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AN EVALUATION OF LIPID PROFILE AND ANTIOXIDANT ACTIVITIES OF *CARICA PAPAYA* SEED OIL IN THE HEART AND LIVER OF FEMALE WISTAR RATS.

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ABSTRACT

Increasing attention is been given to *Carica papaya* seed due to its oil rich nature, and its medicinal value. Four feed diets consisting of 3, 5, 7, and 10 % of the oil were composed and examined for its in-vivo effect on lipid and antioxidants status of female wistar rats compared to those fed with 5 % groundnut oil based diet. The feeds were fed into the rats for 32 days, and examined for their lipid profile and antioxidant status in the liver and heart. Free fatty acids were significantly reduced ($P<0.05$) by 3-10 % diets in the liver, and by 10 % diet in the heart. HMG-CoA/Mevalonate ratio in the liver was significant increased ($P<0.05$) by the 7-10 % diets. All diets significantly increased ($P<0.05$) superoxide dismutase, peroxidase, and reduced glutathione in liver. Lipid peroxidation in the heart was significantly increased ($P<0.05$) by the 3-7 % diets. Lipid peroxidation, and phospholipid in heart were significantly reduced, and increased ($P<0.05$) respectively by 10 % diets. The biochemical status of rats fed with the *Carica papaya* seed oil based diets generally remained comparable to those fed with the groundnut oil diet in the two organs. The *Carica papaya* seed oil exhibited better antioxidant activities, and health benefits than the commonly consumed groundnut oil.

INTRODUCTION

Carica papaya is the most important species within the *Caricaceae* family being cultivated widely for its consumption. Nigeria is one of the major producers of *Carica papaya* in the world [1]. Its seed have antimicrobial activity against *Trichomonas vaginalis* trophozoites [2], bacteriostatic activity on both gram positive and gram negative organisms that could be useful in treating chronic skin ulcer [3]. Air dried papaya seed being consumed offers a cheap, natural, harmless, readily available monotherapy and preventive strategy against intestinal parasitosis [4]. Benzylisothiocynate present in the seeds is the chief or sole antihelmintic [5]. It was recently reported that fermented papaya seed based diets may be good as food condiment, since it had no side effect on the sexual development and reproductive functions of female wistar rats [6].

Lipids are a broad group of naturally-occurring molecules which includes fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and others. The main biological functions of lipids include energy storage, structural components of cell membranes, and as important signaling molecules. However, antioxidants are substances that offer protection to cell membranes and prevent oxidative stress to the tissues of the body by neutralizing toxic oxygen molecules and free radicals [7]. The action of one antioxidant may therefore depend on the proper function of other members of the antioxidant system. The amount of protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts [7]. The relative importance and interactions between these different antioxidants is a very complex question, with the various metabolites and enzyme systems having synergistic and interdependent effects on one another [8]. Earlier work had shown papaya seeds to contain high amount of oil [9]. There is no report yet on the effect of the seed oil consumption in the heart and liver. This work was therefore designed to assess the biochemical benefits of the oil by determining the effects of diets composed from the oil on lipids and the antioxidant status in the heart and liver of wistar rats.

RESULTS

Weight Changes

There were significant reductions ($P<0.05$) in the total weight of rat fed with the 7-10 % *Carica papaya* seed-oil based diets compared to the control (Table 1). However, there were no significant changes ($P<0.05$) in liver and weight of rats fed with all the *Carica papaya* seed diets compared to the control (Table 1).

Antioxidant activities in the liver of the rats fed on the *Carica papaya* seed-oil based diets

Only the 3 % *Carica papaya* seed-oil based diet significantly increased (P<0.05) peroxidase activities in the rat's liver compared to the baseline, and the control diets. The control diet, and all the *Carica papaya* seed-oil based diets significantly increased (P<0.05) lipid peroxidation in the rat's liver compared to the baseline diet fed rats. There was however no significant effect (P > 0.05) of the *Carica papaya* seed oil diets on superoxide dismutase activity, lipid peroxidation, and reduced glutathione level in rat liver compared with the control diets (Table 2).

Antioxidant activities in the heart of the rats fed on the *Carica papaya* seed-oil based diets

Lipid peroxidation in heart of rats fed with all the other *Carica papaya* seed-oil based diet (3-7%) were significantly increased (P<0.05) compared to that of the control diets. It was however only the 10 % *Carica papaya* feed composition that significantly reduced (P<0.05) lipid peroxidation compared to the control diet. (Table 3). Both the control diet, and all the *Carica papaya* seed-oil based diets significantly reduced (P<0.05) the lipid peroxidation in the rat's heart compared with the baseline diet. There were no significant effects (P>0.05) of all the *Carica papaya* seed oil based diets on peroxidase activity, superoxide dismutase activity, reduced glutathione level in the heart compared with the control diet (Table 3).

Table 1: Level of feed intake and weight gain in rat fed with *Carica papaya* seed-oil based diet.

S/N	Biochemical Parameters	Weight Gain (%)				
		Groundnut oil diets (5 %)	*Carica Papaya* Seed Oil Diets			
		Control	3%	5%	7%	10%
1.	Whole body	20.601± 4.500	19.4± 4.0	13.4± 3.1	7.1± 1.8^{*a}	9.9± 2.7^{*a} (%)
2.	Liver (%)	38.507 ±8.914	24.420 ±7.554	43.448 ±10.665	45.150 ±7.842	21.858 ±8.613
3.	Heart (%)	40.637 ±7.593	24.227 ±9.698	31.885 ±8.900	37.650 ±7.924	27.690 ±7.087
4.	Food intake (g/day)†	10.57±0.58	11.43±0.40	11.77±0.84	10.33±1.18	10.03±1.14

1. Values within the same column with superscripts (*a) are significantly different at P < 0.05 compared to the control.

Table 2: Effects of the *Carica papaya* seed-oil based diet on the antioxidant activities in the rat liver.

S/N	Biochemical Parameters	Weight Gain (%)					
		Groundnut oil diets (5 %)		*Carica papaya* seed oil diets			
		Baseline	Control	3 %	5 %	7 %	10 %
1.	Reduced glutathione (mmol/ml)	0.037 ±0.011	0.015 ±0.009	0.017 ±0.006	0.017 ±0.006	0.015 ±0.007	0.038 ±0.014
2.	Lipid	2,884.135 ±380.493^{*b}	3,597.598 ±331.084	4,095.673 ±449.621^{*b}	3,937.503 ±427.445^{*b}	3,294.710 ±661.719^{*b}	3,624.520 ±558.115
3.	Peroxidase activity (unit/ml enzyme)	0.161 ±0.033	0.196 ±0.008	0.231±0.018^{*ab}	0.219 ±0.042	0.209 ±0.017	0.190 ±0.024
4.	Superoxide Dismutase (unit /ml enzyme)	52.500 ±11.080	60.000 ±13.670	60.000 ±7.140	70.000 ±10.140	70.000 ±9.260	50.000 ±8.260

1. Values within the same column with superscripts (*a) are significantly different at P < 0.05 compared to the control.

2. Values within the same column with superscripts (*b) are significantly different at P < 0.05 compared to the baseline.

Table 3: Effects of the *Carica papaya* seed-oil based diet on the antioxidant activities in the rat heart.

S/N	Biochemical Parameters	Weight Gain (%)					
		Groundnut oil diets (5 %)		*Carica papaya* seed oil diets			
		Baseline	control	3%	5%	7%	10%
1.	Reduced glutathione (mmol/ml)	0.088 ±0.045	0.082 ±0.011	0.073 ±0.004	0.090 ±0.009	0.090 ±0.016	0.075 ±0.008
2.	Lipid peroxidation (nmol/ml)	4,048.558 ±552.783 2,	456.733 ±304.304*b	3,368.850 ±457.510*ab	3,510.098 ±481.663*ab	3,547.118 ±147.668*ab	1,975.483 ±459.320*ab
3.	Peroxidase activity (unit /ml enzyme)	0.060 ±0.039	0.083 ±0.032	0.071 ±0.042	0.076 ±0.036	0.065 ±0.035	0.109 ±0.012
4.	Superoxide Dismutase (unit/ml enzyme)	80.000 ±8.16	65.000 ±12.91	81.000 ±0.00	65.000 ±23.80	67.500 ±22.17	65.000 ±12.91

1. Values within the same column with superscripts (*a) are significantly different at P < 0.05 compared to the control.

2. Values within the same column with superscripts (*b) are significantly different at P < 0.05 compared to the baseline.

Lipid Profile in the Rats Liver

All the *Carica* papaya seed oil diets significantly reduced (P<0.05) free fatty acid levels in the liver (Table 4). Also, significant increases (P<0.05) in HMG-CoA/Mevalonate ratio were manifested in liver of rat fed on the 7 % and 10 % *Carica papaya* seed-oil diets compared to control (Table 4). There were no significant effects (P>0.05) of the *Carica papaya* seed oil based diet consumption on cholesterol, triglycerides, and phospholipid levels in the liver compared with the control (Table 4).

Lipid Profile in Rats Heart

The *Carica papaya* seed oil (10%) based diets significantly increased (P<0.05) phospholipid levels in the heart of rats compared with both the baseline, and the control diets (Table 5). The free fatty acids in the heart was mainly significantly reduced (P<0.05) by the 10 % diets. The free fatty acids levels in both control diets, and *Carica papaya* seed oil fed rats were significantly increased (P<0.05) compared to that of the baseline diet. There were no significant effects (P>0.05) of all the *Carica papaya* seed oil based diet consumption on cholesterol, and triglycerides in the heart compared with the control diet (Table 5).

DISCUSSION

Weight changes

The body weight gain (growth) was reduced at high *Carica papaya* seed oil concentration (7-10 %) in diets. This oil can therefore be used not only to control glucose homeostasis in diabetes mellitus but to control dyslipidemia and obesity alike. It is well established that there is a strong link between diabetes mellitus, dyslipidemia, obesity, hypertension and ischemic heart disease [10]. Some side effects of the oil at high concentration (10 %) in the brain that was attributed to the alpha-sitoterol component of the oil limiting cholesterol from gaining access to the brain through the blood brain barrier [11]. We cannot preclude the possibility of toxicity as a result of the high dosage of the *Carica papaya* seed oil, but the level of feed intake/day of the various animal group (Table 1) indicates that there were no loss of appetite as a result of the *Carica papaya* seed oil consumption.

Antioxidant

In mammalian cells, glutathione and the glutathione peroxidases constitute the principal antioxidant defense system [12]. Lipid peroxidation was induced by the Carica *papaya* seed oil in a similar manner as that of groundnut oil in the liver. *Carica papaya* seed oil at high concentration (10 %) could be more effective in reducing lipid peroxidation than groundnut oil. The reverse was however the case at concentration below the 10 % composition. There is low activity of antioxidants in the food or diet when there is high lipid peroxidation. The higher the lipid peroxidation, the lower the antioxidant activity of the *Carica papaya* seed oil. The amount of protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts (*7*). Polyphenols antioxidants

in the oil was earlier reported at a concentration of 119.917 ± 2.626 µg/ml [13]. The *Carica papaya* seed oil manifested similar antioxidant metabolic effect on both superoxide dismutase and peroxidase activities which are

Table 4: Effects of the *Carica papaya* seed-oil based diet on lipid profile in the rat liver.

S/N	Biochemical Parameters	Weight gains (5 %)					
		Groundnut oil diets (5 %)		*Carica Papaya* Seed Oil Diets			
		Baseline	Control	3%	5%	7%	10%
1	Cholesterol (mg/dl)	6.12± 1.95	10.750 ±3.200	13.750 ±3.910	10.500 ±2.850	9.000 ±1.070	6.500 ±1.950
2.	Triglycerides (mg/dl)	77.7± 5.6	64.000 ±13.540	49.500 ±8.410	74.750 ±6.770	83.000 ±6.050	45.500 ±5.510
3.	Phospholipid (mg/dl)	49.80± 4.60	74.20 ±4.81	68.50 ±5.04	77.2 ±13.84	71.75±13.62	63.75±8.69
4.	Free fatty acids (mg/ml)	459.5± 5.1	495.031 ±35.942	306.868 ±32.962^{*ab}	354.792 ±25.380^{*ab}	387.147 ±18.256^{*ab}	348.940 ±48.463^{*ab}
5.	HMG-CoA/ Mevalonate ratio	0.279± 0.089	0.342 ±0.017	0.381 ±0.019	0.394 ±0.031	0.405 ±0.021^{*ab}	0.520 ±0.036^{*ab}

1. Values within the same column with superscripts (*a) are significantly different at P < 0.05 compared to the control.

2. Values within the same column with superscripts (*b) are significantly different at P < 0.05 compared to the baseline.

Table 5: Effects of the *Carica papaya* seed-oil based diet on lipid profile in the rat heart.

S/N	Biochemical Parameters	Weight gains (5 %)					
		Groundnut oil (5 %)		*Carica Papaya* Seed Oil Diet			
		Baseline	Control	3%	5%	7%	10%
1.	Cholesterol (mg/dl)	12.250 ±4.920	15.500 ±3.510	13.250 ±0.960	12.000 ±4.080	15.000 ±1.830	15.750± 2.87
2.	Triglycerides (mg/dl)	86.000 ±11.950	86.000 ±12.760	78.000 ±14.020	86.250 ±7.800	81.000 ±5.290	116.000 ±20.620
3.	Phospholipid (mg/dl)	1,207.500 ±399.480	1,375.500 ±204.530	928.750 ±266.590	1022.500 ±149.660	1,187.750 ±348.480	1,844.250 ±279.130^{*ab}
4.	Free fatty acids (mg/ml)	72.549±38.744	178.997 ±23.747^{*b}	168.618 ±14.133^{*b}	144.081 ±19.888^{*b}	140.663 ±18.759^{*b}	120.473 ±12.521^{*ab}

1. Values within the same column with superscripts (*a) are significantly different at P < 0.05 compared to the control.

2. Values within the same column with superscripts (*b) are significantly different at P < 0.05 compared to the baseline.

enzymatic antioxidants, and on reduced glutathione level in both the rat's heart and liver (Table 2 & 3). Liver is the drug (chemical, nutrients) processing centre. Metabolism of most drug, nutrients, or chemical that is taken in to the body always starts from the liver first before it gets to the other part of the body. This may explain the lack of effect of peroxidase, superoxide dismutase, reduced glutathione in the heart, and the conspicuous effect of the *Carica papaya* seed oil in the liver. The *Carica papaya* seed oil however exhibited potent antioxidant activities.

Lipid profile

Carica papaya seed oil showed similar biochemical effect on cholesterol, triglyceride and phospholipid metabolism to that of groundnut oil in the rat liver. It also manifested similar effect on cholesterol and triglycerides metabolism in the heart. All the *Carica papaya* seed oil diets (3-10 %) consumption however led to reductions on free fatty acids in the liver. The free fatty acid was however mainly reduced in the heart at high concentration (10 %). The reduction of free fatty acids (Table 5) in the heart by the high *Carica papaya* seed oil administration (10 %) could either be due to low supply of plasma free fatty acids due increased usage, or increased oxidation of free fatty acids for ATP production in the heart [14]. However, the reductive effect by the high *Carica papaya* seed oil administration can be limited to that of the increased free fatty acids oxidation since there were no earlier effects of the seed oil administration in the plasma [11]. The reduction of free fatty acids was however more pronounced in the liver (Table 4) as it was manifested even at very low *Carica papaya* seed oil (3 %) administration. *Carica papaya* seed oil may therefore prevent hypercholesterolemia since it showed ability to prevent fatty acid accumulation that could be a primary substrate for cholesterol synthesis [15]. A positive relationship between gluconeogenesis and lipogenesis has been well documented in literature. Lipogenesis is inhibited directly by elevated fatty acids concentration within physiological range [16]. Previous human studies have also shown *papaya* to slow down the heart beat, and reduce blood pressure [17]. Therefore the oil can be considered good for treating hypertensive patients.

Phospholipids are important cellular components, and their metabolism is under several tiers of control. Alterations in the composition of phospholipids in the membrane may affect cellular processes such as the transport of Na^+ and K^+, the transduction of biological signal across the membrane, and the release of arachidonic acid for the production of prostaglandins and leukotrienes [18]. Phospholipid concentration in the heart was however increased at 10 % *Carica papaya* seed oil diet consumption (Table 5). The increased free fatty acids in the heart (Table 5) as a result of the 10 % Carica papaya seed oil consumption could further be attributed to the observed increase in the hearts phospholipid. The oil could also be facilitating the absorption of nutrients (vitamins A, E, and folate) that increased phospholipid biosynthesis in the heart [19]. The increased total phospholipids in hearts of rats could be attributed to the ability of the *Carica papaya* seed oil to facilitate its synthesis by the diphosphocholine-ethanolamine pathway [19].

The higher the HMG-CoA/Mevalonate ratio, the lower the activity of the HMG-CoA reductase in the cholesterol synthesis and vice-versa [20]. Therefore, *Carica papaya* seed oil consumption possessed ability to reduce the accumulation or synthesis of cholesterol in the liver. This ability was earlier reported in the brain [11]. This explains the continual decrease in cholesterol concentration (Table 4) as the papaya seed oil concentration increases in diets. Although, the changes in cholesterol concentration were not remarkably noticed.

In conclusion, the *Carica papaya* seed oil is a potent antioxidant source with ability to improve the lipid and antioxidant status of the rats. The seed oil can also be used for treating obesity, and diseases like hypercholesterolemia due to its reducing effect on cholesterol levels. It can also be effectively used to reduce the lipid peroxidation in both the liver and the heart mainly at high concentration. The oil therefore could be an effective tool for treating liver and heart diseases under controlled administration.

EXPERIMENTALS

Chemicals and Reagents

The following chemicals used: hydrogen peroxide (H_2O_2), pyrogallol, trichloroacetic acid (TCA), 5, 5'- dithio-bis (2-nitrobenzoic acid) (DTNB), epinephrine and 2-thiobarbituric acid were products of Sigma Aldrich Chemicals (USA); Ethylene diamine tetraacetic acid disodium salt (Na_2 EDTA.$2H_2O$), TBA-TCA-HCl reagent, triton X-100, isopropanol, cuprizone reagent, triethanolamine ammonium hydroxide, n-hexane, palmitic acid, sodium arsenate, physiological saline (99 % saline), hydroxylamine hydrochloride ($NH_2OH.HCl$) were products of British Drug Houses chemicals limited, Poole, England; also heparin was a product of Choongwae Pharma Corporation (Korea). Reagent Diagnostic kits for triglyceride and cholesterol were products of Cromatest® diagnostics, Joaquim Costal, Montgat, Barcelona, Spain. All chemicals used were of analytical grade.

Materials

Wholesome ripe and mature *Carica papaya* fruits were purchased at a local fruit market. The fruits were identified at the Applied Biology and Biotechnology Unit of Biological Sciences Department, Covenant University (CU), and deposited (Voucher number: FHI108906) at the Herbarium unit of Forest Research Institute, Ibadan, 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 ^oC 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 ^oC. The extract was then subjected to pure recovery using a rotary evaporator at 69 ^oC. 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 ^oC until when required.

Experimental Animals

Healthy, 24-four-week-old female wistar albino rats (90-120 g) were purchased from the animal house of the University of Agriculture,

Abeokuta, Ogun State, Nigeria. The rats were housed in well ventilated cages at the light proofed CU animal house. The experiment conducted following the Covenant University ethical committee guidelines. 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 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.

Feed Formulation of Experimental Rat Diet

Four *Carica* papaya seed diets (3 %, 5 %, 7 %, and 10 %), and 5 % groundnut based diet (control) were composed as described by Afolabi, Akuiyibo, Rotimi and Adeyemi [11]. 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 Design

Twenty four rats were randomly arranged in cages such that each cages contained four 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 just at the beginning of the feeding experiment (day 0) were termed as 'baseline'. The 'Group 0' served as the baseline control to ascertain the initial biochemical status of the rats. 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'. '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 were sacrificed thirty-two (32) days after feeding the rats with their respective diets. The liver and heart were removed rinsed in potassium chloride solution (0.05 M) and then placed in tabs. They were also excised, weighed and 10 % homogenate was prepared in 0.25 M sucrose buffer. The homogenate was used for antioxidants determination

Lipids were extracted with chloroform–methanol mixture (2:1, v/v) as described by Folch, et al. [21]. Saline/arsenate homogenate was used in the case of the liver as described by Rao and Ramakrlshnan [22]. The extracted samples were thereafter used for further lipid profile analysis.

Weight changes: The weight of each animal and that of the two organs were was determined at day 0 and day 32 of the experiment. The changes in weight were expressed as a percentage of the original weight at day 0.

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 [23]. The reduced glutathione concentration was determined as described by Ellman (*24*). Peroxidase (PER) activity was assayed following the method of Wever, et al. [25]. The PER activity was calculated using the molar extinction coefficient of oxidized pyrogallol (4.5 liter/mol.).

Superoxide dismutase (SOD) activity in homogenised samples was determined by the method of Misra and Fridovich [26]. 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. The protein concentration was determined by means of the biuret reaction, as described by Gornall, et al. [27].

Lipid profiles analysis

Total phospholipids in the organs were extracted with a chloroform–methanol mixture (2:1, v/v) as described by Folch, Lees and Stanley [21]. The phospholipid content was then determined as described by Stewart [28]. However, an aliquot of the phospholipid extract was evaporated to dryness at 60 æ%C. Its absorbance was read at 488 nm, and the 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 [29]. The concentrations of FFA in the plasma samples were extrapolated from a palmitic acid standard curve prepared using the same procedure. Cholesterol concentration was determined spectrophotometrically using 1ml of commercially available cholesterol kit reagent according to the method of Folch, Lees and Stanley [21]. Triglyceride concentrations in the chloroform–methanol extracts of the homogenates were determined spectrophotometrically following the procedure described by Kriketos, et al. [30]. HMG CoA/ Mevalonate ratio was also determined as described by Rao and Ramakrlshnan [22]. The optical density was taken at 540 nm after allowing the mixture to stand for 10 minutes. A similarly treated saline/arsenate solution was used as blank. The same procedure stated above was also carried out for mevalonate determination except that freshly prepared hydroxylamine reagent was used for mevalonate test which is different from that of HMG-CoA test.

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.

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