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Research Paper

In vivo evaluation of lipid and antioxidants qualities of *Carica papaya* seed oil

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ABSTRACT

Oil rich *Carica papaya Linn*. (CPL) seed is currently discarded as waste. This research is aimed at ascertaining the lipid and antioxidant qualities of the oil *in vivo* (compared to groundnut oil). Four diets composed from the oil were fed to wistar rats for 32 days. The lipid profile and antioxidant status of the plasma, erythrocytes and brain were examined. The results showed significant reductions (P<0.05) in body weights (of rats fed with the 7-10 % oil diet), erythrocyte peroxidase activity, as well as cholesterol and phospholipids levels in brain. There was also a significant increase (P<0.05) in the reduced glutathione levels in the erythrocyte of rats fed with the 10 % oil diet. The brain weights were significantly increased (P<0.05) by all the CPL seed oil diets. The CPL seed oil quality is comparable to groundnut oil; but it is a better antioxidant source. Small quantities of 5-7% CPL oil in diets may be appropriate to prevent its deleterious effects on brain.

Keywords: *Carica papaya*; Seeds; Oil quality; Antioxidants; Lipids.

INTRODUCTION

Carica papaya Linn. (CPL) is a perennial, fast-growing, semi-woody tropical herb. Its ripe fruit is cooked as soup with melon seeds and other spices (Gill, 1992). The seeds account for about 16 % of the fresh fruit weight. Each seed is made up of sarcotesta and endosperm (Puangsri, et al., 2005). *Carica papaya* seed extracts have been shown to have several medicinal properties. Among these is the nephro-protective activity of the aqueous seed extract of the unripe mature fruits (Olagunju, et al., 2009). This may involve its antioxidant and/or oxidative free radical scavenging activities. The aqueous seed extract has also been implicated in the treatment of poison-related renal disorders (Olagunju, et al., 2009). The fruit and its seeds have antihelminthic and anti-amoebic activities (Okeniyi, et al., 2007). Crude extracts of *Carica papaya* seed have an antibacterial activity that inhibits the growth of both gram positive (*B cereus, S aureus and S faecalis*) and gram negative (*E coli, P vulgaris and S flexneri*) organisms (Dawkins, et al., 2003), as well as anti-fertility effect on male rats, rabbits and monkeys (Lohiya, et al., 2008). Udoh et al., (2005) also showed that oral

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administration of *C. papaya* seed extract could induce reversible male infertility. Therefore CPL seed extract could be used for the pharmaceutical development of a male contraceptive. This abortifacient property will only occur when high doses of the extract are given (Oderinde, et al., 2002).

The current research is aimed at evaluating the effect of the *Carica papaya* seed oil on lipid and antioxidant profile in the brain, plasma and erythrocytes of albino rats.

MATERIALS AND METHODS

***Chemicals*:** The following analytical grade chemicals were used: hydrogen peroxide (H_2O_2), pyrogallol, trichloroacetic acid (TCA), 5, 5'- dithio-bis (2- nitrobenzoic acid) (DTNB), epinephrine, potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), and 2-thiobarbituric acid were products of Sigma Aldrich Chemicals (USA); Ethylene diamine tetraacetic acid disodium salt (Na_2 EDTA. $2H_2O$) was a product of British Drug Houses chemicals Limited, Poole, England; heparin was a product of Choongwae Pharma Corporation (Korea). Reagent diagnostic kits for cholesterol and triglyceride determinations were products of Cromatest® Diagnostics (Barcelona, Spain). All other chemicals used were also of analytical grade.

***Plant materials*:** Wholesome ripe and mature *Carica papaya* fruits were purchased at a local market. The fruits were identified at the Applied Biology and Biotechnology Unit of Biological Sciences Department, Covenant University (CU), Nigeria. The fruits were cut into longitudinal halves and the wet seeds were separated out. These seeds were then gently but thoroughly rinsed with distilled water, and completely oven dried at 55^{o}C for 40 hours. The dried seeds were pulverized into a fine powder using a mortar and pestle. The oil was extracted from the pulverized *Carica papaya* seeds with a Soxhlet apparatus (J.P. Selecta model) using n-hexane as the extracting solvent at 69^{o}C. The extract was then subjected to pure recovery using a rotary evaporator at 69^{o}C. This yielded fine, clear golden-yellow oil. The oil was kept in an air and water proof container, and stored in a biofreezer at -20^{o}C until when required.

***Feed formulation of experimental rat diet*:** All the rat feed diets were composed with a mixture of flour binder (80g), groundnut cake (50g), fish meal (2.5g), soya meal (65g), wheat offals (7.5g), bone meal (7.5g), pre-mix (1g), salt (1g), methionine (0.25g), and lysine (0.25g). Both the control and the baseline diets also contained white maize (192.5g) and groundnut oil (25g). The 3%, 5%, 7%, and 10% *Carica papaya* seed oil diets contained 202.5g and 15g, 192.5g and 25g, 182.5g and 35g, and 167.5g and 50g of white maize and *Carica papaya* seed oil respectively. Each flour mix was thereafter compressed into a cylindrically shaped cake before air-drying.

***Experimental animals*:** Healthy, 24-four-week-old female wistar albino rats (90-120g) were purchased from the animal house of the University of Agriculture, Abeokuta, Ogun State, Nigeria. The internal guidelines of the University Ethical committee (2009) were taken into consideration prior to, and during this experiment. The rats were housed in well ventilated cages at the light proofed CU animal house. The animal house and its facilities were designed to prevent animals escaping and to prevent wild animals and insects from entering the animal house. The cages were cleaned twice every day. Suffocation in a diethyl ether vapour filled compartment was used to ensure the humane killing of the experimental animals. Animal surgeries were performed in a room different from where the animals were housed. Saw-dust was used as beddings for the animal house. The concentrations of the oil in diets were

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selected such that the recommended 5% composition was accommodated, and the others representing slightly lower (3%), slightly higher (5%), or too higher (10%) concentrations than the recommended.

***Experimental design*:** Twenty four rats were randomly arranged in cages such that each cages contained two rats. All the rats were thereafter left to acclimatize for 2 weeks. They were fed *ad libitum* with their respective diets except for the 24 hours fasting period prior to their being sacrificed. The rats in 'group 0' fed with 5% groundnut oil based diet, but sacrificed at the beginning of the experiment (day 0) were termed as 'baseline'. The rats in 'group 1' fed with 5% groundnut oil based diet, but sacrificed at the end of the 32 days feeding experiment were termed as 'control'. The 'Group 0' served as the baseline control to ascertain the initial biochemical status of the rats. The rats in this group were anesthetized using diethyl ether and sacrificed at Day 0 prior to feeding the rats in the remaining groups with their respective diets. 'Group 1' rats were fed with 5% groundnut oil based diet. 'Group 2' rats were fed with 10%, 'Group 3' rats with 7%, 'Group 4' rats with 5%, and 'Group 5' rats with 3% *Carica papaya* seed oil based diet.

Four (4) rats from each group (groups 1 to 5) were sacrificed thirty-two (32) days after feeding the rats with their respective diets, and blood was collected by cardiac puncture into lithium heparin tubes placed on ice. The plasma was separated from the erythrocyte by centrifuging the whole blood at 3000 rpm for 15 minutes, and the erythrocyte was washed thrice with 0.9% NaCl to remove white blood cells. The erythrocyte and plasma were separately frozen at -20^{o}C until they were analyzed. The brain of each rat was excised, weighed and rinsed in 0.9 M KCl solution. 10% (w/v) homogenate in 0.1M phosphate buffer (pH 7.0) was prepared from the brain tissue, and lipid extracts were prepared in chloroform: methanol as described Folch et al., (1957).

The weight of each animal was determined at day 0 and day 32 of the experiment. The change in weight was expressed as a percentage of the original weight.

Analytical methods

***Bio-makers of oxidative stress*:** The concentration of thiobarbituric acid-reactive substances (TBARS) was determined colorimetrically by the method of Buege and Aust, (1978). The reduced glutathione concentration was determined using Ellman's reagent, according to the method described by Ellman, (1959).

Peroxidase (PER) activity was assayed following the method of Wever et al., (1980). The reaction mixture consisted of 3.0ml of pyrogallol in 0.1 M phosphate buffer (pH 7.0) and 0.5ml of 1% (v/v) H_2O_2. To this was added 0.1ml of the sample, and the change in absorbance was measured at 430 nm at 30-second intervals for 2 minutes. The PER activity was calculated using the molar extinction coefficient of oxidized pyrogallol (4.5 liters/mol.).

Superoxide dismutase (SOD) activity in erythrocyte and the brain was determined by the method of Misra and Fridovich, (1972). 0.5ml of sample was diluted in 4.5ml of distilled water (1:10) dilution factor. An aliquot of 0.2ml of the diluted sample was added to 2.5ml of the 0.05 M carbonate buffer (pH 10.2) in a cuvette and left to equilibrate. The reaction was started by addition of 0.3ml of freshly prepared 0.3 mM epinephrine. The reference cuvette contained 2.5ml of carbonate buffer, 0.3ml of substrate (adrenaline) and 0.2ml of distilled water. The increase in absorbance at 480 nm was monitored and 1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline. SOD levels were expressed as units/mg protein.

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The protein concentration was determined by means of the biuret reaction, as described by Gornall, et al., (1949).

Plasma lipid profiles: Plasma concentrations of total cholesterol and triglycerides were determined with commercial kits (Spin React S.A., Santa Colona, Sant Esteve De Bas, Spain). High density lipoprotein (HDL) cholesterol and triglycerides were determined in plasma with the same commercial kits for total cholesterol and triglycerides after very low density lipoproteins (VLDL) and low density lipoprotein (LDL) were precipitated with heparin–$MnCl_2$ solution, as described by Gidez et al., (1982). LDL cholesterol was calculated through VLDL Cholesterol using the method of Sandkamp et al., (1990). Total phospholipids in plasma were extracted with a chloroform–methanol mixture (2:1, v/v) as described by Folch et al., (1957). The phospholipid content was then determined as described by Stewart, (1980). Briefly, an aliquot of the phospholipid extract was evaporated to dryness at 60 $^{\circ}$C. After residue had cooled, 2ml of chloroform was added to the dried lipid extract and the mixture was vortexed. Ammonium ferrothiocyanate (2ml) was then added and the mixture vortexed for 1 min. It was left for 10 min to allow the phases to separate. The chloroform layer was taken and its absorbance was read at 488 nm. Phospholipid concentrations were then determined using a phospholipid standard as reference.

Free fatty acids (FFA) in plasma were determined according to the method described by Brunk and Swanson, (1981). Briefly, to 100µl of plasma were added 300µl of copper reagent and 2ml of chloroform. This was shaken with a vertical shaker for 10 min and then centrifuged. After centrifugation, the chloroform layer was removed and to this were added 1ml of cuprizone and 100µl of ammonia reagent. The contents were shaken briefly by hand and the absorbance was read at 620 nm 10 min after adding the ammonia reagent. The concentrations of FFA in the plasma samples were extrapolated from a palmitic acid standard curve prepared using the same procedure.

***Erythrocyte lipid profile*:** An improved procedure described by Rose and Oklander, (1965) was employed for the extraction of lipids from erythrocytes using chloroform–isopropanol (7:11, v/v), because the Folch extraction method (Folch, et al., 1957) produced lipid extracts that were highly pigmented. For the determination of cholesterol, an aliquot of the chloroform–isopropanol extract was evaporated to dryness at 60^{o}C. A triton X-100/chloroform mixture (1:1, v/v, 20µl) was added to resolve the lipids; again the solvent was evaporated. Then 1ml of a commercially available cholesterol kit reagent (Spin React S.A., Santa Colona, Sant Esteve De Bas, Spain) was added and the mixture was vortexed. After incubating the mixture in the dark at room temperature for 30 min, the cholesterol content was determined by colorimetry method (Eder and Kirchgessner, 1994). The determination of total phospholipids in the chloroform–isopropanol extract of the erythrocyte followed the same procedure as described for the plasma (Stewart, 1980).

***Brain lipid profiles*:** After washing with 0.05 M KCl solution, aliquots of the chloroform–methanol extract were used for the determination of cholesterol, triglyceride and phospholipid concentrations. Cholesterol was determined in an aliquot of the chloroform–methanol extract of the brain, as described for erythrocytes; the determination of phospholipids followed the same procedure as described for plasma. Triglyceride concentrations in aliquots of the chloroform–methanol extracts of the brain were determined following the procedure described by Kriketos et al., (2003). Briefly, an aliquot of the chloroform–methanol extract in an Eppendorf tube was evaporated to dryness at 60^{o}C. After cooling, 200µl of ethanol (97%) was added to the tube to resuspend the triglyceride. Then 1ml of commercially available

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triglyceride kit (Spin React S. A., Santa Colona, Sant Esteve De Bas, Spain) was added and the mixture vortexed. After incubating the mixture in the dark at room temperature for 20 min, the triglyceride content was determined spectrophotometrically.

***Statistical evaluation*:** Results were expressed as mean ± Standard deviation. One-way analysis of variance (ANOVA) followed by the Duncan Multiple Range Test (DMRT) were used to analyze the results, with $P < 0.05$ considered significant.

RESULTS

***Weight gain*:** All the *Carica papaya* seed oil based diets, including the control diet, generally increased significantly ($P<0.05$) the weight of the rat brain (Table 1) when compared with that of the baseline rats. Furthermore, there was a significant increase ($P<0.05$) in the brain weight of the rats fed on the 10% *Carica papaya* seed oil based diets compared with those fed on the Control diet.

Table- 1: Weight gain in rats fed with *Carica papaya* seed oil based diet.

Biochemical Parameters	Weight Gain (%)					
	Groundnut oil (5%)		*Carica Papaya* Seed Oil Diet			
	Baseline diet	Control diet	3%	5%	7%	10%
Whole body	0	20.6 ± 6.5	19.4± 4.0	13.4± 0.1	7.1± 1.8*	9.9± 2.7*
Brain	0	19.7± 4.4	24.8 ± 3.3	15.6 ±4.7	25.2 ±3.5	39.9± 4.4*

- Values within the same column with superscripts (*) are significantly different at $P < 0.05$.

***The effect of Carica papaya seed oil consumption on antioxidant and lipid profiles of the brain*:** The cholesterol levels (Table 2) significantly increased ($P<0.05$) in the brains of rats fed on 3-5% *Carica papaya* seed diets, or control diet over the 32 days period compared to the baseline. However, increasing concentrations of *Carica papaya* seed oil based diets reduced the cholesterol level in rat brain until the reductive effect became significant ($P<0.05$) in rats fed with 10% *Carica papaya* seed oil based diet when compared to the control diet.

Table- 2: Metabolism of *Carica papaya* seed oil based diet in rat brain.

Biochemical Parameters	Groundnut oil (5%)		*Carica Papaya* Seed Oil Diet			
	Baseline diet	Control diet	3%	5%	7%	10%
Cholesterol (mg/dl)	32.2± 9.3	101.8± 8.2*	113.8± 5.8	90.1± 8.1	83.2± 3.3	86.9± 3.9*
Triglycerides (mg/dl)	67.7± 4.5	74.7± 5.8*	83.4± 5.5*	70.6± 8.5*	67.9± 7.9*	68.3± 5.9*
Phospholipid (mg/g)	183.3± 4.8	236.1± 7.3*	233.7± 4.1	183.5± 2.3	201.0± 10.0	202.4± 4.4*
Reduced glutathione x 10^{-1} (µmol/g)	24.0± 6.2	17.0± 0.4	18.0± 1.0	17.0± 1.0	19.0± 0.2	23.0± 4.0
TBARS (mmol/100g)	9.6± 1.0	7.2± 5.7	9.1± 1.1	11.0± 1.0	10.7± 0.4	10.2± 1.0
SOD (U/mg protein)	4.5± 1.0	4.0± 0.4	4.0± 0.1	4.5± 1.4	4.0± 0.1	4.1± 0.1
PER activity (Unit/mg protein)	8.5± 1.3	7.1± 0.6	9.1± 1.6	6.3± 1.6	5.5± 0.1	6.4± 0.2
Free fatty acids (mg/g)	77.3± 20.0	73.0± 9.0	74.6± 3.3	79.1± 3.5	70.5± 11.1	75.9± 13.5

- Values within the same column with superscripts (*) are significantly different at $P < 0.05$.

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The consumption of control diet or of any of the *Carica papaya* seed oil diets had no significant effect (*P*>0.05) on reduced glutathione, lipid peroxidation, superoxide dismutase, peroxidase, or free fatty acid levels in the rat brain (Table 2) when compared with both the baseline, and the control diets.

***The effect of Carica papaya seed oil consumption on antioxidant and lipid profiles of blood plasma*:** Consumption of both the 5% groundnut oil, and the various *Carica papaya* diets compositing significantly increased (*P*>0.05) plasma cholesterol (Table 3) compared with the baseline diet. Also, the consumption of the 10% *Carica papaya* diet led to a significantly increased *(P*>0.05) plasma triglyceride (Table 3) compared with the baseline diet. However, consumption of 3-7% *Carica papaya* diets significantly reduced (*P*>0.05) plasma phospholipid (Table 3) compared with the baseline. There were no significant effects (*P*>0.05) of consuming *Carica papaya* seed oil diets on plasma cholesterol**,** free fatty acid, triglycerides, phospholipids, HDL-cholesterol, HDL-triglycerides, and LDL-cholesterol levels (Table 3) when compared with the control diets.

Table- 3: Metabolism of *Carica papaya* seed oil based diet in rats plasma.

Biochemical Parameters	Groundnut oil (5 %)		*Carica Papaya* Seed Oil Diet			
	Baseline diet	Control diet	3%	5%	7%	10%
Cholesterol (mg/dl)	70.9± 12.5	102.0± 2.1	102.4 ±3.3	108.5± 8.1	104.2± 7.1	84.6± 6.1
Triglycerides (mg/dl)	93.5± 10.3	101.4± 10.6	85.5± 12.8	91.3± 17.0	109.0± 9.0	121.3± 10.3
Phospholipid (mg/dl)	437.4± 92.9	250.3± 73.4	151.9± 94.7	284.3± 14.7	205.3± 33.5	412.3± 83.5
HDL-Cholesterol (mg/dl)	61.8± 4.3	61.3± 1.2	60.2± 0.3	59.7± 0.9	60.9± 2.6	62.9± 4.5
LDL-cholesterol (mg/dl)	27.9± 8.2	20.5± 5.8	31.3± 5.4	30.5± 5.9	21.5± 6.5	13.3± 2.2
HDL-Triglycerides (mg/dl)	109.1± 35.4	97.9± 37.7	148.3± 5.0	132.7± 44.4	116.0± 41.6	116.7 ±31.1
Free fatty acids (mg/ml)	483.3± 53.6	487.1± 46.1	456.4± 32.4	462.0± 49.9	418.2± 43.3	470.4± 25.2

- Values within the same column with superscripts (*) are significantly different at $P < 0.05$

***The effect of Carica papaya oil consumption on antioxidant and lipid profiles of erythrocyte*:** The consumption of the control diet significantly lowered (*P*<0.05) the reduced glutathione concentration in rat erythrocyte (Table 4) compared to that of the baseline rats. However, the consumption of the 10% the *Carica papaya* seed oil diet significantly increased (*P*<0.05) the reduced glutathione concentrations in rat erythrocytes. Also, thirty two days feeding on the control diet significantly increased (*P*<0.05) the erythrocyte peroxidase activity compared with that of the baseline rats (Table 4). On the contrary, all the *Carica papaya* seed oil diets composition (3-10%) significantly reduced (*P*<0.05) the erythrocyte peroxidase activity when compared with the control diet.

However, the consumption of either the groundnut oil or *Carica papaya* seed oil based diets showed no significant effect (*P*>0.05) on triglyceride, lipid peroxidation, superoxide dismutase, free fatty acid, cholesterol concentration, and

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phospholipids levels in the rat erythrocyte (Table 4) when compared to those of the baseline, and the control diets.

Table -4: Metabolism of *Carica papaya* seed oil based diet in rat erythrocyte.

Biochemical Parameters	Groundnut oil (5%)		*Carica Papaya* Seed Oil Diet			
	Baseline diet	Control diet	3%	5%	7%	10%
Cholesterol (mg/dl)	16.4± 5.1	18.8± 5.7	19.5± 4.2	18.7± 3.1	16.7± 3.4	14.8± 3.2
Triglycerides (mg/dl)	37.7± 15.6	61.2± 16.0	53.2± 16.9	41.2± 17.4	74.9± 19.2	55.9± 19.7
Phospholipid (mg/dl)	129.8 ±11.6	127.8± 12.6	122.8±12.3	157.2± 15.4	113.8± 18.4	118.7± 13.6
Reduced glutathione (µmol/ml)	13.2± 2.0	10.0± 0.3*	12.6± 5.0	14.2± 4.0	10.6± 1.0	23.6± 1.0*
TBARS (nmol/ml)	22.6± 6.4	16.3± 1.2	21.2± 2.7	17.4± 3.1	20.3± 3.8	16.9± 3.0
SOD (Unit/ml)	52.5± 9.6	67.5± 9.1	60.0± 0.0	65.0± 10.0	60.0± 8.1	52.5± 8.6
PER x 10^{-2} (Unit/ml enzyme)	18.4± 7.8	48.6± 7.3	12.7± 2.9*	23.5± 6.3*	5.7± 0.6*	7.5± 5.4*
Free fatty acids (mg/ml)	159.5± 5.1	127.5± 5.5	137.8± 9.5	114.0± 8.8	148.9± 7.3	123.6± 9.0

- Values within the same column with superscripts (*) are significantly different at $P < 0.05$

DISCUSSION

The decrease in weight may be due to the rejection of food or water caused by their reduced palatability, diet-induced anorexia, or systemic toxicity (Abdulazeez, et al., 2008). The inhibition of lipoprotein lipase activity, increased energy expenditure, inhibition of nutrient absorption from the gastrointestinal tract, and suppression of the appetite are some likely reasons for the reductions in body weight upon feeding on the 7-10% *Carica papaya* seed oil diets (Dyer, 1994). The weight of the brain remarkably increased on feeding the rats with the 10% *Carica papaya* seed oil diets when compared to the control diet. The increase in brain weight of the rats fed with the 10% *Carica papaya* seed oil diet can be attributed to a high proportion (10%) of the oil/ fat in the diet compared to the control diet. Consequently, the *Carica papaya* seed oil may contain bioactive constituents that are beneficial to brain development. The brain: whole body weight ratio for all the rats fed with *Carica papaya* oil seed diets were generally increased compared to that of the control diet. Increased brain: body weight ratio was earlier reported in growing rats (Bernal, et al., 1974). The increased ratio observed in this study gives credence to the fact that *Carica papaya* seed oil contains biomolecules beneficial to brain growth. It is evidence that the groundnut oil is limited in performing this role.

The *Carica papaya* seed oil diet reduced cholesterol levels in the brain at 10% composition compared to the control diet. Previous studies indicates that the lipid lowering action of natural products may be mediated through the inhibition of hepatic cholesterol biosynthesis, increased faucal bile acid excretion, enhanced plasma lecithin: cholesterol acyltransferase activity, or reduction of lipid absorption in the intestine (Patil, et al., 2004). The reduction in the brain cholesterol concentration could be the result of reduced lipid absorption in the intestine. Beta-sitosterol, a phytosterol which is present in *Carica papaya* seed oil, inhibits cholesterol absorption in the intestine (Matsuoka, et al., 2008). This may lead to the reduction of cholesterol

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at high concentrations of the papaya oil. A reduction of cholesterol levels could also be as a result of the inhibition of hydroxylmethylglutaryl (HMG) CoA reductase in the liver and brain by a constituent of *Carica papaya* seed oil. Benzylisothiocyanate, a volatile compound present in *Carica papaya* seed oil, has been found to be a genotoxin (Kassie, et al., 1999). Its volatile nature makes it capable of traversing the blood-brain barrier with ease and inhibiting HMG-CoA reductase in the brain thus stalling *in situ* cholesterol synthesis. However, further research is necessary to determine the effect of benzylisothiocyanate on HMG – CoA reductase.

Cholesterol is the most abundant organic molecule in the brain and the brain contains almost a quarter of the unesterified cholesterol present in the entire body (Guyton, 1996). Low concentrations of cholesterol in the brain thus indicate abnormalities in brain cholesterol metabolism. Consequently, the consumption of *Carica papaya* seed oil should be limited to concentrations below 10 % in diets. The *Carica papaya* seed oil diets had no effects on cholesterol levels in the plasma and erythrocytes compared to that of the commercially consumed groundnut oil diet. Thus, *Carica papaya* seed oil should provide the same biochemical benefits in the plasma and erythrocyte as groundnut oil.

Feeding on *Carica papaya* seed oil diets also caused a similar increase in triglyceride concentration even at a concentration as high as 10 % composition. Triglycerides account for approximately 95 % of dietary fat (Denke, 2007). The general increase in triglyceride levels in the rat brain resulting from the consumption of the control diet or *Carica papaya* seed oil diet may be due to the mobilization of fatty acids in the oils into the brain, which are then esterified with glycerol. Increased brain triglyceride levels are beneficial since the brain cannot utilize a fatty acid as an energy source (unless it is first converted to a ketone). The glycerol component of triglycerides can be converted to glucose (via gluconeogenesis) for brain fuel when it is broken down (Denke, 2007). Moreover, feeding on *Carica papaya* seed oil diets resulted in a triglyceride metabolism in rat plasma (Table 3) and in erythrocytes (Table 4) that is similar to that of the control diet.

There was a reduction of phospholipids in the rat brain on consumption of high concentrations of *Carica papaya* seed oil (10 %) for a period of thirty two days. However, the effect of consumption of *Carica papaya* seed oil diets on the phospholipid status may be considered normal in the rat plasma and in erythrocytes since its effect was similar to the phospholipid status of rats fed with control diet over the same feeding period of thirty-two days. Phospholipids are essential molecules found in all cellular membranes (Sanchez, et al., 2006). They are important for optimal brain health and play several roles in the brain. They determine which minerals, nutrients, and drugs go in and out of the cell. They also influence communication between brain cells by influencing the shapes of receptors and promoting the growth of dendrites (Sanchez, et al., 2006). The two most common fatty acids attached to phospholipids in the brain are docosahexaenoic acid (DHA) and arachidonic acid, although the fatty acid content of brain phospholipids can be altered by the composition of diets. The detrimental decrease in the phospholipid concentration in brain tissues due to the consumption of high concentration (10 %) of *Carica papaya* seed oil diet could be the result of increased phospholipase A_2 activity. Phospholipases A_2 (PLA_2; EC 3.1.1.4) form an expanding superfamily of esterases that specifically cleave the acyl ester bond at the *sn*-2 position of membrane phospholipids to produce a free fatty acid (arachidonic acid) and lysophospholipid (Farooqui, et al., 2000). It can be assumed that *Carica papaya* seed oil contains

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compounds that stimulate phospholipase A_2 activity in the brain considering the fact that the brain has several isoforms of the enzyme (Farooqui, et al., 2000). Increased stimulation of PLA_2 could lead to detrimental effects in the brain (Sun, et al., 2004). The consumption of the control diet resulted in lowered levels of reduced glutathione (a natural antioxidant in living organisms) in rat erythrocytes. This effect will increase the susceptibility to the deleterious effect of free radicals in rat erythrocytes (Grey, et al., 2003). However, the consumption of the 10% *Carica papaya* seed oil diet stimulated the reduced glutathione level in rat erythrocytes, thus enhancing its natural ability to minimize or prevent the deleterious effect of free radicals in the erythrocytes. This could indicate that higher concentrations of *Carica papaya* seed oil in a diet would positively affect the reduced glutathione levels in rat erythrocyte. The *Carica papaya* seed oil diets were comparable to the control diet with regard to reduced glutathione activity in rat brain and plasma. Consequently, the *Carica papaya* seed oil is capable of providing biochemical benefits in the brain and plasma that are similar to those of the commonly consumed groundnut oil.

The consumption of the control diet resulted in an increase in the peroxidase activity in rat erythrocytes. However, the consumption of *Carica papaya* seed oil diets (3-10%) produced a decrease in erythrocyte peroxidase activity compared to the control diet. Peroxidase is an inducible enzyme which is expressed only under conditions which permit its gene expression (Hassan and Fridovich, 1978). It catalyzes the breakdown of hydrogen peroxide to water and oxygen, and is induced by the presence of its substrate (hydrogen peroxide) which acts as an inducer. The decreased peroxidase activities arising from the 32 days feeding on *Carica papaya* seed oil indicates the presence of low levels of hydrogen peroxide (a free radical) in the erythrocytes, thus making *Carica papaya* seed oil a good antioxidant source for the depletion of free radicals in erythrocytes.

Carica papaya seed oil diets were comparable to the control diet with regard to lipid peroxidation, superoxide dismutase activity in rat brain, plasma and erythrocytes. This indicates that the quality of *Carica papaya* seed oil is as good as that of groundnut oil with respect to lipid peroxidation, and superoxide dismutase activity in rat. The result of feeding on *Carica papaya* seed oil diets are comparable to those arising from the control diet with regard to HDL-triglyceride, LDL-cholesterol, and HDL-cholesterol metabolism in rat plasma. Also, feeding on *Carica papaya* seed oil diets is also comparable to feeding on control diet with regard to the free fatty acid metabolism in rat plasma, erythrocytes and brain.

CONCLUSIONS

The consumption of *Carica papaya* seed oil in high amounts increases the reduced glutathione concentrations in rat erythrocyte. This makes *Carica papaya* seed oil a good source of antioxidants. The oil is comparable to groundnut oil with regard to the metabolism of cholesterol, triglyceride, phospholipids, and free fatty acids in plasma and erythrocytes. The oil is also comparable to groundnut oil with respect to the metabolism of plasma HDL-cholesterol, LDL-cholesterol, HDL-triglycerides, erythrocyte SOD, and lipid peroxidation. It has comparable results with groundnut oil with regard to brain antioxidants (i.e. superoxide dismutase (SOD), reduced glutathione, peroxidase, and free fatty acids). However, high concentrations of the oil decreased brain cholesterol and brain phospholipids, which could signify a detrimental effect of the oil on the brain. This oil may therefore be preferably used as

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a drug and not as a food condiment, since drugs are used under controlled administration.

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