Control of Hyperlipidemia in Rats By Using Papaya and Stirred Yoghurt

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Abstract

Current research aims to study the effect of feeding on stirred yoghurt only, papaya only and stirred yoghurt supplemented with 20% papaya on hyperlipidemia male rats. The study was conducted on 36 male albino rats divided into two main groups, the first main group 6 rats were fed on basal diet only and contrived as control group, while the second main group 30 rats with induced hypercholesterolemia through nutrition on a diet high in cholesterol content1%, the second group were divided into five equal subgroups and fed for 2 months one subgroup was left as a positive control group (Group 1), whereas group (2) was treated with atorvastatin (10 mg/kg) orally, while groups (3,4,5) have been fed adding stirred yoghurt only, papaya only and stirred yoghurt supplemented with 20% papaya by rate (5ml/kg b.w) orally using a stomach tube daily for 8 weeks. The results revealed that all treated rat groups (experimental groups) showed a significant decrease in TC, TG, LDL-C, VLDL-C homocysteine, TC / HDL-C and LDL-C / HDL-C than positive control group, while HDL-c was increased, in compared with the control (+ve) group. As a result, the best values of these variables appeared in stirred yoghurt supplemented with 20% papaya, atorvastatin and papaya only in compared with the other treated groups. The results revealed that liver enzymes (AST, ALT) showed significant decrease in all treated rat groups, all treated groups showed a significant increase in SOD, GSH and CAT and showed a significant decrease in MDA compared with the control (+ve) group. Histopathological examination of the rat's hearts confirmed this amelioration in rats group that consumed stirred yoghurt supplemented with 20% papaya.

The study recommended the use of stirred yoghurt supplemented with 20% papaya in patients meals suffering from hyperlipidemia.

Keywords: stirred yoghurt - papaya - hyperlipidemia - cholesterol - triglyceride - liver functions - antioxidant enzymes.

Introduction

Recently, probiotic fermented dairy products that provide a healthy functional food are advised for better health. Many previous researches mentioned that, milk fermented by selected culture of lactic acid bacteria (LAB) has high biochemical activity and antioxidant activity (Kullisaar et al., 2003; Sinyayskiy, 2005; Villani et al., 2005). Several early studies demonstrated the ability of supplementing yogurt with fruits (Lurton and Ouattrin, 2003; Skrede et al., 2004; Apostolidis et al., 2006).

Yogurt is fermented milk and is consumed worldwide (Mansour et al., 1994) due to its therapeutic and nutritive values (Perdigon et al., 2002; Karagul, et al., 2004). This “biotechnological” food is rich source of proteins, carbohydrates, vitamins, fats, phosphorous and calcium. The uniqueness of Yogurt is attributed to lactic acid fermentation during its production which makes yogurt easily digestible (Taracki, 2003) and increase the bioavailability of calcium in intestine (Singh and Muthukum, 2008). The health beneficial properties of live lactic acid bacteria in Yogurt includes curing of some intestinal disorders (Shahani and Chandan, 1979), decreased risk of cancer, lowered blood cholesterol and improved digestion of lactose by mal digesters (Shahani and Chandan, 1979; Marona and Pedrigon, 2004). Organoleptic evaluations of fruit juice fortified Yogurts have a marked preference nowadays (Barnes et al., 1991).
Fruits and vegetables are good sources of vitamins, minerals, antioxidants and fibers. So, certain fruits can be used in yoghurt production for improving their nutritional values and sensory properties (e.g. strawberry, apple, cornelian, rosehip, morello cherry, grape, date and other fruit homogenates). (Erdogan, and Zekai, 2003, Vahedi et al., 2008).

Papaya (Carica papaya L.) belongs to the family Caricaceae, one of the most important fruits cultivated throughout the tropical and subtropical regions of the world (Anonymous, 2000). Papaya is regarded as an excellent source of vitamin C (ascorbic acid); a good source of carotene, riboflavin and a fair source of iron, calcium, thiamin, niacin, pantothenic acid, vitamin B-6 and vitamin K (Bari et al., 2006; Adetuyi et al., 2008; Saxholt et al., 2008).

Papaya have a biological activities as immuneostimulating and antioxidant activity (Aruoma et al., 2006; Mehdipour et al., 2006); abortifacient activity (Cherian, 2000; Sarma&Mahanta 2000); post-testicular anti-fertility drug (Lohiya et al., 2000); treating wounds and burns (Mahmood et al., 2005); anthelmintic activity (Stepek et al., 2004); and bacteriostatic activity (Osato et al., 1993). These benefits have been attributed, at least in part, to the amount of antioxidant compounds present in these foods, which reduce the oxidative stress produced by free radicals, and in consequence, cellular damage (Dosil-Diaz et al., 2008). Papaya fruits contain components that can increase the total antioxidant power in blood and reduce the lipid peroxidation level. These components include α-tocopherol, ascorbic acid, beta carotene, flavonoids, vitamin B1, and niacin (Ross, 1999).

Hypercholesterolemia is a significant risk factor for atherosclerosis, which is related to coronary heart disease. It is a condition where abnormally high levels of lipids are found especially low density lipoproteins cholesterol (LDL-C), cholesterol and triglycerides in the blood and leads to hyperlipidemia. This condition is also called hyperlipoproteinemia. In fact, human body is complex machinery and any undesirable change will disturb the balance resulting in diseased state (Gupta, 2011 and Kopparapu, 2011). Hypercholesterolemia may be responsible for oxidative modification of LDL-C, protein glycation, glucose-auto-oxidation with excess production of free radicals and lipid peroxidation products, which represent major risk factors for ischemic heart diseases (Olorunnisla et al., 2012).

The present study was undertaken to investigated the anti-hyperlipidemia activity of papaya, stirred yoghurt and stirred yoghurt with papaya.

**Materials and methods**

**Materials:**

**Ingredients:** Thirty six male albino rats of Sprague Dawley strain were purchased from Laboratory Animal Colonies, Pharmacology Department Faculty of Medicine, Mansoura University. The average weight was 130 ± 5g. The basal diet was performed according to (NRC, 1995). Fresh cow's milk was obtained from the herd of the dairy cattle at Faculty of Agriculture, Mansoura University, Mansoura, Egypt. Skim milk powder made in Poland was obtained from the local market. Fresh fruits of Carica papaya L. were purchased from local market in Mansoura city, Egypt. TClesterol was obtained as a pure white crystalline powder, TClic acid, all chemicals and diagnostic kits were purchased from El-Gomhoria Company, El-Mansoura city, Egypt. TORVAST tablets were obtained from EGYPHAR Pharmaceutical Company, Obour city, Egypt. Each tablet contains 40 mg of atorvastatin. TORVAST drug was dissolved in distilled water in dose 10 mg/kg of rat using a stomach tube according to (Vasu Keshetty et al, 2009). Bacterial Starter Cultures: Yoghurt starter culture (YC-X11 DIP 50u) contains Streptococcus thermophilus and Lactobacillus delbruecki subsp. bulgaricus was obtained from Chr. Hansens Laboratories, Denmark and activated at 42°C using 12% sterilized reconstituted skim milk. After incubation at 42°C for 4-5 h, the obtained working culture was freshly used.
Methods:

Preparation of papaya:

Papaya were washed, the skin was peeled off, seeds were removed and the pulp was cut into smaller pieces and ground to mash using a kitchen blender. The prepared fruit homogenates were used directly.

Production of Stirred Fruit Yoghurt:

Stirred fruit yoghurt were manufactured according to the procedure of (Erdogan, and Zekai, 2003) with some modifications as follows: Cow's milk (fat 3%, protein 3.72%, total solids 12.3% and acidity 0.17%) was used and enriched with 2% skim milk powder to increase solids of milk. The mixture was heated to 85°C for 10 min. and then rapidly cooled to 45°C. The working yoghurt culture was added at the rate of 2% (w/v). The inoculated mix was filled into 2.0 kg plastic cups and incubated at 43°C. The incubation was terminated at pH 4.5. At this point, the yoghurt was stored in a refrigerator (5±1°C) overnight. 20% Fresh fruit homogenates papaya were added at (w/w). The yoghurt mixes were stirred and filled into 250g plastic cups. The resultant stirred yoghurt samples were stored in refrigerator (5±1°C).

Analytical Methods:

Approximate chemical composition of papaya and stirred yoghurt supplemented with 20% papaya moisture, ash, fat, crude protein, crude fibers contents and total solids were determined according to the methods of the (A.O.A.C. 2007). While total carbohydrates were estimated by subtracting the difference from initial weight of the samples as follows: Carbohydrates% = 100 - (% moisture + % protein + % fat +%ash). Total phenols and total carotenoids were determined according to Singleton and Rossi (1965) and Wettstian (1995). Acidity was determined by titration with 0.1N sodium hydroxide solution using the procedure described by Aggarwala and Sharma, (1961).

The sensory evaluation of different stirred yoghurt samples were assessed by regular taste panel of the staff member at Home Economics Dept., Faculty of Specific Education, Mansoura University. Each panelist had an education sheet and was asked to evaluate stirred yoghurt supplemented with 10%, 20% and 30% papaya, stirred yoghurt samples were evaluated using a five point score system (5 excellent, 1 unacceptable) according to (Zekai and Erdogan, 2003).

Induction of hyperlipidemia:

Hyperlipidemia in rats was done according to the method of (Grone et.al 1989) by feeding (20% coconut oil) and cholesterol (1%) for 2 month. The high fat diet contained cholic acid (0.5%) to enhance the central absorption of lipids.

Experimental design:

Animals were kept in the laboratory in plastic cages under constant temperature (24±2 C). All rats were fed on basal diet only for one week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups. The first main group (n=6 rats) was fed on the basal diet only, as a control negative group (C-). The second main group (n=30 rats) was fed for 2 month on the basal diet plus (20% coconut oil), cholesterol (1%) and cholic acid (0.5%) to induce Hyperlipidemia before starting the experiment. After 2 month feeding the second main group of rats was divided into 5 subgroups:

Group I: Hyperlipidemic rats (control +ve).

Group II: Hyperlipidemic rats treated with Atorvastatin (10 mg/kg) orally using a stomach tube daily for 8 weeks.

Group III: Hyperlipidemic rats treated with stirred yoghurt only(5ml/kg body weight) orally using a stomach tube daily for 8 weeks.

Group IV: Hyperlipidemic rats treated with papaya only (5ml/kg body weight) orally using a stomach tube daily for 8 weeks.

Group V: Hyperlipidemic rats treated with stirred yoghurt supplemented with 20% papaya (5ml/kg body weight) orally using a stomach tube daily for 8 weeks.

During the experimental period (8 weeks), the food intake was recorded every day and body weight was recorded every week. Body weight gain (BWG%) was calculated by following formula:
Body weight gain % = \frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{initial weight (g)}} \times 100

Blood sampling:

At the end of experimental period (8 weeks), rats were fasted over night before sacrificing. Blood was collected and centrifuged to separate serum for analysis. Serum was carefully aspirated, transferred into clean cuvet tubes, and stored frozen at -20°C till analysis.

Liver and heart were collected from every rats and weighed to obtain relative weight often being washed and dried using filter paper.

Relative organs weight was calculated as the following:

Relative organs weight = \frac{\text{organ weight (gm)}}{\text{Final body weight (gm)}} \times 100

Heart organs were subjected to histopathological examinations as described by Bancroft et al., (1996).

Chemical analysis:

For each group analysis included the following:

- Total cholesterol (TC) was determined according to Allen, (1974).
- The determination of serum triglycerides (TG) was done according to Fassati and Prencipe, (1982), while high density lipoprotein–cholesterol (HDL-C) was determined according to Lopez, (1977), whereas low density lipoprotein– cholesterol (LDL-C) was determined using the equation according to Friedewable et al., (1972).

\begin{align*}
\text{LDL-c} &= \text{TC} - [\text{HDL-c} + (\text{TG}/5)] \\
\text{VLDL-c} &= \text{TG}/5
\end{align*}

- Athrogenic index was calculated by cholesterol / HDL-c and LDL-c /HDL-c according to Castelli and Levitar (1977). Serum homocysteine levels were estimated using the methods of Frontzen et al., (1998).
- Determination of GOT (AST) and GPT (ALT) were analyzed according to Reitman and Frankel (1957). Serum total protein, Serum uric acid, creatinine and total bilirubin were estimated according to Henry (2001), Fossati et al., (1980), Young (2001) and Jendrassik (1938), respectively. Serum superoxide dismutase (SOD) activity plasma catalase (CAT) activity, reduced glutathione (GSH) and malondialdehyde (MDA) were estimated according to Nishikimi et al., (1972), Aebi, (1984), Beutler et al., (1963) and Satoh, (1978).

Statistical Analysis:

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmasat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan’s multiple range test and p<0.05 was used to indicate significance between different groups (Snedecor and Cochran, 1967).
Results and Discussion

Moisture, ash, fat, Protein, and carbohydrates of papaya and stirred yoghurt supplemented with 20% papaya were determined and tabulated in Table (1). It was clear that moisture, ash, fat, Protein, carbohydrates, dietary fiber, total phenol, total carotenoids, total solids and acidity were 74.92%, 0.47%, 3.65%, 4.16%, 16.8%, 2.14%, 377.9 mg/100g, and 4.98 mg/100g respectively in wet weight for papaya while they were 76.13%, 0.66%, 4.58%, 5.02%, 13.61%, 1.52%, 351.2 mg/100g, and 3.58 mg/100g respectively for stirred yoghurt supplemented with 20% papaya. Other authors reported variable records the total dietary fiber content of ripe fruit varies from 11.9 to 21.5 g/100 g dry matter (Wills et al., 1986; Saxholt et al., 2008). The crude protein content ranges from 3.74 to 8.26 g/100 g dry matter.

Papaya is regarded as an excellent source of vitamin C (ascorbic acid); a good source of carotene, riboflavin and a fair source of iron, calcium, thiamin, niacin, pantothenic acid, vitamin B-6 and vitamin K (Bari et al., 2006; Adetuyi et al., 2008; Saxholt et al., 2008). Carotenoid content (13.80 mg/100 g dry pulp) of papaya (Saran, 2010). amounts varying between the maturation stages (Bari et al., 2006; Hernandez et al., 2006). The total lipid content in ripe papaya fruit varies between 0.92 and 2.2 g/100 g dry matter. Papaya contains a low level of fatty acids. Palmitic acid and linolenic acid are two major fatty acids in papaya. Fatty acid composition change during fruit ripening and no significant difference are observed in lipid composition with maturity of papaya fruits.

The obtained results in Table (2) the sensory evaluation showed non significant differences among the stirred yoghurt samples. In general the use of fruit homogenate for making stirred yoghurt caused an improvement in body and texture properties of the final product. This improvement could be due to the higher content of fibers associated with fruit homogenates added and this may lead to an increase in the viscosity and consequently improve the body and texture. So, addition of some fruit to stirred yoghurt production (such as pineapple and papaya) may be contribute to an increase in the sensory quality of the final product. Erdogan and Zekai (2003) who stated that, fruit additions have an increasing effect on yoghurt consumption. Also, using different fruit additives gives more yoghurt choices to the consumer.

The obtained results in Table (3) showed that the control positive rat group showed a significant decrease in food intake and showed a significant increase in final weight, body weight gain%, relative weight of liver and heart compared with normal control group. All treated groups showed a significant decrease in food intake compared with normal control group while all treated groups had a significant increase in food intake compared with the control +ve group. All treated groups showed a significant increase in final weight compared with normal control group but all treated groups except the group treated with stirred yoghurt only showed a significant decrease in final weight compared with the control +ve group. However, the hyperlipidemic rat groups treated with atorvastatin and stirred yoghurt only showed a significant increase in body weight gain% while the hyperlipidemic rat groups treated with papaya only and stirred yoghurt supplemented with 20% papaya showed a non-significant changes in body weight gain% compared with normal control group but the hyperlipidemic rat groups treated with atorvastatin, papaya only and stirred yoghurt supplemented with 20% papaya showed a significant decrease in body weight gain% compared with the control +ve group except the hyperlipidemic rat group treated with stirred yoghurt only showed non-significant changes.

All treated groups showed a significant increase in relative liver weight compared with normal control group while showed a significant decrease in relative liver weight compared with the control +ve group. The hyperlipidemic rat group treated with atorvastatin showed a significant decrease in relative heart weight while the hyperlipidemic rat group treated with stirred yoghurt supplemented with 20% papaya showed a significant increase in relative heart weight and the hyperlipidemic rat groups treated with papaya only and stirred yoghurt only showed a non-significant change compared with normal control group. The results are in agreement with Esposito et al. (2003) who revealed that dietary fiber demonstrates the ability to regulate energy intake thus enhancing weight loss or maintenance of a healthier body weight.
either through glycemic control or reduced energy intake. Athesh et al., (2012) indicated that the relative weights of the liver and heart were significantly lower in the hyperlipidemic treated groups as compared to the control +ve group. This is probably because of the lower fat content in those tissues.

The obtained results in Table (4) showed that the control positive rat group showed a significant increase in TC, TG, LDL-C and VLDL-C, but showed significant decrease in HDL-C compared with normal control group. The hyperlipidemic rat groups treated with stirred yoghurt only and papaya only showed a significant increase in TC, TG, LDL-C, VLDL-C but showed significant decrease in HDL-C compared with normal control group, except the rat groups treated with atorvastatin and stirred yoghurt supplemented with 20% papaya showed a non-significant changes in TC, TG, LDL-C, VLDL-C and HDL-C compared with normal control group. On the other hand all treated groups showed a significant decrease in TC, TG, LDL-C and VLDL-C but showed a significant increase in HDL-C compared with the control +ve group. The results are in agreement with Aravind et al.,( 2013) who indicated that Papaya lowers high cholesterol levels as it is a good source of fiber. These nutritional values of papaya help to prevent the oxidation of cholesterol. Consumption of fruits and vegetables is associated with lower concentrations of total and low-density lipoprotein cholesterol (Mirmiran et al., 2009). This effect has been ascribed, in part to the antioxidants; polyphenols, tocopherols and carotenoids in plant-based foods. Plant foods rich in antioxidant molecules have received a growing interest because they delay the oxidative degradation of lipids (Nicole et al., 2004). Phenolic compounds and flavonoids have pharmacological properties such as antioxidant, antithrombotic and hypolipidemic effects (Son and Lewis, 2002). Because lifestyle related diseases such as atherosclerosis progress gradually due to unfavorable dietary habits, an improvement of daily nutritional intake is thought to prevent the pathogenesis of such diseases. Examples of plants foods that may possess antioxidant and hypolipidemic effects is Papaya it is a rich source of three powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fiber(Aravind et al 2013). Serum total cholesterol is reduced by inhibiting absorption in the intestine as a result of assimilating and binding of cholesterol as well as bile acids by the lactic acids bacteria. On the other hand, serum lipid improvement seems to be related to the hydrolyase activity of the bile of the bacterial cells that deconjugate about half the bile of the conjugated bile salts that are excreted into the small intestine which would increase the fecal excretion of deconjugated bile salts. To maintain bile salts homeostasis, the bile acid have to be newly synthesized in the liver thus increasing the demand for cholesterol as precursor for bile acids and this leads to lower the level of cholesterol concentration (Taranto et al., 1998 and Pereira & Gibson., 2002).

Agarwal et al. (1992) revealed that decrease in the levels of serum total lipids, serum total cholesterol, LDL-C, VLDL-C, phospholipids and triglycerides in normal rabbits suggesting a remarkable protective and hypolipidemic effects of papaya fruit. Otsukia et al.(2010) indicated that Papaya is a good source of vitamins A, C, E and K as well as folate and fiber, in addition it is fat-free, cholesterol-free and low in sodium, that suggest its potential benefit effects. The results indicated that stirred yoghurt that lowered serum total cholesterol concentrations in rats agree with data from others studies involving various milk product containing selected strains of lactic acid bacteria (Akalin et al.,1997 and Beena & Prasad, 1997).

Data in Table (5) showed that serum levels of the control positive rat group had a significant increase in homocysteine, TC / HDL-C and LDL-C / HDL-C compared with normal control group. All treated groups showed a significant increase in homocysteine compared with normal control group while showed a significant decrease in homocysteine compared with the control (+ve) group. All treated groups showed a significant increase in TC / HDL-C and LDL-C / HDL-C compared with normal control group ,except the rat groups which treated with atorvastatin and stirred yoghurt supplemented with 20% papaya showed a non-significant change in TC / HDL-C and LDL-C / HDL-C compared with normal control group. On the other hand all treated groups showed a significant decrease in TC / HDL-C and LDL-C / HDL-C compared with the control (+ve) group. The results are in agreement with (Aravind et al 2013) who indicated that the folic acid found in papayas is needed for the conversion of homocysteine into amino acids such as.
cysteine or methionine. If unconverted, homocysteine can directly damage blood vessel walls, which is considered as a significant risk factor for a heart attack or stroke. It is now established that the high cholesterol diet and the resultant hyperlipidemia causes an increase in TC, LDL-cholesterol levels to enhance the risk for the development of atherosclerosis (Genest et al., 1992). Additionally, serum TG is also considered a risk factor for atherosclerosis (Jones and Chambliss, 2000). High levels of LDL-cholesterol (bad cholesterol) are also high risk factor of coronary heart diseases (Smith et al., 2004). The association between hyperlipidemia and atherosclerosis has been demonstrated in many studies and trials (Gordon et al., 2007). Hyperlipidemia may be manifested by elevation of total cholesterol, low density lipoprotein and triglycerides concentrations and a reduction in high density lipoprotein concentration. It has also been shown that reducing plasma level of LDL cholesterol sharply reduced the risk of coronary heart disease (Superko and Krauss, 1994).

The results given in Table (6) revealed that the control positive rat group had a significant increase in AST, ALT and creatinine while showed a non-significant change in total protein, total bilirubin and uric acid compared with normal control group. All treated groups showed a significant increase in AST and ALT compared with normal control group. On the other hand all treated groups showed a significant decrease in AST and ALT compared with the control (+ve) group. All treated groups showed a non-significant change in total protein and uric acid compared with normal control group. All treated groups showed a significant decrease in total bilirubin compared with the control (+ve) group. All treated groups showed a significant decrease in creatinine compared with the control (+ve) group, except the rat group treated with papaya only showed a non-significant change in creatinine compared with the control (+ve) group. The results are in agreement with Rajkapoor et al. (2002) who reported that papaya extract improved liver function through the production of structural integrity of hepatocyte cell membrane or regeneration of damaged liver cells. ALT and AST are released into serum when there is severe hepatocellular injury. Elevated serum ALT levels in the absence of viral hepatitis and alcoholism has been reported to lead to a higher risk of cardiovascular disease with greater risk among women (Ioannou, 2006).

Data in Table (7) showed that the control positive rat group had a significant decrease in SOD, GSH and CAT and showed a significant increase in MDA compared with normal control group. All treated groups showed a significant decrease in SOD, GSH and CAT and showed a significant increase in MDA compared with normal control group. On the other hand all treated groups showed a significant increase in SOD, GSH and CAT and showed a significant decrease in MDA compared with the control (+ve) group, except the rat group treated with stirred yoghurt supplemented with 20% papaya showed non-significant change in CAT compared with the control (+ve) group. This explanation is in line with the study of Gutteridge and Halliwell, (2000) who showed that antioxidants play an important role in the body's defence system against reactive oxygen species (ROS). There is growing scientific evidence associating antioxidant rich diets with a lower incidence of cardiovascular disease, cancers, and age-related degenerative processes (Kaliora and Dedoussis, 2007). The main dietary antioxidants in foods include vitamins (vitamin C and E), carotenoids, phytosterols and a wide variety of antioxidant phytochemicals such as simple phenolic compounds, flavonoid glycosides and polyphenols (Kris-Etherton et al., 2002; Pellegrini et al., 2003; Stanner et al., 2004; Guo and Xu, 2005).

Papaya is a powerhouse of nutrients and is available throughout the year. It is a rich source of three powerful antioxidants vitamin C, vitamin A and vitamin E; and the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fiber. All the nutrients of papaya as a whole improve cardiovascular system, protect against heart diseases, heart attacks, strokes and prevent colon cancer. The fruit is an excellent source of beta carotene that prevents damage caused by free radicals that may cause some forms of cancer, Papaya has many phenolic groups which may scavenge free radicals (Aravind et al 2013).

Srikanth et al. (2010) indicated that the aqueous extract of papaya has showed antioxidant activity. Moreover, Imaga et al. (2010) have indicated that the aqueous extract of papaya has shown anti-tumor effect and inhibited the
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proliferative responses of solid and haematopoietic tumor cell lines derived from cervical carcinoma, breast adenocarcinoma, hepatocellular carcinoma, lung adenocarcinoma, pancreatic epithelioid carcinoma and mesothelioma in a dose-dependent manner.

Mahattanatawee et al. (2006) reported that papaya is rich in total ascorbic acid, total dietary fiber, and pectin. These components provide a protection against cellular damage caused by exposure to high levels of free radicals.

Previous reports have indicated that papaya contain α-tocopherol (Ching and Mohamed, 2001), lycopene (van Breemen and Pajkovic, 2008), proteolytic enzymes such as papain and chymopapain (Seigler et al., 2002), alkaloids such as carpain and carpasemine (Iyer et al., 2011), triterpenes, organic acids (Cowan, 1999), cystatin, ascorbic acid, cyanogenic glucosides and glucosinolates (Seigler et al., 2002) and flavonoids (Miean and Mohamed, 2001).

Histopathological Results:

The obtained results are confirmed by the histopathological examination. Heart of control (-ve) group showed normal histological muscular wall of the heart with no evidence of fatty change (Picture 1). While, heart of control (+ve) rat group showed marked cloudy swelling of the muscle with hydropic degeneration and mild fatty changes (Picture 2). Heart of drug treated rats with atorvastatin showed near normal muscular wall of the heart (Picture 3). The heart of rat group treated with stirred yoghurt only showed marked cloudy swelling of the muscle fiber with marked pole cytoplasm (Picture 4). While the heart of rat group treated with papaya showed moderate cloudy swelling of the muscle cells with pole cellular details (Picture 5). The heart of rat group treated with stirred yoghurt supplemented with 20% papaya showed normal muscular wall of the heart with no evidence of fatty change (Picture 6).

Histopathological studies on hyperlipidemia revealed that carica papaya has been used to treat various ailments. To substantiate the claim by traditional practitioners that the extract is medicinally useful in the treatment of some tissue damage, such as kidney and liver damage, this research work attempts to investigate this claim relying on scientific report that fruit of papaya has antioxidant properties. The antioxidant components, alkaloids, flavonoids, phenolics, of the plant contributes to the free radical scavenging ability of C. papaya and thus offer a protection against free radicals induced tissue damage (Ayodele, 2001).

In conclusion, the results of the present study demonstrated new properties of stirred yoghurt supplemented with 20% papaya as a potent lipid lowering agent. Moreover currently available hypolipidemic drugs have been associated with a number of side effects (Brown, 1996), while, stirred yoghurt supplemented with 20% papaya when compared with stirred yoghurt only and papaya only showed comparatively better anti-hyperlipidemic activity.
Table 1:
Chemical composition of papaya and fresh stirred yoghurt supplemented with 20% papaya.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture %</th>
<th>Ash %</th>
<th>Total fat %</th>
<th>Crude protein %</th>
<th>Total carbohydrates %</th>
<th>Dietary fibers %</th>
<th>Total phenol (mg/100g)</th>
<th>Total carotenoids (mg/100g)</th>
<th>Total solids %</th>
<th>Acidity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>papaya only</td>
<td>74.92</td>
<td>0.47</td>
<td>3.65</td>
<td>4.16</td>
<td>16.8</td>
<td>2.14</td>
<td>377.9</td>
<td>4.98</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>stirred yoghurt supplemented with 20% papaya</td>
<td>76.13</td>
<td>0.66</td>
<td>4.58</td>
<td>5.02</td>
<td>13.61</td>
<td>1.52</td>
<td>351.2</td>
<td>3.58</td>
<td>11.66</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Table 2:
Sensory evaluation of stirred yoghurts made with fresh stirred yoghurt supplemented with papaya.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Appearance(5)</th>
<th>Body &amp; texture(5)</th>
<th>Flavour(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>stirred yoghurt only</td>
<td>4.67 a</td>
<td>4.67 ± 0.57</td>
<td>4.00 ± 1.01</td>
</tr>
<tr>
<td></td>
<td>±0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stirred yoghurt supplemented with 10%papaya</td>
<td>4.00 a</td>
<td>4.00 ± 1.00</td>
<td>3.33 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>±1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stirred yoghurt supplemented with 20%papaya</td>
<td>4.65 a</td>
<td>4.66 ± 0.57</td>
<td>4.67 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>±0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stirred yoghurt supplemented with 30%papaya</td>
<td>3.33 a</td>
<td>4.00 ± 0.99</td>
<td>3.00 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>±0.58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean values in each column having different superscript (a, b, c, d) are significantly different. Means with the same letter are insignificantly different.
### Table 3:
Effect of papaya and fresh stirred yoghurt un-supplemented and supplemented with papaya on food intake, initial weight, final weight, weight gain%, relative liver weight and relative heart weight of hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>Food Intake (g)</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
<th>Weight Gain %</th>
<th>Relative liver Weight %</th>
<th>Relative heart Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>19.94 ±0.1</td>
<td>172.49 ±2.54</td>
<td>205.21 ±5.52</td>
<td>18.95 ±1.51</td>
<td>2.69 ±0.08</td>
<td>0.40 ±0.04</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>Control (+ve)</td>
<td>17.52 ±0.02</td>
<td>229.29 ±3.88</td>
<td>303.33 ±3.14</td>
<td>32.32 ±3.36</td>
<td>3.59 ±0.04</td>
<td>0.44 ±0.01</td>
</tr>
<tr>
<td></td>
<td>Treated with</td>
<td>17.72 ±0.02</td>
<td>230.94 ±3.25</td>
<td>287.75 ±1.41</td>
<td>24.61 ±1.16</td>
<td>3.38 ±0.02</td>
<td>0.34 ±0.02</td>
</tr>
<tr>
<td></td>
<td>Atorvastatin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated with</td>
<td>19.51 ±0.08</td>
<td>234.91 ±3.56</td>
<td>304.28 ±1.96</td>
<td>29.53 ±0.67</td>
<td>3.35 ±0.02</td>
<td>0.44 ±0.01</td>
</tr>
<tr>
<td></td>
<td>stirred yoghurt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>only</td>
<td>17.86 ±0.05</td>
<td>228.83 ±1.57</td>
<td>278.10 ±2.69</td>
<td>21.53 ±1.09</td>
<td>3.16 ±0.07</td>
<td>0.42 ±0.01</td>
</tr>
<tr>
<td></td>
<td>papaya only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.61 ±0.01</td>
<td>230.49 ±2.30</td>
<td>278.94 ±2.68</td>
<td>21.03 ±0.70</td>
<td>2.86 ±0.04</td>
<td>0.45 ±0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>stirred yoghurt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>supplemented with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20% papaya</td>
<td>19.94 ±0.1</td>
<td>172.49 ±2.54</td>
<td>205.21 ±5.52</td>
<td>18.95 ±1.51</td>
<td>2.69 ±0.08</td>
<td>0.40 ±0.04</td>
</tr>
</tbody>
</table>

Mean values in each column having different superscript (a, b, c, d) are significantly different. Means with the same letter are insignificantly different.
Table 4:
Effect of papaya and fresh stirred yoghurt un-supplemented and supplemented with papaya on lipid profile of hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>TC mg/d</th>
<th>T.G mg/dl</th>
<th>HDL-C mg/dl</th>
<th>LDL-C mg/dl</th>
<th>VLDL-C mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>152.67</td>
<td>±4.04</td>
<td>127.67</td>
<td>±11.59</td>
<td>48.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>±0.58</td>
<td>78.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±3.06</td>
<td>25.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±2.32</td>
<td></td>
</tr>
<tr>
<td>Control (+ve)</td>
<td></td>
<td>261.33</td>
<td>±3.77</td>
<td>209.00</td>
<td>±10.00</td>
<td>32.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32.67</td>
<td>±3.06</td>
<td>186.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±6.11</td>
<td>41.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±2.00</td>
<td></td>
</tr>
<tr>
<td>Treated with</td>
<td></td>
<td>154.00</td>
<td>±5.57</td>
<td>136.00</td>
<td>±9.64</td>
<td>45.33</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td></td>
<td></td>
<td></td>
<td>45.33</td>
<td>±2.31</td>
<td>81.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±2.00</td>
<td>27.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±1.93</td>
<td></td>
</tr>
<tr>
<td>Treated with</td>
<td></td>
<td>214.33</td>
<td>±4.04</td>
<td>182.33</td>
<td>±4.73</td>
<td>36.67</td>
</tr>
<tr>
<td>stirred yoghurt only</td>
<td></td>
<td></td>
<td></td>
<td>36.67</td>
<td>±1.16</td>
<td>141.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±2.00</td>
<td>36.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±0.95</td>
<td></td>
</tr>
<tr>
<td>Treated with papaya only</td>
<td></td>
<td>189.00</td>
<td>±1.00</td>
<td>161.33</td>
<td>±4.04</td>
<td>39.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39.67</td>
<td>±1.53</td>
<td>117.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±3.22</td>
<td>32.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±0.81</td>
<td></td>
</tr>
<tr>
<td>Treated with</td>
<td></td>
<td>152.33</td>
<td>±4.51</td>
<td>135.33</td>
<td>±15.31</td>
<td>46.33</td>
</tr>
<tr>
<td>stirred yoghurt</td>
<td></td>
<td></td>
<td></td>
<td>46.33</td>
<td>±0.58</td>
<td>78.93</td>
</tr>
<tr>
<td>supplemented with 20%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±1.16</td>
<td>27.07</td>
</tr>
<tr>
<td>papaya</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±3.06</td>
<td></td>
</tr>
</tbody>
</table>

Mean values in each column having different superscript (a, b, c, d) are significantly different.
Means with the same letter are insignificantly different.
Table 5:
Effect of papaya and fresh stirred yoghurt un-supplemented and supplemented with papaya on Homocysteine and Atherogenic indexes of hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Homocysteine (µg/mL)</th>
<th>TC/HDL-C</th>
<th>LDL-C/HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>7.24 ± 0.11</td>
<td>3.16 ± 0.12</td>
<td>1.63 ± 0.05</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td>25.32 ± 0.14</td>
<td>8.04 ± 0.72</td>
<td>5.72 ± 0.63</td>
</tr>
<tr>
<td>Treated with Atorvastatin</td>
<td>8.96 ± 0.07</td>
<td>3.39 ± 0.63</td>
<td>1.79 ± 0.14</td>
</tr>
<tr>
<td>Treated with stirred yoghurt only</td>
<td>10.18 ± 0.14</td>
<td>5.85 ± 0.12</td>
<td>3.85 ± 0.06</td>
</tr>
<tr>
<td>Treated with papaya only</td>
<td>9.27 ± 0.13</td>
<td>4.77 ± 0.20</td>
<td>2.95 ± 0.11</td>
</tr>
<tr>
<td>Treated with stirred yoghurt supplemented with 20% papaya</td>
<td>8.30 ± 0.15</td>
<td>3.29 ± 0.11</td>
<td>1.70 ± 0.04</td>
</tr>
</tbody>
</table>

Mean values in each column having different superscript (a, b, c, d) are significantly different. Means with the same letter are insignificantly different.
Table 6:
Effect of papaya and fresh stirred yoghurt un-supplemented and supplemented with papaya on liver enzymes and kidney functions of hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Variables</th>
<th>AST U/L</th>
<th>ALT U/L</th>
<th>T. Protein g/dL</th>
<th>T Bilirubin mg/dl</th>
<th>Creatinin mg/dl</th>
<th>Uric Acid mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>23.20 f</td>
<td>26.13 e</td>
<td>6.32 a</td>
<td>0.63 a</td>
<td>0.55 b</td>
<td>2.27 a</td>
</tr>
<tr>
<td></td>
<td>±1.31</td>
<td>±0.87</td>
<td>±0.24</td>
<td>±0.08</td>
<td>±0.030</td>
<td>±0.15</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td>76.03 a</td>
<td>61.10 a</td>
<td>6.19 a</td>
<td>0.64 a</td>
<td>0.63 a</td>
<td>2.23 a</td>
</tr>
<tr>
<td></td>
<td>±0.55</td>
<td>±0.87</td>
<td>±0.07</td>
<td>±0.10</td>
<td>±0.01</td>
<td>±0.21</td>
</tr>
<tr>
<td>Treated with Atorvastatin</td>
<td>68.07 b</td>
<td>56.90 b</td>
<td>6.66 a</td>
<td>0.40 cd</td>
<td>0.56 b</td>
<td>2.17 a</td>
</tr>
<tr>
<td></td>
<td>±1.86</td>
<td>±1.39</td>
<td>±0.26</td>
<td>±0.05</td>
<td>±0.02</td>
<td>±0.21</td>
</tr>
<tr>
<td>Treated with stirred yoghurt only</td>
<td>52.83 c</td>
<td>48.47 c</td>
<td>6.28 a</td>
<td>0.48 bc</td>
<td>0.53 bc</td>
<td>2.20 a</td>
</tr>
<tr>
<td></td>
<td>±1.20</td>
<td>±0.47</td>
<td>±0.20</td>
<td>±0.05</td>
<td>±0.03</td>
<td>±0.20</td>
</tr>
<tr>
<td>Treated with papaya only</td>
<td>47.53 d</td>
<td>38.40 d</td>
<td>6.37 a</td>
<td>0.33 cd</td>
<td>0.64 a</td>
<td>2.17 a</td>
</tr>
<tr>
<td></td>
<td>±1.70</td>
<td>±0.82</td>
<td>±0.34</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.20</td>
</tr>
<tr>
<td>Treated with stirred yoghurt supplemented with 20% papaya</td>
<td>68.07 b</td>
<td>56.90 b</td>
<td>6.66 a</td>
<td>0.40 cd</td>
<td>0.55 b</td>
<td>2.17 a</td>
</tr>
<tr>
<td></td>
<td>±1.86</td>
<td>±1.39</td>
<td>±0.26</td>
<td>±0.05</td>
<td>±0.02</td>
<td>±0.20</td>
</tr>
</tbody>
</table>

Mean values in each column having different superscript (a, b, c, d) are significantly different. Means with the same letter are insignificantly different.
Table 7:
Effect of papaya and fresh stirred yoghurt un-supplemented and supplemented with papaya on serum antioxidant enzymes and malondialdehyde (MDA) of hyperlipidemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (u/ml)</th>
<th>GSH (mg/dl)</th>
<th>CAT (u/l)</th>
<th>MDA (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>1018.70 a</td>
<td>16.41 a</td>
<td>4.36 a</td>
<td>2.06 f</td>
</tr>
<tr>
<td></td>
<td>±32.42</td>
<td>±0.44</td>
<td>±0.13</td>
<td>±0.06</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td>451.80 a</td>
<td>11.10 a</td>
<td>0.72 e</td>
<td>8.98 a</td>
</tr>
<tr>
<td></td>
<td>±7.95</td>
<td>±0.71</td>
<td>±0.04</td>
<td>±0.41</td>
</tr>
<tr>
<td>Treated with Atorvastatin</td>
<td>643.92 b</td>
<td>15.49 b</td>
<td>2.71 b</td>
<td>7.75 b</td>
</tr>
<tr>
<td></td>
<td>±8.66</td>
<td>±0.46</td>
<td>±0.03</td>
<td>±0.15</td>
</tr>
<tr>
<td>Treated with stirred yoghurt only</td>
<td>708.00 c</td>
<td>14.07 c</td>
<td>1.88 c</td>
<td>5.80 c</td>
</tr>
<tr>
<td></td>
<td>±8.66</td>
<td>±0.12</td>
<td>±0.04</td>
<td>±0.23</td>
</tr>
<tr>
<td>Treated with papaya only</td>
<td>820.23 d</td>
<td>13.36 d</td>
<td>1.46 d</td>
<td>4.94 d</td>
</tr>
<tr>
<td></td>
<td>±3.15</td>
<td>±0.22</td>
<td>±0.06</td>
<td>±0.14</td>
</tr>
<tr>
<td>Treated with stirred yoghurt supplemented with 20% papaya</td>
<td>928.80 b</td>
<td>12.57 e</td>
<td>0.83 e</td>
<td>3.91 e</td>
</tr>
<tr>
<td></td>
<td>±8.12</td>
<td>±0.21</td>
<td>±0.04</td>
<td>±0.09</td>
</tr>
</tbody>
</table>

Mean values in each column having different superscript (a, b, c, d) are significantly different. Means with the same letter are insignificantly different.
Photo. (1): Heart of rat from control(-ve) group showing normal muscle wall of the heart with no evidence of fatty change. (H and E X 100).

Photo. (2): Heart of rat from control(+ve) group showing marked cloudy swelling of the muscle with hydropic degeneration and mild fatty changes. (H and E X 100).

Photo. (3): Heart of rat from treated with atorvastatin group showing near normal muscular wall of the heart. (H and E X 100).

Photo. (4): Heart of rat from treated with stirred yoghurt only group showing Marked cloudy swelling of the muscle fiber with marked pole cytoplasm. (H and E X 100).

Photo. (4): Heart of rat from treated with papaya only group showing moderate cloudy swelling of the muscle fiber with marked pole cellular details. (H and E X 100).

Photo. (6): Heart of rat from treated with stirred yoghurt supplemented with 20% papaya group showing no histopathological changes. (H and E X 100).
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نتفICK في ارتفاع دهون الدم في الفئران باستخدام الباباب والزيبدي المخفوق

رضا محمد نجيب أحمد وهالة عزت الكاوى
قسم الاقتصاد المنزلي - كلية التربية النوعية - جامعة المنصورة - المنيا - مصر

المنشط العربي

أوضح النتائج المحصلة عليها ان مجموعات الفئران المعالجة ظهرت تحسن بالانخفاض المعنوي في مستوى دهون الدم (الكولسترول - الجليسيدات الثلاثية - الليبروتينات منخفضة الكثافة - الليبروتينات منخفضة الكثافة جدًا - مولشر تصلب الشرايين) وكذلك انخفض مستوى الهيموستاسين بالمقارنة بالمجموعة الضابطة الموجبة. في حين ارتفع مستوى الليبروتينات مرتفعة الكثافة وظهرت النتائج في كل من مجموعة الزيبدي المخفوق المدمج 20% بباباب ومجموعة الارترافاستين بانخفاض معنوي بالمقارنة بالباباب (ALT, AST) بالانخفاض المعنوي بالمجموعة الضابطة الموجبة. كما ظهرت النتائج ارتفاع معنوي (MDA) في مجموعات الفئران المعالجة بالباباب (SOD, GSH, CAT) للانخفاض المعنوي بالمجموعة الضابطة الموجبة. وقد ظهر الفشل الهيستوتنولوجي لخلايا القلب عند وجود تغيرات مرضية في مجموعة الفئران التي تغذى على الزيبدي المخفوق المدمج 20% بباباب.

من خلال النتائج المحصلة عليها في هذا البحث توصي الدراسة بتناول الزيبدي المخفوق المدمج 20% في وجبات مرضى ارتفاع دهون الدم.