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# INVESTIGATING THE PHYSIOLOGICAL CHANGES ON BLOOD SUGAR AND LIPID METABOLITES BY USING VARIOUS *LACTOBACILLUS* SPECIES' IN HYPERCHOLESTEROLEMIA AND DIABETIC MATURE MALE WISTAR RATS

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**ABSTRACT**

In recent years, due to the change in life style, blood sugar and the plasma lipids have increased and the change in the amount of the blood lipoproteins has resulted in more cases of diabetes and cardiovascular diseases. In this condition, due to insulin resistance of triglyceride reserves, Fat cells in blood are hydrolyzed and a great amount of free fatty acids are released into the blood. The rise in the amount of the blood fatty acids increases the absorption of these acids as well as triglyceride synthesis in the liver. The resulted high blood pressure and high plasma triglyceride leads to metabolitic disorders in carbohydrates, fats and proteins and finally a disease. Therefore useful and non-pathogen microbial species were used and given the reducing effects of different species of Lactobacillus on blood sugar, triglyceride, cholesterol, and LDL in In-Vitro, they were used in In-vivo as well.

In this study, white male vistar rats were used. They were first hyperchlostromised and then some of them were made diabetic by streptozotocin (600 mg/kg). Next microbial species were injected into the rats and the amount of cholesterol, triglyceride, LDL, HDL and blood sugar were measured in their serum.

Evaluation of the results revealed that L.*Casei* species compared to the other two species were more effective on reducing cholesterol, triglyceride and other lipid metabolites. Regarding the blood sugar, L.*Fermentum* compared to the other two species was more

effective. Of course, the reduction in cholesterol was more than triglyceride and in LDL was more than HDL. About combined hypercholestrolemic and diabetic rats, L.*Casei* species was more effective than the other ones.

Scientists have concluded that Lactobacillus by high hydrolyzing activity can play a role in decreasing cholesterol level, since free bile salts are not reabsorbed easily in the intestine. Therefore they are excreted as excreta. Following the salts excretion, the need to synthesize bile salts, which have cholesterol as their base, increases and cholesterol, is consumed in this way. As a result of it, bacteria reduce fat metabolites in the body. With regard to the blood sugar: insulin reduction through two mechanisms results in a rise in triglyceride:

First, insulin reduction leads to the activation of fat tissue lipase and the release of fatty acids from the fact tissue. These acids are transferred to the liver. Accumulation of fatty acids in liver results in stimulation of triglyceride synthesis and an increase in VLDL secretion is resulted. Secondly: the reduction of Lipase lipoprotein activity leads to a decrease in taking plasma triglyceride by tissues. As it was reflected in the results, the effect of lactic acid bacteria on reducing cholesterol and triglyceride in diabetic hypercholestrolemic rats was more than decreasing blood sugar which can be intensified by the direct effect of bacteria on the liver and pancreas cells of the rats.

**Keywords: Blood Sugar, Lipid Metabolites, Lactobacillus Species', Hypercholesterolemia, Diabetic**

## INTRODUCTION

Changes in lifestyle, causes an increase in blood sugar and blood plasma lipids which results in diabetes and cardiovascular diseases during recent years while cardiac attacks are the main death causes in diabetic persons. In high fat and blood sugar pathogenesis effects seems that in diabetic resistance, triglyceride storages in adipose cells are hydrolysed and high levels of fatty acids will release in blood circulation.( 1,4)This is the most common abnormal state that seen in the insulin-independent and fat persons. The increase of fatty acids in blood leads to an increase in their absorptions and triglyceride synthesis in liver. (7,10)Triglyceride increases in liver, stimulates Apo proteinβ synthesis and VLDL secretion in liver. (6 )VLDL increase and normal activity of cholesterol ester transfer protein (CETP) leads to VLDL triglyceride exchange with HDL cholesterol. (8,11) So enriched VLDL particles with cholesterol and HDL particles without cholesterol and enriched of triglyceride that both are arterogenics will be created.(2,5) In type I diabetics

persons with high blood pressure and plasma triglyceride, metabolic disorders in carbohydrates, fats and proteins will appear that leads to cardiovascular and kidney diseases.(21)

So in recent days researches for finding efficient drugs have increased that for the first time in this research by using efficient microbial strains and non-pathogens for medication utilization for hyperlipidemia and diabetic persons, new methods are represented that with different species of lactobacillus like *L.Plantarum*, *L.Fermentum*, *L.Casei* and using these microorganisms by considering their decreasing effects on blood sugar, triglyceride and LDL in in-vitro situations. (5,12)

**METHODOLOGY**

In this research at first we prepare bacteria with this method:

1) Lactobacillus species were cultured in MRS broth and preserved in refrigerator for doing the research they were refreshed and made pure culture of them and separate them.
2) Making microbial suspension and determining bacteria numbers by serial dilution and pour plate method in cfu/ml and determination of suspension's concentration in spectrophotometer in λ 600nm.

**Then we prepare the animals with this method:**

In this research we used white male wistar rats that hypercholestromised by highly cholesterol foods and then we created diabetes in some of them by 60 mg/kg of streptozotocin. Then for the tests we did the injections as follows:

First group: 5 rats as a control,

Second group: 35 rats that every day for 14 days which are gavazhed by 2 ml of cholesterol and then 30 rats are gavazhed by 0/02 ml of microbial suspension for 6 days in which 10 rats by *L.Casei*, 10 rats by *L.Fermentum*, 10 rats by *L.Plantarum* with 1:10subtility.

5 rats are considered as control group.

Third group: 35 rats those were diabetic by unit I.P injection from (60 mg/kg) of streptozotocin and then 30 of these rats are gavazhed by 1:10 subtility for 60 days that in each time are gavazhed by 0/02 ml of microbial suspension that 10 rats by *L.Casei*, 10 rats by *L.Fermentum* and10 rats by *L.Plantarum*. 5 rats were in control group.

Forth group: 35 rats that gavazhed by 2 ml of cholesterol for 14 days and were induced by streptozotocin and then 30 rats are gavazhed by 1:10 subtility for 60 days by 0/02 ml of microbial suspension of *L.Casei*(10 rats), *L.Fermentum*(10 rats),

*L.Plantarum*(10 rats) again 5 rats were in control group.

After passing required time, blood samples were collected from all rats and after separating rats' blood serum, cholesterol, triglyceride and other blood serum's lipids metabolites and blood sugar were tested.

Statistical Analysis: all data were investigated by ANOVA (one-way) and TUKEY tests method.

**RESULTS**

The results concerned with measuring the cholesterol levels, triglyceride and other blood lipid metabolits in rats:

Thence, one of the Acid lactic bacteria effects is decreasing cholesterol and triglyceride in in-vitro. In this study, effects of Lactobacillus strains have been evaluated in in-vivo and by evaluating the results, it was seen that cholesterol and triglyceride levels in hypercholestromised and gavazhed rats by Lactobacillus strains (especially *L.Casei*) have a spectacular decrease compared to *L.Plantarum* and *L.Fermentum*. And due to cholesterol, there was a decrease compared to triglyceride and the same case or pattern about LDL and HDL. As specified, in the first group of rats in normal situation cholesterol levels were an average about 90-110 mg/dl and normal triglyceride levels about 100 mg/dl and LDL levels around 40 mg/dl that after treating rats with 2% cholesterol solution in normal silin and 0/05% Acid colic and 30% dextrose for 2 weeks in the second and 4$^{th}$ group and an expressive difference was seen in fat metabolits levels.

**Which the following approximate changes happened:**

The normal cholesterol levels were 90-110 mg/dl then changes to 300 mg/dl and triglyceride levels were 100 mg/dl then change to 160-170 mg/dl and LDL levels were approximately 50-60 mg/dl and then changes to approximately 280 mg/dl and HDL levels were about 40 mg/dl then changes to 43 mg/dl. As a result of considering the mentioned data a remarkable change happened in cholesterol and LDL levels but no specific change had happened in HDL levels. By the next step, after treating the hypercholestromia rats, the second group, infected byLactobacillus strains, in 10 rats which were gavazhed for 20 days by L.Casei strain, these changes happened: the cholesterol levels were 300 mg/dl then decreased to 125 mg/dl, the triglyceride levels were 170 mg/dlthen decreased to 138 mg/dl, HDL levels decreased to 40 mg/dl, and LDL levels were 280 mg/dl then decreased to 54 mg/dl. While in 10 rats which belonged to the second group and were treated by *L.Fermentum* these average data were obtained: In conjunction with cholesterol levels a decrease happened, the amount was

300 mg/dl then changes to 210 mg/dl, the triglyceride levels were 170 mg/dl then decreased to 141 mg/dl, the HDL levels were 43 mg/dl then decreased to 42.5 mg/dl, and the LDL levels were 280 mg/dl then decreased to 63 mg/dl. And in 10 rats of second group, which were treated by *L.Plantarum* strain these approximate data were obtained: the cholesterol levels were 300 mg/dl then decreased to 215 mg/dl, the triglyceride levels were 170 mg/dl then decreased to 148 mg/dl, the HDL levels were 43 mg/dl then decreased to 42mg/dl, and the LDL levels were 280 mg/dl then decreased to 75 mg/dl.

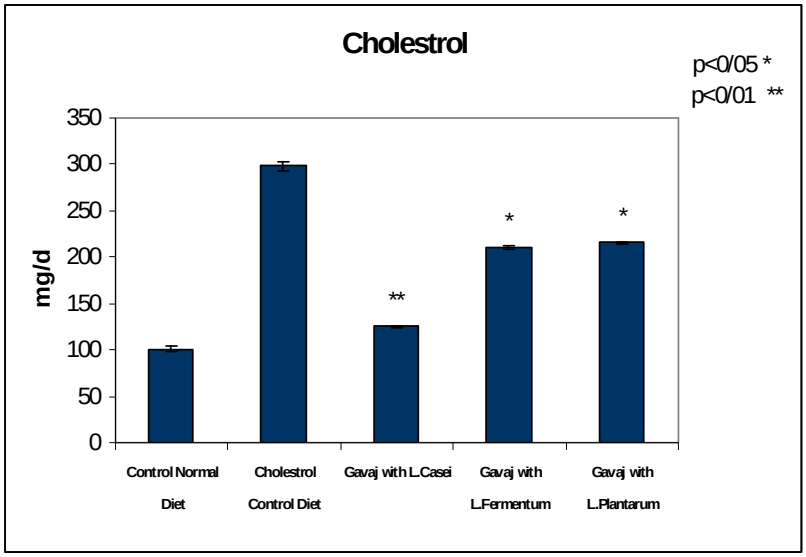



**Figure- Statistical changes of cholesterol in laboratory samples**

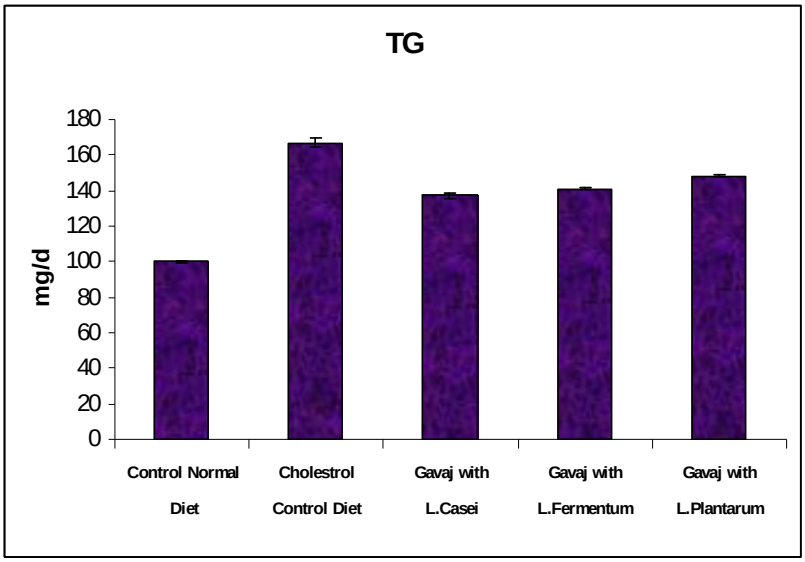



**Figure - Statistical changes of TG in laboratory samples**

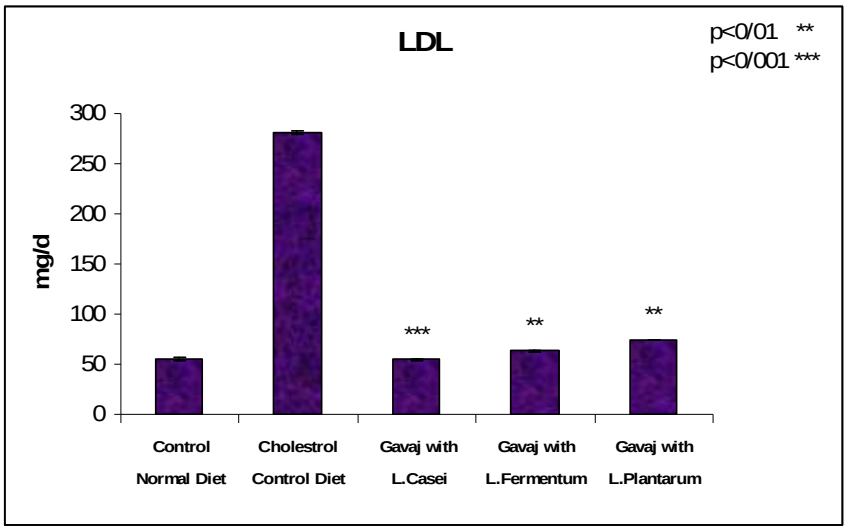



**Figure- Statistical changes of LDL in laboratory samples**

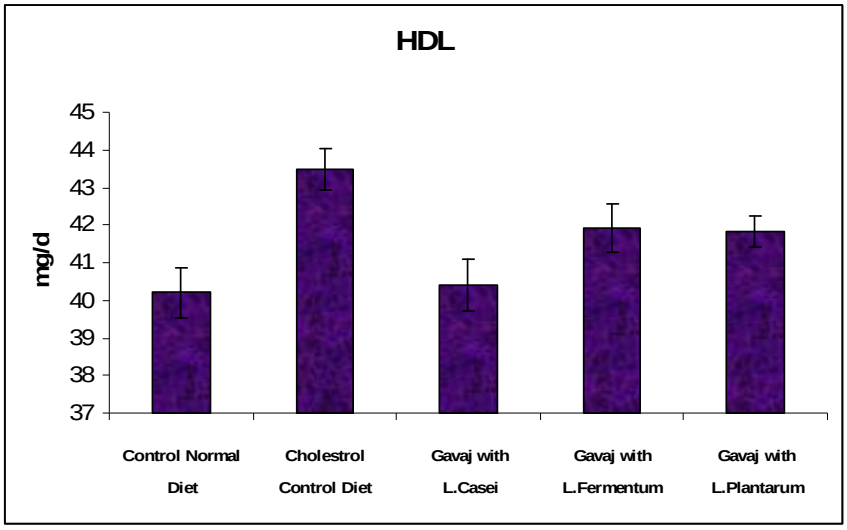


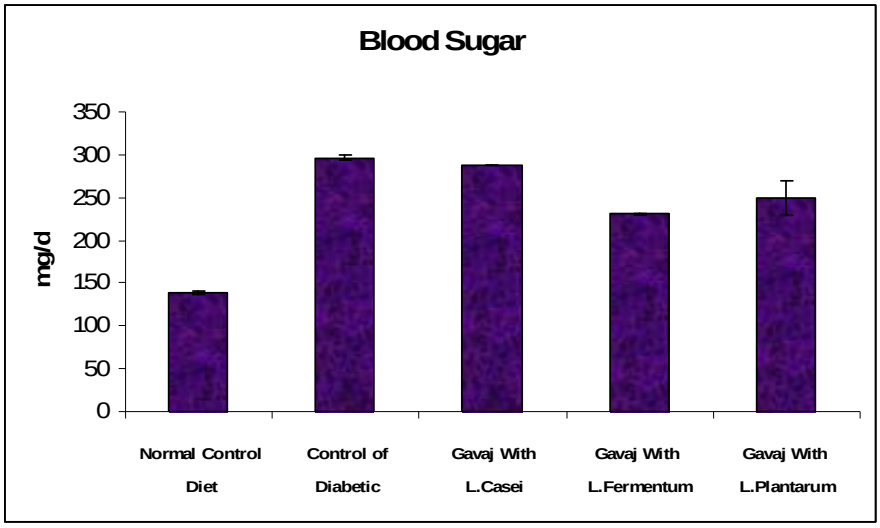

**Figure- Statistical changes of HDL in laboratory samples**

**The results of measuring the blood sugar in diabetic rats**

As one of the results of Acid lactic bacteria is reducing blood sugar in in-vitro situation, so in this study, the effect of Lactobacillus strains in in-vivo situation also tested. By evaluating the results it was seen that the glucose levels in blood serum of diabetic rats which were vaccine by streptozotocin and gavazhed by *Lactobacillus* strains (especially *L.Fermentum*) had an enormous reduction compared to those which were vaccine and gavazhed by *L.Casei* and *L.Plantarum*. In completing the previous paragraph we must say that in first group of rats, in normal situation, BS levels were approximately 135-145 mg/dl that after injecting rats with streptozotocin in third and fourth group a remarkable change was seen in their blood sugar, which rises approximately from 135-145 mg/dl to +300 mg/dl.

After treating the third group by Lactobacillus strains, these average data

were obtained after phlebotomizing the rats and testing their serum by glucose diagnostic kit for blood sugar: In 10 rats which were gavazhed by *L.Fermentum* strain, the glucose levels were approximately 300 mg/dl then decreased to 230 mg/dl, and in 10 rats which were gavazhed by *L.Plantarum* strain, the glucose levels were approximately 330 mg/dl then decreased to 288 mg/dl and after they were gavazhed by *L.Casei* strain, the glucose levels showed a reduction from 320 mg/dl to 269 mg/dl that in this case, *L.Fermentum* was more effective than *L.Plantarum* and *L.Plantarum* was more effective than *L.Casei*.




**Statistical changes of Blood Sugar in laboratory samples**

**The results of lipid metabolits and blood sugar changes in hypercholesterolemia and diabetic rats:**

The effects of Lactobacillus strains on lipid levels in blood serum of rats in the fourth group were very remarkable, otherwise no significant effects were observed on blood sugar levels in rats. It should be noted that changes in cholesterol and LDL were higher than the ones in TG and HDL and in the case of *L.Casei* strain in comparison with other strains is more considerably but by passing time and long-term effects of these strains, this change can be observed in all factors and strains.

In the statistical calculations, at first the data mean obtained from repeated three times and the error rate was measured in each case and in other section, the bacterial strains were compared with each other, as specified in the relevant tables and graphs, a remarkable difference exists in reducing cholesterol levels and triglycerides, and other lipid metabolites and glucose in the blood serum of rats, this fact indicates that the effect of Lactobacillus bacterial strains especially *L.Casei* is more than the other two ones in reducing cholesterol levels and triglycerides and other lipid metabolites and a reducing effect of *L.Fermentum* on

blood sugar is more than the other ones. And in case of combined hypercholesterolemia and diabetic rats, the effect of *L. Casei* was more remarkable than the other strains.

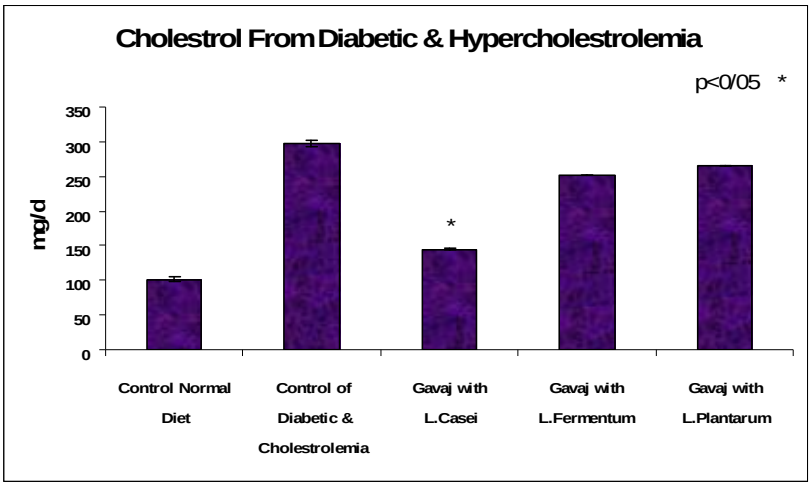



**Figure - Statistical changes of cholesterol in hypercholesterolemia and diabetic samples**

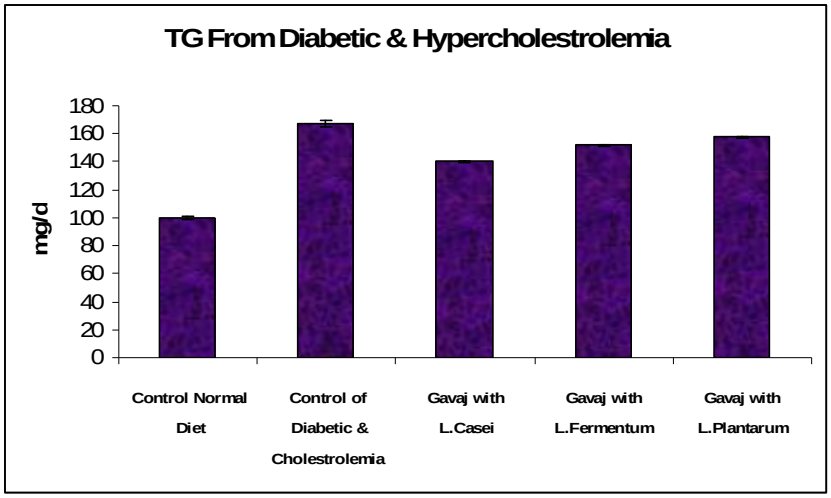



**Figure - Statistical changes of triglycerides, in hypercholesterolemia and diabetic samples**

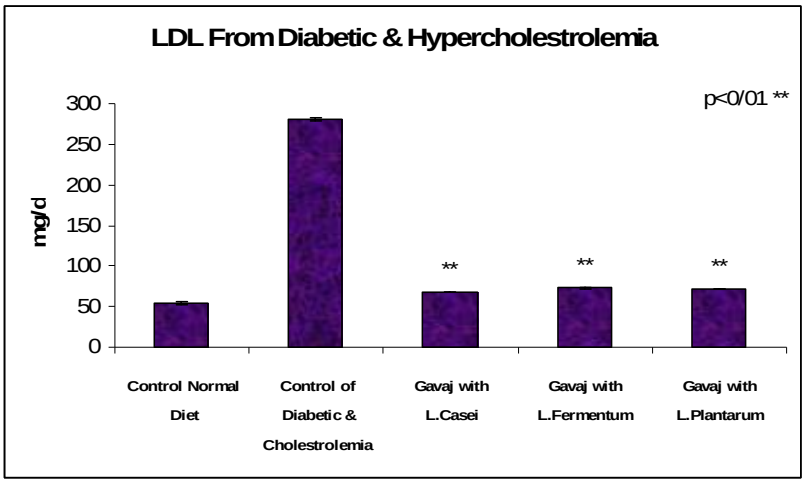



**Figure - Statistical changes of LDL in hypercholesterolemia and diabetic samples**

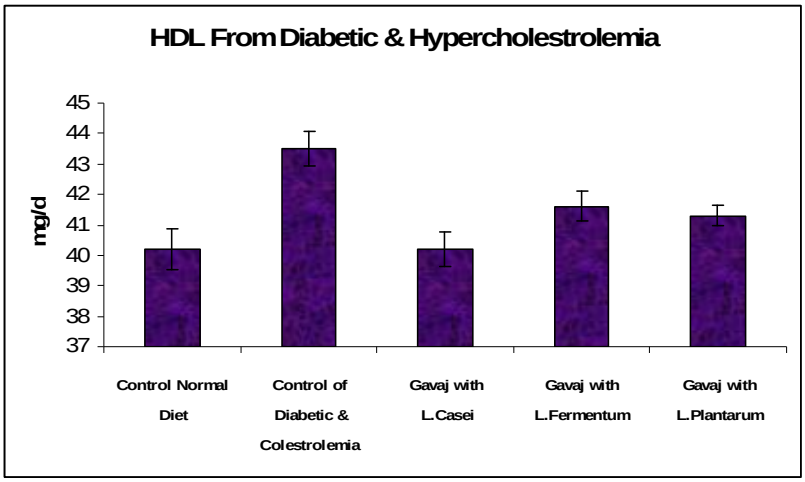



**Figure -Statistical changes of HDL in hypercholesterolemia and diabetic samples**

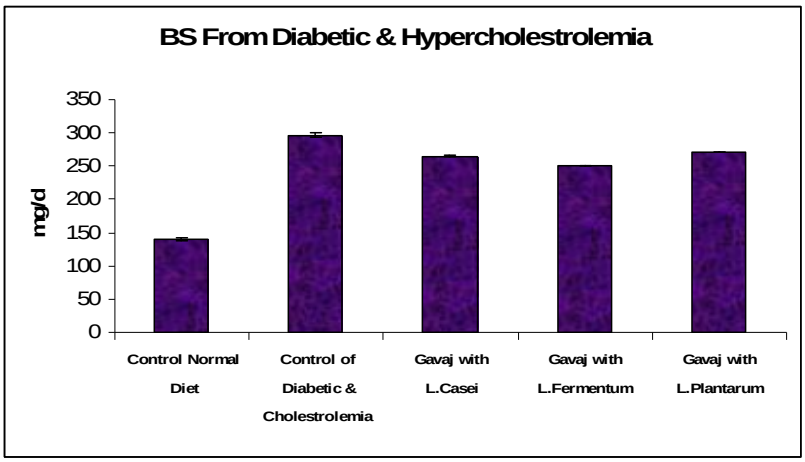



**Figure -Statistical changes of Blood Sugar in hypercholesterolemia and diabetic samples**

## DISCUSSION AND CONCLUSIONS

In various studies which have been conducted so far, different animal have been used to create hyperlipidemia models such as: (Rabbit, Rat, and Guinea pig) that one of the typical and appropriate models for the measurement of lipid metabolite which are similar to humans is rat. In rat's body major parts of IDL before transforming into LDL will be removed by the liver, so the LDL level in normal rats are low and the predominant lipoprotein in their body is HDL.

To create hyperlipidemia models in rats different methods are available that the most common of these methods is cholesterol-rich and colic aciddiets, in this study this common method were used. Because it causes an increase in levels of triglyceride, cholesterol and LDL compared to control group rats. Indicates the best model to create hyperlipidemia. Several studies have shown that diets rich in fat increases plasma lipids, especially triglycerides (Arsov, 1995)this phenomenon happens due

totransformationof extra energizing foods into fatty acids and eventually triglyceride(3).On the other hand, the cholesterol in this diet increases the cholesterol content of liver cells. This process due to the SREB path leads to a decrease of endogenous cholesterol synthesis in decreasing LDL receptor expression. Reduction of LDL receptors also decreases LDL hepatic uptake and thereby increases blood cholesterol and LDL (Michetti, 1999). Colic acid in the diet by inhibiting the enzyme -7alpha-hydroxylase (cypA1) causes a decrease in catabolism of cholesterol, while it increases plasma cholesterol (18).

As assessing the results of this study it was seen that the cholesterol levels and triglyceride in hypercholesterolemia rats which were gavazhed by, Lactobacillus strains particularly *L.Casei* had significantly reduced compared to *L.Fermentum* and *L.Plantarum* ones, the reduction in cholesterol levels was more remarkable than triglyceride and about LDL the reduction was more remarkable than HDL, which is consistent with the results of researchers. The reviews of some researches related to this study about the accuracy of work schedule are listed below:

Ota and colleagues (1999) showed that the antimicrobial effect of lactic acid bacteriamay be due to production of lactic and acetic which results a decrease in pH lumen and these bacteria may produce compounds such as hydrogen peroxide or bacteriocins that these compounds may act as an antibiotic substance [19].

De Smet and colleagues (1995) have suggested that Lactobacillus with high hydrolase activity canparticipate in reducing cholesterol levels because free bile salts, cannot be easily reabsorbed in the intestine. So they will be exorcised in stool form [14].

Marshall and colleagues (1998), in order to test the effect of cholesterol by hydrolase activity of lactic acid bacteria theory, conducted a study; the consumption of probiotic products in the samples decreases serum cholesterol but the absence of consuming these compounds in two weeks, returning cholesterol levels to a high level [17].

De Rodas et al (1996) noted that the digestion and absorption of cholesterol and conjugating of bile salts involved in lowering cholesterol are associated with hepatic intestinal cycling. [13]

Klaver and colleagues (1999) stated that the hydrolase activity of Lactobacillus deposits cholesterol in acidic conditions. Although this explanation is convincing but it should be considered that in the part of small intestine which bile salts and cholesterol absorption happens, pH below 6 is not so

common therefore, this assumption may not be as effective in lowering cholesterol as the sole mechanism [16].

Marshall & Taylor (1998) reported that the removal of cholesterol by Lactobacillus takes place in the presence or absence of bile salts and cholesterol deposition at low pH cannot be completely a cause of eliminating cholesterol in in-vitro. They mentioned that there may be a physical relationship between bacteria and cholesterol levels that by the ability of bacteria to conjugate bile salts, this will increase further [17]. Studies indicate that in the culture of these bacteria even in the absence of bile salts, levels of Lactobacillus are seldom able to bind to cholesterol which is added to the environment and with increasing concentrations of free bile salts, the amount of coupling between cholesterol and bacterial levels increases and It has been said that this is a specific binding thus, these bacteria play their role in reducing cholesterol.(20)

The third factor is the properties of Lactobacillus, these bacteria species are resistant to stomach acid and bile and intestine ducts and they are tolerant to levels of pH (2 to 3) and high concentrations of bile (2/0 to 2 percent) and there is also a material called hydroxy methyl glutamate in probiotic products That are produced by microorganisms during fermentation and inhibits cholesterol synthesis in the body and thereby reduces cholesterol levels in the blood.(15)

And the other point is that beta-galactosidase enzyme in Lactobacillus, breaks down the lactose in animals' meals to produce positive lactic acid so in this way prevents the fat storage and adipose tissue in the animals' body.

Another factor in the reduction of cholesterol and triglycerides and lipid metabolites by Lactobacillus is the hydrolase enzyme activity of Lactobacillus which is conjugated in the cycle of the intestine - liver thus, influence on the metabolism of bile salts which are the final products of cholesterol metabolism.(9) In this way that we separated the steroids from glycine and taurine enzymes then bile salts make released that lowers cholesterol and LDL in the blood serum of the animal. The released salts will not flow into the bloodstream because they will be exorcised through stool.

So following salt excretion, the need to synthesis of bile salts that cholesterol is a precursor of them is reinforced and the cholesterol may be used by this method and hydrolase activity caused by bacteria, resulting in reducing animal fat metabolites.

Generally, the consumption of Lactobacillus products can be considered as

an effective medicine to reduce cardiovascular diseases and cholesterol, blood triglyceride, practically in the community. And can be applied as a drug with very few side effects as well as other useful effects such as anti-microbial activity in the gastrointestinal tract, curing acute inflammation of the stomach and intestines, curing diarrhea, resistance to digestive enzymes and etc. Hopefully by producing these bacteria product and promoting its use in our community many potential risks of myocardial infarction, thrombosis and increase of cholesterol and triglyceride in blood and the consequences of them may be prevented.

**The effect of lactic acid bacteria on the amount of BS in the blood serum of rats:**

Regarding streptozotocin injection significantly increased triglyceride only. Slight increase in cholesterol levels and LDL can be seen that from the statistical viewpoint is not significant. Plasma triglycerides increase by streptozotocin is due to a decrease in insulin secretion. Decrease in insulin secretion leads to an increase in blood triglyceride by two mechanisms:

Firstly: Decrease in insulin leads to activation of lipase in adipose tissue and release of fatty acids from adipose tissue, these fatty acids may be transferred to liver, accumulation of fatty acids in the liver leads to stimulation of triglyceride synthesis and finally increases VLDL secretion.

It should be noted that in long period insulin will modulate the production of extra VLDL by decreasing the production of Apo protein involved in the VLDL structure.

Secondly: Reduction of lipoprotein lipase activity leads to reducing uptake of plasma triglycerides by peripheral tissues.

Reports indicate that increases the expression and activity of intestinal ACAT which is a key protein in cholesterol absorption through intestine. Injecting streptozocin after10 weeks also reduces the expression and activity of hepatic LDL receptors. However, using streptozotocin only in long period is capable of causing hypercholesterolemia in rats and using it in short period (4 weeks) is not able to create hypercholesterolemia and only facilitates the creation of hypercholesterolemia in hyperlipidemic rats.

Injections during animals' feeding with cholesterol and colic acid-containing foods may increase triglycerides, cholesterol and LDL levels effectively. The results coordinate numerous reports showing that cholesterol-containing dietary in diabetic animals may increase plasma cholesterol. Healthy rats are resistant to cholesterol-containing foods but Streptozotocin

facilitates the creation of hypercholesterolemia in animals by increasing the expression of ACAT, the results of this study confirm the findings of other researchers. So that by evaluating the results it was observed that glucose levels in blood serum of diabetic rats inoculated with streptozotocin and gavazhed by Lactobacillus strains especially *L.Fermentum* have reduced remarkably compared to *L.Casei* and *L.Plantarum* ones. The effect of lactic acid bacteria on reducing cholesterol and triglycerides in hypercholesterolemia diabetic rats is more than blood sugar reduction this could be intensified by direct effect of bacteria on the cells of the liver and pancreas of rats.

Damage to pancreatic beta cells, which is associated with reducing beta-cell regeneration capacity, can lead to diabetes. Increase of oxidative pressure may be one of the causes of diabetes and increases constantly during diabetes. Due to natural weakness of the antioxidant system in pancreatic beta cells any agent which increases oxidative pressure causes an increase in progressive destruction of pancreatic beta cells. Conversely, strong antioxidant agents rebuild pancreatic beta cells and increase insulin production.

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