Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(2): 2062-2067

Received: 11-01-2018
Accepted: 12-02-2018

Adil Abbas Alsultani
MSc Student, Department of Physiology & Pharmacology & Biochemistry College of Veterinary Medicine/University of Baghdad-Iraq

Luma Waleed Khalil
PhD, MSc, Department of Physiology & Pharmacology & Biochemistry College of Veterinary Medicine/University of Baghdad-Iraq

Sabah Mahdi Hadi
PhD, Department of Biotechnology MSc, College of Science/University of Alnahrrien- Iraq

Correspondence
Adil Abbas Alsultani
MSc Student, Department of Physiology & Pharmacology & Biochemistry College of Veterinary Medicine/University of Baghdad-Iraq

Role and importance of casein hydrolysate in reducing hyperlipidemia and DNA fragmentation induced by fructose in adult male rats

Adil Abbas Alsultani, Luma Waleed Khalil and Sabah Mahdi Hadi

Abstract
This study was designed to assess ameliorative role of casein Hydrolysate specially bioactive peptide on hyperlipidemia that induced by fructose in adult male rats. Thirty two male rats were randomly and equally divided into four groups for 42 days. Experimental groups were as following: group one (G1) control group, group two (G2) treated with casein Hydrolysate (1g/kg/day) orally, group three (G3) treated with fructose (40%) in drinking water and group four (G4) given casein Hydrolysate and fructose. Blood sample collection from orbital sinus technique by using capillary tube in case anesthetized rats after 42 days of the experimental period for measuring the following parameters: triglyceride, total cholesterol, high density lipoprotein cholesterol, insulin-like growth factor-1, and DNA fragmentation. Results exhibited that there is a significant decrease ($p < 0.05$) in triglyceride and total cholesterol in casein Hydrolysate group and casein Hydrolysate with fructose group as compared with other groups. At the same time, high density lipoprotein cholesterol increase significantly ($p < 0.05$) in casein Hydrolysate group compared with other groups. DNA fragmentation of white blood cells of fructose group showed induction of chromosomal damage compared to casein Hydrolysate with fructose that ameliorates this damaging effects. In conclusion, the results of this study documented that casein Hydrolysate is a good hypolipidemic agent that will ameliorates the deleterious effects of fructose in adult male rats.

Keywords: casein hydrolysate, fructose, hyperlipidemia, total cholesterol

1. Introduction
Hyperlipidemia is considered one of the main risk factors that cause cardiovascular diseases (CVDs). These pathological conditions compose threats for more than one third of total peoples around the world, and consider the major cause of death and failure throughout the world by the year 2020 [1, 2]. Hyperlipidemia is the status an increase in lipid content of the body that probably includes the high-rise in one or more of triglycerides, cholesterol, fatty acids and lipoproteins levels [3, 4]. Lipids are usually present in the blood stream that are often classified as triglycerides and cholesterol. The cholesterol moves around the blood stream and it is usually contributory in the organization of cells in the body. It is also contributor in the functioning of cells as well. While the triglycerides, in general, are either utilized immediately or gets accumulated in the adipose cells [5, 6]. Alteration and/or abnormality in the metabolism of lipids and lipoproteins is a very common state that taken place within general population, and it consider as one of the main risk factor in the happening of CVD due to their effect on atherosclerosis [7].
Fructose is a highly lipogenic sugar that has strong metabolic effects. Fructose becomes a main ingredient of our modern diet. Fructose consumption has steadily increased over the past 30 years in parallel to the growth of the obesity/ metabolic syndrome epidemic. Fructose is constituent in many commercially produced food products [8]. Excess consumption of fructose is an important contributor to the metabolic syndrome. It has been hypothesized that fructose consuming in our diet may be among the factors that contribute to the epidemic of the metabolic syndrome and, consequently, to the epidemic of chronic renal disease [9]. This hypothesis is supported by the preliminary evidence demonstrating that high fructose consumption induces damage of kidney in both rats [10] and rodents [11]. Reduction of cellular energy cause Increase fructose catabolism, this lead to increase the cells capability to lipid peroxidation [12]. Furthermore, it has been postulated that increased fructose catabolism can accelerate free radical production similar to glucose and impairs the free radical defense system leading to oxidative stress [13, 14, 15]. Fructose is found in a many of foods.

In table sugar, it is bound to glucose to form the disaccharide sucrose, whereas in honey it occurs in monosaccharide form. In fruit, berries, and vegetables, fructose occurs in both monosaccharide and disaccharide forms [16].

Bovine milk is a prime source of proteins of high biological value, it is characterized by a complete amino acids (AA) profile and high digestibility. Apart from their nutritional value, milk proteins and peptides have a wide range of biological functions [17]. Casein is the major proteinaceous component of milk, where it accounts for about 80% of the total protein inventory. Casein has made us interest due to its numerous using in the food, drug, and cosmetic industries in addition to its importance as an investigation material for illustrating essential questions as regards to the protein chemistry [18, 19]. Milk proteins like casein are among the most important sources of bioactive peptides; these remain inactive within the sequence of the parent protein until they are released either by gastrointestinal digestion or by food processing. Bioactive peptides are specific protein fragments that have a positive effect on body functions or conditions and may ultimately influence health [20]. Their beneficial health effects are classified as antimicrobial, immunomodulatory, lowering of hypertension, antithrombotic, and antioxidant (AO), in addition to lowering of cholesterol and enhancing absorption of mineral and bioavailability [21, 22].

2. Materials and Methods

2.1 Experimental animals and care

A total number of experimental animals are 32 male rats, their weights between (325 – 375) g and their ages between (3.0 - 3.5) months. Animals were housed in plastic cages in conditions(room temperature, 26 ± 3ºC) in the animal house of department of physiology and pharmacology at the college of Veterinary Medicine – University of Baghdad, with controlled lightening of twelve hours (7.00-19.00) and twelve hours night cycle. They were left for thirty days for adaptation, Animals had free access to water and standard pellet diet along the experimental period.

2.2 Preparation of casein hydrolysate

After the pilot study, the dose of casein hydrolysate was calculated according to dose (1g/kg/day) [23] by using gastric intubation, dissolving 6 grams of casein hydrolysate in 18 ml of distilled water and orally administered to rats twice a day according to body weight of each rat. Casein hydrolysate purchased from Direvo CO. (Germany).

2.3 Preparation of fructose

Fructose was dissolved in distilled water and was given to rats with drinking water at a dose of 40% (40 g/100 ml of water) **(24)**. Fructose purchased from Direvo CO. (Germany).

2.4 Blood samples collection

During fasting (for 8-12 hrs) the blood samples were collected after (42 days) of the experiment. Blood sample collection from orbital sinus technique by using capillary tube in case anesthetized rat by intramuscular injection of xylazine (40mg/kg B. W.) and ketamine (90 mg/kg B.W.). Blood sample were kept in gel tube, and centrifuged at 3000 rpm for 15 minutes, then serum sample were isolated and frozen at (-20 Cº) until analysis.

2.5 Experimental design for the experiment

Thirty two adult male rats were used in this experiment, divided equally to four groups and treated as following: Group one (control): eight rats were received distal water daily; Group two: eight rats were received (1g/kg/day) casein Hydrolysate daily by orally intubation; Group three: eight rats were received (40%) high fructose daily in drinking water; and Group four: eight rats were received (1g/kg/day) casein Hydrolysate and (40%) high fructose daily.

2.6 Determination of parameters of the experiment

Triglyceride (g/dl), total cholesterol (g/dl), and high density lipoprotein cholesterol (g/dl) concentrations were measured by using serum blood and calculated by using a special kit (Biosystem, Spain). Insulin-like growth factor-1(µl /ml) was measured by using serum blood and calculated by using Elisa kit (Elabscience, USA). Extraction and gel electrophoresis of DNA fragmentation measured by using blood according to Saini and Das (2014) and Couto *et al*., (2013) [25, 26].

2.7 Statistical analysis

Statistical analysis of data was performed according for One-Way Analysis of Variance (ANOVA) utilizing a significant level of ($P < 0.05$) and Least significant differences (LSD) to assess significant differences among means of the groups by using the SAS [27].

3. Results and Discussion

3.1 Effect of casein Hydrolysate and fructose on serum triglyceride, total cholesterol and high density lipoprotein cholesterol concentrations

Table (3-1) indicated that mean values of serum triglyceride concentration after (42) days of experiment significantly ($P \leq 0.05$) decrease after oral intubation of casein Hydrolysate (G2) comparing to control group, this indicating hypolipidemic effect of casein Hydrolysate. The result also showed that TAG increased significantly ($P \leq 0.05$) in fructose group (G3) comparing to the control group and (G2, G4) groups. at the end of experiment, the mean values of this parameter were (68.46±0.55), (53.68±0.55) and (81.46±0.67) for groups G4, G2 and G3 respectively comparing to control group with a mean value (66.66±0.83) at the same period.

The results also showed significant ($P \leq 0.05$) increase in serum total cholesterol concentration in rats received fructose (after 42 days) with mean value (81.65±0.71) comparing to the observed values in other treated groups and control. While rats received casein Hydrolysate (G2) and rats received (casein Hydrolysate +fructose) (G4) caused significant ($P \leq 0.05$) decrease in serum TC concentration with mean values (61.23±0.39) and (67.37±0.60) respectively comparing to the value (77.51±0.64) in control group (G1).

There was a significant ($P \leq 0.05$) increase in serum HDL-Cholesterol in the group (G2) treated with casein Hydrolysate (53.88±0.67) in comparison with other groups (Table 3-1). It also revealed significant ($P \leq 0.05$) decrease in fructose group (G3) with mean value (34.46±0.79) comparing to other groups.

Fructose consumption can promote hepatic lipogenesis because, first, the liver is the main site of fructose metabolism [28]; second, entry of fructose into glycolysis via fructose-1-phosphate bypasses the main rate-controlling step of glycolysis catalyzed by phosphofructokinase, thus providing unregulated amounts of lipogenic substrates acetyl-CoA and glycerol-3-phosphate, and, third, fructose can activate sterol receptor element binding protein-1c (SREBP-1c) independently of insulin, which then activates genes involved in DNL [29, 30].

A high flux of fructose to the liver, the main organ capable of

metabolizing this simple carbohydrate, perturbs glucose metabolism and glucose uptake pathways, and leads to a significantly enhanced rate of de novo lipogenesis and triglyceride (TG) synthesis, driven by the high flux of glycerol and acyl portions of TG molecules from fructose catabolism [31].

There is a considerable evidence supporting the ability of high fructose diets to upregulate the lipogenesis pathway, leading to increased TG production [32]. Insulin and glucose are known to directly regulate lipid synthesis and secretion. Insulin controls hepatic sterol regulatory element binding protein (SREBP) expression, which is a key transcription factor responsible for regulating fatty acid and cholesterol biosynthesis. SREBP binds to sterol responsive elements (SRE) found on multiple genes, and can activate a cascade of enzymes involved in cholesterol biosynthetic pathways, such as HMG-CoA reductase [33] and fatty acid synthase (FAS) [34].

Fructose diets also upregulate hepatic SREBP-1, SCD1 and FAS expression in both the human and in rodents, which increases the level of lipogenesis in the liver. This in turn increases the synthesis and secretion of VLDL and apoB-containing lipoproteins [35].

We have previously reported that rodents fed the hydrolyzed casein diet had reduced fed state plasma concentrations of glucose relative to those fed control diet [36]. Therefore redirection of glucose from glycolysis could also potentially remove substrates used in de novo lipogenesis. Many studies measured liver lipid contents and hepatic gene Expression levels of enzymes involved in de novo lipogenesis showed that rodents fed hydrolyzed casein were characterized by a decreased expression of genes involved in de novo lipid synthesis and decreased content of free fatty acids and triacylglycerols. expression of lipogenic genes Sterol regulatory element binding transcription factor 1(Srebf1), Acetyl-Coenzyme A carboxylase alpha (Acaca), Fatty acid synthase (Fasn), and Stearoyl Coenzyme A desaturase 1 (Scd1) was significantly decreased after feeding with hydrolyzed casein, indicating that de novo lipid synthesis was depressed. Furthermore, liver free fatty acids (FFA) and lysophosphocholine were reduced in rodents fed hydrolyzed casein relative to those fed control diets. Therefore, we speculate that ingestion of extensively hydrolyzed casein in rodents facilitates reduction of glucose concentrations by increasing glycogen deposition [37], as well as by increasing conversion of glucose to D-glucuronic acid, which subsequently can lead to decreased deposition of lipids [38].

The rodents fed hydrolysed casein diets had lower body mass gain and adipose tissue mass than rodents fed the intact casein diets [36]. As seen, faeces from rodents fed the hydrolysed casein diets contained a higher fat concentration. Moreover, the content of lipids in skeletal muscle, liver and spleen was reduced and the plasma content of both lipid and choline was decreased in the rodents given hydrolysed casein diets. The combination of higher faecal and lower tissue and plasma lipid contents could indicate a lower uptake of lipids from the feed in the gastrointestinal tract of rodents given hydrolysed casein diets. Thus, it is likely that reduced fat absorption might be one of the factors contributing to the reduced energy efficiency associated with intake of hydrolysed casein [38].

Table 3.1: Effect of casein hydrolysate and fructose on some lipid profiles

Groups Parameters	G1 Intact Rats Received distilled water	G2 Rats received Casein hydrolysate	G3 Rats received Fructose	G4 Rats received Casein hydrolysate + Fructose	LSD
Total cholesterol (mg/dl)	77.51±0.64 B	61.23±0.39 D	81.65±0.71 A	67.37±0.60 C	1.750
HDL-Cholesterol (mg/dl)	45.81±0.72 B	53.88±0.67 A	34.46±0.79 D	43.68±0.60 C	2.040
Triglyceride (mg/dl)	66.66±0.83 B	53.68±0.55 C	81.46±0.67 A	68.46±0.55B	1.929

Values represent mean ± SE (N=8). Different capital letters denote a significant difference between groups ($p \leq 0.05$).

3.2 Effect of oral intubation of casein Hydrolysate and fructose for (42) day on serum insulin- like growth factor-1 (IGF-1) concentration

The results showed significant decrease ($P \leq 0.05$) in this parameter was observed in fructose treated group (G3) (1547.25±3.95), the result also showed significant ($P \leq 0.05$) difference between treated group (G4) (1654.50±6.55), as compared to each other at the end of the experiment table (3-2).

Growth hormone (GH) generates IGF-I at various target tissues in autocrine and paracrine fashion [39], but most circulating IGF-I is produced in hepatocytes [40]. Liver-specific deletion of the GH receptor in rodents (GHRLD) resulted in a 90% reduction in serum IGF-I levels [41].

Regarding the role of IGF-I in the liver, low serum levels of IGF-I have been observed in patients with chronic liver disease, and malnutrition, despite normal or elevated GH secretion [42], because the hepatocytes produce most of the serum IGF-I, and GH resistance generally occurs in chronic liver disease [43]. It has been considered that IGF-I does not affect hepatocyte function directly because the hepatocytes express few IGF-I receptors (IGF-IR) in a normal condition [44].

Table 3-2: Effect of casein hydrolysate and fructose on IGF-1 hormone

Groups Parameters	G1 Intact Rats Received distilled water	G2 Rats received Casein hydrolysate	G3 Rats received Fructose	G4 Rats received Casein hydrolysate + Fructose	LSD
IGF-1 (mg/dl)	1767.38±7.49 A	1751.63±6.33 A	1547.25±3.95 C	1654.50±6.55 B	18.022

Values represent mean ± SE (N=8). Different capital letters denote a significant difference between groups ($p \leq 0.05$).

3.3 Effect of casein Hydrolysate and fructose for (42) day on DNA fragmentation

The results in figure (3-1) showed that animals treated with casein hydrolysate showed non-significant damage in DNA bands, while in figure (3-2) that animals treated with fructose showed a significant increase in DNA fragmentation appeared as strongly damaged, the results also showed in figure (3-3) that animals treated with casein hydrolysate and fructose together showing improvement in DNA by decreasing the fragmentation, also appeared a significant increasing in the concentration of nucleic acid as compared with casein hydrolysate and control groups.

However, increased oxidative stress and its downstream effects can lead to various conditions such as cardiovascular diseases [45], Alzheimer's disease [46], aging [47], and cancer [48]. Dietary intake of antioxidant compounds can reinforce the

body's oxidant status and help to maintain a balanced condition in terms of oxidant/antioxidant in the body. Given this background, there is increasing interest in food proteins and their constituent peptides as potential candidates for use as antioxidants.

Milk proteins also contribute much in the context of antioxidant peptides. YFYPEL, a hexapeptide isolated from pepsin hydrolysate of bovine casein, showed antioxidant activity by scavenging superoxide, DPPH, and hydroxyl radicals in vitro [49, 50].

There are many researches mention that fractions of acidic peptide had higher bioavailability and a higher residual ratio of antioxidant activity [51].

Another recent study refers that charge properties of casein peptides on absorption stability, antioxidant activity, and cytoprotection were evaluated. This study suggested that negatively charged peptide fractions had greater bioavailability (BA) and antioxidant activities after digestion and absorption. In addition, all peptides that transfer through cell membrane increase of cell viability, elevated catalase activity, and decreased superoxide dismutase activity [52].

Consistent with that is demonstration here casein Hydrolysate may prevent DNA damage that produced from the oxidative stress which result from exposure to fructose.

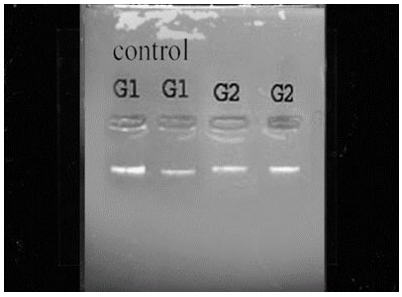

Fig (3-1): Gel electrophoresis DNA fragmentation in DNA of WBCs of rats treated casein hydrolysate. In which band (G1) represent control group and (G2) represent rats treated with casein hydrolysate

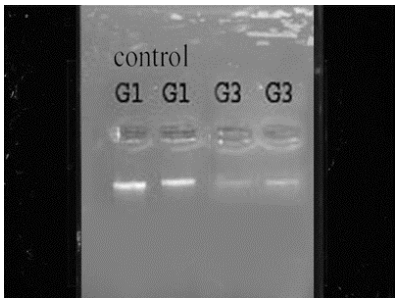

Fig (3-2): Gel electrophoresis DNA fragmentation in DNA of WBCs of rats treated fructose. In which band (G1) represent control group and (G3) represent rats treated with fructose

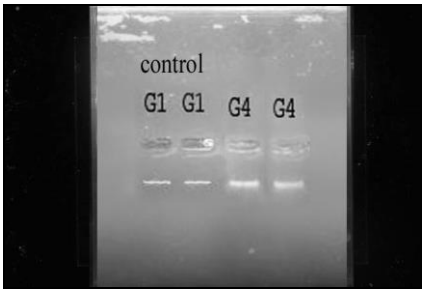

Fig (3-3): Gel electrophoresis DNA fragmentation in DNA of WBCs of rats casein hydrolysate and fructose. In which band (G1) represent control group and (G4) represent rats treated with fructose and casein hydrolysate together

4. Conclusion

In conclusion, supplementation of the casein hydrolysate ameliorate the adverse effects of fructose on insulin- like growth factor-1, total cholesterol, triglyceride, HDL-C concentrations and DNA fragmentation of WBC, and protection the body against the deleterious effect of hyperlipidemia induced by fructose.

5. Acknowledgments

Authors would like to express our sincere thanks and Praise to almighty Allah, the most gracious, the most merciful. Glory and praise be to Allah and his blessing upon Prophet Muhammad (peace be upon him and his family) and his household especially Imam Mossa AL. Kadhim (peace be upon him) for giving us the strength for accomplish this work, we acknowledge Dr. Muhammad Riyadh Alkenany for his moral support.

6. References

1. Jorgensen T, Capewell S, Prescott E, Allender S, Sans S, Zdrojewski T. Population-level changes to promote cardiovascular health. European Journal Preventive Cardiology. 2013; 20(3):409-21.
2. Shattat G. A Review Article on Hyperlipidemia: Types, Treatments and New Drug Targets. Biomedical & Pharmacology Journal. 2014; 7(2):399-409.
3. Guo F, Huang C, Liao X, Wang Y, He Y, Feng R, *et al*. Beneficial effects of mangiferin on hyperlipidemia in high-fat-fed hamsters. Molecular Nutrition and Food Research. 2011; 55:1809-18.
4. Braamskamp MJ, Wijburg FA, Wiegman A. Drug therapy of hypercholesterolemia in children and adolescents. Drugs. 2012; 72:759-72.
5. Iughetti L, Bruzzi P, Predieri B. Evaluation and management of hyperlipidemia in children and adolescents. Current Opinion in Pediatrics. 2010; 22:485-93.
6. Talath F, Ansari M, Naaz H, Banu H, Mehveen Z. Hyperlipidemia- A Critical Pathological Condition. International journal of pharmacy and pharmaceutical research. 2017; 8(3):110-124.
7. Hassan B. Overview on Hyperlipidemia. Journal Chromat Separation Techniq. 2013; 4:3.
8. Chen J, Muntner P, Hamm LL, Jones DW, Batuman V, Fonseca V, *et al*. The metabolic syndrome and chronic kidney disease in US adults. Annals of Internal Medicine. 2004; 140:167-74.
9. Nakagawa T, Hu H, Zharikov S, Tuttle KR, Short RA, Glushakova O, *et al*. A causal role for uric acid in fructose-induced metabolic syndrome. Amercan Journal of Physiology. Renal Physiology. 2006; 290:625-31.
10. Oudot C, Lajoix AD, Jover B, Rugale C. Dietary sodium restriction prevents kidney damage in high fructose-fed rats. Kidney International. 2013; 83:674-83.
11. Aoyama M, Isshiki K, Kume S, Chin-Kanasaki M, Araki H, Araki SI, *et al*. Fructose induces tubule interstitial injury in the kidney of mice. Biochemical and Biophysical Research Communications. 2012; 419: 244–9.
12. Punitha IS, Rajendran K, Shirwaikar A, Shirwaikar A. Alcoholic stem extract of *Coscinium fenestratum* regulates carbohydrate metabolism and improves antioxidant status in streptozotocin-nicotinamide induced diabetic rats. Evidence- Based Complementary Alternative Medicine. 2005; 2:375-81.

13. Kumar S, Anandan R. Biochemical studies on the cardioprotective effect of glutamine on tissue antioxidant defense system in isoprenaline-induced myocardial infarction in rats. Journal of Clinical Biochemistry and Nutrition. 2007; 40:49-55.
14. Reddy SS, Ramatholisamma P, Karuna R, Saralakumari D. Preventive effect of Tinospora cordifolia against high-fructose diet-induced insulin resistance and oxidative stress in male Wistar rats. Food and Chemical Toxicology. 2009; 47:2224-9.
15. Samraa H, Abdel-Kawi A, Kamel MA, Hassanin B, Khalid S, Hashem C. The effect of high dietary fructose on the kidney of adult albino rats and the role of curcumin supplementation: A biochemical and histological study. Beni-suef university journal of basic and applied science. 2016; 5:52-60.
16. White JS. Challenging the fructose hypothesis: new perspectives on fructose consumption and metabolism. Advances in Nutrition. 2013; 4(2):246-256.
17. Szwajkowska M, Wolanciuk A, Barłowska J, Król J, LitwiĔczuk Z. Bovine milk proteins as the source of bioactive peptides influencing the consumers' immune system—A review. Animal Science Papers and Reports. 2011; 29(4):269-280.
18. Frisher H, Meisel H, Schlimme E. OPA method modified by use of N, N-dimethyl-2-mercaptoethylammonium chloride as thiol components. Fresenius Z Analytical Chemistry. 2011; 330:631-633.
19. Wang J, Su Y, Jia F, Jin H. Characterization of casein hydrolysates derived from enzymatic hydrolysis. Chemistry Central Journal. 2013; 7:62.
20. Kitts DD, Weiler K. Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. Current Pharmaceutical Design. 2003; 9:1309-1323.
21. Plaisancié P, Claustrec J, Estiennea M, Henryd G, Boutroud R, Paqueta A, *et al*. A novel bioactive peptide from yoghurts modulates expression of the gel-forming MUC2 mucin as well as population of goblet cells and Paneth cells along the small intestin. The Journal of Nutritional Biochemistry. 2013; 24:213-221.
22. Awad S, El-Sayed MI, Wahba A, El Attar A, Yousef MI, Zedan M. Antioxidant activity of milk protein Hydrolysate in alloxan-induced diabetic rats. Journal of Dairy Science. 2016; 99(11):8499-8510.
23. Chan YK, MCgill AT, Kanwar RK, Krissansen GW, Haggarty N, Xin N, *et al*. Povine peptic casein hydrolysate ameliorates cardiovascular risk factors in amodel of apoe-deficient mice but not overweight, mildy hypercholesterolaemic men. Current research in nutrition and food science. 2014; 2(1):8-19.
24. Lateef FA. Role of acrylamide in metabolic syndrome induction relative to fructose in adult male rat. MSc thesis, College of Veterinary Medicine –University of Baghdad-Iraq. 2014, 1-121.
25. Saini D, Das B. Usefulness of nucleic acids (DNA/RNA) from buccal cells isolated from mouthwashes using a modified method. Forensic Research. 2014; 5(4):1-5
26. Couto MC, Sudre AP, Lima MF, Bromfim TC. Comparison of techniques for DNA extraction and agarose gel staining of DNA fragments using samples of Cryptosporidium. Veterinarni Medicina. 2013; 58(10):535-542.
27. SAS®. SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA, 2010.
28. Stanhope K, Havel P. Fructose consumption: potential mechanisms for its effects to increase visceral adiposity and induce dyslipidemia and insulin resistance. Current Opinion in Lipidology. 2008; 19(1):16-24.
29. Matsuzaka T, Shimano H, Yahagi N, *et al*. Insulin-independent induction of sterol regulatory element-binding protein-1c expression in the livers of streptozotocin-treated mice. Diabetes. 2004; 53:560-569.
30. Nagai Y, Nishio Y, Nakamura T, *et al*. Amelioration of high fructose-induced metabolic derangements by activation of PPAR alpha. Amercan Journal of Physiology. Endocrinology and Metabolism. 2002; 282:E1180-E1190.
31. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. Nutrition & Metabolism. 2005; 2:5.
32. Kok N, Roberfroid M, Delzenne N. Dietary oligofructose modifies the impact of fructose on hepatic triacylglycerol metabolism. Metabolism. 1996; 45:1547-1550.
33. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell. 1997; 89:331-340.
34. Bennett MK, Lopez JM, Sanchez HB, Osborne TF. Sterol regulation of fatty acid synthase promoter. Coordinate feedback regulation of two major lipid pathways. The Journal of Biological Chemistry. 1995; 270:25578-25583.
35. Biddinger SBA, Hernandez-Ono C, Rask-Madsen JT, Haas JO, Aleman R, Suzuki EF. *et al.* Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. Cell Metabolism. 2008; 7:125-34.
36. Lillefosse HH, Tastesen HS, Du ZY, Ditlev DB, Thorsen FA, Madsen L, *et al*. Hydrolyzed casein reduces dietinduced obesity in male C57BL/6J mice. Journal of Nutrition. 2013; 143(9):1367-1375.
37. Yde CC, Clausen MR, Ditlev DB, Lillefosse H, Madsen L, Kristiansen K, *et al*. Multi-block PCA and multi-compartmental study of the metabolic responses to intake of hydrolysed versus intact casein in C57BL/6J mice by NMR-based metabolomics. Metabolomics. Available, 2014; 10.1007/s11306-014-0623-4.
38. Clausen M, Zhang X, Yde C, Ditlev D, Lillefosse H, Madsen L, *et al*. Intake of Hydrolyzed Casein is Associated with Reduced Body Fat Accretion and Enhanced Phase II Metabolism in Obesity Prone C57BL/6J Mice. PLoS ONE. 2015; 10(3):e0118895.
39. Liu JL, Yakar S, LeRoith D. Conditional knockout of mouse insulin-like growth factor-I gene using the cre/loxp system. Proceedings of the Society for Experimental Biology and Medicine. 2000; 223:344-351.
40. Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu JL, *et al*. Circulating levels of IGF-I directly regulate bone growth and density. The Journal of Clinical Investigation. 2002; 110:771-781.
41. Fan Y, Menon RK, Cohen P, Hwang D, Clemens T, DiGirolamo DJ, *et al*. Liver-specific deletion of the growth hormone receptor reveals essential role of growth hormone signaling in hepatic lipid metabolism. the Journa of Biological Chemistry. 2009; 284:19937-19944.
42. Donaghy A, Ross R, Gimson A, Hughes SC, Holly J, Williams R. Growth hormone, insulinlike growth factor-I, and insulinlike growth factor binding proteins 1 and 3 in chronic liver disease. Hepatology. 1995; 21:680-688.

43. Yakar S, Liu JL, Stannard B, Butler A, Accili D, Sauer B, *et al*. Normal growth and development in the absence of hepatic insulin-like growth factor I. Proceedings of the National Academy of the Scinces USA. 1999; 96:7324-7329.
44. Takahashi Y. The Role of Growth Hormone and Insulin-Like Growth Factor-I in the Liver. International Journal of Molecular Sciences. 2017; 18:1447.
45. Singh U, Jialal I. Oxidative stress and atherosclerosis. Pathophysiology. 2006; 13(3):129-142.
46. Smith MA, Rottkamp CA, Nunomura A, Raina AK, Perry G. Oxidative stress in Alzheimer's disease. Biochimica et Biophysica Acta. 2000; 1502(1):139-144.
47. Hyun DH, Hernandez JO, Mattson MP, de Cabo R. The plasma membrane redox system in aging, Ageing Research Reviews. 2006; 5(2):209-220.
48. Kinnula VL, Crapo JD. Superoxide dismutases in malignant cells and human tumors. Free Radical Biology and Medicine. 2004; 36(6):718-744.
49. Suetsuna K, Ukeda H, Ochi H. Isolation and characterization of free radical scavenging activities peptides derived from casein. Journal of Nutritional Biochemistry. 2000; 11(3):128-131.
50. Chakrabarti S, Jahandideh F, Wu J. Food-Derived Bioactive Peptides on Inflammation and Oxidative Stress. BioMed Research International, 2014. Article ID 608979, 11 pages.
51. Wang C, Wang B, Li B. Bioavailability of peptides from casein hydrolysate in vitro: Amino acid compositions of peptides affect the antioxidant efficacy and resistance to intestinal peptidases. Food Research International. 2016a; 81:188-196.
52. Wang B, Xie N, Li B. Charge properties of peptides derived from casein affect their bioavailability and cytoprotection against H_2O_2-induced oxidative stress. Journal of Dairy Science. 2016b; 99(4):2468-2479.