IMPACT OF PROBIOTICS LACTOBACILLUS_RHAMNOSUS  AND LACTOBACILLUS_CASEI ON LIPOGLYCEMERIC AND ANTIOXIDATIVE STATUS OF DIABETIC RATS

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ABSTRACT: Probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit. There are very limited studies on antidiabetic effect of lactic acid bacteria despite their potentiality in reducing the risk of diabetes onset. The present study explored the effect of administering live culture of L. rhamnosus [MTCC No 8712] and L. casei [MTCC No 1408] in STZ induced diabetic albino male rats. Animals (approximately aged 6 months) were grouped into three in a randomized fashion that average weight of animals in each group would be 150-250 gms. Normal feed pellets and water ad libitum were provided to animals throughout the experiment. As Group 1-Control Group, Group 2-Positive control Group were on only insulin treatment (1 unit /day) and the Group 3-Treated Group were on probiotics treatment. The treatment was continued for 30days. Body weight, blood glucose, lipid profile, antioxidative status was analyzed by standard protocol. After subculturing L. rhamnosus and L. casei and standardizing with Mac Ferlan's device, the mixture culture was administered to the treatment group of animals by intraperitoneal injection (~73x10^6 cfu/g). With respect to body weight of the three groups after the treatment period, the treated group was found to have a drastic reduction in the body weight. While before treatment there was no significant difference. Glucose levels after treatment in both the probiotic and insulin treated groups were similar and comparable. Administration of the selected LAB Species L. rhamnosus and L. casei ameliorated glycemic status of the treated animals. Only Glutathion level was markedly reduced in positive control groups (p<0.001) (insulin treated) and there was only a substantial reduction in the GSH level of probiotic treated (p<0.05). There was a significant reduction in total cholesterol, TGL, VLDL (P<0.001) in the probiotic treated group after treatment whereas there was no impact on LDL, HDL concentration. Hence, LAB strains of Lactobacillus rhamnosus and Lactobacillus casei may represent potential therapeutic age in reducing glycemic level, body weight and lipid profile but not much on antioxidative status except preventing considerable depletion of GSH.

INTRODUCTION: Probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit.

In the past few years, scientific and commercial interest in probiotics has grown rapidly as these microorganisms have shown potential benefits, primarily in prevention, in health conditions such as diarrhea, necrotizing enterocolitis, and allergies. Probiotics are “live microbial food ingredient that is beneficial to health”. Also Live microorganism or a live food based microbial culture that is beneficial to health is known as Probiotics.
Inflection of human health through metabolic effects of probiotics gains momentum as various studies revealed that particular strains with specific metabolic traits can offer tangible health benefits

- Consumption of Lactobacillus probiotic strains would increase gut lactobacilli microflora and may enhance lactose digestibility in lactose intolerance individuals.

- There exists emerging evidence that probiotic strains in animal models with specific traits can enable gut bioactivation of phytoestrogens as well has substantiated that probiotics exert metabolic effects: halitosis, oxalate metabolism.

- Chronic hyperglycemia and hyper insulimia primarily stimulates the formation of advanced glucose endproducts (AGEs), which lead to an overproduction of reactive oxidative species(ROS). Protein glycation and increased oxidative stress are the two main mechanisms involved in biological aging. Oxidative stress plays a major role in the pathogenesis and progression of diabetes.

- Mofid et al. 2011 conducted an experimental study on diabetic rats using the supplementation of probiotic yogurt containing Lactobacillus acidophilus La5 and Bifido-bacterium lactis Bb12 and the results reavealed that Probiotic yogurt significantly decreased fasting blood glucose and increased erythrocyte superoxide dismutase and glutathione peroxidase activities and total antioxidant status.

- A significant delay in the elevation of glucose intolerance and hyperglycemia was observed by Tabuchi et al. (2003) after administering Lactobacillus GG during progression of streptozotocin induced diabetes in rats.

Among various functional foods with an antioxidant effect, probiotic foods have been reported to repress oxidative stress. The recent best known examples of functional foods is fermented dairy products containing lactic acid bacteria. However, there are very limited studies on antidiabetic effect of lactic acid bacteria despite their potentiality in reducing the risk of diabetes onset.

A study conducted by Matsuzaki et al. showed that oral administration of Lactobacillus casei in KK-Ay mice significantly brought down plasma glucose levels and inhibited the production of cell specific CD4+T cells and cytokines (interferon-gamma and interleukin-2) that are leading factors for induction of autoimmune diabetes.

Yet another study demonstrated that autoimmune destruction of pancreatic β-cells was also inhibited by the gavage feeding of L. casei in NOD mice. In contrast to these observations, in the present study direct administration of cultures was used.

Considering the facts in the above studies, the present study explored the effect of administering live culture of L. rhamnosus [MTCC No 8712] and L. casei [MTCC No 1408] in STZ induced diabetic albino male rats.

MATERIALS AND METHODS:

Animals: Male albino rats (6months) of average weight of 150-250 gms were used for the study. Animals were bred at PSGIMS&R (PSG Institute of Medical Science and Research) animal house and protocol has been approved by Institutional Animal Ethical Committee (IAEC). Animals were brought to the lab and allowed the animals to acclimatize for 14 days. Animals (approximately aged 6 months) into three were grouped in a randomized fashion that average weight of animals in each group would be 150-250 gms. Normal feed pellets and water ad libitum were provided to animals throughout the experiment. Animals were maintained 12 hrs light/dark condition. Animals (6 in a cage) were housed with husk as a bedding material under the controlled temperature of around 25±2°C and 50% humidity condition according to CPCSEA guidelines.

Thus 18 Male Albino rats were divided into 3 groups( 6 in each group ) as Group 1-Control Group, Group 2-Positive control Group were on only insulin treatment (1 unit /day) and the Group 3-Treated Group were on probiotics treatment such as Lactobacillus casei and Lactobacillus rhamnosus. The insulin and probiotic treatment was continued for 30days. According to plan of study, animal body weight screening, weekly assessment of blood glucose and post treatment
blood was drawn to check lipid profile and antioxidative status such as Super oxide dismutase, Glutathione and thiobarbituric acid reactive substances by standard methods.

**DRUG AND PROBIOTICS:**

- **Streptozotocin – 60mg/kg body weight.** The drug STZ was obtained from P.S.G College Of Pharmacy - Coimbatore-India
- **Probiotics-** *Lactobacillus casei and Rhamnosus* (~73x10⁸ cfu/g)

Probiotic strains were obtained from IMTECH – Chandigarh, India.

**Induction of Diabetes:** Rats were fasted for 12-hr before diabetes was induced using Streptozotocin. Two sets of rats received a single intraperitoneal injection of 50mg/rat of STZ (Sigma, St.Louis, MO, USA). STZ was freshly dissolved in 0.05 M citrate buffer, PH 4.5. For the intraperitoneal injection of STZ, the rat was held in one hand in dorsal position, the injection site was swabbed using povidone– iodine solution and the designated amount of STZ was injected in the caudal abdominal cavity using sterile 25guage needle. However, after 48 hours when the blood sugar was analysed it was observed that all the 12 of the rats were hyperglycemic.

**Administration of Probiotics:** After subculturing *L. rhamnosus* and *L. casei* and standardizing with Mac Ferlan’s device, the mixture culture was administered to the treatment group of animals by intraperitoneal injection (~73x10⁸ cfu/g).

**Preparation of Bacterial Cultures:** The two strains used in this study, *Lactobacillus rhamnosus* [MTCC NO 8712] and *Lactobacillus casei* [MTCC NO 1408], were obtained from IMTECH, Chandigarh, and stored at -80°C. After recovery at 35°C for 30 minutes in a biochemical incubator, the two strains were separately inoculated into MRS liquid broth and placed in an anaerobic workstation at 35°C for 24 hours. The strains were harvested by centrifugation at 2000 × g for 20 minutes, washed twice with normal saline (0.9% NaCl), and resuspended at (~73x10⁸ cfu/g) in sterile normal saline. Subsequently, 2 mL of the solution was administered intragastrically to the rats daily.

**Animal body weight:** All the rats were fed on standard feed pellets and water ad libitum. On the zero-th day before treatment, body weight of all the animals was measured which was then followed by regularly noting the changes in body weight on every third consecutive days for a month by using a digital weighing scale.

**Measuring Blood Glucose Level:** Sampling from the tail tip is appropriate when only small volumes of blood are needed.

Blood glucose levels were being measured from the animal tail tip, it is a simple procedure that was carried out in P.S.G animal house where 1-2 mm of tissue is cut from the tail tip distal to the bone with a scalpel, and then blood is obtained by direct flow or by gently massaging (‘milking’) the tail and collecting 0.5ml of blood on glucose test strips. Immediately after the sample collection on glucose strips it is inserted into the glucometer to measure the blood glucose. Following the initial cut, a 2-hour recovery period is recommended and then rat was placed back into its home cage. The blood was drawn before inducing diabetes, after inducing diabetes and finally after the treatment but before sacrificing the animals.

**Cardiac Puncture:** The rats were held by the scruff of skin above the shoulders so that its head is up and its rear legs were down. 1 ml syringe and a 22 gauge needle were used. 5 mm needle was inserted from the center of the thorax towards the animal’s chin, 5-10 mm deep, holding the syringe 25-30 degrees away from the chest.

- Animal was laid on back and syringe was pushed vertically through sternum
- Animal was laid on side and needle was inserted perpendicular to chest wall

0.5 cc of air was withdrawn to create a vacuum in the syringe. Needle must be removed from under the skin and try a slightly different angle or direction. When blood appeared in the syringe, it was held still and gently plunger was pulled back to obtain the maximum amount of blood available. Pulling back on the plunger too much was avoided inorder to prevent the heart to collapse. Once the blood flow stopped, the needle was pulled out slightly.
Estimation of Antioxidative Properties: MDA is the end – product of lipid peroxidation, which is a process where reactive oxygen species degrade polyunsaturated lipids. This compound is a reactive aldehyde and is one of the many reactive electrophilic species that cause toxic stress in cells and forms advanced glycation end products.

Lipid peroxidation in terms of thiobarbituric acid reactive substances was measured by the method of Ohkawa et al. Reduced glutathione was determined according to the method of Ellman. The SOD was estimated by using Marklund and Marklund Method modified by Nandhi.

Plasma Lipids: Total cholesterol, triacylglycerol, and HDL-C levels in plasma were determined by enzymatic kits that were procured from Bayer’s Diagnostics Pvt. Ltd., Baroda, India. Low-density lipoprotein (LDL-C) and very low-density lipoproteincholesterol (VLDL-C) levels were calculated by using Friedewald’s equation.

Statistical Analysis: All the data has been expressed as Mean ± SD. One way ANOVA followed by Tukey's multiple comparison test used to find statistical difference between probiotics, drug treated group and control group. SPSS – Version 12 software was used for all statistical calculations. Statistical significance was set as p<0.001 and p<0.05.

With reference to food intake of the animals of positive control group was found to have a food intake significantly less (p<0.001) than the normal control and probiotic treated groups.

With regard to the quantity of water consumed by all the three groups, it was found that quantity varied significantly high (p<0.001) particularly, the diabetes induced animals of positive control group and treated group were found to consume more water (70.98 ± 2.14 and 66.74 ± 2.7) respectively. Whereas with respect to initial body weight of the animals before the treatment, all the groups were found to have no difference.

However with respect to body weight of the three groups after the treatment period, the treated group was found to have a drastic reduction in the body weight.

Blood glucose level indicate glycemic status of the body. Glucose value initially assessed before inducing diabetes in positive control and treated group and the normal control group (who were not to be induced with diabetes) were found to be uniformly similar across groups.

Glucose levels after inducing diabetes in the animals of positive control group and treated group were fairly similar but whereas glucose value of the normal control group was significantly lower (p<0.001) as probably it was not induced with diabetes.

However, glucose levels after the study period that is final glycemic level of control group was almost comparable with their initial level whereas it was differing significantly (p<0.001) between pre and post treatment glucose values of the control group compared with counterparts namely untreated and treated groups.

Nevertheless, with regard to antioxidative status of experimental rats and their counter part of control group, it was found to have SOD and TBARS fairly uniform across all three groups. While that of positive control group was found to have a marked reduction in glutathione level in comparison with control group (p<0.001). Similarly probiotic treated group was also found to have a substantial reduction in their glutathione level (p<0.05) in comparison with their counter group.

Lipid parameters of the animals may vary according to the metabolic alteration warranted by disease condition. In the present, study total cholesterol, triglycerides, VLDL vary significantly (p<0.001) among the three groups namely control group, positive control group, treated groups after the treatment period. After the treatment period lipid parameters namely, total cholesterol, TGL, VLDL, LDL were found to be on higher side (p<0.001) in the positive control group, which could be attributed to hyperglycemia and just because as they were treated with one unit of insulin per day. However HDL levels of all the three groups were not having any significant variations.

As represented in table 2, it is understood that the body weight of all the three groups vary significantly.
Especially the body weight of treated group was significantly lower in comparison with normal control group (p<0.001) and positive control group (with diabetes) but who received 1 unit of insulin/day may be insulin being an anabolic steroid would have mitigated the body weight maintenance that was comparable with non diabetic control group.

Also the same table shows a significant difference in the blood glucose levels after the treatment period. Treated animals were found to have a significantly a higher levels when compared to both positive control and normal control group (p<0.001).

**DISCUSSION:** Diabetes is a chronic pathological disorder that is influenced by various life style factors. Diet plays a pivotal role in influencing the glycemic status. Functional foods are probably understood to influence the glucose homeostasis. Probiotics have been defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” 8. Addition of probiotics is a mean to make a food functional (FAO/WHO, 2001) 1. Fermented dairy products containing probiotics lactic acid bacteria and bifido bacteria are presently well known examples of functional foods 18. Dahi, a traditional Indian fermented milk product and yogurt, a western fermented diary product are considered a functional food due to its nutritional and therapeutic benefits 19. Probiotics can be found in dairy and non diary products 24.

However there are few studies available to show the potential benefits of LAB in diabetes. In the present study, the LAB species culture containing *Lactobacillus rhamnosus* and *Lactobacillus casei* was administered intraperitoneally. The results revealed that the administration of above mentioned probiotics lead to impart significant benefits in the STZ induced rats.

The diabetes induced rats were found to consume standard diets differently from that of control group initially, however the diabetes per se in the positive control group at the end of the study lead to a significant poor intake. Similarly the mean water intake at the end of the study was significantly between three groups especially, diabetes induced rats who were not treated with probiotics but only with 1 unit of insulin/rat per day to control diabetes were found to consume more water and their body weight (Table 2) was comparable with that of control group. Whereas the body weight of the treated animals who were not on insulin were having a markedly lower body weight.

Such drastic reduction in body weight of the treated group may be attributed to the reason that as these animals were not receiving any insulin for controlling glycemia. Actually, insulin was given at 1 unit/rat/day to positive control group inorder to prevent major morbidity and sudden mortality. And as insulin being anabolic steroid, which could have increased the weight of the insulin treated diabetic rats.

However glucose levels after induction of diabetes were similar across both the diabetic rat groups while post treatment glucose level were also almost similar, Such a similarity in the blood glucose levels indicate that LAB (*Lactobacillus rhamnosus* and *Lactobacillus casei*) were able to control glucose level of the experiment group relatively equal to the group treated with minimum level of insulin. However, blood glucose level of normal control group remained almost consistant throughout the study period.

Ning xie *et al* (2011) 25 in their study, observed that rats fed with *Lactobacillus plantarum* 9-41-A lost weight significantly (p<0.05) in comparison with model groups rats and decreased in liver and adipose tissue weight contributed to this finding. In our study also, the treated animals lost weight (table 1) which could be ascribed to both untreated diabetes per se and may be reduction in the weight of visceral organs and adipose tissue.

Kalavathy *et al* (2003) 26 had reported that administration of a mixture of 12 strains of LAB at 1% in the basal diet of broilers significantly increased body weight gain however lowered serum cholesterol level. In contrary to this, in our study the treatment with LAB (2 strains) decreased body weight of experimental group which may probably ascribed to diabetes status that too without any devastating morbidity or sudden mortality for 4 weeks till they were sacrificed.
When homeostasis of gut microflora could be considered to be environment factors involved in the development of obesity and its associated metabolic disorders\textsuperscript{26}.

**TABLE 1: FOOD AND WATER INTAKE AND BODY WEIGHT GAIN IN EXPERIMENTAL AND CONTROL RATS**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Positive control group</th>
<th>Treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/d)</td>
<td>17.73 ± 0.57\textsuperscript{a}</td>
<td>16.43 ± 0.67 \textsuperscript{***}</td>
<td>17.87 ± 0.74 \textsuperscript{a}</td>
</tr>
<tr>
<td>Water intake (mL/d)</td>
<td>58.86 ± 3.31 \textsuperscript{***}</td>
<td>70.98 ± 2.14 \textsuperscript{***}</td>
<td>66.74 ± 2.7 \textsuperscript{***}</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>156 ± 18.54 \textsuperscript{a}</td>
<td>160 ± 19.14 \textsuperscript{a}</td>
<td>163 ± 11.37 \textsuperscript{a}</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>248.62 ± 32.47 \textsuperscript{a}</td>
<td>241.97 ± 39.00 \textsuperscript{a}</td>
<td>200.12 ± 26.98 \textsuperscript{***}</td>
</tr>
</tbody>
</table>

* Values are presented as Mean ± SD. Values with different superscript letters are significantly different \textsuperscript{*} (P < 0.05) and \textsuperscript{**} * (P < 0.001)

**TABLE 2: BODY WEIGHT, BLOOD GLUCOSE, BLOOD LIPIDS AND ANTI-OXIDATIVE LEVELS AFTER STUDY PERIOD**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Positive control group</th>
<th>Treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>248.62 ± 32.47 \textsuperscript{a}</td>
<td>241.97 ± 39.00 \textsuperscript{a}</td>
<td>200.12 ± 98 \textsuperscript{***}</td>
</tr>
<tr>
<td>Glucose Initial Values before inducing diabetes</td>
<td>87.39 ± 7.03 \textsuperscript{NS}</td>
<td>89.02 ± 7.87 \textsuperscript{NS}</td>
<td>87.77 ± 7.14 \textsuperscript{NS}</td>
</tr>
<tr>
<td>Glucose after inducing Diabetes</td>
<td>87.60 ± 7.48 \textsuperscript{***}</td>
<td>287.04 ± 44.94 \textsuperscript{a}</td>
<td>295.32 ± 34.97 \textsuperscript{a}</td>
</tr>
<tr>
<td>Glucose post treatment</td>
<td>90.87 ± 8.75 \textsuperscript{***}</td>
<td>196.09 ± 25.19 \textsuperscript{a}</td>
<td>206.85 ± 42.75 \textsuperscript{a}</td>
</tr>
<tr>
<td>TBARS</td>
<td>0.29 ± 0.15 \textsuperscript{NS}</td>
<td>0.33 ± 0.12 \textsuperscript{NS}</td>
<td>0.31 ± 0.11 \textsuperscript{NS}</td>
</tr>
<tr>
<td>GSH</td>
<td>42.50 ± 9.3 \textsuperscript{NS}</td>
<td>24.28 ± 3.9 \textsuperscript{***}</td>
<td>30.16 ± 3.7 \textsuperscript{a}</td>
</tr>
<tr>
<td>SOD</td>
<td>2.66 ± 1.08 \textsuperscript{NS}</td>
<td>2.35 ± 0.96 \textsuperscript{NS}</td>
<td>2.95 ± 1.20 \textsuperscript{NS}</td>
</tr>
<tr>
<td>TOTAL CHOLESTROL</td>
<td>78.60 ± 5.66 \textsuperscript{***}</td>
<td>107.81 ± 5.28 \textsuperscript{***}</td>
<td>90.27 ± 4.66 \textsuperscript{NS}</td>
</tr>
<tr>
<td>TGL</td>
<td>45.73 ± 2.15 \textsuperscript{***}</td>
<td>87.80 ± 5.62 \textsuperscript{***}</td>
<td>63.89 ± 5.60 \textsuperscript{***}</td>
</tr>
<tr>
<td>LDL</td>
<td>58.58 ± 3.77 \textsuperscript{***}</td>
<td>87.93 ± 5.89 \textsuperscript{NS}</td>
<td>65.90 ± 4.96 \textsuperscript{NS}</td>
</tr>
<tr>
<td>HDL</td>
<td>23.11 ± 1.96 \textsuperscript{NS}</td>
<td>20.07 ± 2.74 \textsuperscript{NS}</td>
<td>23.19 ± 2.22 \textsuperscript{NS}</td>
</tr>
<tr>
<td>VLDL</td>
<td>9.14 ± 0.42 \textsuperscript{***}</td>
<td>17.56 ± 1.12 \textsuperscript{***}</td>
<td>12.77 ± 1.11 \textsuperscript{***}</td>
</tr>
</tbody>
</table>

*** Values differ from control group at (P < 0.001)
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FIGURE 5: GLUCOSE AFTER INDUCING DIABETES

FIGURE 6: THIOBARBITURIC ACID REACTIVE SUBSTANCES

FIGURE 7: GLUTATHIONE

FIGURE 8: SUPEROXIDE DISMUTASE

FIGURE 9: TOTAL CHOLESTROL

FIGURE 10: TRIGLYCERIDES

FIGURE 11: LOW DENSITY LIPOPROTEIN

FIGURE 12: HIGH DENSITY LIPOPROTEIN
Modulation of the intestinal microbiota by supplementation with certain LAB strain may lead to body weight reduction²¹. Probably the administration of LAB strains in the present study would have also rendered a dramatic weight loss.

Yet, when we looked at the difference between the glucose levels immediately after induction of diabetes and post treatment period, administered animals had a substantial reduction (31.68%) even without insulin administration whereas, upon insulin treatment the positive control group was able to accomplish only 29.95% decrease in their mean glucose level after the study period.

Conversely, in the study conducted by Yadav et al. (2007)²⁷ revealed that high fructose feeding lead to a accumulation of significantly more glycogen in a livers of rats fed with high fructose diet than the animals fed with high fructose and Indian curds (Dahi). However the mechanism by which dahi in their study reduced the accumulation of glycogen in the liver was not known or reported. In the present study also the mechanism of Lactobacillus rhamnosus and Lactobacillus casei in lowering the glucose levels after the treatment period was not known.

Chronic hyperglycemia may progressively lead to glucotoxicity which in turn may ameliorate lipid metabolism and its peroxidation. Thus, chronic hyperglycemia may exacerbate oxidative stress as a consequence of formation of amoleoride compounds. Many medical therapies include antioxidants like vitamin E, C and the mineral Selinium for preventing metabolic syndrome and CVD.

In an effort to explore the effect of LAB strains Lactobacillus rhamnosus and Lactobacillus casei on oxidative stress, we observed that the probiotics significantly decreased glutathione (p<0.005) while these strains did not have any impact on thiobarbituric acid-reactive substances and superoxide dismutase. The mean SOD and Tbars levels were on par with each other among the three groups even after the treatment period. Conversely, consistent hyperglycemia could bring in oxidative stress which in turn can exacerbate GCS phosphorylation and consequently decrease GCS transcription or activity²³.

The loss or depletion GSH could also be possible due to influence of inducible Nitric oxide synthase and Nitric oxide production²⁴. And inducible Nitric oxide synthase and Nitric oxide production may be augmented by oxidative stress. When more RoS are generated in the case of uncontrolled diabetes, more glutathione actively involved in scavenging free radicals removing hydrogen and lipid peroxides. Because of the cysteine residue, GSH is readily oxidized non-enzymatically to glutathione disulfide (GSSG) by electrophilic substances (e.g. free radicals and reactive oxygen/nitrogen species). The GSH efflux from cells contributes to a net loss of intracellular GSH.

Cellular GSH concentration are reduced markedly in response to protein malnutrition. Oxidative stress and many physiological conditions³⁰. In line with Wu’s (2003)²⁹ report, the diabetes animals in the present study had a significant reduction in the GSH values which could be ascribed to oxidative stress that would have depleted the GSH levels. However, though the treated animal was not treated with any antidiabetic drug, still they have a significant reduction of GSH (P<0.005) which is suggestive of influence due to probiotic administration. Therefore, though the mechanism is not clear yet it is understood that probiotics may be helpful in preventing the loss of GSH.

NO Hypercholesterolemia is widely recognized as a devoting risk factor for the development of cardiovascular diseases (CVD) such as atherosclerosis, coronary heart diseases and stroke. It has been demonstrated that even a 1% reduction in serum cholesterol could reduce the risk of coronary heart disease by 2-3%³¹.
Focus of marked interest in the relationship between serum cholesterol and lactic acid bacteria was begun since Mann and Spoerry(1974) discovered the hypocholesterolemic effects of fermented milk ingested by the Massai tribes people. In the present study, in line with El-Shafie etal, (2009) the reduction in serum cholesterol level was observed due to the administration of LAB, however the strains of LAB used by them were of Lactobacillus plantarum and Lactobacillus paracasei.

Fazeli et al(2010) have also shown that the administration of Lactobacillus plantarum A7(10^8 CFU mL^-1) for 14 days is effective in lowering serum lipid parameters in rats. Such study indicates that probiotics may also be used as a cost effective modality of treatment in controlling lipid parameters.

LDC-C is the important component of serum cholesterol. Hence, reduction of the LDL-C level may be an indispensible factor for lowering serum total cholesterol level. The mixture of two strains(Lactobacillus rhamnosus and Lactobacillus casei) used in the study appeared to lower TGL, LDC –C, VLDL –C but did not affect the HDL-C of all the three groups including control group. Indeed, there was no significant difference between HDL-C levels of all the three groups .In a study conducted by Park et al (2007) it was observed that the hepatic LDR-R mRNA expression of rats fed on either a normal diet or a high cholesterol diet increased with L-Acidophilus ATCC 43121 administration and the serum LDL-C level lowered accordingly, but by what means the LAB affect the hepatic LDR-R mRNA expression of rats further in-depth investigation.

Further, in a randomized, Placebo controlled and parallel designed study conducted by Abd EI-Gawada et al (2005) demonstrated that feeding 50gm of buffalo milk-yoghurt fortified with Bifidobacterium longum Bb-46 for 35 days significantly reduced concentration of total cholesterol by 50.3%, LDL cholesterol by 56.3% and triglycerides by 51.2% compared to the control (p<0.05). Similarly, Taranto et al (2000) reported that L.reuteri administration was effective in inhibiting hypercholesterolemia in mice.

Further, he noticed a decrease in total cholesterol (22%) and TGL(33%) too. Similarly, as we expected, TGL was significantly reduced in the treated group (with Lactobacillus rhamnosus and Lactobacillus casei) in comparison with the positive control (Diabetic group but without LAB) and normal control group(without disease and supplementation).

Arhar and Prajapathi(2001) demonstrated the hypocholesterolemic effect of probiotic diet in humans and proposed that lactic acid fermenters of yogurt can lower the levels of total cholesterol, HDL-C and LDL-C in a well balanced way by invtro assimilation of cholesterol by bacteria yogurt.

In our study, after the administration of two LAB strains (Lactobacillus rhamnosus and Lactobacillus casei) in the treated group did not have any significant change in their HDL-C. Likewise, even the other two groups were also having a HDL-C levels on par with the treated and positive control groups. In the same way, Ning Xie etal (2011) have also observed that there was no change in the HDL-C concentration of rats administered with two strains LAB (Lactobacillus fermentum MIL16 and lactobacillus plantarum 9-41-a).

Further, Ibrahim et al (2005) and St-onge et al (2002) also reported similar results regarding the effect of fermented milk on the HDL-C level in rats or humans. However, Hashimoto et al (1999) observed that a diet containing Lactobacillus casei TMC 0409 could increase the level of HDL-C in rats. A promising explanation for these contradictory findings remain indistinct, although distinguished characteristics of the bacterial strains could be considered.

Thus, as there are several hypothesis proposed to explain these findings, we acknowledge one of the following mechanisms that could be involved in bringing out such a mitigating effect on the lipid profile when probiotics are used;

1) Cholesterol may be bound to the bacterial cellular surface or incorporated into the bacterial cellular membrane or converted into coprostanol by cholesterol reductase which is produced by strains of Lactobacelli.
2) Consumption of cholesterol by intestinal bacteria, thus reducing the amount of cholesterol available for absorption.

3) Inhibition of micelle formation by certain probiotic strains.

4) Some bacterial species excrete bile salt hydrolase, leading to increased bile excretion in faeces.

5) Short chain fatty acids produced upon selective fermentation of food by intestinal bacterial microflora may lower plasma cholesterol levels.

CONCLUSION: In summary, the LAB strain Lactobacillus rhamnosus and Lactobacillus casei exerted a favourable impact on glycemic status and typically these strains extended a role in reducing bodyweight of the diabetic wistar rats induced by STZ. Further it was noted that strains resulted in low fat storage and morbidless healthier life. However, probiotics used in this study did not bring out a major change in the antioxidative property except preventing much depletion of GSH in the treated animals. Thus, the strains of Lactobacillus rhamnosus and Lactobacillus casei may represent potential therapeutic agents for controlling lipid and lowering excessive body weight gain. Further, indepth invivo studies are required to analyse the exact mechanisms behind these beneficial effects of the probiotics.

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