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EFFECT OF PROBIOTIC *LACTOBACILLUS FERMENTUM* MCC3216 ON PROGRESSION OF TYPE 2 DIABETES WITH THEIR ANTI-INFLAMMATORY ACTIVITY IN HIGH FRUCTOSE FED DIABETIC RATS

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Keywords:

Probiotic, *Lactobacillus fermentum* MCC3216, Antidiabetic, Type 2 diabetes, Anti-inflammatory

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ABSTRACT: The antidiabetic and anti-inflammatory effects of probiotic *Lactobacillus fermentum* MCC3216 was investigated in high fructose-fed type 2 diabetic rats. Type 2 diabetes was induced by feeding high fructose diet to male albino Wistar rats. A single daily dose of 2×10^8 CFU/ml of *L. fermentum* MCC3216 was given to Wistar rats for 8 weeks. The fasting blood glucose, body weight, oral glucose tolerance test, glycosylated hemoglobin, insulin, liver glycogen, lipid profile, and oxidative stress parameters were measured. The level of Interleukin-6 and Interleukin-10 were also measured in serum. Histopathology analysis of pancreas and liver injury were performed. At 8 week *L. fermentum* MCC3216 significantly ($P<0.05$) decrease FBG, body weight, glucose intolerance, HbA1c, insulin, liver glycogen, total cholesterol, triacylglycerol, LDL-C, VLDL-C, lipid peroxidation and IL-6 level as compared to diabetic rats. The values for HDL-C, reduced glutathione, superoxide dismutase, catalase and IL-10 were significantly ($P<0.05$) increased in *L. fermentum* MCC3216 treated group as compared to diabetic rats. Islets of Langerhans and hepatic cells in *L. fermentum* MCC3216 treated group were protected from destruction as compared to the diabetic rats. *L. fermentum* MCC3216 exerts the antidiabetic and anti-inflammatory effect on high fructose-fed type 2 diabetic rats and significantly improves hyperglycemia, hyperinsulinemia, glucose intolerance, dyslipidemia, oxidative stress and regulates immunological responses. These results suggest that further research is required in other diabetic models for clinical application of *L. fermentum* MCC3216 in progression and delay of T2DM.

INTRODUCTION: Diabetes mellitus is a progressive metabolic disease which is linked with the abnormal increase in blood glucose level. The prevalence of metabolic syndrome mainly type 2 diabetes is increasing rapidly due to major changes in dietary habits, lack of physical activity and obesity.

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According to recent report of WHO 382 million cases were found with diabetes in 2013 worldwide which will rise to about 592 million by 2035 [1]. Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance, hyperglycemia, dyslipidemia, the relative decrease in insulin secretion from *β*-cells and *β*-cell failure [2].

Various pathophysiological conditions are involved in the progression of type 2 diabetes. Body weight gain and body mass can increase the amount of NEFA, hormones, a proinflammatory cytokine, glycerol that are involved in insulin resistance [3]. Lifestyle improvement that reduces obesity can minimize the progression of type 2 diabetes.

Probiotic supplementation can improve glycemic control in T2DM [4]. Dyslipidemia is a major complication of T2DM which is associated with serious clinical conditions such as high triglyceride levels, hypertension, increased low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) which may lead to cardiovascular diseases [5]. Oxidative stress plays an important role in the development of diabetic complications such as dyslipidemia, insulin resistance, high blood pressure, and inflammation. Antioxidant rich diet and lifestyle improvement in the early stage of diabetes can reduce its complication [6]. Chronic hyperglycemia, activation of the immune system, increased the level of circulating Proinflammatory cytokines has been involved in the pathophysiology of insulin resistance and T2DM. Interleukin-6 and Interleukin-10 are considered to be a potential biomarker in the diagnosis of T2DM. Levels of IL-6, IL-10 and single nucleotide polymorphisms in the IL-10 gene are associated with the subclinical inflammation and greater risk of developing T2DM [7].

The major classes of oral antidiabetic drugs include sulfonylureas, meglitinide, biguanides, α-glucosidase inhibitors, thiazolidinedione (TZD), sodium-glucose cotransporter (SGLT2) inhibitors and dipeptidyl peptidase 4 (DPP-4) inhibitors [8]. The prolonged use of these antidiabetic drugs has various side effects such as impaired hypoglycemia, diarrhea, lactic acidosis, flatulence, bloating and leads to multiple organ failure [9]. Probiotic lactic acid bacteria do not have these side effects so they can be a better alternative to these drugs. Probiotics are considered as beneficial bacteria which provide a health benefit when consumed in sufficient amount. Lactic acid bacteria are a major group which contains most of the probiotic bacteria used in human health management [10]. Recently, individual studies reported the potential use of probiotics in T2DM. They exert the beneficial effect by improving FBG and HDL-C in T2DM [11]. Multi-strain probiotic modestly improves the fasting insulin and HbA1c in people with type 2 diabetes reported by recent finding [12]. *L. casei* prevent the onset and development of type 2 diabetes via altering gut microbiota and microbiota-based bile acid-chloride exchange mechanism in high-fat sucrose (HFS) diet-induced rat models [13]. One study reported that *L. casei* significantly ameliorates glucose tolerance and oxidative stress in high-fat diet and streptozotocin-induced type 2 diabetes in mice [14].

We have isolated *L. fermentum* MCC3216 from curd in our lab. In our previous in-vitro study we have found that *Lactobacillus fermentum* MCC3216 exhibited good probiotic properties such as acid-bile salt tolerance, antimicrobial and antioxidative properties. Therefore in this *in-vivo* study, we investigated the antidiabetic and anti-inflammatory properties of *L. fermentum* MCC3216 in type 2 diabetes rats induced by a high-fructose diet.

MATERIAL AND METHODS:

Preparation of Bacterial Suspension: *L. fermentum* MCC3216 (Gene bank accession no.-KY392986) used in the present study were isolated from a dairy sample (curd) and characterized for their probiotic properties. *L. fermentum* MCC3216 were cultured in de Mann Rogosa Sharpe (MRS) broth at 37 °C in anaerobic condition. Colony counting was performed before the animal experiments begin to assess the numbers of surviving bacteria were adjusted to 2.0×10^8 CFU/ml. The bacterial dose was prepared in double distilled water (ddH_2O) for oral administration to rats.

Animal Maintenance and Experiment Design: Thirty, male albino Wistar rats 6-8 week of age (175 ± 25 gm of body weight) were used for all experiments in present study. Animals were housed in a group of two in separate cages under controlled conditions of temperature (22 ± 2 °C) with a 12-h light/dark cycle. All animals were given a standard diet (golden feed, New Delhi) and water *ad libitum*. The animals were randomly divided into four groups (n=6 per group) as follows:

Normal control group (NCG): rats without diabetes receiving only standard chow diet and normal drinking water; (Diabetic) High fructose-fed control group (HFCG): rats with diabetes but no treatment receiving standard chow diet and a 21% fructose solution with drinking water; Metformin and high fructose treated group (MTHFG): rats with diabetes and treated with metformin (a drug for treating diabetes) receiving standard chow diet supplemented with Metformin 100 mg/kg of body

weight and a 21% fructose solution with drinking water; and the *L. fermentum* MCC3216 and high fructose treated group (LFHFG): rats with diabetes and treated with *L. fermentum* MCC3216 receiving standard chow diet supplemented with *L. fermentum* CIB17 (MCC3216) with 2×10^8 cfu/ml and a 21% fructose solution with drinking water for 8 weeks except for the days before oral glucose tolerance tests (OGTTs) and blood collection. Food and water intake was recorded every 2 days, and body weight was measured once a week. This study was approved by the Institutional Animal Ethics Committee of PBRI, Bhopal. (Reg. no. -1824/PO/ ERe/S/15/CPCSEA/ IAEC/PN-17018)

Oral Glucose Tolerance Test: Oral glucose tolerance tests were performed after 4 weeks of the experimental period on overnight fasted diabetic rats followed by an oral glucose load (2 g/kg of body weight). Blood samples were collected from the tail vein of each animal just after oral glucose administration at 0, 30, 60, 90 and 120 min for the assay of glucose. Glucose concentration was measured with an Accu-Check active blood glucose meter (Roche Group, Indianapolis, IN, USA).

Blood and Tissue Sample Collection: At the end of the experiments (8^{th} week), blood samples were collected from the retro-orbital puncture of 12-h fasted and anesthetized animals. A portion of whole blood samples was used for determination of glycosylated hemoglobin (HbA1c). The remaining blood samples were centrifuged at 4000 × *g* for 10 min at 4 °C and plasma was collected to analyze insulin level. Furthermore, serum was separated and stored at −20 °C for the analysis of IL-6 and IL-10. All animals were sacrificed by under mild ether anesthesia. The whole pancreas and liver from each animal was removed after sacrificing the animal and was collected in 10% formalin solution.

Determination of Fasting Blood Glucose Level, HbA1c, Insulin, Liver Glycogen, and Lipid Profile: Fasting blood glucose level was measured by Accu-Check active blood glucose meter (Roche Group, Indianapolis, IN, USA). Glycosylated hemoglobin (HbA1c) was measured using the Euro diagnostic system kit. Plasma insulin was determined with an Insulin Rat ELISA kit (Thermo Scientific, USA). Liver glycogen was assayed using Glycogen assay kit (Abcam, UK). Total cholesterol (TC), triacylglycerol (TG), and High-density lipoprotein cholesterol (HDL-C) levels in blood serum were determined by manufacture protocol of enzymatic kits purchased from (Span Diagnostic Reagent Kit, India). Friedewald's formula calculated Low-density lipoprotein (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) levels in blood serum.

Determination of Oxidative Stress Parameters in Pancreas: The activity of oxidative stress parameters were measured in the pancreas. LPO was measured according to the method of Ohkawa *et al.* [15]. Superoxide dismutase (SOD) was measured according to the method of Kakkar *et al.* [16] Catalase (CAT) was estimated as described by Sinha [17]. Reduced glutathione (GSH) was estimated as described by Ellman [18].

Determination of Inflammatory Cytokines IL-6 and IL-10: Determination of IL-6 (Pro-inflammatory cytokines) and IL-10 (Anti-inflammatory cytokines) was done in serum sample separated from blood. Levels of Interleukin-6 and Interleukin-10 were measured according to the recommendations of the manufacturer using ELISA kits purchased from RayBio®.

Histopathologic Analysis: The pancreases and liver were fixed for 48 h in 10% formalin saline and processed by the paraffin technique. Sections of 5-micron thickness were cut and stained by hematoxylin and eosin (H & E) for histological examination. Observation of slides was done under a light microscope (DM2000; Leica, Bensheim, Germany).

Statistical Analysis: The data were analyzed by One way ANOVA followed by Bonferroni test using sigmastat32, India. $p<0.05$ was considered as a level of significance. The resulting values were expressed as mean ± standard error of the mean.

RESULTS:

***Lactobacillus fermentum* MCC3216 Improved Body weight: Table 1** shows the change in body weight. The food and water intakes were found to be quite the same across groups (*i.e.*, NCG, HFCG, MTHFG, and LFHFG) of rats tested. Extra energy received by HFCG, MTHFG, LFHFG in the form of high fructose diet. The body weights of all the rats rapidly increased in high fructose diet

receiving groups except NCG. The one-way ANOVA for repeated measurements revealed a significant (P<0.05) increase in body weights of HFCG than that of NCG group while body weight gain in MTHFG and LFHFG animals was non-significant (P>0.05) than that of NCG group.

TABLE 1: CHANGE IN BODY WEIGHTS OF RATS ADMINISTERED *LACTOBACILLUS FERMENTUM* MCC3216 AND HIGH FRUCTOSE DIET DURING AN 8-WK EXPERIMENTAL PERIOD

Parameters	Initial body weight (g)	Final body weight (g)	Body weight gain (g)
NCG	179.4±8.502	222.3±9.357	41.9±29.627
HFCG	181.6±8.480	239.4±11.213*	57.8±40.870
MTHFG	177.5±6.715	225.6±7.344	48.1±34.011
LFHFG	183.3±7.934	228.1±9.382	43.8±30.971

NCG, Normal control group; HFCG, High fructose-fed control group; MTHFG, Metformin, and high fructose treated group; LFHFG, *Lactobacillus fermentum* MCC3216, and high fructose treated group. All groups contain six animals; Values are expressed as MEAN±SEM, One way ANOVA followed by Bonferroni test. *Significantly different from normal control group (NCG) (P<0.05).

***Lactobacillus fermentum* MCC3216 Alleviated Oral Glucose Tolerance Test: Fig. 1** shows the experimental results of the OGTT at week 4. Glucose tolerances were impaired at 4 wk in the HFCG group. The area under the curve (AUC) glucose value in the HFCG group was significantly higher by 63.5% than that in the NCG group (P<0.05). The AUC glucose value in the MTHFG, LFHFG group, was significantly (P<0.05) lower (57.8% and 54.8% respectively) than that in the HFCG group.

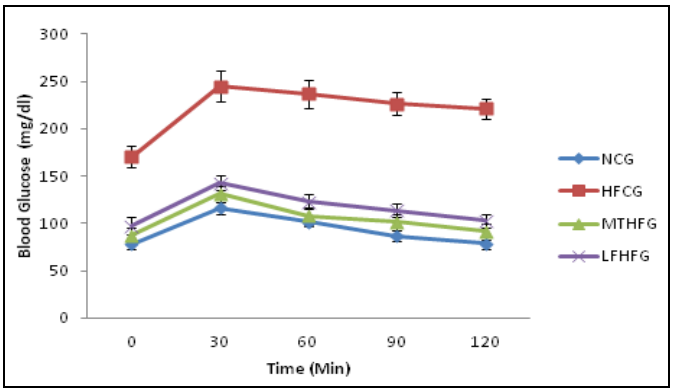

FIG. 1: EFFECT OF *LACTOBACILLUS FERMENTUM* MCC3216 ON ORAL GLUCOSE TOLERANCE TEST AT 4 WEEK IN HIGH FRUCTOSE DIET INDUCED DIABETIC RATS. NCG, Normal control group; HFCG, High fructose-fed control group; MTHFG, Metformin and high fructose diet treated group; LFHFG, *Lactobacillus fermentum* MCC3216 and high fructose diet treated group.

***Lactobacillus fermentum* MCC3216 Ameliorated Fasting Blood Glucose, Glycosylated Hemoglobin, Insulin, Liver Glycogen, Lipid Profile, and Oxidative Stress Parameters:** As shown in **Table 2** the FBG levels in the HFCG group were significantly increased (P<0.05, 67.14%) as compared to NCG group it shows the diabetic state of HFCG animals. Levels of FBG in the MTHFG and LFHFG group were significantly (P<0.05) increased (27.98% and 42.95% respectively) as compared to NCG group but significantly decreased (54.37% and 42.40% respectively) as compared to HFCG animals after 8 weeks. HbA1c levels in the HFCG group were significantly increased (P<0.05, 45.34%) as compared to the NCG group. No significant difference (P>0.05) was observed in HbA1c level in MTHFG group, and significant increase ((P<0.05), 20.70%) was observed in LFHFG group as compared to the NCG group, but significant (P<0.05) decrease (43.04% and 31.07% respectively) was found in MTHFG and LFHFG group as compared to HFCG group animals.

Liver plasma insulin was significantly higher (P<0.05, 70.22%) in the HFCG group than that of NCG animals after 8 wk of fructose administration; however, plasma insulin was also significantly higher (P<0.05, 19.58% and 38.09% respectively) in the MTHFG and LFHFG as compared to NCG group animals. Liver glycogen level was significantly higher (P<0.05, 20.70%) in HFCG group than that of NCG animals, however, No significant difference (P>0.05) were observed in MTHFG and LFHFG group than that of NCG group but found to be significantly different (P<0.05, 17.86% and 16.83% respectively) from HFCG group animals.

There was a significant increase (P<0.05, 23.95%, 37.02%, 31.43% and 34.40%, respectively) in the level of serum TC, TG, LDL-C, and VLDL-C and significant (P<0.05, 27.29%) decrease in serum HDL-C in HFCG animals as compared to NCG animals. However, significant (P<0.05) decrease (17.33%, 28.52%, 26.11% and 28.45%, respectively) in TC, TG, LDL-C, VLDL-C and a significant increase in serum HDL-C (P<0.05, 27.61%) was observed after *L. fermentum* MCC3216 administration in LFHFG animals than that of HFCG at 8 wk of experimental period.

Moreover, there was a significant (P<0.05) decrease (20.21%, 32.25%, 28.76%, 31.66% respectively) in TC, TG, LDL-C, VLDL-C and a significant increase in serum HDL-C (25.63%) was found in MTHFG as compared to HFCG animals.

Level of LPO in pancreatic tissue was significantly increased (P<0.05, 42.70%) in HFCG group as compared to the NCG group. Administration of Metformin and *L. fermentum* MCC3216 significantly decreased the LPO level (P<0.05, 33.57% and 43.06% respectively) in MTHFG and LFHFG group as compared to HFCG group. The concentration of GSH in pancreatic tissue was significantly decreased (P<0.05, 66.24%) in HFCG group as compared to the NCG group. Administration of Metformin and *L. fermentum* MCC3216 significantly increased the GSH conc. (P<0.05, 63.06% and 61.51% respectively) in MTHFG and LFHFG group as compared to HFCG group. Level of SOD in pancreatic tissue was significantly decreased (P<0.05, 53.09%) in HFCG group as compared to the NCG group. Administration of Metformin and *L. fermentum* MCC3216 significantly increased the level of SOD (P<0.05, 49.62% and 44.73% respectively) in MTHFG and LFHFG group as compared to HFCG group. The concentration of CAT in pancreatic tissue was significantly decreased (P<0.05, 34.83%) in HFCG group as compared to the NCG group. Administration of Metformin and *L. fermentum* MCC3216 significantly increased the CAT concentration (P<0.05, 28.29% and 23.25% respectively) in MTHFG and LFHFG group as compared to HFCG group.

TABLE 2: FBG, HbA1c, INSULIN, LIVER GLYCOGEN, LIPID PROFILE AND OXIDATIVE STRESS PARAMETERS ANALYSIS IN RATS ADMINISTERED *LACTOBACILLUS FERMENTUM* MCC3216 AFTER 8 WEEKS

Variables	Groups*			
	NCG	HFCG	MTHFG	LFHFG
FBG (mg/dL)	78.5±4.607	238.9±13.877†	109.0±6.2611†,‡	137.6±8.519†,‡,**
HbA1c (%)	4.52±0.1114	8.27±0.3123†	4.71±0.1184‡	5.70±0.1930†,‡,**
Plasma insulin (ng/ml)	0.78±0.0302	2.62±0.0812†	0.97±0.0452†,‡	1.26±0.0567†,‡,**
Liver glycogen (mg/g) tissue	9.23±0.71	11.64±1.62†	9.56±0.84‡	9.68±0.96‡
Total cholesterol (mg/dL)	93.15±6.12	122.5±10.17†	97.74±7.01‡	101.26±6.14‡
Triacyglycerol (mg/dL)	53.32±6.41	84.67±11.02†	57.36±9.89‡	60.47±9.15‡
HDL-C (mg/dL)	35.98±4.10	26.16±4.47†	35.18±4.13‡	36.14±4.68‡
LDL-C (mg/dL)	54.11±7.31	78.92±9.52†	56.22±8.30‡	58.31±7.99‡
VLDL-C (mg/dL)	11.25±0.92	17.15±3.02†	11.72±2.11‡	12.27±2.08‡
LPO (n mol/mg/protein)	3.14±0.17	5.48±0.22†	3.64±0.16†,‡	3.12±0.17‡,**
GSH (mg/dl)	4.78±0.13	1.47±0.17†	3.98±0.18†,‡	3.82±0.58†,‡
SOD (U/mg) protein	12.75±0.07	5.98±0.08†	11.87±0.06†,‡	10.82±0.014†, ‡, **
CAT (U/mg) protein	30.14±1.21	19.64±0.32†	27.39±1.13†,‡	25.59±1.10†, ‡, **

NCG, Normal control group; HFCG, High fructose-fed control group; MTHFG, Metformin and high fructose treated group; LFHFG, *Lactobacillus fermentum* MCC3216 and high fructose treated group; FBG, Fasting blood sugar; HbA1c, Glycosylated hemoglobin; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; VLDL-C, Very low-density lipoprotein cholesterol; LPO, Lipid peroxidation; GSH, reduced Glutathione; SOD, Superoxide dismutase; CAT, Catalase

* All groups contain six animals, Values are expressed as MEAN±SEM, One way ANOVA followed by Bonferroni test

†Significantly different from the normal control group (P<0.05).

‡ Significantly different from High fructose-fed control group (P<0.05).

** Significantly different from Metformin drug-treated control group (P<0.05).

***Lactobacillus fermentum* MCC3216 Ameliorated IL-6 and IL-10: Fig. 2** shows the results of Interleukin- 6 (IL-6) and Interleukin- 10 (IL-10) measurement in rats administered *L. fermentum* MCC3216 for 8 weeks. There was a significant (P<0.05) increase (80.32%) was found in the values of IL-6 in HFCG group than those in the NCG group. However, these values were significantly (P<0.05) decreased (69% and 54.78% respectively) in the MTHFG and LFHFG group compared with those in the HFCG group; moreover these values were found to be significantly (P<0.05) increased (36.54% and 56.49% respectively) in the MTHFG and LFHFG group than those in the NCG group. There was a significant difference (P<0.05, 31.43%) were observed in IL-6 level between the MTHFG and LFHFG groups. **Fig. 2** shows that there was a significant (P<0.05) decrease (31.97%) was found in the values of IL-10 in HFCG group than those in the NCG group.

However, these values were significantly (P<0.05) increased (28.74% and 28.22% respectively) in the MTHFG and LFHFG group compared with those in the HFCG group. No significant differences were observed in IL-10 level in the MTHFG and LFHFG group than those in the NCG group; however, there is also no significant difference was found between the MTHFG and LFHFG groups.

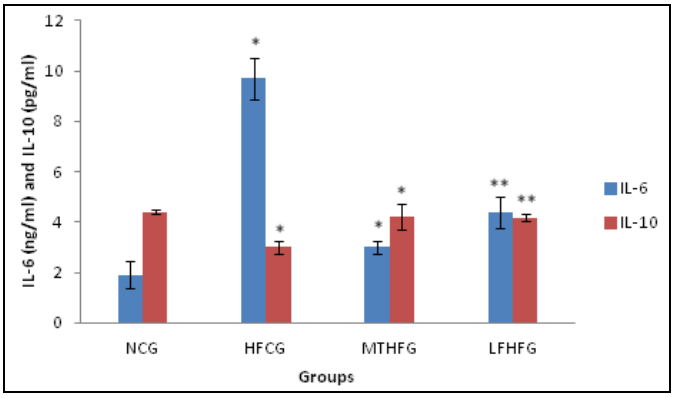

FIG. 2: THE LEVELS OF IL6 AND IL-10 IN BLOOD SERUM OF RATS ADMINISTERED *LACTOBACILLUS FERMENTUM* MCC3216 AT 8WK. (A) IL-6 (B) IL-10. IL, interleukin; NCG, Normal control group; HFCG, High fructose-fed control group; MTHFG, Metformin, and high fructose treated group; LFHFG, *Lactobacillus fermentum* MCC3216 and high fructose treated group. All groups contain six animals; values are expressed as MEAN ± SEM, One way ANOVA followed by Bonferroni test. * Significantly different from normal control group (P<0.05). ** Significantly different from High fructose-fed control group (P<0.05).

Effect of *Lactobacillus fermentum* MCC3216 on Histology of Pancreas and Liver: Histological analysis of the pancreases of the four experimental groups shows the significant differences in the structural characteristics, number and pattern of islets of Langerhans **Fig. 3**. **Fig. 3A** illustrates the normal histological structure of Wistar rat pancreas with normal islet in the normal control group (NCG).

The islets cells appeared round or oval, elongated and evenly dispersed in pancreatic acinar or cytoplasm with clear boundaries around the cells. **Fig. 3B** illustrates the histological structure of diabetic control rat pancreas in HFCG group. The islets cells cluster were found to be irregular in shape, undefined and necrosis of the cells was seen. **Fig. 3C** illustrates the histological structure of rat pancreas in MTHFG group. Metformin-treated rat pancreas showing adequate protection from HFD-induced changes in the pancreatic islets cells. **Fig. 3D** illustrates the histological structure of rat pancreas in LFHFG group. Probiotic *Lactobacillus fermentum* MCC3216 treated diabetic rat pancreas showed adequate regeneration and protection of pancreatic β-cells when compared with the diabetic control group (HFCG).

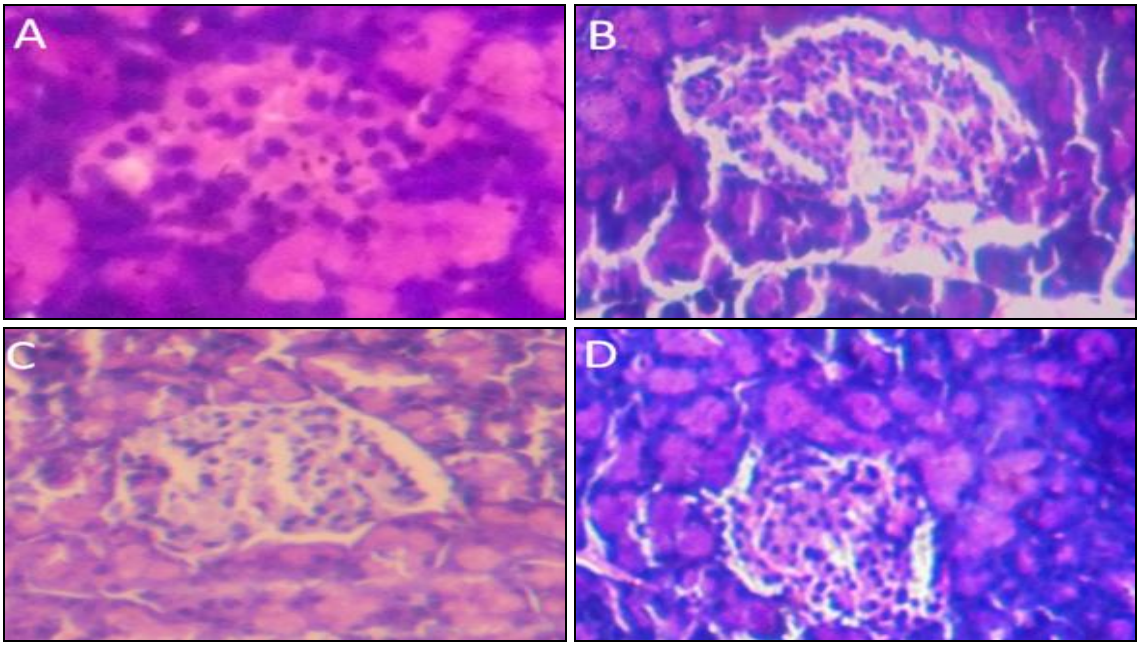

FIG. 3: EFFECT ON HISTOLOGICAL CHANGES OF PANCREAS IN HIGH FRUCTOSE DIET INDUCED DIABETIC RATS. H&E STAINING OF PANCREAS WAS PERFORMED FOLLOWING 8 WEEKS OF ADMINISTRATION, MAGNIFICATIONS, X100 (A) PANCREATIC SECTIONS OF RATS IN THE NORMAL CONTROL GROUP (NCG); (B) PANCREATIC SECTIONS OF RATS IN THE HIGH FRUCTOSE FED CONTROL GROUP (HFCG); (C) PANCREATIC SECTIONS OF RATS IN THE METFORMIN AND HIGH FRUCTOSE TREATED GROUP (MTHFG); (D) PANCREATIC SECTIONS OF RATS IN THE, *LACTOBACILLUS FERMENTUM* MCC3216 AND HIGH FRUCTOSE TREATED GROUP (LFHFG)

Histological analysis of the liver in four experimental groups of the rat was shown in **Fig. 4**. As described in **Fig. 4A** the normal histological structure of liver cells in the NCG group with a small number of cells undergoing a degenerative process. **Fig. 4B** illustrates the histological structure of diabetic control rat liver in HFCG group. Liver of HFCG rat showing highest pathological changes like derangements of cords, vascular changes, cellular infiltration, degeneration, necrosis in cells and some hepatocytes with microvesicular cytoplasm undergoing steatosis process. **Fig. 4C** illustrates the histological structure of rat liver in MTHFG group. Liver of metformin-treated rat shows ample protection from HFD-induced changes in the normal structure of hepatocytes. **Fig. 4D** illustrates the histological structure of rat pancreas in LFHFG group. Liver of diabetic rat treated with Probiotic *Lactobacillus fermentum* MCC3216 shows the highest restoration of liver cells and minimal necrosis with normal appearance of hepatocytes.

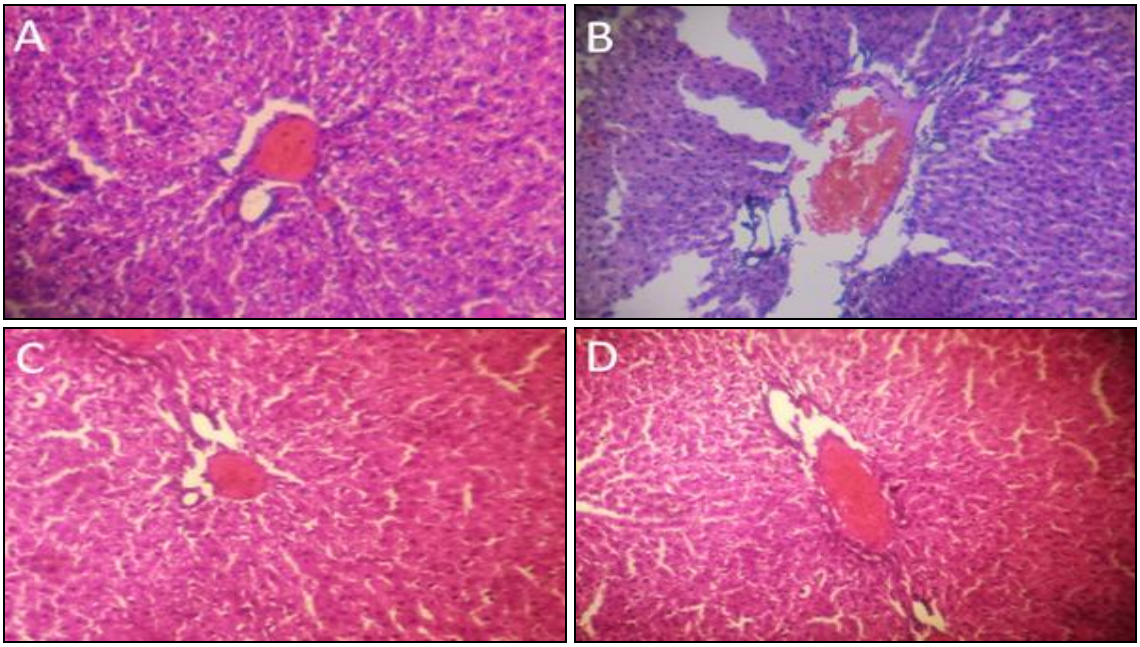

FIG. 4: EFFECT ON HISTOLOGICAL CHANGES OF LIVERS IN HIGH FRUCTOSE DIET INDUCED DIABETIC RATS. H&E STAINING OF PANCREAS WAS PERFORMED FOLLOWING 8 WEEKS OF ADMINISTRATION, MAGNIFICATIONS, X100 (A) LIVER SECTIONS OF RATS IN THE NORMAL CONTROL GROUP (NCG); (B) LIVER SECTIONS OF RATS IN THE HIGH FRUCTOSE FED CONTROL GROUP (HFCG); (C) LIVER SECTIONS OF RATS IN THE METFORMIN AND HIGH FRUCTOSE TREATED GROUP (MTHFG); (D) LIVER SECTIONS OF RATS IN THE, *LACTOBACILLUS FERMENTUM* MCC3216 AND HIGH FRUCTOSE TREATED GROUP (LFHFG)

DISCUSSION: Probiotics are 'live micro-organism' which when consumed in adequate amount confers the health benefits to the host. The most common genera in probiotic are *Lactobacillus* and *Bifidobacterium* which usually consumed as microbial food supplements. Various clinical conditions and diseases including diabetes mellitus can be improved by the probiotic bacteria [19]. Various research findings indicate that specific strains of lactic acid bacteria can be useful in the management of type 2 diabetes [20, 21]. Yadav *et al*. reported that dahi containing *L. acidophilus* and *L. casei* significantly decrease the high fructose diet-induced diabetic symptoms in wistar rats [22]. In the present study, we examine the antidiabetic and anti-inflammatory effect of *Lactobacillus fermentum* MCC3216 in Wistar rats for the 8 weeks of the experimental period. We have found that *L. fermentum* MCC3216 administration prevents and delays the onset of high fructose-induced type 2 diabetes symptoms in wistar rats. Body weight gain in all experimental groups was significantly ($P<0.05$) affected by the time, diet, dose of the drug and probiotic bacteria. Administration of *L. fermentum* MCC3216 prevents the excessive body weight gain in LFHFG group. Metformin and *L. fermentum* MCC3216 both prevented the body weight gain in diabetic rats as compared to LFHFG group. The values were found to be close to the normal control group values in both MTHFG and LFHFG group after 8 weeks. Yun *et al*., reported that *Lactobacillus gasseri* BNR17 controls the

body weight gain in db/db mice with T2DM [23]. Results of OGTT reveals that *L. fermentum* MCC3216 improves the glucose tolerance in LFHFG group while diabetic rats of HFCG group shows impaired glucose tolerance at 4 weeks. Administration of Probiotic *Lactobacillus gasseri* improves the glucose tolerance in rats with body weight control reported by the shirouche *et al*. [24]

Glycemic control is one of the most important measures for the diagnosis of the diabetic condition. Fasting plasma glucose (FPG), postprandial plasma glucose (PPG) and glycated hemoglobin (HbA1c) are the three important aspects for the measurement of plasma glucose in diabetic condition. Increased Fasting blood glucose level is a very common marker for the diagnosis of T2DM [25]. In the present study, we observed that FBG was significantly increased in diabetic control (HFCG) group at 8 weeks of the experimental period which reflects the onset of T2DM condition. Administration of *L. fermentum* MCC3216 *and* metformin in rats significantly decreased the FBG in LFHFG and MTHFG group than that of HFCG group and prevented the onset of T2DM. The value FBG in LFHFG and MTHFG was close to the NCG group values. These results reveal the antidiabetic potential of *L. fermentum* MCC3216 in rats. Probiotics supplementation in the sufficient amount improves the glucose metabolism within the 8 weeks of experimental period [26].

HbA1c is one of the very important markers of glycemic control in T2DM. The concentration of HbA1c indicates the no. of glucose molecules binds with the hemoglobin in RBC which provide average blood glucose level of individual of about preceding two-three months. It is helpful in the prediction of severity of diabetic complications[27]. HbA1c was significantly increased in HFCG animals during 8 week of an experiment which is may be due to the increase in blood glucose level of rats. Administration of *L. fermentum* MCC3216 (LFHFG) and metformin (MTHFG) in rats prevents the increase of HbA1c molecules which indicates the reduction of blood glucose level for a longer period. There was a significant reduction of HbA1c level was found in the high fructose-induced diabetic rats after administration of *L. fermentum* MCC3216. Probiotics supplementation significantly improves the HbA1c in type 2 diabetes patients [28]. Dahi containing *L. acidophilus* and *L. casei* significantly reduce the HbA1c in high fructose-induced type 2 diabetic rats reported by Yadav *et al.* [29]

Insulin is the main hormone that involves blood glucose management. Insulin resistance occurs because of visceral adiposity, dyslipidemia, and obesity in liver and muscle cells. Inflammation, oxidative stress, habitual and genetic factors play an important role in insulin resistance. In the present study, high fructose diet-induced insulin insensitivity significantly improved by *L. fermentum* MCC3216 and metformin in LFHFG group and MTHFG group respectively. Li *et al*., reported that administration of *Lactobacillus casei* ameliorates insulin resistance and protects the islets of Langerhans in the type 2 diabetic mice. The basic mechanism behind these finding may be PI3K/Akt signaling pathway and SCFA/intestinal microbiota pathways [30].

The liver plays a crucial role in the management of normal blood glucose homeostasis, by enhancing the rate of glycogen synthesis postprandially [31]. In the present study, diabetic control group rats (HFCG) showed an elevated level of glycogen during 8 wk of the experimental period. Normally liver glycogen is beneficial to the body, but additional sugar may lead to release bad cholesterol which can cause diabetic dyslipidemia. Treatment of *L. fermentum* MCC3216 reduces the glycogen accumulation in LFHFG group than that of HFCG group at 8 weeks.

Abnormalities in lipid metabolism produce in diabetic condition may lead to dyslipidemia that can be a major risk factor to increase cardiovascular disease [32]. In the present study, we have observed the concentration of serum lipids such as TC, TG, LDL-C, and VLDL-C were significantly (P<0.05) high and HDL-C concentration was significantly (P<0.05) low in diabetic control animals (HFCG) as compared to the normal control group animals (NCG). Significant (P<0.05) decrease was observed in the level of TC, TG, LDL-C, VLDL-C and a significant increase in serum HDL-C (P<0.05) level were observed in LFHFG animals after the administration of *L. fermentum* MCC3216 than that of HFCG group animals.

However, there was a significant (P <0.05) decrease in TC, TG, LDL-C, VLDL-C and a significant increase in serum HDL-C was found in MTHFG as compared to HFCG. Probiotics ameliorate cholesterol level by several mechanisms such as integration of cholesterol into the cell membrane of probiotic bacteria, production of SCFAs from various oligosaccharides, conversion of cholesterol into the coprostanol and precipitation of cholesterol with deconjugated bile [33].

Increased oxidative stress may lead to cardiovascular diseases and plays an important role in the progression of diabetes. Oxidative stress induces many diabetic complications such as retinopathy, nephropathy, neuropathy, and stroke. Probiotics ameliorate the host redox status through their ability to chelate metal ion. They also regulate various signaling pathways of antioxidant systems [34]. In the present study administration of *L. fermentum* MCC3216 significantly decreased the LPO level (P<0.05) in LFHFG group as compared to HFCG group. The concentration of GSH, SOD, and CAT was significantly increased (P<0.05) in LFHFG group as compared to HFCG group which indicates that *L. fermentum* MCC3216 posses good antioxidative properties in the pancreas.

Interleukin 6 and Interleukin 10 cytokine plays an important role in the pathophysiology of type 2 diabetes. IL-6 is a multifunctional cytokine, and their elevated circulating level is significantly connected with T2DM. It is also involved in the enhancement of insulin resistance, β-cell dysfunction and inflammation [35]. In this study, we have observed the significant (P<0.05) increase in the level of IL-6 in diabetic rats of HFCG group as compared to normal rats of NCG group. However, there was significant (P<0.05) decrease was found in the level of IL-6 in LFHFG group after administration of *L. fermentum* MCC3216. IL-6 level was also decreased in the metformin-treated group (MTHFG). *L. fermentum* MCC3216 and metformin treatment lower the IL-6 level in high fructose-fed rats. IL-10 considered as an anti-inflammatory cytokine which lowers the production of another inflammatory cytokine such as IL-12 and inhibits the antigen presentation to MHC cells. We have found in our previous research *L. paracasei* MCC3195 exerts an antidiabetic and anti-inflammatory effect in high fructose type 2 diabetic rats [36]. Yaghini *et al*. reported that a low serum level of IL-10 might be a major risk factor for T2DM [37]. In the present study, we have observed the significant (P < 0.05) decrease in the level of IL-10 in diabetic rats of HFCG group as compared to the normal rats of NCG group.

However, there was significant (P<0.05) increase was found in the level of IL-10 in the LFHFG group after administration of *L. fermentum* MCC3216. IL-10 level was also increased in metformin-treated group MTHFG. *L. fermentum* MCC3216 and metformin treatment increase the IL-10 level in high fructose-fed rats. It has been suggested that the antidiabetic effect of *L. fermentum* MCC3216 may be a character of its immunoregulatory attribute. Histological analysis of pancreas shows the protection of islet of Langerhans structure by *L. fermentum* MCC3216 in type 2 diabetic rats. Similarly, histological analysis of the liver also confirms the protection of hepatic cell structure by *L. fermentum* MCC3216 in type 2 diabetic rats.

CONCLUSION: This study demonstrates that *Lactobacillus fermentum* MCC3216 administration in high fructose induced type 2 diabetic Wistar rats significantly protect and improves the glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, oxidative stress, and immuno-regulatory properties by ameliorating the IL-6 and IL-10 levels. Histopathological analysis of pancreas and liver confirms the protective effect of *L. fermentum* MCC3216 on these organs in type 2 diabetic rats. *L. fermentum* MCC3216 exerts the antidiabetic, antioxidative and anti-inflammatory effect in high fructose fed type 2 diabetic rats and protects the diabetes induced complication. These results suggest that oral administration of *L. fermentum* MCC3216 can be used for the prevention and delay the onset of T2DM. It may also have application as a functional food for the management of T2DM. Furthermore, studies are required to determine these activities in another diabetic models for the clinical application of *L. fermentum* MCC3216 in the prevention of T2DM.

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