were frozen immediately. Levels of human DA from human saliva were measured using an Enzyme-linked immunosorbent assay (ELISA) kit (Genway, CA, USA).

#### Statistical analyses

Statistical analysis was performed using GRAPH PRISM software. Experiments were done in triplicate. All data are presented as mean  $\pm$  standard error of mean (SEM). Statistical analyses were done using a Dunnett's multiple comparison one-way analysis of variance (ANOVA) test to compare multiple experimental groups, and using an unpaired  $t$ -test for results shown in the upper row of Figure 2. Results were considered significant when  $*p \leq 0.05$ ,  $**p \leq 0.01$  or  $***p \leq 0.001$ .

## RESULTS

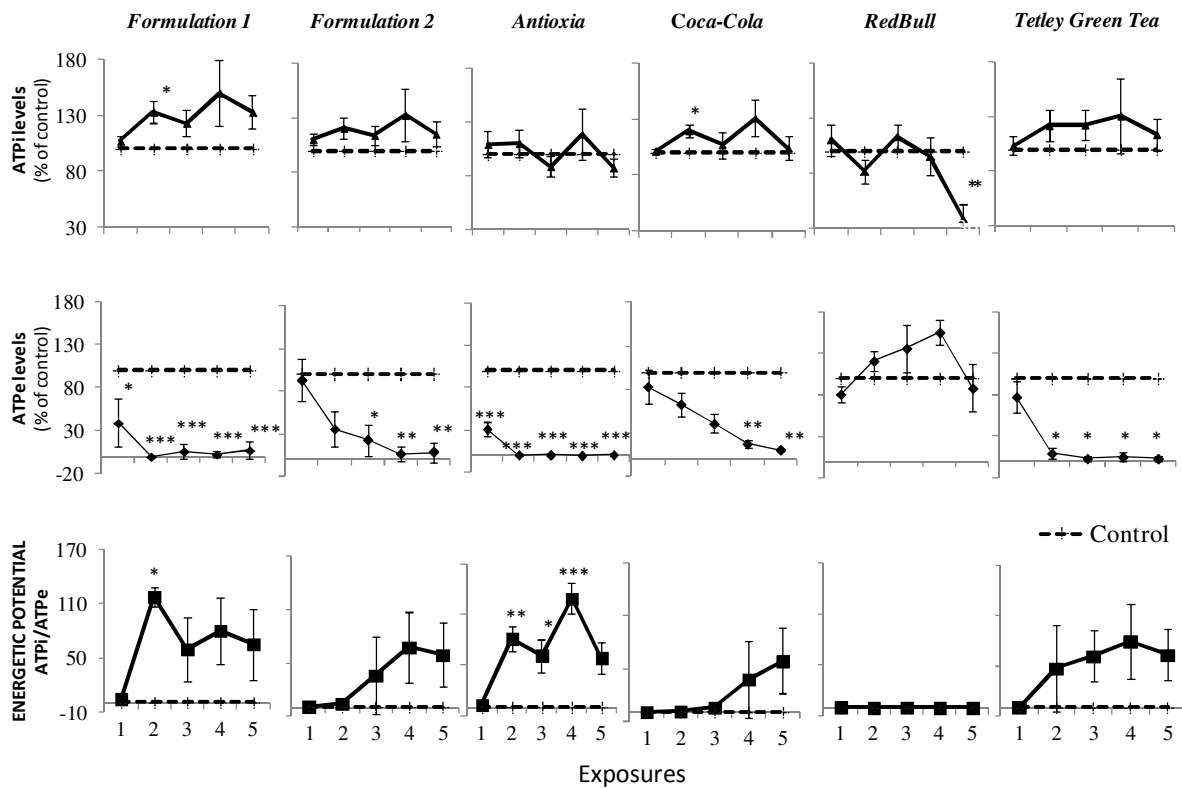
### F1 and F2 are rich in polyphenols

As a result of polyphenols being potential functional molecules, levels of polyphenol of F1 and F2 for one

dose were measured and their levels were compared to other beverages. Figure 1A shows that F1 (381 mg/dose) contained the highest levels of polyphenols, followed by Antioxia (258 mg/dose), a wild-berry juice already known to be polyphenol-rich and F2 (180 mg/dose). Tetley green tea (90 mg/dose) had low levels of polyphenols whereas Red Bull and Coca-Cola contained no detectable levels of polyphenols. Anthocyanins levels were detected in F1 and F2. The amounts of anthocyanins per dose present in various beverages are shown in Figure 1B. F1 and Antioxia had the highest levels of anthocyanins with 17 and 19 mg/dose, respectively. F2 had very low but detectable levels of anthocyanins, whereas Red Bull, Coca-Cola, and Tetley green tea did not contain anthocyanins.

### Polyphenol-rich beverages regulate ATP responses

The ability to maintain a rate of ATP production in the intracellular environment is a crucial parameter of cellular homeostasis and the cell viability. We tested the capacity of various beverages to generate ATPi in oral human



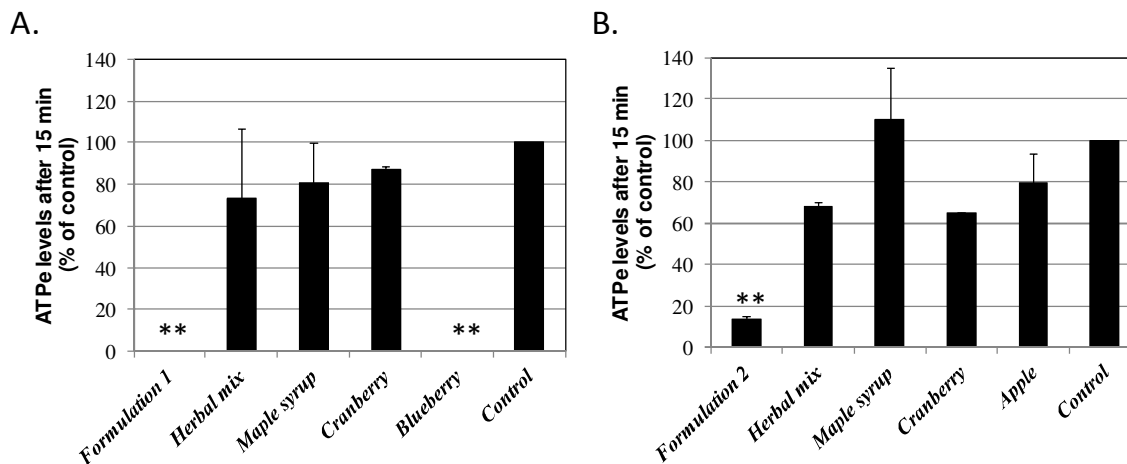
**Figure 2.** Effects of various beverages on ATP responses in CAL27 oral mucosal cells. Impact of beverages on ATPi (upper row) of CAL27 cells exposed up to 5 times to various beverages is shown. Data from three separate experiments ( $n = 3$ ) are expressed as percent of HBSS-treated control cells. Values were normalized to control levels (100%), are expressed as mean of normalized ATP levels  $\pm$  SEM. Paired t-test were performed to determine the level of significance as shown by \* $p \leq 0.05$ . The middle row shows levels of ATPe obtained following exposures to beverages. Values of three separate experiments ( $n = 3$ ) were normalized to control levels, and are expressed as mean  $\pm$  SEM of normalized ATP levels produced. In the bottom row, ATPi/ATPe ratios were calculated to show the impact of various beverages on the overall energetic potential of CAL27 cells. Results in middle and bottom row were significantly different from control as shown by \* $p \leq 0.05$ , \*\* $p \leq 0.01$  or \*\*\* $p \leq 0.001$ .

CAL27. ATPi levels were normalized to control, and values are expressed as percentage of control. Figure 3 shows in the upper line that ATPi levels of CAL27 were significantly enhanced by F1 after a second exposure and this increase was sustained, suggesting that F1 induces sustainable ATPi production. Coca-Cola significantly enhanced intracellular ATP after two exposures, but this response was not sustained. F2, Antioxia, and Tetley green tea did not significantly modulate ATPi levels. ATPi levels were strongly reduced by five exposures of Red Bull, indicating that it is an inhibitor of ATP production.

ATPe levels were measured from the conditioned culture media collected, following exposure to beverages tested. ATPe levels were normalized to control and values are expressed as percentage of control. Results in Figure 2 show in the middle row that F1 and Antioxia, the beverages containing the highest concentrations of

polyphenols and anthocyanins, inhibited ATP secretion of CAL27 upon their first exposure to the beverages. This inhibition was dose-dependent and was maintained after 5 exposures. Blocking ATP secretion may be important in energy conservation. F2, as well as Coca-Cola and Tetley green tea, inhibited ATP secretion from CAL27 cells in a dose-dependent manner, suggesting that these beverages may also play a role in energy conservation. In contrast, Red Bull enhanced ATPe levels in a dose-dependent manner, and the increase was significant after four exposures, indicating that Red Bull induces ATP secretion from CAL27 cells, draining cells from ATPi required for intracellular reactions.

To measure the overall energetic potential, ATPi levels were divided by ATPe levels to obtain ATPi/ATPe ratios. Figure 2 shows in the bottom row that F1 and Antioxia, both polyphenol-rich beverages, are best at promoting the energetic potential of mucosal cells, causing a



**Figure 3.** Blueberry of F1 inhibits ATP secretion. (A) The impact of the individual ingredients of F1 on ATP secretion of CAL27 cells was compared to that of F1 itself. (B) Ingredients of F2 were exposed to CAL27 to measure ATP secretion. Ingredients act in synergy to inhibit ATP secretion. ATPe levels were normalized to untreated. Values are expressed as mean  $\pm$  SEM of  $n = 2$ . Results were significantly different from control as shown by  $**p \leq 0.01$ .

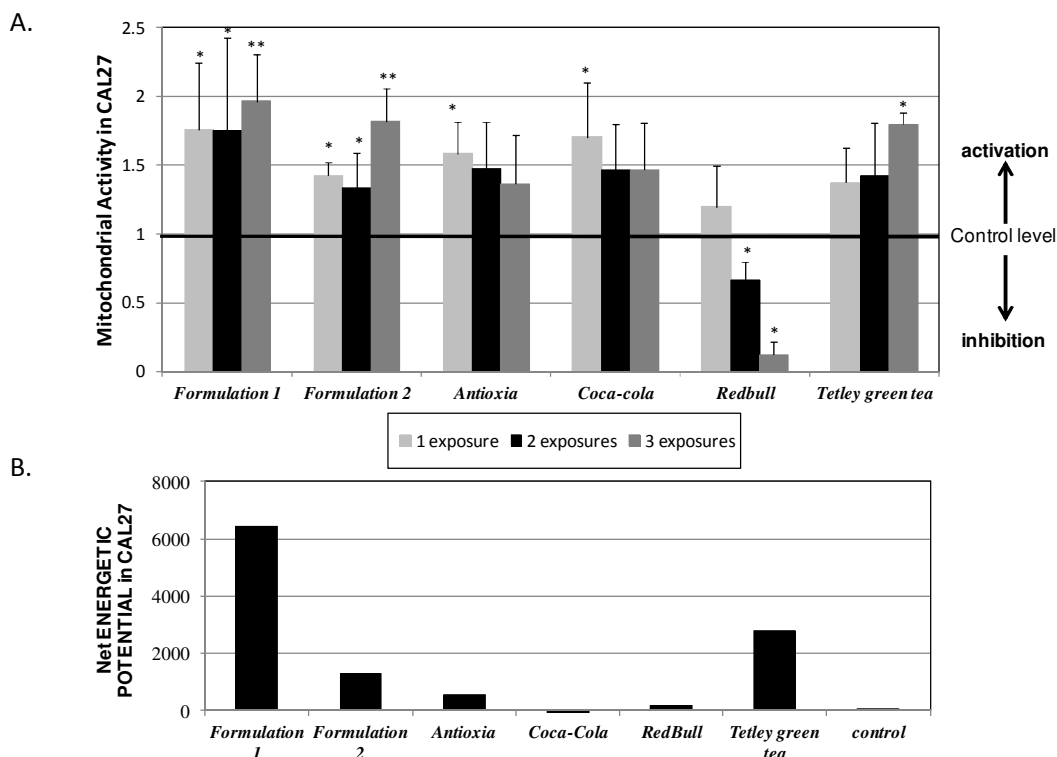
significant and sustainable energy lift. After 4 exposures, F2 and Tetley green tea were comparable at enhancing the energetic potential of mucosal cells. By inducing ATP secretion and blocking ATP production, Red Bull clearly prevented cells from boosting their energetic potential, suggesting that this beverage cannot be a sustainable solution for long-term energy needs. Finally, Coca-Cola also slightly enhanced the energetic potential of mucosal cells, but only after their fifth exposure. In summary, Figure 2 demonstrates that polyphenol-rich beverages could be sustainable energy boosters.

### Blueberries promote energy conservation

The effect of individual ingredients of F1 on ATP secretion was investigated. Figure 3A shows that the blueberry concentrate inhibits ATP secretion as much as the whole beverage (F1) when compared to control (equal to 100%), suggesting that blueberry is responsible for the ATP secretion inhibition of F1. Because F1 contains the highest concentration of anthocyanins, this data suggests that anthocyanins from blueberries may play an important role in energy conservation. Results obtained with ingredients of F2 (Figure 3B) demonstrate that the combination of the different ingredients is required for the ATP secretion inhibition by F2. Despite the presence of low anthocyanin levels in F2, probably due to the cranberry concentrate, the cranberry concentrate was not enough to inhibit ATP secretion. In contrast to blueberry, cranberry is not a potent inhibitor of ATP secretion.

### F1 is best at inducing a sustainable energy lift in stressed cells

In CAL27 cells treated with 40 mM of AAPH, Figure 4A shows that F1 and F2 significantly enhanced mitochondrial activity of stressed cells after one exposure only, and this impact was sustained up to three exposures. Antioxia and Coca-Cola also enhanced mitochondrial activity after one exposure, but this was not sustained after subsequent exposures (Figure 4A). Tetley green tea increased mitochondrial activity only after the third application of the beverage, suggesting that it acts more slowly than F1 and F2, or that a higher dose of this drink is required to enhance mitochondrial activity in cells undergoing oxidative stress. In contrast, Red Bull inhibited mitochondria activity of stressed CAL27 cells in a dose-dependent manner. Since MTT value is also measure of cell viability (Mosmann, 1983), this result suggests that Red Bull causes mucosal toxicity by reducing cell viability of stressed CAL27 cells. To determine the effect of oxidative stress on the energetic potential of CAL27, ATPi/ATPe from non-treated cells was subtracted from that of AAPH-treated cells to obtain the net energetic potential of cells. Data shown in Figure 4B indicates that F1 is best at inducing an energy lift in stressed cells after 5 exposure, followed by Tetley green tea and F2. In contrast to what was observed in normal cells (Figure 2B), Antioxia had much lower impact than F1 on the energetic potential of stressed cells (Figure 4B). Red Bull and Coca-Cola had little or no impact on the sustained energetic potential of stressed cells.



**Figure 4.** F1 is best at inducing an energy lift in cells undergoing oxidative stress. (A) The impact of various beverages on mitochondria activity of cells treated with 40 mM AAPH was determined. MTT results were normalized to control levels. Values are expressed as mean  $\pm$  SEM of  $n = 3$ . Results were significantly different from control as shown by \* $p \leq 0.05$ , or \*\* $p \leq 0.01$ . (B) The net energetic potential was obtained by subtracting the energetic potential of healthy cells (without AAPH treatment) to that of stressed cells (treated with 40 mM AAPH) after 5 exposures. Data was normalized to control, and values are expressed as differences of mean values for  $n = 3$ .

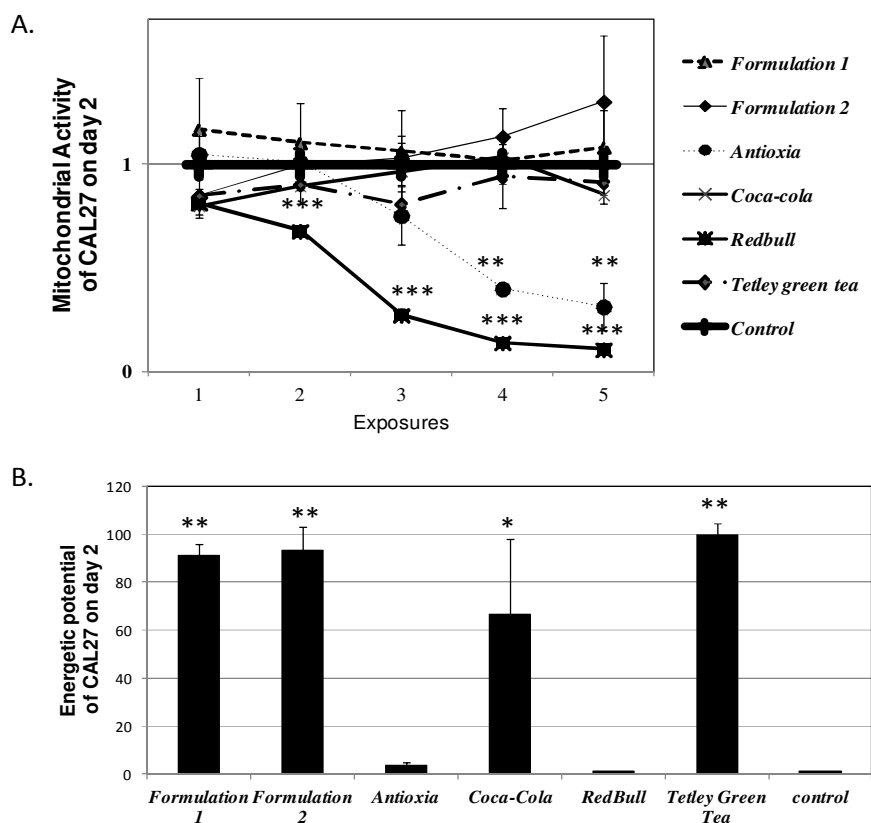
### Herbal extracts promote a renewable generation of energy

To measure the impact on the renewable generation of energy, mitochondrial activity and ATP responses were measured from the same CAL27 cells receiving two treatments of various beverages on two consecutive days. Results were normalized to control levels post-treatment on the second day. F1, F2, Coca-Cola or Tetley green tea had no effect on mitochondrial activity (Figure 5A). However, Antioxia and Red Bull both inhibited mitochondrial activity in a dose-dependent manner (Figure 5A), suggesting their toxic effect in mucosal cells. Polyphenol levels present in beverages do not correlate with the impact on mitochondrial activity since F1 and Antioxia, containing the highest levels of polyphenols (Figure 1), had differential effects on mitochondrial activity (Figure 5A). This data suggest that herbal extracts present in F1 may be responsible for mucosal protection. The impact for the repeated use of beverages on the energetic potential of CAL27 cells was

also determined using ATP<sub>i</sub>/ATP<sub>e</sub> ratio. The data clearly shows that Antioxia and Red Bull inhibit the generation of renewable ATP (Figure 5B), whereas F1, F2, Coca-Cola and Tetley green tea enhanced the energetic potential of mucosal cells. Thus, we conclude that the addition of herbal extracts in polyphenol-rich beverages may be required to promote a renewable generation of ATP and to prevent mucosal toxicity.

### Polyphenol-rich beverages protect dopaminergic cells

ROS produced from oxidative stress plays an important role in mitochondria-mediated cells death (Orrenius, 2007) Thus, ROS levels can serve as markers of cell toxicity. ROS production of dopaminergic SH-SY5Y neurons treated with 40 mM AAPH was measured after being exposed to conditioned media of CAL27 cells treated with various beverages. Results demonstrate that F1, F2, and Antioxia provided neuroprotection to dopaminergic cells



**Figure 5.** The impact of various beverages on renewing energy. MTT results measuring mitochondrial activity from CAL27 cells treated twice on two consecutive days with various beverages were obtained. MTT results were normalized to control levels. MTT results are directly proportional to cell viability. Values are expressed as mean  $\pm$  SEM of  $n = 3$ . (B) To measure the energetic potential, ATPi/ATPe ratio were obtained on the second day of treatment of CAL27 cells after 5 exposures with various beverages. Values are expressed as mean  $\pm$  SEM of  $n = 3$ . In both A and B, results were significantly different from control as shown by \* $p \leq 0.05$ , \*\* $p \leq 0.01$  or \*\*\* $p \leq 0.001$ .

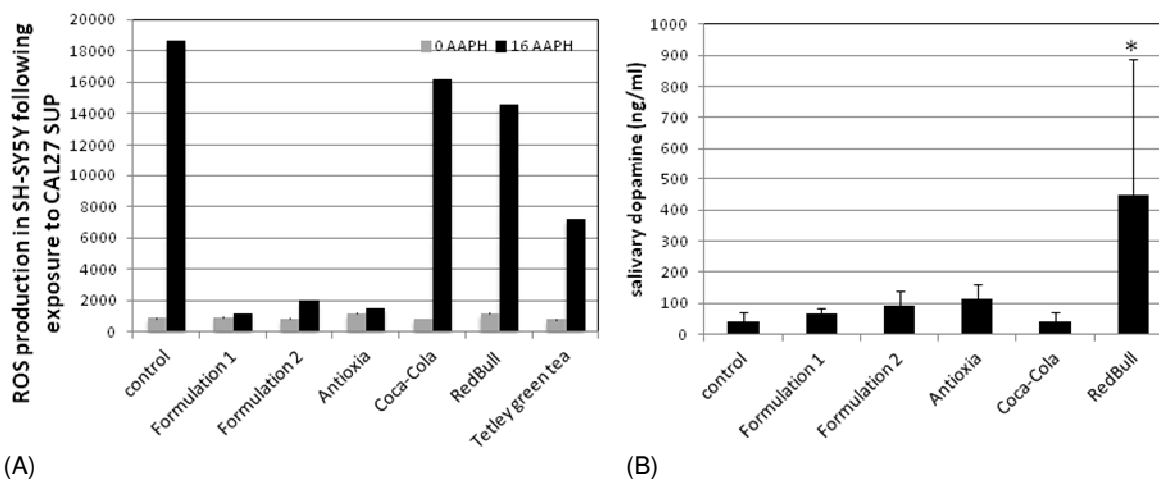
by inhibiting ROS production, while Coca-Cola and Red Bull did not (Figure 6A). Tetley green tea had a low neuroprotective activity compared to the other polyphenol-rich beverages. To measure the impact of beverages on the human submucosal dopaminergic system, levels of DA were detected in saliva of human subjects five minutes after drinking beverages. Figure 6B show that Red Bull significantly enhanced levels of salivary DA compared to control (water), whereas all other beverages tested did not. This data suggests that, in contrast to Red Bull, polyphenol-rich beverages do not induce an addictive response.

## DISCUSSION

We provide evidence for the first time that polyphenols

regulate ATP responses by inducing, through the oral intake of polyphenol-rich beverages, a sustainable energy lift in mucosal cells. Results show that polyphenol-rich can enhance ATP production and block ATP secretion simultaneously. The impact dietary intake of polyphenols on energy regulation has been proposed through a link with the hypothalamic neuropeptide systems (Panickar, 2013). Here, we propose that polyphenols can directly regulate energy regulation by modulating ATP responses. It is possible that the stimulation of ATP production results in the acceleration of ATP synthesis by increasing mitochondrial electron transport and/or decrease ATP consumption (or degradation). The optimization of body functions such as muscle contraction and recovery, mental focus, and maintenance of the immune system require a constant energy supply. Baicalein and baicalin are two major





**Figure 6.** Polyphenol-rich beverages protect the dopaminergic system. (A) Human dopaminergic cells were treated with conditioned media from CAL27 cells exposed to various beverages for 24 h. ROS production of SH-SY5Y was induced with 16mM AAPH ( $n = 1$ ) or without AAPH ( $n=3$ ) to determine the impact when cells are undergoing oxidative stress. Data show that mucosal exposure of polyphenol-rich beverages (F1, F2, Antioxia) inhibited ROS production in dopaminergic cells that may be present submucosally. (B) Levels of salivary DA detected 5 min after drinking 20 ml of various beverages in healthy human individuals are shown. Values are expressed as mean  $\pm$  SEM of salivary DA ng/ml of at least  $n = 6$ . Results were significantly different from control as shown by  $*p \leq 0.05$ .

flavonoids of skullcap (*Scutellaria baicalensis* Georgi or *Scutellaria lateriflora*). A study suggests that baicalin or baicalein increases cellular ATP levels of HIT-T15 cells (Li et al., 2012). Thus, skullcap extracts contained in F1 is likely to regulate ATP responses.

To understand the energy potential of F1, it would be also interesting to study ATPi production using other cell types. Wong et al. (2011) investigated the energizing properties of plants used in modern Chinese medicine, using H9c2 cardiomyocytes, and they demonstrated properties of 'Yang-invigorating' herbs to stimulate their mitochondrial activity. Red Bull decreases ATPi after 5 doses (Figure 2), causing cellular energy depletion. Certain factors can cause ATP depletion, including severe oxidative stress (Ahmad et al., 2004). There is also increasing evidence for the physical alteration of the biological membrane as a major factor in the evolution of irreversible injury during ATPi depletion (Florine-Casteel et al., 1991). It has been shown that there is a sequence of specific intracellular events linked to cell death by necrosis (Figure 1) (Golstein and Kroemer, 2006). This sequence includes the first signs of mitochondrial dysfunction such as ROS production by mitochondria and mitochondrial swelling, the decrease in intracellular ATP, loss of  $Ca^{2+}$  homeostasis, perinuclear clustering of organelles, activation of some proteases lysozymes rupture, and finally rupture of the plasma membrane.

A role for polyphenols in energy conservation is proposed because polyphenol-rich beverages have the

ability to block ATP secretion. Mechanisms of energy conservation are not well understood, but it is known that energy conservation can be triggered by various meditation techniques. For example, it is known that highly experienced yogi have the capacity to meditate for very long periods of time without eating, possibly by enhancing their energy conservation capacity. Meditation is known to affect the autonomic nervous system, causing heart rate variability (Servant et al., 2009) and to activate the sympathetic nervous system, and subsequent catecholamine/cortisol release for controlled stress responses. The innate immune system can also be impacted by meditation, and this modulates inflammatory responses (Kox et al., 2012). With its ability to restore and maintain a general state of homeostasis, meditation can influence the fate of diseases such as the metabolic syndrome and coronary heart disease (Paul-Labrador et al., 2006).

Red Bull induces ATP secretion (Figure 2) and enhances mucosal cell death (Figure 3), suggesting a role in mucosal toxicity. It has long been known that ATPe may be a mediator of cytotoxic cell-dependent lysis (Francesco et al., 1990). Since massive extracellular release of ATP often occurs after metabolic stress, brain ischemia and trauma, ATPe may be involved in the etiopathology of many neurodegenerative conditions. Studies on immune cells have demonstrated that ATPe can act as a potent stimulus for the maturation and release of interleukin-1 $\beta$  via activation of purinergic P2X7

receptors (Le Feuvre et al., 2002). ATPe is also known to be toxic to primary neuronal dissociated cells and organotypic neuronal cultures from cortex, striatum and cerebellum. Therefore, it is likely that excess amount of ATPe can be damaging to surrounding neuronal cells.

Protective effects of mitochondrial activity by polyphenol-rich beverages can be explained by their antioxidant properties or their capacity to boost ATP generation. First, mitochondria are the major sites of cellular ROS production and also targets of ROS. Mitochondrial DNA, proteins, and lipids in the inner membrane of mitochondria are thus vulnerable to oxidative damage by ROS. Maintaining a balance between ROS levels and antioxidant molecules concentrations prevent development of generalized mitochondrial dysfunction and poor energy metabolism (Halliwell, 1996). Antioxidants protect cell membrane integrity, contributing to the resistance against assaults by ROS or by autolytic enzymes during the process of irreversible cell injury (Wu et al., 1996). Interestingly, our laboratory has demonstrated that F1 and F2 have strong antioxidant properties by decreasing ROS concentration produced by CAL27 cells (unpublished observation). These antioxidant properties could be explained by the presence of polyphenols in *Melilotus officinalis*, which was shown to have the highest antioxidant activities when compared to other plant extracts (Picada Pereira et al., 2009). Further investigation is necessary to determine the mechanisms underlying the link between this antioxidant capacity and the modulation of ATPi production.

Results show that the blueberry concentrate alone inhibits ATP secretion to the same extent as F1 (Figure 3A). The effect of blueberry extract on ATP secretion has been demonstrated in a neurodegenerative model induced by amyloid- $\beta$  peptide where acute ATP leakage was prevented (Fuentealba et al., 2011). Blueberries (*Vaccinium* spp), blackberries (*Rubus* L. hybrids), and black currants (*Ribes nigrum* L.) are rich sources of dietary anthocyanins. High levels of anthocyanins in berries are thought to play an important role in human health and disease prevention due to their powerful antioxidant activity (Zafra-Stone et al., 2007).

Indeed, anthocyanins are increasingly studied for their physiological roles in protecting higher plants against destructive oxidative damage (Wang et al., 2009; Prior et al., 2003). The amounts and distribution of anthocyanins in berries differ, depending on their plant species, cultivation conditions, and producing districts.

Consequently, the antioxidant activity may be different among various berry extracts (Connor et al., 2002). For example, the degree of ripeness differently affects the concentrations and proportions of the various polyphenols: generally phenolic acid concentrations decrease during ripening, whereas anthocyanin concentrations

increase (D'Archivio et al., 2007). Moreover, several studies have shown that the content and antioxidant activities of total anthocyanins and total phenolics in various fruits are highly correlated (Moyer et al., 2002; Wang and Lin, 2000). However, studies using extracts from different fruits and vegetables have suggested that there may be synergic or additive biological effects due to unique combinations of anthocyanins and other phenolics (Bagchi et al., 2004). These differences can depend on the type of cultivar used, since many cultivars and native species of these berries exist, some with substantially higher antioxidant levels than others.

The neurotransmitter dopamine plays a major role in reward processing, regulating reinforcement and motivational behavior, in addition to other functions in motor, mood, stress and addictive behavior. We show that polyphenol-rich beverages, including F1 and F2, provided mucosal protection and subsequent neuroprotection by preventing dopaminergic cells from undergoing oxidative stress (Figure 6A). F1 and F2 are composed of skullcap, known to contain baicalein, which can protect PC12 cells of 6-hydroxydopamine (6-OHDA)-induced damage (Zhang et al., 2012). Thus, it is possible that baicalein plays a role in the neuroprotection of F1 and F2. In contrast to the neuroprotective effect of F1 and F2, Red Bull caused mucosal toxicity and did not protect dopaminergic cells from oxidative stress (Figure 6A). Neurotoxicity of SH-SY5Y by an impairment of mitochondrial ATP synthesis was previously shown using methadone (Perez-Alvarez et al., 2010), suggesting that Red Bull may act through the same pathway to cause neurotoxicity.

Interestingly, DA levels in human saliva were enhanced only after drinking a sip of Red Bull, but not after drinking polyphenol-rich beverages (Figure 6B), suggesting that Red Bull causes the activation of the dopaminergic system that plays a role in addiction. We conclude that polyphenol-rich beverages may protect the dopaminergic system, while Red Bull does not. Further studies in a larger group of individuals will be required to prove this point, and to better understand the impact of polyphenol-rich beverages on the dopaminergic system.

## Conclusion

Mucosal ATP responses can be regulated through the intake of polyphenol-rich beverages to ensure a constant supply of energy. Taken together, results summarized in Table 2 shows that F1 is best, compared to other beverages tested, at promoting a sustainable and renewable generation of energy, and at providing mucosal protection and neuroprotection of dopaminergic cells. On the other hand, the well-known energy drink Red Bull did not promote a sustainable and renewable production of ATP

**Table 2.** Summary of results.

Parameter	F1	F2	Antioxia	Coca-Cola	Red Bull	Tetley green tea
Polyphenol-rich	++++	++	+++	-	-	++
Anthocyanin-rich	+++	+	++++	-	-	-
Sustainable energy lift in healthy mucosal cells	+++	-	++++	-	-	-
Sustainable energy lift in damaged mucosal cells	++++	++	+	-	-	++
Renewable energy lift in healthy mucosal cells	++	++	-	+	-	++
Energy conservation	+++	++	++++	++	-	++
Mucosal protection	+++	++	-	+	-	++
Neuroprotection of dopaminergic cells	+++	+++	+++	-	-	++
Total score	25	14	19	4	0	12

and instead caused an energy crash. We propose that oral intake of polyphenol-rich beverages, especially F1, can act as energy drinks to promote energy homeostasis. Therefore, one should consider polyphenol-rich beverages as alternatives to commercial caffeine-rich energy drinks, such as Red Bull, with toxic effects.

## ACKNOWLEDGEMENTS

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**Abbreviations:** **ATPe**, Extracellular ATP; **ATPi**, intracellular ATP; **DA**, dopamine; **F1**, formulation 1; **F2**, formulation 2; **MLDS**, mesolimbic dopaminergic system; **ROS**, reactive oxygen species; **SEM**, standard error of the mean, **ATP**, adenosine triphosphate.

## REFERENCES

- Acquaviva R, Russo A, Galvano F (2003). Cyanidin and cyanidin 3-O-beta-D-glucoside as DNA cleavage protectors and antioxidants. *Cell Biol. Toxicol.* 19:243-252.
- Ahmad S, Ahmad A, Ghosh M, Leslie CC, White CW (2004). Extracellular ATP-mediated signaling for survival in hyperoxia-induced oxidative stress. *J. Biol. Chem.* 279:16317-16325.
- Bagchi D, Sen CK, Bagchi M, Atalay M (2004). Anti-angiogenic, Antioxidant, and Anti-carcinogenic Properties of a Novel Anthocyanin-Rich Berry Extract Formula. *Biochemistry* 69:75-80.
- Boyer PD (1998). Energy, Life, and ATP. *Biosci. Rep.* 18:97-117.
- Burnstock G (2006). Historical review: ATP as a neurotransmitter. *Trends Pharmacol. Sci.* 27:66-176.
- Connor AM, Luby JJ, Hancock JF, Berkheimer S, Hanson EJ (2002). Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage. *J. Agric. Food Chem.* 50:893-898.
- D'Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C, Masella R (2007). Polyphenols, dietary sources and bioavailability. *Ann. Ist Super Sanità.* 43:348-361.
- Fitz JG (2007). Regulation of cellular ATP release. *Trans. Am. Clin. Climatol. Assoc.* 118:199-208.
- Florine-Casteel K, Lemasters JJ, Herman B (1991). Lipid order in hepatocyte plasma membrane blebs during ATP depletion measured by digitized video fluorescence polarization microscopy. *J. FASEB* 5:2078-2084.
- Francesco DV, Pizzo P, Zanollo P, Bronte V, Collavo D (1990). Extracellular ATP as a possible mediator of cell-mediated cytotoxicity. *Immunol. Today* 11:274-277.
- Fuentealba J, Dibarrart AJ, Fuentes-Fuentes MC, Saez-Orellana F, Quiñones K, Guzmán L, Perez C, Becerra J, Aguayo LG (2011). Synaptic failure and adenosine triphosphate imbalance induced by amyloid-beta aggregates are prevented by blueberry-enriched polyphenols extracts. *J. Neurosci. Res.* 89:1499-1508.
- Golstein P, Kroemer G (2006). Cell death by necrosis: towards a molecular definition. *Trends Biochem. Sci.* 32:37-43.
- Gordon JL (1986). Extracellular ATP: effects, sources and fate. Review article. *J. Biochem.* 233:309-319.
- Grubešić JR, Vuković J, Kremer D, Vladimir-Knežević S (2005). Spectrophotometric method for polyphenols analysis: Prevalidation and application on *Plantago* L. species. *J. Pharm. Biomed. Anal.* 39:837-842.
- Halliwel B (1996). Commentary Oxidative Stress, Nutrition and Health. Experimental Strategies for Optimization of Nutritional Antioxidant Intake in Humans. *Free Radic. Res.* 25:57-74.
- Heim KE, Tagliaferro AR, Bobilya DJ (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem.* 13:572-584.
- Huang YA, Dando R, Roper SD (2009). Autocrine and paracrine roles for ATP and serotonin in mouse taste buds. *J. Neurosci.* 29:13909-13918.
- Korkina LG, Pastore S, Dellambra E, De Luca C (2012). New Molecular and Cellular Targets for Chemoprevention and Treatment of Skin Tumours by Plant Polyphenols : A Critical Review. *Curr. Med. Chem.* 20(7):852-68.
- Kox M, Stoffels M, Smeekens SP, van Alfen N, Gomes M, Eijvogels TM, Hopman MT, van der Hoeven JG, Netea MG, Pickkers P (2012). The influence of concentration/ meditation on autonomic nervous system activity and the innate immune response: a case study. *Psychosom. Med.* 74:489-94.
- Le Feuvre S, Brough D, Rothwell N (2002). Extracellular ATP and P2X7 receptors in neurodegeneration. *Eur. J. Pharmacol.* 447:261-269.
- Lefevre M, Howard L, Most M, Ju Z, Delany J (2008). Gene expression microarray analysis of the effects of grape anthocyanins in mice: a test of a hypothesis-generating paradigm. *Metabolism* 57:S52-7.
- Li YP, Su H, Pi XF, Gong YC, Xiong XY, Wu GJ, Li ZL (2012). The Stimulatory Activities of Baicalin and Baicalin Compounds Derived from *Scutellaria baicalensis* on insulin secretion in vitro. *Adv. Mater. Res.* 554-556: 1673-1676.

- Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65:55-63.
- Moyer RA, Hummer KE, Finn CE, Frei B, Wrolstad RE (2002). Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribes. *J. Agric. Food Chem.* 50:519-525.
- Orrenius S (2007). Reactive Oxygen Species in Mitochondria-Mediated Cell Death. *Drug Metab. Rev.* 39:443-455.
- Ostrom RS, Gregorian C, Insel PA (2000). Cellular release of and response to ATP as key determinants of the set-point of signal transduction pathways. *J. Biol. Chem.* 275:11735-11739.
- Panickar KS (2013). Effects of dietary polyphenols on neuroregulatory factors and pathways that mediate food intake and energy regulation in obesity. *Mol. Nutr. Food Res.* 57:34-47.
- Paul-Labrador M, Polk D, Dwyer JH, Velasquez I, Nidich S, Rainforth M, Schneider R, Merz CN (2006). Effects of a randomized controlled trial of transcendental meditation on components of the metabolic syndrome in subjects with coronary heart disease. *Arch. Intern. Med.* 166:1218-24.
- Perez-Alvarez S, Cuenca-Lopez MD, Melero-Fernandez de Mera RM, Andonis Karachitos EP, Bednarczyk P, Kmita H, Aguirre N, Galindo MF, Jordan J (2010). Methadone induces necrotic-like cell death in SH-SY5Y cells by an impairment of mitochondrial ATP synthesis. *Biochim. Biophys. Acta* 1802:1036-1047.
- Picada Pereira R, Fachineto R, Souza Prestes A, Puntel RL, Santos da Silva GN, Heinzmann BM, Boschetti TK, Athayde ML, Bürger ME, Morel AF, Morsch VM, Rocha JBT (2009). Antioxidant effects of different extracts from *Melissa officinalis*, *Matricaria recutita* and *Cymbopogon citratus*. *Neurochem. Res.* 34:973-983
- Prior RL, Hoang H, Gu L, Wu X, Bacchiocca M, Howard L, Hampsch-Woodill M, Huang, D, Ou B, Jacob R (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC<sub>FL</sub>)) of plasma and other biological and food samples. *J. Agric. Food Chem.* 51:3273-3279.
- Rossi A, Serraino I, Dugo P, Paola RD, Mondello L, Genovese T, Morabito D, Dugo G, Sautebin L, Caputi AP, Cuzzocrea S (2003). Protective effects of anthocyanins from blackberry in a rat model of acute lung inflammation. *Free Radic. Res.* 37:891-900.
- Sears B, Rocordi C (2012). Role of fatty acids and polyphenols in inflammatory gene transcription and their impact on obesity, metabolic syndrome and diabetes. *Eur. Rev. Med. Pharmacol. Sci.* 16:1137-54.
- Servant D, Logier R, Mouster Y, Goudemand M (2009). Heart rate variability. *Appl. Psych. Encephale.* 35:423-8.
- Stevenson DE (2012). Polyphenols as Adaptogens – The Real Mechanism of the Antioxidant Effect? Iraj Rasooli (Ed). *Bioactive Compounds in Phytomedicine.*
- Volonte C, Amadio S, Cavaliere F, D'Ambrosi N, Vacca F, Bernardi G (2003). Extracellular ATP and Neurodegeneration. *Curr. Drug Targets* 2:403-412.
- Wang H, Zhou M, Brand J, Huang L (2009). Inflammation and taste disorders: mechanisms in taste buds. *Ann. NY Acad. Sci.* 1170:596-603.
- Wang SY, Lin HS (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.* 48:140-146.
- Wong HS, Leung HY, Ko KM (2011). 'Yang-Invigorating' Chinese tonic herbs enhance mitochondrial ATP generation in H9c2 cardiomyocytes. *Chinese Med.* 2:1-5.
- Wu Y, Sun FF, Tong DM, Taylor MM (1996). Changes in membrane properties during energy depletion-induced cell Injury studied with fluorescence microscopy. *J. Biophys.* 71:91-100.
- Xie C, Kang J, Chen JR, Nagarajan S, Badger TM, Wu X (2011). Phenolic acids are in vivo atheroprotective compounds appearing in serum of rats after blueberry consumption. *J. Agric. Food Chem.* 59:10381-7.
- Zafra-Stone S, Yasmin T, Bagchi M, Chatterjee A, Vinson JA, Bagchi D (2007). Berry anthocyanins as novel antioxidants in human health and disease prevention. *Mol. Nutr. Food Res.* 51:675-83.
- Zhang Z, Cui W, Li G, Yuan S, Xu D, Hoi MP, Lin Z, Dou J, Han Y, Lee SM (2012). Baicalein protects against 6-OHDA-induced neurotoxicity through activation of Keap1/Nrf/HO-1 and involving PKCalpha and PI3K-AKT signaling pathways. *J. Agric. Food Chem.* 60:8171-82.

*Full Length Research Paper*

# Exclusive breastfeeding: Mothers' awareness and healthcare providers' practices during antenatal visits in Mvomero, Tanzania

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In Tanzania, about 42% of children below five years are stunted due to chronic malnutrition. Exclusive breastfeeding (EBF) may be an effective strategy to protect infants from malnutrition. Therefore, it is important to disseminate accurate information on breastfeeding recommendations to pregnant women attending antenatal visits. The aim of the study was to assess the awareness of exclusive breastfeeding among first time pregnant women attending antenatal clinics and breastfeeding counselling practices of healthcare providers, for alignment with the World Health Organizations (WHO) recommendations. A cross sectional study of eighty first time pregnant women attending antenatal clinics at Mzumbe Health Centre and Tangeni dispensary, and six nurses providing care in these facilities was undertaken. Questionnaires were used to evaluate women's breastfeeding knowledge and future intentions to breastfeed and nurses' breastfeeding knowledge and counselling practices. Results indicated that women's knowledge in exclusive breastfeeding was generally poor; there were no differences in breastfeeding knowledge between the two facilities. About 94% of women had never received breastfeeding counselling at the antenatal clinics, 61% received breastfeeding information from their mothers, 37.5% said glucose water should be given immediately after delivery, only 23.8% planned to introduce solids at six months, the majority indicating that they would start solids at a younger age. Common reasons for introducing solids were; baby will be old enough (55%), baby will be hungry (32.5%), advised by the nurse (7.5%). Only one nurse had received training on breastfeeding, nurses' knowledge of WHO breastfeeding recommendations was poor; however nurses had satisfactory knowledge of how to solve breastfeeding problems. Only three nurses said they educate mothers about exclusive breastfeeding. In conclusion, findings highlight a need to focus on delivering information and education to women and nurses.

**Key words:** Breastfeeding, pregnant women, healthcare providers, knowledge, practices.

## INTRODUCTION

The under-five child mortality rate is very high in Tanzania. In the period of 2006 to 2010, it was reported

to be 81 per 1,000 live births (National Bureau of Statistics (NBS) and ICF Macro, 2010). A major factor

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contributing to infant and child mortality is malnutrition. The current Tanzania Demographic and Health Survey (TDHS) reports that approximately 42% of children below five years are stunted as a result of chronic under nutrition (NBS and ICF Macro, 2010). Malnutrition of the foetus starts during intra-uterine life when the mother has inadequate food intake as a result of food insecurity, poor caring practices, lack of information on proper diet (Lartey, 2008), and unhealthy living environments (NBS and Opinion Research Corporation (ORC) Macro, 2005). Other factors which contribute to maternal malnutrition in sub-Saharan Africa include micronutrient deficiencies, high rates of HIV infection and malaria (United Nations Children's Fund (UNICEF), 2005). The situation becomes worse after delivery when the infant is not exclusively breastfed and due to early introduction of nutritionally-poor complementary foods. The Tanzania National Strategy for Growth and Reduction of Poverty Strategy paper of 2011 targets to increase the prevalence of exclusive breastfeeding from 50% (2010) to 60% by 2015 (International Monetary Fund, 2011). Increasing rates of exclusive breastfeeding is a global goal which is urgently required as an intervention for child survival (UNICEF, 2009).

Exclusive breastfeeding means giving infants only breast milk with no addition of other foods or drinks, including water (WHO/UNICEF, 2003). The WHO recommends an early initiation of breastfeeding of one hour after birth and exclusive breastfeeding for six months (WHO, 2001b; WHO/UNICEF, 2003). Exclusive breastfeeding for the first six months of life is estimated to lower infant death by 13% (Jones et al., 2003). Other dangers associated with not breastfeeding as recommended include high infant death rates caused by lowered protection against harmful bacteria and other gastrointestinal infections and slow recovery from illnesses (WHO, 2001b).

In Africa, the majority of mothers fail to practice exclusive breastfeeding as recommended (UNICEF, 2006b). There are cultural, social and economic barriers to exclusive breastfeeding including practices of pre-lacteal feeding, giving drinking water and herbal teas (Shirima et al., 2001). In Tanzania, studies report early introduction of non milk foods such as thin maize porridge and animal milk as early as a few weeks or months after birth (NBS and ICF Macro, 2010; Shirima et al., 2001). In some societies like the Yoruba of Nigeria, exclusive breastfeeding is regarded as threatening to the infant because they believe the infant needs drinking water to suppress thirst and accelerate faster growth (Davies-Adetuyogbo, 1997). Despite the well documented benefits of exclusive breastfeeding both to the mother and the baby (Kramer and Kakuma, 2002; WHO, 2002), and recommendations by the WHO (WHO, 2001b; WHO/UNICEF, 2003), only 50% of women in Tanzania breastfeed their infants exclusively for six months (NBS and ICF Macro, 2010).

Available evidence suggests that breastfeeding practices

may not meet the current recommendations for exclusive breastfeeding (Shirima, 2000, 2001; NBS and ICF Macro, 2010). Antenatal visits may provide a platform for healthcare providers to give information about the exclusive breastfeeding practices. The information and counselling provided by healthcare providers is usually taken to be the most influential because mothers see them as their role models in matters related to breastfeeding (Hillenbran and Larsen, 2002). Therefore so as to understand barriers and limitations to exclusive breastfeeding, the purpose of this study was to assess the awareness of exclusive breastfeeding among first time pregnant women attending antenatal clinics and to assess breastfeeding counselling practices of healthcare providers in comparison with the WHO recommendations. Knowledge of exclusive breastfeeding among pregnant women and healthcare providers was also assessed.

## MATERIALS AND METHODS

A cross sectional study by using questionnaires to evaluate exclusive breastfeeding knowledge of mothers and health care providers and also to determine breastfeeding counselling practices of health care providers was conducted in Mvomero district in Morogoro region of East Central Tanzania. Mvomero is a rural district with a total population of 260,535. The study was conducted at antenatal care clinics of two health facilities in Mvomero district namely, Mzumbe Health Centre and Tangeni dispensary. This area was selected because it is within a new district formed from the existing Morogoro rural district a few years ago. Due to this fact, there are very few studies already done in the district, and breastfeeding in this group as a district unit of population has not been studied. The study was designed to include two kinds of respondents: First time pregnant women in their 16+ week of pregnancy attending antenatal clinics and a convenience sampling method was used where any woman available and meeting the inclusion criteria was interviewed to assess their knowledge of exclusive breastfeeding. Nurses providing care to pregnant women during antenatal visits were also included in the study. Data were collected between June to July, 2011.

All pregnant women attending antenatal clinics during the days of data collection were approached in the waiting area of the antenatal clinics. Those meeting the study criteria of being first time mothers and above 16 weeks pregnant were explained the purpose of the study verbally, and confidentiality of response was assured. Those willing to participate gave oral consent. Interviews were conducted privately after their clinic appointments. Also, health care providers providing antenatal care read information sheets gave written consent. A convenience sample of eighty pregnant women, forty from each health facility was interviewed. This target number for each health facility was pre-determined based on past attendance records.

Six out of eight nurses providing antenatal services in the two health facilities were included. Four of these were from Mzumbe health centre and two from Tangeni dispensary. These were chosen because in Tanzania, nurses are the main providers of antenatal and delivery care in all health facilities. These consist of nurses who have been trained at various levels of the health system for at least two to four years in maternal and child health aiding, nursing and midwifery care.

Two questionnaires were developed for collecting the data of the study. One questionnaire was developed to determine pregnant women's knowledge of breastfeeding and future intentions to

**Table 1.** Demographic characteristics of women (n = 80).

Characteristic	n (%)
<b>Literacy level</b>	
Not able to read and write	25 (31.2)
Can read and write to some extent	24 (30.0)
Can read and write	31 (38.8)
<b>Education level</b>	
No formal education	25 (31.2)
Standard 1-7	27 (33.8)
Secondary school	12 (15.0)
Above secondary school	16 (20)
<b>Occupation</b>	
Housewife	18 (22.5)
Casual worker	36 (45.0)
Formally employed	11 (13.8)
Self employed	15 (18.8)
<b>Stage of pregnancy (weeks)</b>	
16-20	18 (22.5)
21-25	11 (13.8)
26-30	25 (31.2)
Above 30	26 (32.5)
<b>Started antenatal clinic (weeks)</b>	
At 6-15	26 (32.5)
At 16-20	49 (61.2)
At 21-25	5 (6.2)

breastfeed. The questionnaire was pre-tested on six pregnant women attending MCH clinics at Mzumbe health centre to ensure that questions are worded clearly and changes were incorporated. Interviews were conducted after women's clinic appointments to assess their knowledge in areas of breastfeeding such as: Importance of colostrum and initiation of breastfeeding, duration of feeding, practical aspects of breastfeeding, effective feeding, advantages to the baby and the mother, breastfeeding problems and future intentions to breastfeed. The questionnaire also included socio-demographic characteristics of the group. The second self administered questionnaire was developed to determine the current practices and breastfeeding knowledge of nurses providing antenatal care.

Questions were formulated based on WHO breastfeeding recommendations, study instruments used in similar and previous research and published literature of studies on breastfeeding knowledge, attitudes and practices. In addition, nurses were asked to identify a correct picture indicating positioning and attachment of infants to the breast. This study was approved by the Massey University Human Ethics Committee and permission was granted by the Morogoro Regional Medical Office. Data were analysed using statistical package for social sciences (SPSS) package version 17 (SPSS Inc., Chicago, IL, USA). All variables were categorical and were described by using the frequencies and percentages.

## RESULTS

### Pregnant women

Demographic characteristics of participating women are presented in [Table 1](#). Results indicated that although the majority of women (58.8%) knew that immediately after birth a baby should only be given breast milk, there was a high percentage of the mothers (37.5%) who believed that babies should be given glucose water and 3.8% who did not know what a baby should be given immediately after delivery. When asked about how soon a baby should be put to breast after delivery, 52.8% knew that it is within 1 h after delivery. The majority of the women (63.8%) said colostrum was important and among these, 33.8% said colostrum is important because it provides babies with protection against infection and illness. More than half of pregnant women (57.5%) knew that a baby should be breastfed on demand. Only 28.8% were aware that breast milk alone is sufficient for the baby for six months while 41.3% thought 4 to 5 months is the appropriate age to start solid foods. About 93.8% of the women were aware that breastfeeding should be continued up to two years. More than half of the women (52.5%) believed that it is right to give water to a baby after every breastfeed.

When asked when they intend to introduce solid foods, 35% said 4 months, 25% said 3 months, 23.8% said six months, 12.5% said 5 months, 2.5% said 2 months and 1.2% said they will introduce solids at less than 1 month. Reasons given for introducing solids at the mentioned age included; baby will be old enough to start solids (55%), baby will be hungry (32.5%), advised by the MCH nurse (7.5%), advised by relatives and friends (2.5%) and that's the age their mother introduced solids to them (2.5%).

Women's responses to questions of knowledge of advantages of breastfeeding both to the mother and the baby are presented in [Table 2](#). The people most frequently mentioned to help with problems related to breastfeeding were other family members (33.8%), mother (31.2%), MCH nurse (27.5%), friends (6.2%) and husband or partner (1.2%). When asked about their future intentions to breastfeed, (93.8%) said 'yes'. Of those who intended to breastfeed, 66.2% intended to do so for 1 to 2 years, and 28.8% for 7 to 12 months. Those who did not intend to breastfeed (3.8%) claimed to have health problems that did not allow them to do so and 1.2% said their relatives never breastfed because they had no milk, so they assume the same will happen to them. Women gave different responses regarding breastfeeding in various circumstances. These are shown in [Table 3](#). More than half of the women (65%) had received support and information regarding breastfeeding from various sources. Of these, 22.5% received this information from their mothers and 35% preferred other family members for support regarding breastfeeding.

**Table 2.** Frequency of women's responses to questions regarding advantages of breastfeeding to mother and baby and effective breastfeeding (n = 80).

Questions asked	*Agree [n (%)]	Neither agree nor disagree [n (%)]	*Disagree [n (%)]
Breastfeeding reduces the risk of lung infection among babies	23 (28.7)	47 (58.8)	10 (12.5)
Baby who received breastfeeding is less prone to get diarrhoea	68 (85)	10 (12.5)	2 (2.5)
Breast milk provides baby with more protection from allergy compared to formula milk	53 (66.2)	26 (32.5)	1 (1.2)
Breastfeeding causes good development of baby's teeth and gum	36 (45)	39 (48.5)	5 (6.2)
Breastfeeding is beneficial for the mother	68 (85)	12 (15)	0 (0)
Exclusive breastfeeding is beneficial in spacing birth	64 (80)	16 (20)	0 (0)
Breastfeeding helps to stimulate uterine contraction	7 (8.8)	58 (72.5)	15 (18.8)
Mothers who practiced breastfeeding may achieve pre-pregnancy weight faster	20 (25)	58 (72.5)	2 (2.5)
Mother who practiced breastfeeding is less likely to experience breast problems	38 (47.4)	15 (18.8)	27 (33.8)
Babies will gain weight if they receive effective breastfeeding	78 (97.5)	2 (2.5)	0 (0)
Correct positioning helps to achieve effective breastfeeding	41 (51.2)	38 (47.5)	1 (1.2)
Babies sleep well after they receive adequate breastfeeding	78 (97.5)	2 (2.5)	0 (0)

\*Strongly agree' and 'agree' were combined, and 'strongly disagree' and 'disagree' were combined.

Spearman's rank order correlation was run between level of literacy, highest level of education attained, occupation and monthly family income and various variables of breastfeeding knowledge. The correlation analysis revealed various positive and inverse significant correlations between demographics and knowledge variables. For example, the level of literacy was found to be positively correlated with the variable 'how often a baby should be breastfed' (R = 0.517; P < 0.01). Therefore, knowing how to read and write increased a woman's knowledge of breastfeeding on demand. Highest level of education attained was significantly correlated with the variable 'when mothers intend to introduce solid foods' (R = 0.418; P < 0.01). This means that women with higher education of at least secondary school intended to introduce solid foods at a right age of six months. Occupation was positively correlated with 'giving water is encouraged after every breastfeeding' (R = 0.621;

P = 0.039). That is, women who were employed were more likely to continue breastfeeding for longer duration than unemployed women. Monthly family income was inversely correlated with 'how soon should a baby be put on the breast after delivery' (R = -0.535; P = 0.025). Therefore, women with higher family monthly income were more knowledgeable in this variable compared to those with lower income.

### Nurses

Six nurses participated in this study. All nurses were female and had breastfed children of their own. The majority were public health nurses (66.7%), the remaining were nursing officers and maternal and child health aides. Half of them had reached Diploma level as their highest education level. When asked about sources of their breastfeeding knowledge, two nurses said it was

from feeding their own children, two said clinical experience and the remaining two mentioned personal reading. The nurses indicated a deficit in breastfeeding training, for example only one nurse had undergone training in matters related to breastfeeding, and the rest had never undergone any training regarding breastfeeding. Regarding breastfeeding recommendations, all six nurses were aware of the recommendations on exclusive breastfeeding for six months and the use of bottles, teats and pacifiers.

Only 3 nurses knew the recommendation for demanded breastfeeding. When asked if they educate mothers about these breastfeeding recommendations, only 2 nurses said 'yes'. When asked if they discuss importance of colostrum with pregnant women all nurses said 'no'. Nurses indicated high knowledge on breastfeeding during certain circumstances that may affect breastfeeding. For example when asked whether breastfeeding should continue during maternal



**Table 3.** Responses given by women on questions about breastfeeding in certain circumstances (n = 80).

Question	Response	n (%)
In your opinion, should breastfeeding continue during:	Yes	29 (36.3)
	No	31 (38.8)
	Does not know	20 (25)
Pregnancy	Yes	62 (77.5)
	No	10 (12.5)
	Does not know	8 (10)
Maternal sickness	Yes	80 (100)
	Yes	80 (100)
Child sickness	Yes	29 (36.3)
	No	29 (36.3)
	Does not know	22 (27.5)
Menstruation	Yes	27 (33.8)
	No	40 (50)
	Does not know	13 (16.3)
Mother on medication	Yes	36 (45)
	No	26 (32.5)
	Does not know	18 (22.5)
HIV infected	Yes	27 (33.8)
	No	40 (50)
	Does not know	13 (16.3)
Alcohol drinking	Yes	36 (45)
	No	26 (32.5)
	Does not know	18 (22.5)

and maternal and child illness and menstruation, all of them said 'yes'. However, nurses did not have enough knowledge on other breastfeeding scenarios; for example when asked if breastfeeding should continue when the mother is on medication and during alcohol drinking only one nurse said 'yes'. Regarding if women should continue breastfeeding during pregnancy, 5 nurses said 'yes', when a mother is human immunodeficiency virus (HIV) infected, 2 nurses said she should continue breastfeeding, 2 nurses said she should not continue to breastfeed and the remaining 2 did not know whether an HIV positive mother should breastfeed or not.

All the nurses agreed that breast milk is the ideal food for the baby. Participants indicated different levels of knowledge when asked whether mixing breastfeeding with formula feeding reduces breast milk supply, 2 agreed, 2 were neutral and 2 disagreed with this statement. All the 6 nurses agreed that counselling by healthcare providers is effective in encouraging more women to breastfeed and were aware that a baby should be put to the breast within the first hour after delivery. When asked if they counsel pregnant women about exclusive breastfeeding, 3 nurses said 'yes' and that they do it 'sometimes'.

Regarding options that may help to resolve sore nipples, all the nurses said that seeking expert assistance with positioning and attachment helps resolve sore nipples while 2 nurses thought to stop feeding on the affected side will help resolve problem of sore nipples and 2 said advising mothers to apply breast milk to nipples will help resolve sore nipples. None of the nurses suggested checking symptoms of nipple thrush or advising mothers to apply lanolin to help resolve sore nipples. When asked about symptoms that could indicate poor attachment to the breast, nurses suggested the following; baby feeding unsettled (4), sore and cracked nipples (5), repeated engorgement (5) and mastitis (3).

On the side of the options to help resolve the problem of breast milk insufficiency, all nurses mentioned increasing frequency of breastfeeding and seeking expert assistance with positioning and attachment. One nurse mentioned advising mothers to drink more fluid will help to resolve breast milk insufficiency. Regarding the advice to be given to women with mastitis, 3 nurses said it is better to continue breastfeeding when a woman has mastitis, 3 nurses also said that women with mastitis should stop feeding on the affected side while 2 nurses said women with mastitis should stop feeding altogether.

## DISCUSSION

Previous studies in Tanzania reported high prevalence of pre-lacteal feeding and discarding of colostrum (Shirima et al., 2000, 2001; Nkala and Msuya, 2011). However, this study indicates most mothers knew that babies should receive the first milk. There appeared to be different views about the health benefits of colostrum for an infant. About 64% of respondents said that colostrum is important and they knew that colostrum and breast milk was the best food and that only colostrum should be given to the baby immediately after a safe delivery. This positive result could be explained by the positive breastfeeding culture of the Tanzanian women.

Early initiation of breastfeeding is important for the health of the infant and successful establishment and maintenance of breastfeeding (Mikiel-Kostyra et al., 2002). Shirima et al. (2001) reported 84% of rural mothers and 93% of urban mothers in Tanzania initiated breastfeeding within six hours after delivery which reflected their knowledge of the importance of early initiation of breastfeeding. A delay in the onset of breastfeeding has been reported to have an increased risk of neonatal mortality (Edmond et al., 2006).

In the current study, 52.8% of mothers were aware of the need to initiate breastfeeding within 1 h of birth. The difference between results of the current study and those of Shirima et al. (2001) could be due to the women in that study being informed either during the antenatal or postnatal period on the benefits of early initiation. In this study, participants had three misconceptions about why

babies should not be put to the breast within the first hour. These incorrect ideas could be influenced by tradition and cultural values of this semi-rural population. In order to solve this problem of such misconceptions, educating the public on the current breastfeeding recommendations, particularly through breastfeeding campaigns, which not only target the mother but the general community and social networks may be useful. This result also supports the need for breastfeeding education during the antenatal period.

### **Duration of breastfeeding**

More than half of the women had the perception that it is good to give water to a baby after every breastfeeding. Shirima et al. (2001) also reported high prevalence of water supplementation in rural and urban areas of Morogoro region. Only 28.8% of pregnant women interviewed were aware that babies less than six months of age should not be given water or anything else to eat or drink, and that breast milk alone is sufficient for the infant until six months of age. The WHO recommends no water supplementation in exclusively breastfed babies (WHO, 2001b). This is because breast milk contains enough average daily fluid requirements for healthy infants which are in the range of 80 to 100 ml/kg in the first week of life to 140 to 160 ml/kg between 3 to 6 months of life (Pan American Health Organization (PAHO)/WHO, 2003), if breastfed exclusively and on demand. The content of breast milk is 88% water and it contains very low amounts of solutes, therefore there is no need of water to flush out the excess solutes (PAHO/WHO, 2003). The WHO recommends continuation of breastfeeding for up to two years and beyond (WHO/UNICEF, 2003). In the current study, women were well aware of the age to stop breastfeeding altogether. About 94% of pregnant women believed that breastfeeding should continue until the child is two years of age. This is a very encouraging result which may mean a long duration of breastfeeding as recommended in this population.

The findings of this study showed that more than half of women knew that babies should be breastfed on demand. Both Shirima et al. (2000 and 2001) reported results which are similar to those of the current study that breastfeeding on demand was highly practised in both rural and urban areas of Morogoro region. This practice is in line with the WHO recommendations which emphasize demand feeding (WHO/UNICEF, 2003).

### **Introduction of solids**

Negative effects of early introduction of solid foods continue to be an important concern for the health of the Tanzanian infants. In this study, only 31.3% of women

thought six months was the appropriate age to start giving solid foods. The results from this study are in line with findings from the TDHS (NBS and ICF Macro, 2010), which identified that most babies in Tanzania are not exclusively breastfed for the first six months of life. The lack of knowledge about the recommended age for introduction of solid food among the first time mothers in our sample is probably the major contribution to the large number of women who intended to introduce solid foods at less than six months. Similar results were reported by Shirima et al. (2000) that introduction of solid foods in rural areas was done at a median age of 2 months and 2.5 months in urban areas of Morogoro. This study indicates that the majority of women intend early initiation and long duration of breastfeeding, but very few intend to breastfeed exclusively. Therefore, as an essential target, future interventions in Tanzania must target exclusivity, beginning in the antenatal period.

### **Future intentions to breastfeed**

In Tanzania, breastfeeding rates are very high at 94% (NBS and ORC Macro, 2005). This was also reflected in this study where the proportion of pregnant women who intended to breastfeed their babies was high at 93.8%. Although the participants were pregnant women who had not started breastfeeding, their early decision would bring a positive outcome after childbirth. These results are similar to those of a study by Hoyer and Pokorn (1998), which showed that the time of decision to breastfeed was important to determine the duration of breastfeeding and that the intention to breastfeed was a good indicator of the actual initiation and duration of breastfeeding. In that study, 77.3% of pregnant women intended to breastfeed. This early choice of women allowed them to have better intellectual and physical preparation. Therefore, targeting education in the antenatal period may be a crucial strategy to increase rates of exclusive breastfeeding.

Although participants in our study viewed breastfeeding as the best nutrition for infants, the majority did not support exclusive breastfeeding, but rather, supplementation of breast milk with water and home-made foods. Only 28.8% of mothers were aware that breast milk is sufficient for the baby for the first six months without addition of any other food or drink. A study by Davies-Adetugbo (1997) reported similar findings after assessing the knowledge and attitudes of breastfeeding in poor, rural communities of Nigeria. Mothers in that study mentioned breastfeeding as the best nutrition for their babies, on the other hand, they did not practice exclusive breastfeeding but rather, supplemented breast milk with other fluids and formula. Similar practices were reported by Shirima et al. (2001) in Tanzania that early introduction of thin porridge was common among mothers in Morogoro region. These practices can lead to reduced breast milk production, early cessation of breastfeeding and bottle feeding (Hill et al., 1997).

### **Advantages of breastfeeding to mother and baby**

Mothers in this study did not fully understand all the health benefits of breastfeeding, both to the infant and the mother, suggesting a need to emphasize this information in antenatal breastfeeding education in the population studied. Although the majority of the pregnant women knew that breast milk provided the best nutrition for the baby as seen in the results, they were not aware of other benefits such as breastfeeding reduces the risk of lung infection among babies, breastfeeding causes good development of baby's teeth and gum, and that a mother who practiced breastfeeding is less likely to experience breast problems. These gaps in women's knowledge provide an important opportunity to further promote breastfeeding. Shirima et al. (2000) reported that the advantages of breastfeeding mentioned by mothers were only those related to the infant and none to the mother. This reflects another area for stressing breastfeeding education and information to pregnant women. However, majority of women were aware of some advantages as they strongly agreed that babies sleep well after they receive adequate breastfeeding and that babies will gain weight if they receive effective breastfeeding.

### **Sources of breastfeeding information and support**

More than half (65%) of pregnant women mentioned receiving breastfeeding information from sources other than the MCH nurses. Although health care providers' advice is not the only expected source of information, it is interesting to note that a higher number of the respondents received breastfeeding information from other sources such as their mothers (largest proportion), grandmothers, friends and relatives rather than the physician and the media. This result may be explained by the Tanzania culture that pregnant women are usually supported and taken care of by their mothers, grandmothers or other female relatives, and the way they practice breastfeeding is usually due to the information they receive from such people. Contrary to this finding, a study done in Uganda among antenatal and postnatal women reported health facilities as the major source of breastfeeding information (Petit, 2008). This could be because participants in that study included mothers who had already given birth, who might have been informed about breastfeeding before and after discharge from the hospital.

The effect of receiving information from people other than healthcare providers was reported by Shirima et al. (2001) who identified that mothers who received breastfeeding information from traditional birth attendants or someone other than healthcare providers reported a shorter duration of exclusive breastfeeding. The fact that more respondents received information about breastfeeding from the family and almost none from other

sources provides an important basis for targeting future interventions, as education and communication activities on exclusive breastfeeding in Tanzania are only targeting mothers, usually in their antenatal visits during pregnancy or for child health care. Therefore, it would be important that the Tanzanian national strategy on the promotion, protection, and support of breastfeeding contain a component of information, education, and communication activities aimed at these influential family members to improve their knowledge of the need for exclusive breastfeeding in infants up to six months.

### **Breastfeeding in certain circumstances**

There are certain circumstances that may affect breastfeeding and reduce the rates of early initiation, exclusivity and duration of breastfeeding. The women in this study were asked about some certain circumstances which may affect breastfeeding. The respondents in this study were generally quite knowledgeable about breastfeeding in these circumstances, such as whether breastfeeding should continue during maternal illness, child illness and during menstruation. These were encouraging findings among these first time mothers to be. However, the majority of women did not know whether breastfeeding should continue during alcohol drinking, pregnancy, HIV infection and when the mother is on medication. Similar to these results, Shirima et al. (2001) reported that 84% of mothers from rural areas and 61% from urban areas of Morogoro believed that a woman should stop breastfeeding as soon as she becomes pregnant. This could be due to cultural beliefs surrounding breastfeeding in these populations. Breastfeeding campaigns and education programmes should be more focused on the special conditions where breastfeeding should or should not continue.

To date there have been many studies conducted regarding mothers' knowledge of breastfeeding but they did not tackle women's knowledge of alcohol during breastfeeding. However, Jones et al. (2011) found that both midwives and pregnant women were comfortable to discuss the topic but they were not knowledgeable regarding the recommendations and risks associated with alcohol drinking during pregnancy and breastfeeding. Generally, little is known regarding how women perceive and understand the issue of alcohol consumption while breastfeeding in Tanzania. In the current study, 45% of the respondents were aware that breastfeeding should continue during alcohol drinking. When a breastfeeding mother consumes alcohol, a small amount of alcohol passes freely into her breast milk and passes freely out of breast milk after approximately two hours after alcohol consumption for women of average weight. It is therefore more desirable to breastfeed the infant when no alcohol is remaining in breast milk and blood (WHO, 2001b). However, the WHO recommends avoiding alcohol or at

least restricting alcohol consumption (drink occasionally) during lactation (WHO, 2001b).

All six nurses involved in the study indicated that they have never demonstrated correct positioning and attachment of the baby to the breast to women during antenatal visits. Also, only 2 nurses were able to identify a correct picture demonstrating correct positioning of the baby to the breast. This result was contrary to that of a recent study carried out in South Africa to assess knowledge and practices of nursing staff regarding the Baby Friendly Hospital Initiative (BFHI). That study found out that 89% of nurses involved in the study were able to demonstrate the correct positioning of the baby to the breast (Daniels and Jackson, 2011). This difference could be caused by the nurses in the latter study having received training on breastfeeding and infant feeding issues during their time in service to update their knowledge.

However, the results of the current study were similar to a study carried out in Nigeria to assess the knowledge, attitude and practices of health workers in local government facilities regarding BFHI. That study which also involved nurses without prior breastfeeding training indicated that only 5.2% of the nurses were able to demonstrate the correct positioning and attachment of the baby to the breast (Okolo and Ogbonna, 2002). A study which used ultra sound to indicate the way a baby removes milk from the breast indicated that correct positioning and attachment of the baby at the breast is vitally important for the effective transfer of milk and may be the most important measure to prevent a number of breastfeeding problems (Woolridge, 1986). Therefore it is important for healthcare providers providing antenatal care to know this and correctly demonstrate it to the women in order to make those who would otherwise not breastfeed due to breast problems caused by improper attachment breastfeed successfully.

Healthcare providers have been believed to be in the appropriate place to promote and support breastfeeding; even so, unavailability of practical instruction and direction of proper breastfeeding technique may have effects on the positive results of breastfeeding, thus training is greatly needed (Leavitt et al., 2009). By gaining suitable knowledge and skills, healthcare providers can help increase the rate of exclusive breastfeeding and the duration of breastfeeding. Staff training together with refresher courses on breastfeeding recommendations should be introduced to all health facilities to enable nurses providing antenatal care to obtain necessary skills and knowledge. The preparation and distribution of pamphlets and posters that contain major breastfeeding recommendations could be taken into consideration.

Three nurses said that they would recommend breastfeeding until four months, contradicting the WHO recommendations which require infants to be exclusively breastfed up to six months (WHO/UNICEF, 2003). This could be due to not receiving any training on breastfeeding. These nurses did not know the recommended age for

introduction of solid foods. They suggested introducing solid food before six months. Evidence shows that infants who were exclusively breastfed for six months suffered less from gastrointestinal infections and experienced no growth deficits compared to infants who were exclusively breastfed for less than six months (Kramer and Kakuma, 2002).

All nurses knew the recommendation against the use of pacifiers. Step 9 of the ten steps to successful breastfeeding recommends no use of bottles, teats or pacifiers to enable mothers to establish and sustain breastfeeding (WHO/UNICEF, 1989). This could be because the use of pacifier is not a common practice in Tanzania.

Although only three nurses said they train mothers about breastfeeding recommendations, all the six nurses recognised that counseling by healthcare providers is effective in encouraging more women to breastfeed. This reflects nurses' personal choice to breastfeed their own children. Other studies also reported the majority of nurses do not train women about breastfeeding but they do recognize the need for practical and emotional support (Ebersold et al., 2007; Sheehan et al., 2009). The current study found out that only three nurses reported to be counselling pregnant women about breastfeeding and only in response to being asked. Aspects of breastfeeding which were reported to be discussed included the importance of colostrum, effective feeding and advantages of breastfeeding to the baby. These results were consistent with those of other studies that reported that a number of healthcare providers did not have enough breastfeeding knowledge and they were not prepared to give proper breastfeeding counseling to mothers (Amir and Ingram, 2008; Izatt, 1997). Even though these results were expected, they have drawn attention to the need to reinforce the advantages and importance of breastfeeding among healthcare providers.

### **Breastfeeding problems**

Nurses in this study responded well to the question which asked them to state signs of poor attachment of the baby to the breast. Among the reasons that were mentioned by most of the nurses were: baby feeding unsettled, sore and cracked nipples, repeated engorgement and mastitis. With regard to management of breastfeeding for women with mastitis, three nurses said that women with mastitis should stop feeding on the affected side while two nurses said women with mastitis should stop feeding altogether. This indicated poor knowledge of mastitis management among nurse participants. Even though these nurses are not the ones to look after mothers when they have given birth, this result is a cause for concern. For the appropriate management of mastitis, it is advised that breastfeeding mothers should not give the breast a rest; instead they should continue breastfeeding on demand so that the milk is removed from the breast (Savage-King, 1998).

## Breastfeeding education

The major sources of breastfeeding knowledge reported by nurses were gained from clinical experience, breastfeeding their own children and personal reading. The reason for this could be because there is no internal, routine training of healthcare providers in health facilities. A study carried out in Australia showed similar findings where doctors reported personal experience as their major source of breastfeeding knowledge (Brodribb et al., 2009). The nurses indicated lack of formal training, only one nurse had undergone training in breastfeeding, and the rest had never undergone formal training on breastfeeding after graduation. Initial training could be a good source of breastfeeding information to the nurses. This result is contrary to the requirement of the BFHI. Both facilities involved in this study are declared 'baby friendly'. The BFHI policy requires breastfeeding education courses to be provided for all nurses and other healthcare providers. Healthcare providers can be at risk of being a negative influence, especially when they give women incorrect, unsatisfactory and contradictory breastfeeding information and recommendations (Nelson, 2006). The lack of on-going training for nurses dealing with pregnant women indicated a gap which requires to be looked at by the health facilities. The findings of this study were similar to a previous study that healthcare providers and nurses were not knowledgeable enough to provide breastfeeding counselling to women (Freed et al., 1995).

In other countries, provision of education and training regarding breastfeeding in the form of programs was reported to be successful to improve the knowledge and practices of healthcare providers and to promote breastfeeding (Kronborg et al., 2008). This study supports the need for healthcare providers to acquire more knowledge regarding breastfeeding and to improve their education in order to be able to encourage exclusive breastfeeding for women during pregnancy. Educating women on breastfeeding during pregnancy helps to prepare them mentally to do so and has been shown to increase rates of exclusive breastfeeding (Su et al., 2007).

The findings of this study indicated inadequate knowledge and breastfeeding practices among the nurses despite the fact that the health facilities are supposed to be 'baby friendly'. This is a major obstacle to recommended breastfeeding practices. It indicates the need for baby friendly hospital initiative training for the nurses of these health facilities to respond to the concern and growing need for recommended breastfeeding practices.

## Conclusion

There were many areas in which breastfeeding knowledge was incomplete, and nurses and the women were not fully aware of the WHO breastfeeding recommendations.

Also it was observed that there was a high frequency of antenatal attendance, but nearly all these attendees had not received information and counselling regarding breastfeeding which may have contributed to mothers' lack of knowledge.

The study has observed that even though a small number of women would initiate breastfeeding within 1 h after delivery, the majority intended to breastfeed for up to 1 to 2 years thus long duration of breastfeeding. However, it is observed that exclusive breastfeeding for six months would be rare as the majority of women intended to introduce solid foods before six months. The major sources of information on breastfeeding for pregnant women were mothers, grandmothers and mothers-in-law which may explain the perceptions of pregnant women which are not in agreement with the current breastfeeding recommendations.

Although nurses were knowledgeable in some aspects of breastfeeding, they appeared not to be ready to educate and counsel women. Also, nurses' knowledge deficits have been identified in some important areas of breastfeeding such as the duration of exclusive breastfeeding, correct attachment and positioning of the infant to the breast and breastfeeding in special situations. These deficits can negatively affect the quality of information provided to pregnant women during antenatal visits.

Accurate breastfeeding counselling and advice by healthcare providers with emphasis on the current breastfeeding recommendations can improve the breastfeeding knowledge of first time pregnant women thus increase rates of exclusive breastfeeding which in turn may reduce infant morbidity and mortality rates.

Potentially, the findings of this study indicate various important policy implications for breastfeeding interventions. Strategies to encourage mothers to follow breastfeeding recommendations should concentrate on improving their knowledge and understanding of the recommendations and must target exclusivity, beginning in the antenatal period. Also, efficient on-going training about breastfeeding knowledge and skills for all healthcare providers providing antenatal and postnatal care is recommended.

## ACKNOWLEDGEMENT

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## REFERENCES

- Amir LH, Ingram J (2008). Health professionals' advice for breastfeeding problems: Not good enough. *Int. Breast. J.* 11, 22.
- Brodribb W, Fallon AB, Jackson C, Hegney D (2009). Breastfeeding knowledge-the experiences of Australian general practice registrars. *Aus. Fam. Phy.* 38:26-29.
- Daniels L, Jackson D (2011). Knowledge, attitudes and practices of nursing staff regarding the Baby-Friendly Hospital Initiative in non-

- accredited obstetric units in Cape Town. *S. Afr. J. Clin. Nutr.* 24:32-38.
- Davies-Adetugbo A (1997). Socio-cultural factors and the promotion of exclusive breastfeeding in rural Yoruba communities of Osun State, Nigeria. *Soc. Sci. Med.* 45:113-125.
- Ebersold SL, Murphy SD, Paterno MT, Sauvager MD, Wright EM (2007). Nurses and breastfeeding: Are you being supportive? *Nurs. Women Health* 11:482-487.
- Edmond KM, Zandoh C, Quigley MA, Amenga-Etego S, Owusu-Agyei S, Kirkwood BR (2006). Delayed breastfeeding initiation increases risk of neonatal mortality. *Pediatrics* 117:380-386.
- Freed G, Clark S, Sorenson J, Lohr J, Cefalo R, Curtis P (1995). National assessment of physicians' breastfeeding knowledge, attitudes, training and experience. *J. Am. Med. Assoc.* 273:472-476.
- Haider R, Kabir I, Huttly SRA, Answorth A (2002). Training peer counselors to promote and support exclusive breastfeeding in Bangladesh. *J. Hum. Lact.* 18:7-12.
- Hill P, Humenick S, Brennan M, Woolley D (1997). Does early supplementation affect long-term breastfeeding? *Clin. Pediatr.* 36:345-350.
- Hillenbran KM, Larsen PG (2002). Effect of an educational intervention about breastfeeding on the knowledge, confidence, and behaviors of pediatric resident physicians. *Pediatrics* 110:59.
- Hoyer S, Pokorn D (1998). The influence of various factors on breastfeeding in Slovenia. *J. Adv. Nurs.* 27:1250-1256.
- IMF (2011). Tanzania: Poverty Reduction Strategy Paper. National Strategy for Growth and Reduction of Poverty. Dar es Salaam, Tanzania: Tanzania Ministry of Economics and Economic Affairs.
- Izatt SD (1997). Breastfeeding counselling by health care providers. *J. Hum. Lact.* 13:109-113.
- Jones G, Steketee RW, Black RE, Bhutta ZA, Morris SS, Bellagio Child Survival Study Group (2003). How many child deaths can we prevent this year? *Lancet* 362:65-71.
- Jones SC, Eval M, Telenta J, Cert G, Shorten A, Johnson K (2011). Midwives and pregnant women talk about alcohol: What advice do we give and what do they receive? *Midwifery* 27:489-496.
- Kramer MS, Kakuma R (2002). The optimal duration of exclusive breastfeeding. A systematic review. Department of Child and Adolescent Health and Development, Geneva.
- Kronborg H, Vaeth M, Olsen J, Harder I (2008). Health visitors and breastfeeding support: Influence of knowledge and self-efficacy. *Eur. J. Public Health* 18:18283-18288.
- Lartey A (2008). Maternal and child nutrition in Sub-Saharan Africa: Challenges and interventions. *Proc. Nutr. Soc.* 67:105-108.
- Leavitt G, Martinez S, Orti N, Garcia L (2009). Knowledge about breastfeeding among a group of primary care physicians and residents in Puerto Rico. *J. Commun. Health* 34:1-5.
- Mikiel-Kostyra K, Mazur J, Boltrusko I (2002). Effect of early skin-to-skin contact after delivery on duration of breastfeeding: A prospective cohort study. *Acta Pediatr.* 91:1301-1306.
- National Bureau of Statistics (NBS) [Tanzania] and ORC Macro (2005). Tanzania Demographic and Health Survey (TDHS) 2004-05. National Bureau of Statistics and ORC Macro, Dar es Salaam, Tanzania.
- National Bureau of Statistics (NBS) [Tanzania] and ICF Macro (2010). Tanzania Demographic and Health Survey (TDHS) 2009-10. National Bureau of Statistics and ICF Macro, Dar es Salaam, Tanzania.
- Nelson AM (2006). A meta-synthesis of qualitative breastfeeding studies. *J. Midw. Women's Health* 51:13-20.
- Nkala TE, Msuya SE (2011). Prevalence and predictors of exclusive breastfeeding among women in Kigoma region, Western Tanzania: A community based cross sectional study. *Int. Breastfeed. J.* 6:17.
- Okolo SN, Ogbonna C (2002). Knowledge, attitudes and practice of health workers in Keffi Local Government hospitals regarding Baby Friendly Hospital Initiative practices. *Eur. J. Clin. Nutr.* 56:438-441.
- Pan American Health Organization (PAHO)/World Health Organization (WHO) (2003). Guiding principles for complementary feeding of the breastfed child. PAHO, WHO, Washington, DC.
- Petit AI (2008). Perception and knowledge on exclusive breastfeeding among women attending antenatal and postnatal clinics. A study from Mbarara hospital – Uganda. Official Publication of the Tanzania Medical Students' Association.
- Savage-King F (1998). Helping mothers to breastfeed (Rev. ed.). African Medical and Research Foundation. Nairobi, Kenya.
- Sheehan A, Schmied V, Barclay L (2009). Women's experiences of infant feeding support in the first 6 weeks post-birth. *Matern. Child Nutr.* 5:138-150.
- Shirima R, Greiner T, Kylberg E, Gebre-Medhin M (2000). Exclusive breast-feeding is rarely practised in rural and urban Morogoro, Tanzania. *Public Health Nutr.* 4:147-154.
- Shirima R, Gebre-Medhin M, Greiner T (2001). Information and socio-economic factors associated with early breastfeeding practices in rural and urban Morogoro, Tanzania. *Acta Pediatr.* 90:936-942.
- Su LL, Chong YS, Chan YH, Chan YS, Fok D, Tun KT (2007). Antenatal education and postnatal support strategies for improving rates of exclusive breastfeeding: Randomized controlled trial. *Br. Med. J.* 355:596-612.
- United Nations Children's Fund (2005). The state of the world's children 2006. UNICEF, New York.
- United Nations Children's Fund. (2006b). Progress for children: A world fit for children statistical review. Retrieved from [http://www.unicef.org/progressforchildren/2007n6/index\\_41511.htm](http://www.unicef.org/progressforchildren/2007n6/index_41511.htm)
- United Nations Children's Fund. (2009). Tracking progress on child and maternal nutrition: A survival and development priority. UNICEF, New York.
- World Health Organization/ United Nations Children's Fund. (1989). Protecting, promoting and supporting breastfeeding: The special role of maternity services. WHO, Geneva.
- World Health Organization. (2001b). Global Strategy for infant and young child feeding: The optimal duration of exclusive breastfeeding. Fifty World Health Assembly. WHO, Geneva.
- World Health Organization. (2002). Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life. WHO, Geneva, Switzerland.
- World Health Organization and United Nations Children's Fund. (2003). Global strategy for infant and young child feeding. WHO, Geneva, Switzerland.
- Woolridge M (1986). The anatomy of infant sucking and aetiology of sore nipples. *Midwifery* 2:164-176.

## Full Length Research Paper

# A preliminary study on the estimation of nutrients and anti-nutrients in *Oedaleus abruptus* (Thunberg) (Orthoptera: Acrididae)

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Natural protein is being overexploited gradually because of a huge population boom. Grasshoppers may be a good food resource to overcome this problem. The present study evaluated the nutritional quality and anti-nutritional factors that is, the proximate composition, vitamins, minerals, fatty acids and amino acid contents of *Oedaleus abruptus*. Results revealed that this species contains about 60% crude protein and nearly 587 kcal/100 g of energy. A total of six minerals were detected, where calcium and magnesium had the highest content. Among the fatty acids, palmitic acid, oleic acid and eicosenoic acid was present in quite good amount and a very high amount of linoleic and linolenic acid was also detected. Eighteen amino acids have been reported, among which the values of threonine, proline and tyrosine were more than 10%. Results of vitamins were also encouraging as a good amount of retinol, ascorbic acid and niacin were detected. The anti-nutritional factors showed a very low value. The results indicated the insects to be a good source of food that could be considered as an alternative to fish and meat and/or a supplement for both human and livestock consumption.

**Key words:** Nutrients, anti-nutrients, alternative food, *Oedaleus abruptus*.

## INTRODUCTION

Most developing and under developed countries are having difficulties to provide sufficient food for their people, and consequently an insufficient intake of protein is leading to malnutrition (Aylward and Morgans, 1995). Sometimes calories are considered to be more important than protein supplements. However, in some instances, authors like Ramos-Elorduy et al. (1984) opined that the deficiency of high quality of protein might be the major problem. In the developing countries of Asia, Latin America and Africa, a gradual "population boom" is an additional problem that is causing over-exploitation of natural food resource and importing more and more of

food is quite expensive (Breman and Debrah, 2003). In this scenario, there is an urgent need to conduct experiments for the search of indigenous alternative protein supplements worldwide. Various researchers reported that insects could be an attractive alternative as they are natural food of many vertebrates including human (DeFoliart, 1999).

Among these edible insects, short-horned grasshoppers of the order *Orthoptera* are proving to have immense potential. Some of them are known to contain high amount of protein, fat, carbohydrate and energy (Anand et al., 2008a), and many of them are easy to culture

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in mass scale (Haldar et al., 1999; Anand et al., 2008b), and are suitable for building “acridid farms” as they could yield a huge annual biomass with a very low maintenance cost (Anand et al., 2008b). Hence, farming these grasshoppers in controlled conditions could constantly supply a low cost alternative protein rich diet for human and livestock consumption.

Although in various parts of the world, insects are considered as an important protein source, literature is almost absent in the Indian point of view. In this context, Anand et al. (2008a) did a preliminary study on the proximate composition and mineral content of four acridid species commonly found in India. However, further studies are needed to look for the most suitable species throughout the world. Moreover, for a complete picture on the suitability of any material as food, there is an obvious need to further explore the contents of fatty acids, amino acids, vitamins and anti-nutritional factors. Keeping this in mind, the present study aimed to explore the nutritional value in terms of proximate composition, minerals, vitamins, fatty acids, amino acids, energy and anti-nutritional factors of a multivoltine grasshopper species: *Oedaleus abruptus* (Thunberg) from family Acrididae under the order Orthoptera.

## MATERIALS AND METHODS

### Collection and preparation of sample species

Nine hundred eighty seven (987) adult individuals of *O. abruptus* were collected from the nearby grasslands and croplands of Santiniketan (23° 39'N, 87° 42'E), Birbhum, West Bengal, India by sweeping technique, using standard insect nets. They were freeze killed prior to the estimation of wet body weight. Then they were oven dried (Indian instrument manufacturing company, Kolkata, India) at 60 °C until the dry body weight became constant. Moisture was estimated using the formula:

$$\text{Moisture (M\%)} = 100 - [(\text{dry weight} \times 100) / \text{wet weight}]$$

Legs and wings were removed from the dried matter to get rid of excess chitin, and after that they were crushed to powder form before subjected to further analyses.

### Estimation of proximate composition of the acridids

Nutrient composition was estimated by standard procedures according to Helrich (1990) on dry matter basis. Percentage nitrogen content was estimated by Micro-Kjeldahl method using Tecator Kjeltec system (Sweden). Nitrogen content was converted into crude protein (%) using the factor  $N \times 6.25$ . Crude fat was estimated by ether extraction method, using soxhlet apparatus (Indian instrument manufacturing company, Kolkata, India). Percentage content of crude fibre was chemically determined by repeated treatment of dilute  $H_2SO_4$  and dilute NaOH, and washed with distilled water. Ash contents were obtained by keeping the dried samples in a muffle furnace (Indian instrument manufacturing company, Kolkata, India) at 550 °C for 6 h. Nitrogen free extract (NFE) and carbohydrate content (%) was calculated by difference method. Energy was estimated using oxygen bomb calorimeter (Rajdhani Scientific Instruments Co., New Delhi, India) and

expressed as kcal/100 g.

### Estimation of minerals, amino acids, fatty acids and vitamins

Mineral contents such as Ca, Fe, Zn, Mg, Cu and Mn were estimated by Varian Techtron atomic absorption spectrophotometry (Victoria, Australia) using standard reference chemicals according to Anand et al. (2008b). The extraction of fatty acids from the acridid samples and their methyl ester preparation were performed according to the method of Bettelheim and Landesberg (1997). The purified methyl esters of fatty acids were subjected to Agilent 6890N gas chromatographic (Palo Alto, USA) analysis. Percent compositions of the samples were computed from the GC peak areas. Amino acids were analyzed according to Ghosh et al. (1995) and Wang et al. (2007). Samples were first hydrolyzed with 6 N HCl containing 1% phenol for 22 h at 105 °C, then the amino acid contents were determined by a PICO.TAG system according to PICO.TAG operation manual (Waters, USA) at 38 °C. Quantitative estimation of tryptophan could not be done by afore mentioned method, so it was determined by AIMIL Photochem colorimeter (Photochem Electric Instruments, Jodhpur, India) following the strategies proposed by Fischl (1960). Vitamins like retinol, ascorbic acid, niacin, riboflavin and thiamin were also estimated by colorimetric method according to Helrich (1990).

### Estimation of anti-nutritional factors

Anti-nutritional factor of phenolic polymers like tannin was determined chemically with vanillin-HCl reagent and catechin solution according to Gupta et al. (1988). Content of oxalate was determined by simple titration using methyl red as indicator following the procedures proposed by Gupta et al. (1988). Titration was again used to measure the amount of phytin phosphorus using ferric chloride ( $FeCl_3$ ) as indicator according to Agbede and Aletor (2004). Phytin content was calculated with a multiplication of the value of phytin phosphorus by 3.55 (Agbede and Aletor, 2004).

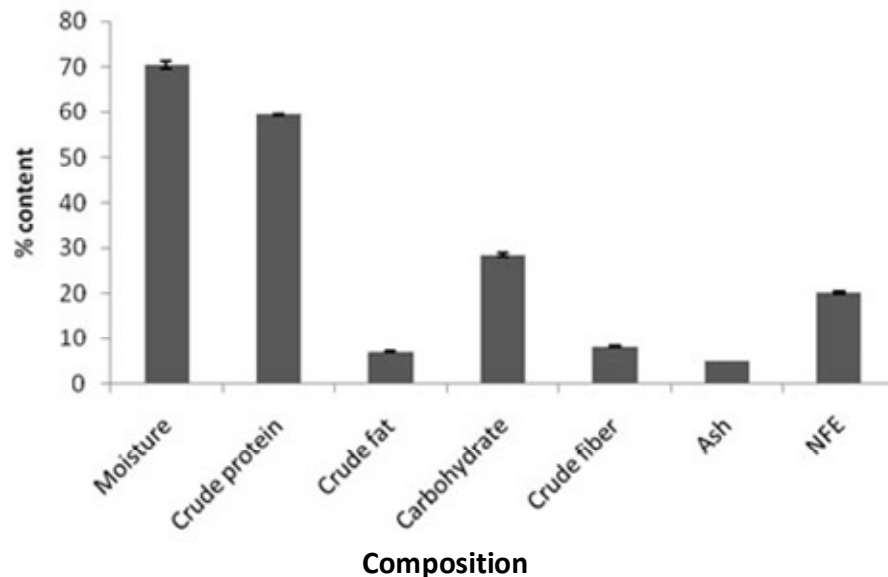
### Statistical analysis

Data are presented as means  $\pm$  standard deviation (SD). The experiments were carried out in three replicates. For completely randomized designs, all the data were statistically analyzed by one-way analysis of variance (ANOVA) using ‘S Plus’ (version 4.0) software. Results were subjected to Duncan’s multiple range test (DMRT) to understand the significant difference between the data within a sample group.

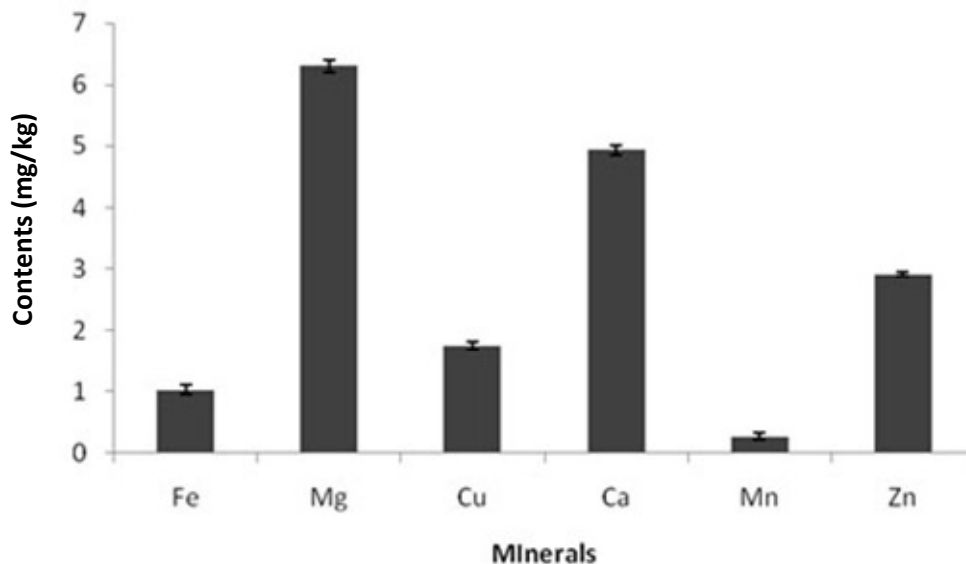
## RESULTS

Proximate composition revealed about 70% moisture content in *O. abruptus* (Figure 1). The species had a quite high level of crude protein (about 60%). On the other hand, the percentage of crude fat, crude fiber and ash were reasonably low (less than 10%), however, nitrogen-free extract (NFE) and carbohydrate contents were of moderate level (nearly 20 and 30%, respectively). The energy content of the species was  $587.48 \pm 2.14$  kcal/100 g (mean  $\pm$  SD) and the protein to energy (P/E) ratio was  $100.97 \pm 1.08$  mg protein/kcal (mean  $\pm$  SD), and these were quite high in this acridid (data not presented in graphical or tabulated forms).





**Figure 1.** Proximate composition of *O. abruptus*. Data are presented as means  $\pm$  SD.



**Figure 2.** Mineral contents (mg/kg) in the body tissues of *O. abruptus*. Data are presented as means  $\pm$  SD.

A total of six minerals were estimated. Among them, magnesium (Mg) was present in the highest amount (more than 6 mg/kg), followed by calcium (Ca) and zinc (Zn) (Figure 2). Iron (Fe) and copper (Cu) contents were moderately low (1.03 and 1.74 mg/kg, respectively), and manganese (Mn) was found in the lowest amount (about 0.27 mg/kg) (Figure 2). Eight fatty acids were detected in *O. abruptus* (Table 1). The content of linolenic acid was found to be highest (39.45%), followed by linoleic acid (15.34%). The content of palmitic acid, oleic acid and

eicosenoic acid were present in good amounts (4.55, 4.27 and 7.91%, respectively), but myristic acid was extremely low (0.37%).

Among the 18 amino acids detected in this study, threonine, proline and tyrosine were present in very high amount (more than 10%), whereas aspartic acid and cysteine were detected to be very low (0.65 and 0.48%, respectively). Five vitamins, namely: retinol, thiamine, riboflavin, niacin and ascorbic acid were detected in the body tissues of the selected acridid (Table 1). Thiamine

**Table 1.** Fatty acid, amino acid and vitamin contents of the acridid species.

Property	<i>O. abruptus</i>
<b>Fatty acid (%)</b>	
Myristic acid	0.37
Palmetic acid	4.55
Oleic acid	4.27
Stearic acid	1.12
Arachidonic acid	1.62
Eicosenic acid	7.91
Linoleic acid	15.34
Linolenic acid	39.45
<b>Amino acid (%)</b>	
Aspartic acid	0.65
Glutamic acid	4.51
Serine	5.08
Glycine	7.92
Histidine	7.58
Arginine	8.03
Threonine	15.99
Alanine	3.02
Proline	15.34
Tyrosine	10.31
Valine	6.24
Methionine	1.98
Cysteine	0.48
Isoleucine	1.45
Leucine	5.21
Phenylalanine	4.10
Lysine	2.04
Tryptophan	2.25
<b>Vitamin (mg/100 g)</b>	
Retinol	4.64
Thiamine	0.50
Riboflavin	1.01
Niacin	6.00
Ascorbic acid	6.25

was found to be present in very low amount (0.50 mg/100 g), whereas retinol, niacin and ascorbic acid were quite high (4.64, 6.00 and 6.25 mg/100 g, respectively).

Four anti nutritional factors, tannin, oxalate, phytin bound phosphorus (phytin P) and phytin were analyzed for the acridid. All of them were found to be present in very low amount. Among the analyzed anti-nutritional factors, tannin was present in the highest amount, which was around 2.45%, followed by oxalate (around 0.6%). On the other hand, phytin P and phytin was around 0.025 and 0.085%, respectively (data not presented in graphical or tabulated forms).

## DISCUSSION

Insects are rich in protein, and many scientists have reported the protein qualities of insects from different parts of the world. Chen and Feng (1999) reported that in China, nearly hundred kinds of edible insects had been analyzed till date. According to them, at the egg, larva, pupa and adult stages, the raw protein content generally is 20 to 70%, with average higher protein content in the order *Hemiptera* (around 42 to 73%). Banjo et al. (2006) analyzed fourteen edible insect species from south-western Nigeria and found that twelve of them have protein content of 20% and above, where the highest amount of protein (29.62%) was observed in *Analeptes trifasciata* (Fabricius, 1775). Ramos-Elorduy (1998) worked on the edible insects of Mexico and found those insects to have very high crude protein content. According to this study, the highest amount of protein was found in the red legged locusts (75.30%), whereas most of them contained around 40 to 60% crude protein. Our results revealed that the selected acridid species was also high in protein content (about 60%) and have a very high P/E ratio (more than 100 mg of protein/kcal) which proved this group of insects to be a good protein and energy supplement.

Protein is composed of mainly twenty amino acids. Among these, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and histidine are essential for human adults (Young, 1994). Analysis of more than hundred edible insects in China showed that they contain all the necessary amino acids (Chen et al., 2009). Wang et al. (2007) reported a total of seventeen amino acids in the Chinese acridid *Acrida cinerea* (Thunberg, 1815). Comparing their data with the acridid species of our interest revealed that nine of those seventeen amino acids were present in higher amount in *O. abruptus*. Two of them were almost similar and six were present in lower amounts. Although edible insects are good in amino acid content, reports indicated that some of them are deficient in some amino acids. As an example, studies on other edible insects like mormon cricket (*Anabrus simplex* Haldeman 1852) and house cricket (*Acheta domesticus* Linnaeus 1758) showed that they were deficient in methionine (DeFoliart et al., 1982; Finke et al., 1985; Nakagaki et al., 1987). Similarly, as reported by Landry et al. (1986), the essential amino acids of six lepidopteran species were also found to be deficient in the same amino acid. Our result corresponds with the above stated reports as the content of methionine, cysteine and lysine were found to be quite low (below 3%). Therefore we support the conclusion of Wang et al. (2007) that insects might be unsatisfactory as the only source of dietary protein due to limiting amino acids, but would be extremely beneficial as a supplement.

Fat is one of the major nutrients for any living organism. Authors like Feng et al. (2000a, b), Chen and Feng (1999) and He et al. (1999) reported that insects are

higher in fat content at the larva and pupa stages, while adults contained relatively lower amount of fat. Among grasshoppers, the fat content of adult *Oxya chinensis* (Thunberg 1815) was found to be only 2.2% (Chen et al., 2009); likewise Melo et al. (2011) reported that the same of *Sphenarium purpuracens* (Charpentier 1841-45) was also quite low (5.75%). Our study also showed lower fat content (nearly 7%) in the experimental acridid. Though the fat content of most of the edible insects is lower than other animal food sources but still they could play an important role for human nutrition.

Chen and Feng. (1999) reported that unlike other animal fat, edible insects have a higher essential fatty acid content which is necessary for human body, and hence edible insect fat has a high nutritive value. The results of fatty acid content of our species when compared to that of the variegated grasshopper *Zonocerus variegatus* (Linnaeus 1758), it was revealed that *O. abruptus* contained a total of eight fatty acids compared to five of *Z. variegatus* (Adeyeye, 2011), however in the fatty acids *Z. variegatus* is richer in palmitic acid (13.5%), stearic acid (5.7%), oleic acid (4.27%) and linoleic acid (9.4%). On the other hand, Wang et al. (2007) also reported the existence of eight fatty acids in *A. cinerea*. The palmitoleic and lauric acid that they found was not observed in our results; instead we detected arachidonic and eicosenoic acids. According to Chen and Feng (1999), edible insects are rich in protein and fat but not so rich in carbohydrate content; however the authors also added that carbohydrates of edible insects differ from species to species ranging from 1 to 10% in China. On the other hand, larva of *Cirina forda* (Westwood 1849) which is relished as a human food in Nigeria contains about 38% of carbohydrate (Akinnawo and Ketiku, 2000). Our species of interest also showed a moderately high amount of carbohydrate content (about 30%).

The gross amount of energy content of the food comes mainly from protein, fat and carbohydrate. As edible insects are quite rich in these three ingredients, in general they mostly contain quite high energy. For example, Sogbesan and Ugwumba (2008) reported energy value of 587.13 kcal/100 g in the sexual forms of the African termite (*Macrotermes subhyalinus* Rambur 1842), while Oyarzun et al. (1996) reported 688 kcal/100 g of energy in the arboreal termites, *Nasutitermes* spp. Braide et al. (2010) reported a calorific value of 433.8 kcal/100 g for the caterpillar *Bunaea alcinoe* (Stoll 1780) in Nigeria. Literature survey showed that the energy content of most of the edible insects varied from nearly 217 to 777 kcal/100 g (Ramos-Elorduy, 2008). Energy content of *O. abruptus* also supported this observation which was more than 550 kcal/100 g.

Edible insects have been found to contain minerals also. Among them, Na, K, Ca, Zn, Fe and Mg are prevalent in literature (Akinnawo and Ketiku, 2000; Ramos-Elorduy et al., 2002; Ojewola and Udom, 2005; Ekop et al., 2010). Ramos-Elorduy (2005) reported that edible

insects under the orders *Orthoptera*, *Lepidoptera* and *Hymenoptera* have much lower variation in mineral contents. The author continued stating that these insects are mostly low in Na, and sometimes high in Ca, Zn, Fe, K and Mg. Our results supported this view as Mg is present in the highest amount (more than 6 mg/kg), followed by Ca and Zn. Fe was observed in a moderate amount.

Though Na and K were not detected, a small trace of Mn (less than 1 mg/kg) was obtained. There were also reports that indicated edible insects to be rich in vitamin contents too. For example, Kodondi et al. (1987) reported rich vitamin content in some edible caterpillar in Zaire. Ramos-Elorduy et al. (1988) observed that edible insects are rich in vitamin B group such as thiamine, riboflavin and niacin. The presence of these three vitamins was also observed in case of *O. abruptus*, where niacin was present in high amount but the rest of the two were a little lower. Furthermore, two other vitamins, that is ascorbic acid and retinol were also present in quite high amount.

Up to this level, it has been discussed that the grasshopper of our interest is nutritionally comparable to the other available food insects. However, it is also important to know whether this species can compete with the other conventional protein sources like fish, meat, soybean etc. Surveying literature in this regard showed that the protein content of *O. abruptus* is higher than that of grain and soybean and almost comparable to fishmeal but lower than meat (Hasanuzzaman et al., 2010; Baker et al., 2011; Adeniyi et al., 2011). Hasanuzzaman et al. (2010) and Adeniyi et al. (2011) also reported crude fat content of fish and meat varies between 4 and 9% which is also similar to that of *O. abruptus*. On the other hand, Baker et al. (2011) observed crude fat of soybean meal to be about 1 to 1.5%, quite lower than that obtained in the present study.

According to Koumi et al. (2011), the carbohydrate content of fish and soybean could be around 23 and 27%, respectively, again very close to the value of the carbohydrate content of nearly 30% observed in the grasshopper of the this study. Among vitamins, this insect has a higher proportion of thiamine than wheat germ, peas, bread, beans, rice, soybean, milk and egg; whereas for riboflavin it is richer than aged cheese, bread, beef liver, milk, eggs, yogurt, pork, spinach, trout and chicken and niacin is in greater amount than bread, grouper fish, peas, beans, corn, wheat, milk, bacon and eggs, and in general is poor in terms of ascorbic acid (Ramos-Elorduy and Pino, 2001). On the other hand, mineral contents were quite lower in comparison with meat, fish, soybean or corn (Batal et al., 2010; Koumi et al., 2011; Adeniyi et al., 2011).

Anti-nutritional factors are usually present in plant materials but many phytophagous insects have been identified to retain these materials in quite a good amount (Berenbaum, 1993). Hence, it is recommended to analyze these anti-nutrients if a phytophagous insect is being considered as food, though this kind of study is not

much frequent in the literature. Among the anti-nutritional factors, tannin, oxalate and mineral bound phytate (that is, phytin P and phytin) were detected in the grasshopper of our interest. Adeduntan (2005) reported tannin percentage in grasshoppers of Ondo state of Nigeria to be 1.05%, which is a little lower than the amount (about 2.5%) observed in *O. abruptus*. However, this amount is itself quite lower than cereal grains and other plant food materials for example in tannin content *Pachira glabra* (Bombacaceae) (3.53%) and in *Azalia africana* (Fabaceae) (2.65%) (Ogunlade et al., 2011; Hassan et al., 2011). On the other hand the percentage of phytate was reported to be quite high in grasshoppers by Adeduntan (2005), which was about 1.1% compared to quite lower amount of nearly 0.025% of phytin P and 0.09% of phytin in the present study.

We could not find any literature describing the oxalate content of any grasshopper species but it has been reported in some edible beetles such as adults of *Oryctes monoceros* (Olivier 1789) and *Cirina forda* (Omotoso, 2006; Ifie and Emeruwa, 2011). Both of these papers revealed that those beetles contain less than 0.005% oxalate, much lower than the insect of our interest. However, this amount is also under the tolerance limit because much higher amounts have been observed in various plant food materials (Kalita et al., 2007).

## Conclusion

Nutrient composition of the present study reveals that *O. abruptus* is a nutritious insect with high protein and energy. Amino acids, fatty acids, and vitamins were present in high amount. Though anti-nutritional factors were present, they were found to be in very low amount and within the tolerance limit. It has been also discussed that this insect is nutritionally comparable to fish, soybean and various other conventional food ingredients in terms of proximate composition and/or vitamins. Thus it can be safely stated that acridids could be a good alternative source of not only protein and energy but also important as source of vitamins. This proves that an effort should be made so that these grasshoppers could be mass reared in controlled conditions in farms as a mini-livestock. This insect could be used as a supplement in formulated feed for various animal species such as fish, chickens, ostriches, pigs or some pets and for humans, it could be used in the preparation of snacks, creams, salads, cookies, casseroles, flours, breads, desserts, sauces, seasonings, soups, etc.

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**Abbreviations:** NFE, Nitrogen free extract; P/E, protein to energy ratio.

## REFERENCES

- Adeduntan SA (2005). Nutritional and anti-nutritional characteristics of some insects foraging in Akure Forest Reserve, Ondo State, Nigeria. *J. Food Technol.* 3:563-567.
- Adeniyi OR, Ademosun AA, Alabi OM (2011). Proximate composition and economic values of four common sources of animal protein in South-western Nigeria. *Zootecnia Trop.* 29:231-234.
- Adeyeye EI (2011). Fatty acid composition of *Zonocerus variegatus*, *Macrotermes bellicosus* and *Anacardium occidentale* kernel. *Int. J. Pharm. Bio. Sci.* 2:135-144.
- Agbede JO, Aletor VA (2004). Chemical characterization and protein quality evaluation of leaf protein concentrates from *Glyricidia sepium* and *Leucaena leucocephala*. *Int. J. Food Sci. Tech.* 39:253-361.
- Akinawo O, Ketiku AO (2000). Chemical composition and fatty acid profile of edible larva of *Cirina forda* (Westwood). *Afr. J. Biomed. Res.* 3:93-97.
- Anand H, Ganguly A, Haldar P (2008a). Potential value of Acridids as high protein supplement for poultry feed. *Int. J. Poult. Sci.* 7:722-725.
- Anand H, Das S, Ganguly A, Haldar P (2008b). Biomass production of acridids as possible animal feed supplement. *J. Environ. Sociobiol.* 5:181-190.
- Aylward F, Morgans J (1995). Protein and nutrition policy in low-income countries. Charles Knight and Co. Ltd., London, UK.
- Banjo AD, Lawa OA, Songonuga EA (2006). The nutritional value of fourteen species of edible insects in Southwestern Nigeria. *Afr. J. Biotechnol.* 5:298-301.
- Baker KM, Utterback PL, Parsons CM, Stein HH (2011). Nutritional value of soybean meal produced from conventional, high-protein, or low-oligosaccharide varieties of soybeans and fed to broiler chicks. *Poult. Sci.* 90:390-395.
- Batal AB, Dale NM, Saha UK (2010). Mineral composition of corn and soybean meal. *J. Appl. Poult. Res.* 19:361-364
- Berenbaum MR (1993). Sequestered Plant Toxins and insect palatability. *Food Insects Newslett.* 6:1-9.
- Bettelheim FA, Landesberg JM (1997). Laboratory experiments for general, organic and biochemistry. Saunders College Publishing, USA.
- Braide W, Sokari TG, Hart AD (2010). Nutritional quality of an edible caterpillar of a lepidopteran, *Bunaea alcinoe*. *Adv. Sci. Technol.* 4:49-53.
- Breman H, Debrah K (2003). Improving African food security. *SAIS Rev.* 23:153-170.
- Chen XM, Ying F, Zhiyong C (2009). Common edible insects and their utilization in China. *Entomol. Res.* 39: 299-303.
- Chen XM, Feng Y (1999). Edible insects in China. China Science and Technology Press, Beijing.
- DeFoliart GR (1999). Insects as food: why the Western attitude is important. *Annu. Rev. Entomol.* 44:21-50.
- DeFoliart GR, Finke MD, Sunde ML (1982). Potential value of the Mormon cricket (Orthoptera: Tettigoniidae) harvested as a high-protein feed for poultry. *J. Econ. Entomol.* 75:848-852.
- Ekop EA, Udoh AI, Akpan PE (2010). Proximate and anti-nutrient composition of four edible insects in Akwa Ibom State, Nigeria. *World*

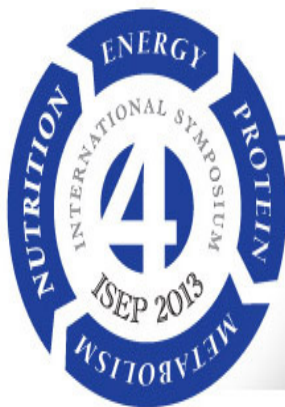
- J. Appl. Sci. Technol. 2:224-231.
- Feng Y, Chen XM, Wang SY, Ye SD, Chen Y (2000a). The nutritive elements analysis of bamboo insect and review on its development and utilization value. *Forest Res.* 13:188-191.
- Feng Y, Chen XM, Wang SY, Ye SD, Chen Y (2000b). The common edible insects of Hemiptera and its nutritive value. *For. Res.* 13:612-620.
- Finke MD, Sunde ML, DeFoliart GR (1985). An evaluation of the protein quality of mormon crickets when used as high protein feedstuff for poultry. *Poult. Sci.* 64:708-712.
- Fischl J (1960). Quantitative colorimetric determination of tryptophan. *J. Biol. Chem.* 235:999-1001.
- Ghosh AK, Naskar AK, Jana ML, Khwala S, Sengupta S (1995). Purification and characterization of an amyloglucosidase from *Termitomyces clypeatus* that liberates glucose from xylan. *Biotechnol. Prog.* 11:452-456.
- Gupta PC, Khatta VK, Mandal AB (1988). Analytical techniques in animal nutrition. Haryana Agricultural University, Hisar.
- Haldar P, Das A, Gupta RK (1999). A laboratory based study on farming of an Indian grasshopper *Oxya fuscovittata* (Marschall) (Orthoptera: Acrididae). *J. Orth. Res.* 8:93-97.
- Hasanuzzaman MAF, Hossain SZ, Das M (2010). Nutritional potentiality of earthworm (*Perionyx excavatus*) for substituting fishmeal used in local feed company in Bangladesh. *Mesopot. J. Mar. Sci.* 25: 25-30.
- Hassan LG, Umar KJ, Dangoggo SM, Maigandi AS (2011). Anti-nutrient composition and bioavailability prediction as exemplified by calcium, iron and zinc in *Melocia corchorifolia* leaves. *Pak. J. Nutr.* 10:23-28.
- He JZ, Tong Q, Huang XH, Zhou ZH (1999). Nutritive composition analysis of moths of *Dendrolimus houi* Lajongquiere. *Entomol. Knowl.* 36:83-86.
- Helrich K (1990). Official methods of analysis of the Association of Official Analytical Chemists. A.O.A.C. Philadelphia, USA.
- Ifie I, Emeruwa CH (2011). Nutritional and anti-nutritional characteristics of the larva of *Oryctes monoceros*. *Agric. Biol. J. N. Am.* 2:42-46.
- Kalita P, Mukhopadhyay PK, Mukherjee AK (2007). Evaluation of the nutritional quality of four unexplored aquatic weeds from north east India for the formulation of cost-effective fish feeds. *Food Chem.* 103:204-209.
- Kodondi KK, Leclerc M, Gaudin HF (1987). Vitamin estimations of three edible species of Attacidae caterpillars from Zaire. *Int. J. Vitam. Nutr. Res.* 57:333-334.
- Koumi AR, Koffi KM, Atsé BC, Kouame LP (2011). Growth, feed efficiency and carcass mineral composition of *Heterobranchus longifilis*, *Oreochromis niloticus* and *Sarotherodon melanotheron* juveniles fed different dietary levels of soybean meal-based diets. *Afr. J. Biotechnol.* 10:14990-14998.
- Landry SV, DeFoliart GR, Sunde ML (1986). Larval protein quality of six species of Lepidoptera (Saturniidae, Sphingidae, Noctuidae). *J. Econ. Entomol.* 79:600-604.
- Melo V, Garcia M, Sandoval H, Jiménez HD, Calvo C (2011). Quality proteins from edible indigenous insect food of Latin America and Asia. *Emirate J. Food Agric.* 23:283-289.
- Nakagaki BJ, Sunde ML, DeFoliart GR (1987). Protein quality of the house cricket, *Acheta domesticus*, when fed to broiler chicks. *Poult. Sci.* 66:1367-1371.
- Ogunlade I, Ilugbiyin A, Osasona AI (2011). A comparative study of proximate composition, antinutrient composition and functional properties of *Pachira glabra* and *Azelia africana* seed flours. *Afr. J. Food Sci.* 5:32-35.
- Ojewola GS, Udom SF (2005). Chemical evaluation of the nutrient composition of some unconventional animal protein sources. *Int. J. Poult. Sci.* 4:745-747.
- Omotoso OT (2006). Nutritional quality, functional properties and anti-nutrient composition of the larva of *Cirina forda* (Westwood) (Lepidoptera: Saturniidae). *J. Zhejiang Univ. Sci. B.* 7:51-55.
- Oyarzun SE, Crawshaw GJ, Valdes EV (1996). Nutrition of the Tamandua: I. Nutrient composition of termites (*Nasutitermes* spp.) and stomach contents from wild tamanduas (*Tamandua tetradactyla*). *Zool. Biol.* 15:509-524.
- Ramos-Elorduy J (1998). Creepy crawly cuisine: the gourmet guide to edible insects. Park Street Press, Rochester VT, USA.
- Ramos-Elorduy J (2005). Insects a hopeful food. In: Ecological implications of mini-livestock, ed. M. Paoletti, pp. 263-291. Oxford IBH Publishing, Enfield, New Hampshire, USA.
- Ramos-Elorduy J (2008). Energy supplied by edible insects from Mexico and their nutritional and ecological importance. *Ecol. Food Nutr.* 47:280-297.
- Ramos-Elorduy J, Pino JM. (2001). Contenido de vitaminas de algunos insectos comestibles de México. *Rev. Soc. Quím. Méx.* 45: 66-76.
- Ramos-Elorduy J, Pino JM, Rincón F, Marquez C, Escamilla E, Alvarado M (1984). Protein content of some edible insects in Mexico. *J. Ethnobiol.* 4:61-72.
- Ramos-Elorduy J, de Morales LJ, Pino JM, Nieto Z (1988). Contenido de tiamina riboflavin y niacin en algunos comestibles de Mexico. *Rev. Technol. Alimen.* 23:21.
- Ramos-Elorduy J, Gonzalez EA, Hernandez AR, Pino JM (2002). Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler. *J. Econ. Entomol.* 95:214-220.
- Sogbesan AO, Ugwumba AAA (2008). Nutritional evaluation of termite (*Macrotermes subhyalinus*) meal as animal protein supplements in the diets of *Heterobranchus longifilis* (Valenciennes, 1840) fingerlings. *Turk. J. Fish. Aquat. Sci.* 8:149-157.
- Wang D, Zhai S, Zhang C, Zhang Q, Chen H (2007). Nutritional value of the Chinese grasshopper *Acrida cinerea* (Thunberg) for broilers. *Anim. Feed Sci. Technol.* 135:66-74.
- Young VR (1994). Adult amino acid requirements: the case for a major revision in current recommendations. *J. Nutr. (Suppl.)* 124:1517S-1523S.

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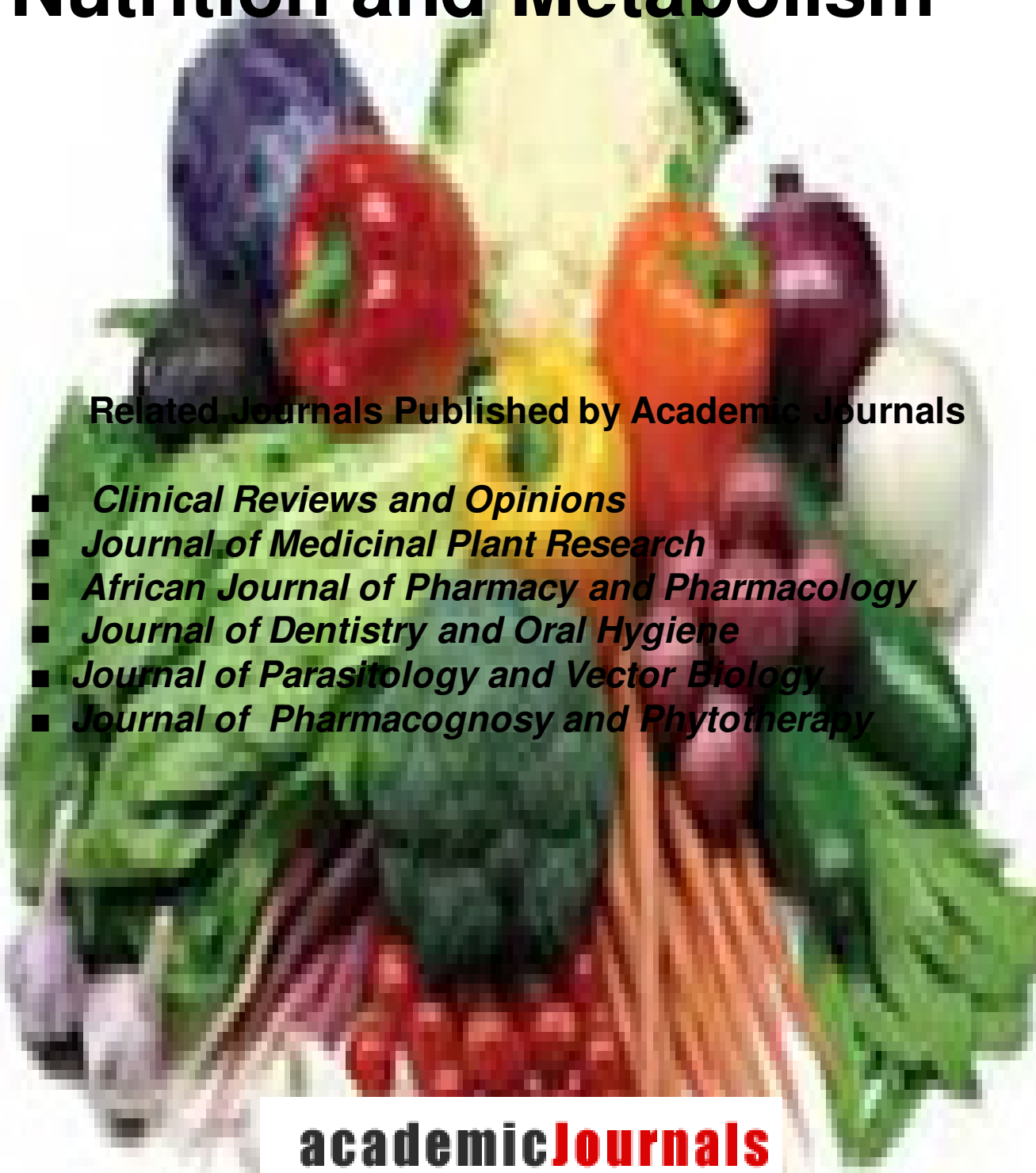
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