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Biochemical Evaluation of Hypolipidemic effect of probiotics on Hepatic Lipid Metabolism experimentally induced Non Alcoholic Fatty Liver Disease in Rats

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ABSTRACT

The main study applied on the biochemical effects of probiotics on Hepatic Lipid Metabolism experimentally induced Nonalcoholic fatty liver disease (NAFLD) in rats. Thirty male albino rats were divided into three groups (10 rats each). The first group fed a normal diet and represents the control group. The second group (NAFLD) fed normal diet enriched with1% cholesterol and 2% coconut oil and act as positive control (+ ve control). The third group fed on normal diet enriched with1% cholesterol and 2% coconut oil and probiotics(BIO-BC™) at a dose of 1-2g/liter at a rat dose (0.5-1ml/kg. body. weight). Samples collected after 2,4and6 weeks after induction from treatment. serum was collected for estimation of Total Cholesterol (TC) ,Triacyleglecerol (TAG), Low density lipoprotein cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL-C), High density lipoprotein cholesterol (HDL-C), Total lipids (TL), phospholipids, Nonesterified fatty acids(NEFA),Apo-lipoprotein -B (Apo-B),Our Results revealed a. significant increase in serum TC, TAG, LDL-C, VLDL-C,T L, phospholipids, NEFA, Apo-B While serum HDL-C showed a significant decrease. The behavioral biochemical results indicated treatment with probiotics showed significant changes and improves these parameters.

Keywords: NAFLD, probiotics, Lipid profiles.

1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents a wide spectrum of disorders, the hallmark of which is hepatic steatosis. NAFLD was considered a benign condition, but is now increasingly recognized as a major cause of liver-related morbidity and mortality. The fundamental derangement in nonalcoholic fatty liver disease is insulin resistance, a key component of the metabolic syndrome, which includes type 2 diabetes mellitus, hypertriglyceridemia, essential hypertension, low circulating high-density lipoprotein, and obesity [17]. Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host" seems to be the most accurate [28]. The aim of this study to evaluate the Hypolipidemic Effect of probiotics on Hepatic Lipid Metabolism in experimentally induced NAFLD in rats.

2. MATERIALS AND METHODS

2.1 Animals and chemicals:

Male white albino rats, 6weeks age and weighting (150– 180g) were used in the experiment. Rats were housed in separate metal cage with free access to water. Rats were kept under constant and nutritional environmental condition throughout the experiment. Rats were left for15 days before beginning of experiment for acclimatization. Cholesterol and

coconut oil were purchased from El-Goumhouria Co. for Trading Chemicals, Egypt. Hepatic lipid metabolism induced NAFLD by continuous supplementation of high fat diet (HFD) was prepared by High Cholesterol (1% wt/wt) and (Coconut oil 2%wt/wt) to normal ration according to [22].

2.2 Probiotic (BIO-BC™):
Improve growth this product was kindly supplied from Animal Health Division by KanzyMedipharm (™),EgyptDaone chemical co. Ltd ./Da405, :Daonsihwa industrial complex , 1252.6,Jeongwang-dong, siheung-si, kyonggi-do, korea . it was given orally in dose and duration as mentioned below:

*2.2. A Composition (*Each kg contain): It composed of :
StreptococcusFaecalis 1billionCFU/kg.
BacillusSubtitles0.2billionCFU/kg.
Clostridiumbutyricum0.2billionCFU/. kg
Carrier glucose 1kg

2.2. B Preparation and dosage of probiotic:
It is a powder added to drinking water in a dose of 1-2g/liter (KanzyMedipharm***)*** as manufactures instructions and at a rat dose (0.5-1ml/kg. body. weight) orally .according to (Paget and Barnes J.M.1964)[24].

2.3 Experimental design:
Rats were divided into 3 groups (10 per each).
Group I was fed on normal diet and served as control group.
Group II was fed on high fat diet (Normal NAFLD) for 12 weeks.
Group III was fed on normal diet (NAFLD) and probiotics at a dose of1-2g/liter (KanzyMedipharm)as manufactures instructions and at a rat dose (0.5-1ml/kg. body. weight) daily according to [24] orally.

2.4 Sampling:
After overnight fasting blood samples was collected from all animal groups (control and experimental groups) after12weeksfordetectionof hyperlipidemia .then samples were collected after 2,4and6 weeks from onset of treatment.

*2.4.1 Blood samples***:**
Blood samples were collected from medial canthes of eye and collected in dry, clean and screw capped tubes then rats decapitated for liver tissue removal containing serum were separated by centrifugation at 2500 r.p.m for 15 minutes. The clean clear serum was separated by Pasteur pipette and kept in a deep freeze at -20C till used for determination of the biochemical Parameters:serum lipid profiles as TC[34], TAG [35],LDL-C[10],VLDL-C [38],HDL-C [37],Total lipids [36], phospholipids [39], NEFA [30], Apo-B [30].

2.4.2 Tissue Sample:
Liver specimens were preserved in 10% buffered neutral formalin and subjected for Histopathological Examination according to the technique described by [2].

2.5 Statistical analysis:
The obtained data were analyzed represented using the statistical package for social science (SPSS, 13.0 software, 2009)[33], for obtaining mean and standard deviation and error. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping.

Table 1. Effect of Probiotics administration on lipid profiles after 2weeks on Hepatic Lipid Metabolism Experimentally Induced Nonalcoholic fatty liver disease in rats

Parameter/ group	**T C mg/dl**	**TG (mg/dl**	**LDL-C mg/dl**	**VLDL-C mg/dl**	**HDL-c mg/dl**	Totallipid **mg/dl**	Phosholipid **mg/dl**	**NEFA ng/ml**	**Apo(B) (ng/ml)**
Normal Control group	89.18 ^{e} **±**1.59	131.39^{de} ±14.55	19.28^{C} ±2.63	26.28^{d} ±2.91	40.40^{C} ±0.48	353.49^{cd} ±23.78	77.06^{bc} ±2.97	64.00^{cd} ±3.47	2.43^{cde} ±0.11
control NAFLD group	149.05^{bc} ±22.35	204.62^{ab} ±13.29	59.25^{ab} ±16.09	35.72^{a} ±4.50	37.92^{c} ±2.33	490.73^{abc} ±35.40	106.84^{a} ±12.32	76.94^{cd} ±7.93	2.67^{bcd} ±0.21
NAFLD Treated probiotics group	95.96^{de} ±2.70	171.55^{abc} ±9.80	31.17^{bc} ±2.52	31.66^{ab} ±4.56	39.25^{c} ±2.84	386.04^{bc} ±19.98	79.94^{bc} ±4.14	56.08^{e} ±8.13	2.23^{de} ±0.16

Data are presented as (Mean ± S.E).S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at *(P<0.05*)

Table 2. Effect of Probiotics administration on lipid profiles after 4weeks on Hepatic Lipid Metabolism Experimentally Induced Nonalcoholic fatty liver disease in rats.

Parameter/ group	**T C mg/dl**	**TG mg/dl**	**LDL-C mg/dl**	**VLDL-C mg/dl**	**HDL-c mg/dl**	**Totallipid mg/dl**	**Phosholipid mg/dl**	**NEFA ng/ml**	**Apo(B) ng/ml**
Normal Control group	83.04^{e} ±0.85	113.72^{e} ±8.69	16.49^{C} ±3.45	22.76^{e} ±1.74	41.65^{C} ±1.19	335.25^{cd} ±8.06	71.67^{bc} ±4.90	41.44^{e} ±2.46	2.16^{e} ±0.08
control NAFLD group	192.29^{a} ±27.68	185.69^{abc} ±14.49	66.97^{a} ±16.84	34.47^{ab} ±3.49	35.69^{c} ±2.01	446.84^{ab} ±20.32	103.41^{a} ±9.24	61.87^{de} ±2.31	2.40^{cde} ±0.17
NAFLD Treated probiotics group	64.67^{e} ±1.07	152.75^{cd} ±14.57	17.56^{c} ±3.83	26.58^{de} ±1.98	37.45^{c} ±2.00	347.18^{cd} ±14.64	102.17^{a} ±5.28	51.80^{bc} ±7.49	2.20^{de} ±0.11

Data are presented as (Mean ± S.E).S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at *(P<0.05*)

Table 3. Effect of Probiotics administration on lipid profiles after 6weeks on Hepatic Lipid Metabolism Experimentally Induced Nonalcoholic fatty liver disease in rats.

Parameter/ group	T C mg/dl	TG mg/dl	LDL-C mg/dl	VLDL-C mg/dl	HDL-C mg/dl	Totallipid mg/dl	Phosholipid mg/dl	NEFA ng/ml	Apo(B) ng/ml
Normal Control group	82.87^{e} ±6.75	159.02^{abc} ±5.46	24.18^{c} ±2.15	33.80^{ab} ±1.09	32.87^{c} ±0.70	353.19^{cd} ±4.88	66.01^{c} ±1.50	67.59^{bc} ±2.48	2.53^{cd} ±0.08
control NAFLD group	173.12^{ab} ±32.31	176.84^{abc} ±20.44	75.87^{ab} ±24.94	39.58^{a} ±4.20	25.69^{cd} ±1.82	364.44^{cd} ±23.58	90.15^{ab} ±23.58	63.98^{bc} ±9.41	2.65^{bcde} ±0.27
NAFLD Treated probiotics group	93.19^{de} ±1.17	130.42^{de} ±13.97	31.07^{bc} ±3.27	27.59^{de} ±1.50	37.87^{C} ±2.14	329.24^{cd} ±8.95	75.31^{bc} ±3.52	60.57^{cd} ±4.47	2.20^{a} ±0.06

Data are presented as (Mean ± S.E).S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at *(P<0.05*)

3. RESULTS AND DISCUSSION

The obtained results demonstrated in tables (1,2 and 3)revealed that a significant increase in TC, TAG, LDL-C, VLDL-C Total lipids, phospholipids, NEFA, Apo-B While a significant decrease in HDL-C observed in hepatic lipid metabolism Experimentally induced NAFLD in rats after 2, and 6weeks compared with the normal control group These Results were nearly similar to [20] showed that may be due to the increase of plasma triglyceride rich lipoproteins (TRLs) is associated with multiple alterations of other lipoproteins species that are potentially atherogenic has expanded the picture of diabetic dyslipidaemia [6].

These results in accordance with [4]a significant increase in FFA are generally modified by fatty acyl-CoA synthetases almost immediately on entry into cells and thus are unavailable for transport or diffusion. The majority of excess FFA is likely converted to TG, which may be stored [3]**.** More ever these results were agree with[31]revealed that a significant increase in serum to total lipids, total cholesterol, TAG, phospholipids, LDL, VLDL this increase due to catabolic effect of serum cholesterol. Hyperglycemia causes oxidative stress due to increased mitochondrial production of the superoxide anion, non-enzymatic glycation of proteins [5].

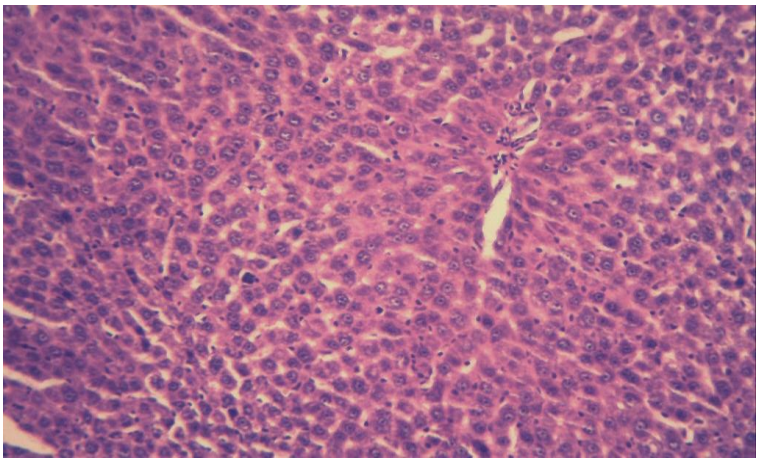
Also these results were nearly similar to [21]showed that a significant increase in serum FFA, TAG due to An important source of lipid for the hepatocyte is circulating FFAs. The various sources of these fatty acids include lipolysis of stored TGs in a dipocytes and dietary fat. These hydrophobic substances travel in the circulation bound to albumin, which increases the concentration of FFA the serum can carry and maintains a very low unbound concentration. The importance of FFAs may lie in the fact that their concentration in plasma is often significantly increased in the various disorders associated with hepatic steatosis [26].

However these results were nearly similar to [7] determined that a significant increase in TC ,TAG, LDL-C,VLDL-C concentration caused by nonalcoholic fatty liver disease the inhibition of acyl-coenzyme A:diacylglycerol acyltransferase, the enzyme that catalyzes the final step in triglyceride synthesis, results in improvement in hepatic steatosis and insulin sensitivity. hepatic specific inhibition of acyl-coenzyme A:diacylglycerol acyltransferase with antisense oligonucleotides improves hepatic steatosis in obese, diabetic mice but, unexpectedly, exacerbated injury and fibrosis in that model of progressive nonalcoholic fatty liver disease. hepatocyte triglyceride synthesis was inhibited, free fatty acids accumulated in the liver, leading to induction of fatty acid oxidizing systems that increased hepatic oxidative stress and liver damage. These findings suggest that the ability to synthesize triglycerides may, in fact, be protective in obesity.hyperlipidaemia and hyperglycaemia are among a myriad of risk factors that may contribute to the pathogenesis of many cardiovascular diseases, such as hypertension, diabetes and atherosclerosis [11].

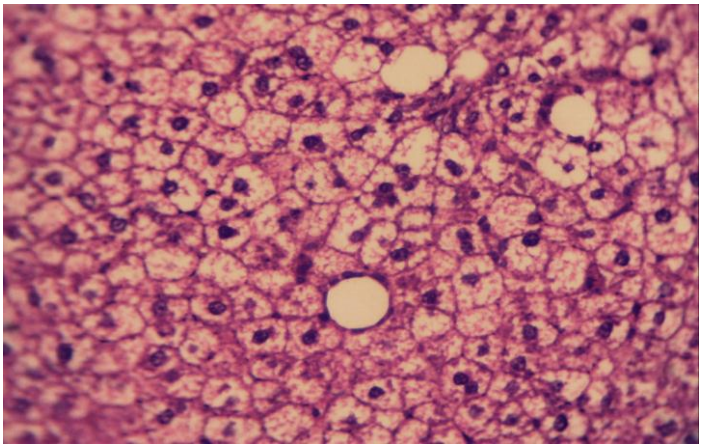
Also these results were in accordance with [18] found that a significant increase in Total lipids, Apo-B concentration interpreted the Elevated levels of certain blood lipids the principal cause of cardiovascular disease and other disabilities in developed countries. a positive association between cholesterol levels and the risks of coronary heart disease. In addition to a significant decreased in HDL-Cl observed in hepatic lipid metabolism Experimentally induced NAFLD in rats after 2,4 and 6weeks compared with the normal control group[14].

Furthermore [27] found that the causal relationship between hepatic fat accumulation, insulin resistance, liver damage and the etiological role of hepatic fat accumulation in pathogenesis of extra- and intra-hepatic manifestations. Treatment with probiotics showed a significant decrease in serum (TC ,TAG, LDL-C ,VLDL-C, Total lipids, phospholipids, NEFA, Apo-B while a significant increase HDL-C compared with the hepatic lipid metabolism Experimentally induced NAFLD in rats after 2,4 and 6weeks These Results were nearly similar to [13]revealed that a significant decrease in serum lipid profiles level may due to the probiotics may have positive effect on blood lipid level. Other lipolytic and proteolytic enzymes produced in small intestine can adversely affect survival of the bacteria. Conjugated bile salts, too, have a devastating effect on the bacteria. They can ruin plasmatic

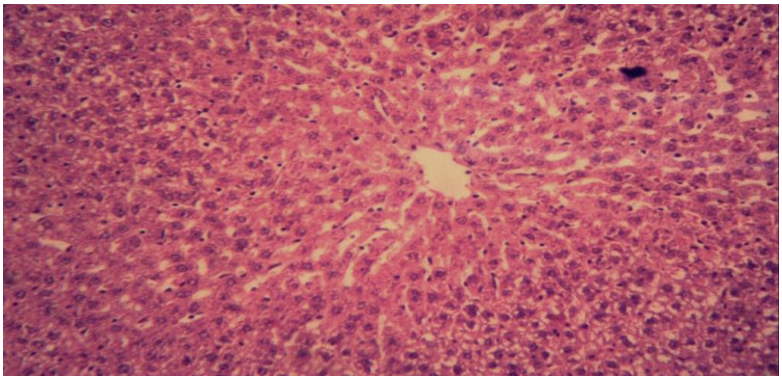
membrane's structure through emulsification. These greatly reduce the adhesive properties and the viability of the bacteria as they move through the gastro intestinal tract [23].



Group (1) Figure (1): Liver of Normal control rats showing Normal Histological structure of the liver, Normal Hepatic Lobules and Hepatocytes.



Group(2)Figure (2): Liver of NAFLD rats, the microscopical examination of liver showing sever congestion of the central vein and portal blood vessels. the hepatocytes showing sever degenerative changes in the form of vacuolar degeneration in the cytoplasm of hepatocytes in which the hepatocytes showing the presence fibrillated threads in the cytoplasm, mild degree of fatty change should be seen. in some of hepatocytes in which the hepatocytes giving signet ring shape as it contained flattened squeezed nucleus to one side.



Group(3)Figure (3): Liver of Probiotics treated rats, showing mild hydropic degeneration of hepatocytes and mild congestion of hepatocytes central vein portal blood vessels mononuclear leucocytic infiltration in the portal area. Some of hepatocytes showing scatter hepatocytes severed from fatty change.

However [25] observed that the benefit of probiotics is its ability to reduce serum lipid. probiotic bacteria may have a positive effect on the level of lipids in blood. probiotics' effect on cholesterol assimilation was "strain-dependent". Since in vitro studies demonstrated certain bacteria could take out cholesterol from culture media[9] .
on the other hand [18]found that a significant decrease in serum TC, TAG, LDL-C ,VLDL-C may be due to the Supplementation of diet with lactic acid bacteria the potential to reduce serum cholesterol levels. Various approaches have been used to alleviate this issue, including the use of probiotics, especially *Bifidobacterium*spp. and *Lactobacillus* spp. the hypocholesterolemic effects. Several possible mechanisms for cholesterol removal by probiotics are assimilation of cholesterol by growing cells, binding of cholesterol to cellular surface, incorporation of cholesterol into the cellular membrane, deconjugation of bile via bile salt hydrolase, coprecipitation of cholesterol with deconjugated bile, binding action of bile by fibre, and production of short-chain fatty acids by oligosaccharides. the mechanisms of action of anti-cholesterolemic potential of probiotic microorganisms and probiotic food products, with the aim of lowering the risks of cardiovascular and coronary heart diseases [15].

These results were agree with [12] found that in rats fed on high cholesterol diet containing probiotics leading to decrease in total lipids, TC, TAG , phospholipids, LDL, VLDL, NEFA, Apo-B after 2 weeks of experiment, this decrease because highly significant after 8 weeks of experiment. This decrease is due to low activity of hydroxyl methyl glutaryle co-enzyme A in the liver, which responsible for cholesterol synthesis.

Furthermore the treatment of probiotics in the HDL-C a significant increase these results were in accordance with [31]found that a significant increase in serum HDL. This increase due to catabolic effect of serum cholesterol.Also[8]observed that regular exercise contributes to the prevention of cardiovascular dysfunction by controlling traditional cardiovascular risk factors, including HDL- and LDL (low-density lipoprotein)-cholesterol, improving antioxidant factors, such as SOD (superoxide dismutase which permits unrestricted non-commerical use, distribution and reproduction in any medium [19].

More over these results in accordance to [32] found that a significant decrease in serum FFA may be due to FFA and other lipids in hepatocytes are involved in production of reactive oxygen species, mitochondrial dysfunction and endoplasmic reticulum stress. They have pro-apoptotic capacity and can stimulate pro-inflammatory signaling pathways.An abnormal leptin secretion may contribute to switch from insulin sensitivity to IR [29].

The present study demonstrated that probiotics treatment provided an effective treatment against NAFLD in rats since these compounds were able to ameliorate serum biochemical parameters lipid profiles.

4. CONCLUSION

We conclude that, administration of diet rich in the natural products as probiotics is very important for treatment of different body organs, especially liver against NAFLD (Hyperlipidemia).

5. REFERENCES

1. Andreas L. Birkenfeld andGerald I. Shulman. 2014. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 Diabetes. A Phytonutrients Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, United States Department of Agriculture, Hepatology.Volume 59, Issue 2, pages 713–723
2. Bancroft, J.D. and Stevens, S.A. 1996. Theory and Practice of Histological Techniques. Churchill-Livingstone, New York. 435-470.
3. Baynes,W.2003. Chemical modification of protein by lipids in diabetes. Clin. Chem. Lab. Med. 41: 1159-1165.
4. Bradbury MW and Berk PD.2004. Cellular uptake of long chain free fatty acids: the structure and function of plasma membrane fatty acid binding protein. AdvMolCell Biol 33: 47–81.
5. Brownlee, M. 2001. Biochemistry and molecular cell biology ofdiabeticcomplications.Nature. 414 (6865): 813–820.
6. ChenlinJi, Yanyan Dai, Weiwei Jiang, Juan Liu, Miao Hou, Junle Wang, Jonas Burén, Xiaonan Li .2014. Postnatal overfeeding promotes early onset and exaggeration of high-fat diet-induced nonalcoholic fatty liver disease through disordered hepatic lipid metabolism in rats . The Journal of Nutritional Biochemistry.
7. Choi, Steve Sa,b; Diehl, Anna Maea. 2014. Hepatic triglyceride synthesis and nonalcoholic fatty liver disease. Lipid metabolism:Current Opinion in Lipidology:juneVolume19-Issue3-P5-300.
8. deLemos, E. T., Reis, F., Baptista, S. 2007. Exercisetraining is associated with improved levels of C-reactiveprotein and adiponectin in ZDF (type 2) diabetic rats. Med. Sci. Monit. 13, BR168–BR174.
9. deRoos NM, Katan MB.2000. Effects of Probiotic Bacteria on Diarrhea, LipidMetabolism,and Carcinogenesis: American Journal of Clinical Nutrition 71(2): 405-11.
10. Friedewald, W.T. 1972. Colorimetric determination of serum LDL cholesterol Clin.Chem. 18, 499.
11. Hanrui Zhang Yoonjung Park Junxi WU, Xiu ping Chen,Sewon Lee, Jiyeon Yang Kevin C. Dell Sperger and Cuihua Zhang. 2009. Role of TNF-α in vascular dysfunction.
12. Ibrahim, A.A.; ELsayed, E.M.; Hafez, S.A.; El-Zeini, H.M. and Salah F.A. 2005. The hypocholesterdaemic effect of milk youghurt and soy- youghurt containing bifido bacteria inrat fed on acholestrol enriched diet. International Dairy Jouranl, 15(1): 37-44.
13. Isolauri E, Salminen S.2008.Probiotics: use in allergic disorders: a Nutrition, Allergy, Mucosal Immunology, and Intestinal Microbiota (NAMI) Research Group Report. J ClinGastroenterol, 42(Suppl):2:S91-96.
14. Janet D. Sparks, Walter N. Shaw, James P. Corsetti, Mary Bolognino,Joseph F. Pesek, Charles E. Sparks.2000 Insulin-treated Zucker diabetic fatty rats retain the hypertriglyceridemia associated with obesity Metabolism.Volume 49, Issue 11, November, Pages 1424–1430.department of Pathology and Laboratory Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY; and Genetic Models, Inc, Indianapolis.
15. Kim Y, Whang JY, Whang KY, Oh S, Kim SH.2008.Characterization of the Cholesterol reducing activity in a Cell-free Supernatant of Lactobacillus acidophilus ATCC 43121. Bioscience Biotechnology Biochemistry 72: 1483-1490.
16. King, G.L. and Loeken, M.R. 2004. Hyperglycemia-induced oxidative stress in diabetic complications. Histochem.Cell.Biol. 122: 333-338.

17. Lotze MT, Zeh HJ, Rubartelli A, Sparvero LJ, Amoscato AA, Washburn NR.2007. The grateful dead damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. Immunol Rev .220;60–8110.1111/j.1600-065X.00579.x
18. Manoj Kumar, RavinderNagpal, Rajesh Kumar, R. Hemalatha, VinodVerma, Ashok Kumar, Chaitali Chakraborty, Birbal Singh, Francesco Marotta, Shalini Jain, and Hariom Yadav.2012. Cholesterol-Lowering Probiotics as Potential Biotherapeutics for Metabolic Diseases .the Creative Commons Attribution License, Hindawi Publishing Corporation Experimental Diabetes Research. 2012; Article ID 9029.
19. Marketou, M. E., Zacharis, E. A., Nikitovic, D. 2006.Early effects of simvastatin versus atorvastatin onoxidative stress and proinflammatory cytokines in hyperlipidemic subjects. Angiology 57, 211–218
20. M.-R. Taskinen.2003. Diabetic dyslipidaemia: from basic research to clinical practice.Department of Medicine, Division of Cardiology, University of Helsinki, Helsinki, Finland Diabetologia ; 46:733–749.
21. Murray R.K, D. K. Granner, P. A. Mayes, and V. W.2002. Rodwell, Harper's Biochemistry, Appleton & Lange.
22. NRC (National Requirments of Laboratory animals) fourth revised edition.1995. subcommittee on laboratory Animal Nutrition , National Research Conuncil, National Academy Press,Washington , D.C.Of the lipid – lowering agents clofibrate and BM15.075
23. Olejnik A, Lewandowska M, Obarska M, Grajek W.2005.Tolerance of Lactobacillus and *Bifidobacterium* strains to low pH, bile salts and digestive enzymes.
24. Paget, G.F. and Barnes J. M.1964. Evaluation of drud activities Academic Press, London And New York , Chapter 6 pp.133-166.
25. Parvez S, Lee HC, Kim DS, Kim HY.2005. Bile salt hydrolase and chlolesterol removal effect by *Bifidobacterium bifidum* NRRL. World J MicrobiolBiotechnol;22(5):455-459.
26. P. Tessari A. Coracina, A. Cosma, A. Tiengo., 2009. Hepatic lipid metabolism and non-alcoholic fatty liver disease
27. Quercioli,Alessandra; Montecucco,Fabrizio; Mach,Francois. 2009. The role of hepatic fat accumulation in pathogenesis of non-alcoholic fatty liver disease (NAFLD). Update on the treatments of Non alcoholic fatty liver Disease (NAFLD). Cardiovascular& Hematological Disorders-Drug Targets. 9(4) 261-270
28. Sanders ME.2003. Probiotics: Considerations for human health. NutrRev 3(61):91-99.
29. Schlee M, Harder J, Köten B, Stange EF, Wehkamp J .2008. Probiotic lactobacilli and VSL3 induce enterocyte beta-defensin 2. ClinExpImmunol 151: 528-535.
30. Schuster. 1979. Estimation of free fatty acids Clini. Biochem S:24
31. Shenana, M.E., Hafez, M.E., AHia, H.F, Gafour, W.A. and Omnia, M. 2006. Effects of probiotic Kunsu-Younghurtoncholesterolemia and histological changes in cholesterol.Fed rats. Journal of Biological chemistry & Environmental sciences 2006: 1(4): 807-823.
32. Stanton MC, Chen SC, Jackson JV, Rojas-Triana A, KinsleyD, Cui L, Fine JS, Greenfeder S, Bober LA, Jenh CH.2011.InflammatorySignals shift from adipose to liver during high fatfeeding and influence the development of steatohepatitisin mice. J Inflamm (Lond); 8: 8
33. Statistical Analysis system (S.A.S).2002. Version 9.00 .S.A.S Institute Inc., cary , NC,USA
34. Young DS.2001.Effect of disease on clinical lab .Tests , 4th ed.AACC2001.Calorimetric determination of total cholesterol .
35. Young DS.2001. Effect of disease on clinical lab Tests , 4thed.AACC. Calorimetric determination of triacyleglycerol .
36. Young DS.2001..Effect of disease on clinical lab Tests , 4thed.AACC. Calorimetric determination of total lipids.
37. Young DS.2001.Effect of disease on clinical lab Tests , 4thed.AACC.Calorimetric determination of HDL Cholesterol.
38. Young DS.2001.Effect of disease on clinical lab Tests , 4thed.AACC. Calorimetric determination of VLDL Cholesterol.
39. Young DS.2001.Effect of drUgs on clinical lab Tests, 4thed.AACC Calorimetric determination of phospholipids.

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