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ANTIDIABETIC AND ANTI-INFLAMMATORY EFFECT OF PROBIOTIC *LACTOBACILLUS PARACASEI* MCC3195 IN HIGH FRUCTOSE FED TYPE 2 DIABETIC RATS

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Keywords:

Lactobacillus paracasei MCC3195, Probiotic, Antidiabetic, Anti-inflammatory, Type 2 diabetes

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ABSTRACT: The aim of this study was to investigate the antidiabetic and anti-inflammatory effects of probiotic *Lactobacillus paracasei* MCC3195 in high fructose fed type 2 diabetic rats. High fructose diet was used to induce type 2 diabetes in male albino wistar rats. *L. paracasei* MCC3195 was administered to rats at a single dose of 2×10^8 cfu/ml per rat for 8 week. The body weight, fasting blood glucose, oral glucose tolerance test, glycosylated hemoglobin, insulin, Liver glycogen level, lipid profile and oxidative stress parameters were analyzed. Interlukin-6 and Interlukin-10 were also estimated in serum. Histopathology analysis of pancreas and liver injury were done. At 8 week body weight, FBG, glucose tolerance, HbA1c, insulin, liver glycogen level, total cholesterol, triacylglycerol, LDL-C, VLDL-C, lipid peroxidation and IL-6 were significantly increased in diabetic rats, while administration of *L. paracasei* MCC3195 significantly decreased these factors. The level of HDL-C, reduced glutathione, superoxide dismutase, catalase and IL-10 were significantly decreased in diabetic rats, whereas administration of *L. paracasei* MCC3195 significantly increased these factors. Administration of *L. paracasei* MCC3195 protects the Islets of langerhans and hepatic cells from destruction as compared to the diabetic rats. Probiotic *L. paracasei* MCC3195 exerts the antidiabetic and anti-inflamatory effect in high fructose-fed type 2 diabetic rats, by significantly improving glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, oxidative stress and immunoregulatory properties as well as protects the type 2 diabetes induced complications. Further, research is needed in other diabetic models for clinical application of *L. paracasei* MCC3195 in delay or prevention and progression of T2DM.

INTRODUCTION: Diabetes mellitus is one of the most prevalent metabolic diseases in almost every country. Worldwide 90% cases were reported of type 2 diabetes mellitus and it is increasing day by day due to major changes in lifestyle such as decreased physical activity and increased cases of obesity [1].

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Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia, dyslipidemia, peripheral insulin resistance, impairment in insulin secretion by beta cells and β - cell failure [2]. The major complication of T2DM leads to other severe clinical conditions including hypertension, increase in low-density lipoprotein cholesterol (LDL-C) and decrease in high-density lipoprotein cholesterol (HDL-C) which involve in the incidence of cardiovascular diseases [3].

Life style changes that involve body weight control and obesity management can improve the glycaemic control and delay the progression of

type 2 diabetes [4]. Glucose homeostasis can be maintained by preserving beta cell dysfunction and insulin signaling in receptor cells of glucose [5]. Oxidative stress played a major role in the onset and pathogenesis of T2DM which increase the risk of high blood pressure, insulin resistance, dyslipidemia, inflammation and other diabetic complication [6].

Activation of the immune system and chronic inflammation has been involved in the pathophysiology of insulin resistance and type 2 diabetes mellitus. Rodrigues *et al*., reported that the IL-6 and IL-10 levels and single nucleotide polymorphisms in IL-10 gene are linked with the subclinical inflammation in the type 2 diabetes mellitus [7]. Several research investigation reported that higher levels of inflammatory markers includes interleukin-6 (IL-6), C reactive protein (CRP), tumor necrosis factor-α (TNF-α) was present in T2DM patients [8].

The most common oral antidiabetic agents available for diabetes treatment are sulfonylureas, biguanides, *α*-glucosidase inhibitors, thiazolidine-dione (TZD), meglitinide, sodium-glucose cotrans-porter (SGLT2) inhibitors and dipeptidyl peptidase 4 (DPP-4) inhibitors [9]. These antidiabetic agents have high cost for treatment and their prolonged use has various side effects such as diarrhea, impaired hypoglycemia, flatulence, lactic acidosis, bloating and leads to multiple organ damage [10]. Probiotic Lactic acid bacteria does not have these side effects, so they can be considered in management of T2DM.

Probiotics are considered as live microorganism which exerts the health benefits to the host when consumed in adequate amount. Most of the strain of Lactic acid bacteria group have been used in human health management [11]. In recent years several studies reported the potential use of probiotic *lactobacilli* in the management of type 2 diabetes mellitus by improving blood glucose level and lipid metabolism [12]. One study found that *L. rhamnosus* improves the gut function by ameliorating intestinal microbial balance, oxidative stress and inflammation in high-fat diet fed rats as well as in streptozotocin treated rat [13]. In a recent finding found that administration of *Lactobacillius casei* improves the blood glucose balance, glucose tolerance, lipid metabolism and protects islets of langerhans in the type 2 diabetic mice. The underlying mechanism behind these finding may be PI3K/Akt signalling pathway and SCFA/intestinal microbiota pathways [14]. In another study they have found that *L. casei* improves the type 2 diabetes in mice by gut flora and GLP-1 mechanism [15].

We have isolated *L. paracasei* MCC3195 from cheese in our lab. In our previous in-vitro study we have found that *Lactobacillus paracasei* MCC3195 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. paracasei* MCC3195 in type 2 diabetes rats induced by high-fructose diet.

MATERIAL AND METHODS:

Preparation of Bacterial Suspension: *L. paracasei* MCC3195 (Gene bank accession no.-KY392984) used in the present study were isolated from dairy sample (cheese) and characterized for their probiotic properties. *L. paracasei* MCC3195 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. Bacterial dose were 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 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 *Lactobacillus. paracasei* MCC3195 and high fructose treated group (LPHFG): rats with diabetes and treated with *L. paracasei* MCC3195 receiving standard chow diet supplemented with *L. paracasei* CH_3 (MCC3195) 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. All the animal experiments were approved by the Institutional Animal Ethics Committee of PBRI, Bhopal. (Reg.No.-1824/PO/ERe/S/15/CPCSEA/IAEC/PN-17018)

The four experimental groups with six rats each were prepared as per given schedule.

Group I (NCG): Normal control group
Group II (HFCG): High fructose fed control group
Group III (MTHFG): Metformin and high fructose treated group
Group IV (LPHFG): *L. paracasei* MCC3195 and high fructose treated group

Oral Glucose Tolerance Test: Oral glucose tolerance tests were performed after 4 weeks 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 were used for determination of glycosylated haemoglobin (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 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 cholestrol (HDL-C) levels in blood serum were determined by manufacture protocol of enzymatic kits purchased from (Span Diagnostic Reagent Kit, India). Low-density lipoprotein (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) levels in blood serum were calculated by Friedewald's formula.

Determination of Oxidative Stress Parameters in Pancreas: The activity of oxidative stress parameters were measured in pancreas. LPO was measured according to the method of Ohkawa *et al.*, [16]. Superoxide dismutase (SOD) was measured according to the method of Kakkar *et al.*, [17]. Catalase (CAT) was estimated as described by Sinha [18]. Reduced glutathione (GSH) was estimated as described by Ellman[19].

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 Interluekin-6 and Interlukin-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 haematoxylin and eosin (H & E) for histological examination. Observation of slides was done under 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 level of significance. The resulting values were expressed as mean±standard error of the mean.

RESULTS:

Effect on Body Weight: Table 1, presents change in body weight. The food and water intakes were found to be quite same across groups (*i.e.*, NCG, HFCG, MTHFG, and LPHFG) of rats tested. Extra energy received by HFCG, MTHFG, LPHFG 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 ANOVAs 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 LPHFG animals was non significant than that of NCG group.

TABLE 1: CHANGE IN BODY WEIGHTS OF RATS FED WITH NORMAL AND HIGH FRUCTOSE DIET DURING A 8 WEEK EXPERIMENTAL PERIOD

Parameters	Initial body weight (g)	Final body weight (g)	Body weight gain (g)
NCG	179.5±8.503	221.2±11.356	41.7±29.48635
HFCG	180.5±8.479	238.2±10.147*	57.7±40.800
MTHFG	176.5±6.716	226.5±7.342	50±35.319
LPHFG	184.2±8.998	234.0±9.099	49.8±35.213

NCG, Normal control group; HFCG, High fructose fed control group; MTHFG, Metformin and high fructose treated group; LPHFG, *Lactobacillus paracasei* MCC3195 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)

Effect on Oral Glucose Tolerance Test: Fig. 1 shows the experimental results of the OGTT at week 4. Glucose tolerances were impaired at 4 week in the HFCG group.

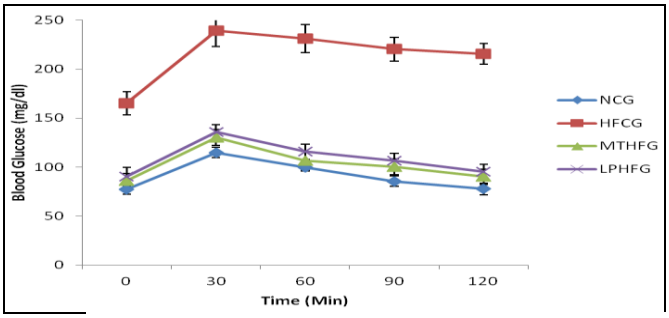

FIG. 1: EFFECT OF *L. PARACASEI* MCC3195 TREATMENT ON ORAL GLUCOSE TOLERANCE TEST AT 4 WEEK IN HIGH FRUCTOSE DIET INDUCED DIABETIC RATS. NCG, Normal control group; HFCG, High fructose fed control group; MTHFG, Metformin and high fructose treated group; LPHFG, *Lactobacillus paracasei* MCC3195 and high fructose treated group

The area under the curve (AUC) glucose value in the HFCG group was significantly higher 64% than that in the NCG group (P<0.05). The AUC glucose value in the MTHFG, LPHFG group was significantly ($P < 0.05$) lower (58% and 56% respectively) than that in the HFCG group.

Effect on Fasting Blood Glucose, Glycosylated Hemoglobin, Insulin, Liver Glycogen, Lipid Profile and Oxidative Stress Parameters: Table 2 shows that the fasting blood glucose levels in the HFCG group were significantly higher ($P < 0.05$, 67%) than those in the NCG group it confirms the diabetic state of HFCG animals. Fasting blood glucose levels of the MTHFG and LPHFG group were significantly ($P < 0.05$) higher (27.20% and 40.36% respectively) as compared to normal group but significantly lower (54.42% and 44.29% respectively) than that of HFCG animals after 8 week. HbA1c levels in the HFCG group were significantly higher ($P < 0.05$, 45.19%) than those in the NCG group. No significant difference was observed in HbA1c level in MTHFG and significant increase (17.32%) in HbA1c level was observed in LPHFG group than that of NCG group, but significantly ($P < 0.05$) decrease (41.82% and 33.7% respectively) in MTHFG and LPHFG group than that of HFCG group.

Liver plasma insulin significantly increased ($P < 0.05$, 56%) in the HFCG group as compared with NCG animals after 8 week of fructose administration; however, plasma insulin was also significantly increased ($P < 0.05$, 15% and 20% respectively) in the MTHFG and LPHFG than that of NCG group. Liver glycogen level were significantly increased ($P < 0.05$, 19.90%) in HFCG group as compared to NCG animals, however, No significant difference were found in MTHFG and LPHFG group as compared to NCG group but found to be significantly different ($P < 0.05$, 20.20% and 19.45% respectively) from HFCG group. There was a significant ($P < 0.05$) increase (18%, 35%, 32% and 34%, respectively) in the level of serum TC, TG, LDL-C and VLDL-C and significant ($P < 0.05$, 19%) decrease in serum HDL-C in HFCG animals as compared to NCG animals. However, significant ($P < 0.05$) decrease (12.60%, 26.90%, 26.43% and 28.18%, respectively) in TC, TG, LDL-C, VLDL-C and a significant increase in serum HDL-C ($P < 0.05$, 20.56%) was

observed after *L. paracasei* administration in LPHFG animals than that of HFCG at 8 week. Moreover, there was a significant ($P < 0.05$) decrease (15.04%, 31.41%, 29.17%, 31.06%) in TC, TG, LDL-C, VLDL-C and a non significant increase in serum HDL-C (17.01%) was found in MTHFG as compared to HFCG animals.

Level of LPO in pancreatic tissue was significantly increased ($P < 0.05$, 49.23%) in HFCG group as compared to NCG group. Administration of Metformin and *L. paracasei* MCC3195 significantly decreased the LPO level ($P < 0.05$, 39.82% and 35.66% respectively) in MTHFG and LPHFG group as compared to HFCG group. Concentration of GSH in pancreatic tissue was significantly decreased ($P < 0.05$, 66.45%) in HFCG group as compared to NCG group. Administration of Metformin and *L. paracasei* MCC3195 significantly increased the GSH concentration ($P < 0.05$, 60.35% and 58.66% respectively) in MTHFG and LPHFG group as compared to HFCG group. Level of SOD in pancreatic tissue was significantly decreased ($P < 0.05$, 52.09%) in HFCG group as compared to NCG group. Administration of Metformin and *L. paracasei* MCC3195 significantly increased the level of SOD ($P < 0.05$, 47.33% and 44.69% respectively) in MTHFG and LPHFG group as compared to HFCG group.

Concentration of CAT in pancreatic tissue was significantly decreased ($P < 0.05$, 37.33%) in HFCG group as compared to NCG group. Administration of Metformin and *L. paracasei* MCC3195 significantly increased the CAT concentration ($P < 0.05$, 29.19% and 23.99% respectively) in MTHFG and LPHFG group as compared to HFCG group.

TABLE 2: FBG, HBA1C, INSULIN, LIVER GLYCOGEN, LIPID PROFILE AND OXIDATIVE STRESS PARAMETERS ANALYSIS IN RATS ADMINISTERED *LACTOBACILLUS PARACASEI* MCC3195 AFTER 8 WEEKS

Variables	Groups*			
	NCG	HFCG	MTHFG	LPHFG
FBG(mg/dL)	78.0±4.604	234.8±13.877†	107.0±6.2611†, ‡	130.8±8.519†, ‡, **
HbA1c (%)	4.39±0.1025	8.01±0.3094†	4.66±0.1179‡	5.31±0.1183†, ‡, **
Plasma insulin (ng/ml)	0.76±0.0295	1.74±0.0795†	0.89±0.0446†, ‡	0.95±0.0411†, ‡, **
Liver glycogen (mg/g) tissue	8.21±0.61	10.59±1.54†	8.45±0.82‡	8.53±0.91‡
Total cholesterol (mg/dL)	91.85±5.45	112.5±9.58†	95.57±6.73‡	98.32±5.69‡
Triacyglycerol (mg/dL)	52.26±5.32	80.54±10.54†	55.24±9.26‡	58.87±8.86‡
HDL-C (mg/dL)	34.87±3.12	28.23±3.80†	34.02±3.24	35.54±3.51‡
LDL-C (mg/dL)	53.08±6.25	77.81±8.44†	55.12±7.25‡	57.24±7.97‡
VLDL-C (mg/dL)	10.12±0.85	15.29±2.59†	10.54±1.28‡	10.98±1.25‡
LPO (n mol/mg/protein)	2.32±0.15	4.57±0.18†	2.75±0.14†, ‡	2.94±0.15†, ‡
GSH (mg/dl)	4.62±0.12	1.55±0.16†	3.91±0.17†, ‡	3.75±0.55†, ‡
SOD (U/mg) protein	13.61±0.06	6.52±0.07†	12.38±0.05†, ‡	11.79±0.012†, ‡, **
CAT (U/mg) protein	29.92±1.13	18.75±0.27†	26.48±1.07†, ‡	24.67±1.04†, ‡, **

NCG, Normal control group; HFCG, High fructose fed control group; MTHFG, Metformin and high fructose treated group; LPHFG, *Lactobacillus paracasei* MCC3195 and high fructose treated group; FBG, Fasting blood sugar; HbA1c, Glycosylated haemoglobin; 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 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 ($P < 0.05$).

Effect on IL-6 and IL-10: Fig. 2 shows the results of Interlukin- 6 (IL-6) and Interlukin- 10 (IL-10) measurement in rats administered *L. paracasei* MCC3195 for 8 week. **Fig. 2A** shows that there was a significant ($P <0.05$) increase (80.64%) was found in the values of IL-6 in HFCG group than those in NCG group. However, these values were significantly ($P < 0.05$) decreased (69.46% and 58.69% respectively) in the MTHFG and LPHFG group compared with those in the HFCG group; moreover these values were found to be significantly ($P < 0.05$) increased (36.61% and 53.13% respectively) in the MTHFG and LPHFG group than those in the NCG group. No significant differences were observed in IL-6 level between the MTHFG and LPHFG groups.

Fig. 2B shows that there was a significant ($P < 0.05$) decrease (28.73%) was found in the values of IL-10 in HFCG group than those in NCG group. However, these values were significantly ($P < 0.05$) increased (25.30% and 24.75% respectively)

in the MTHFG and LPHFG group compared with those in the HFCG group. No significant differences were observed in IL-10 level in the MTHFG and LPHFG group than those in the NCG group; however, there is also no significant difference was found between the MTHFG and LPHFG groups.

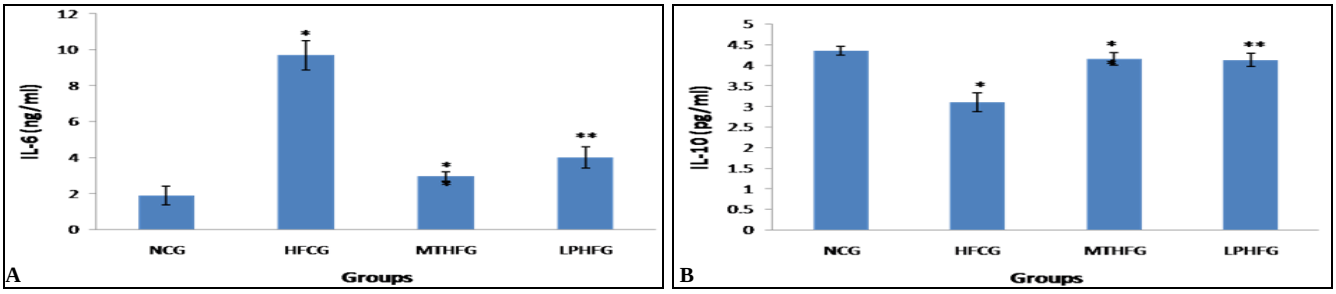

FIG. 2: THE LEVELS OF IL6 AND IL-10 IN BLOOD SERUM OF RATS ADMINISTERED *LACTOBACILLUS PARACASEI* MCC3195 AT 8 WEEK. (A) IL-6 (B) IL-10. IL, interleukin; NCG, Normal control group; HFCG, High fructose fed control group; MTHFG, Metformin and high fructose treated group; LPHFG, *Lactobacillus paracasei* MCC3195 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 on Histology of Pancreas and Liver: Fig. 3 shows the results of histology analysis of the pancreases of the four experimental groups which reveals considerable differences in the structure, number and pattern of islets of Langerhans. **Fig. 3A** shows the normal histological structure of rat pancreas with normal islet in normal control group (NCG). The islets cells were round or oval, elongated and evenly distributed in pancreatic acinar or cytoplasm with clear boundaries around the cells. **Fig. 3B** shows 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 clearly seen. **Fig. 3C** shows the histological structure of rat pancreas in MTHFG group. Metformin treated rat pancreas showing good protection from HFD-induced changes in the pancreatic islets. **Fig. 3D** shows the histological structure of rat pancreas in LPHFG group. Probiotic *Lactobacillus paracasei* MCC3195 treated rat pancreas showed adequate regeneration and protection of *β*-cells when compared with diabetic control group (HFCG).

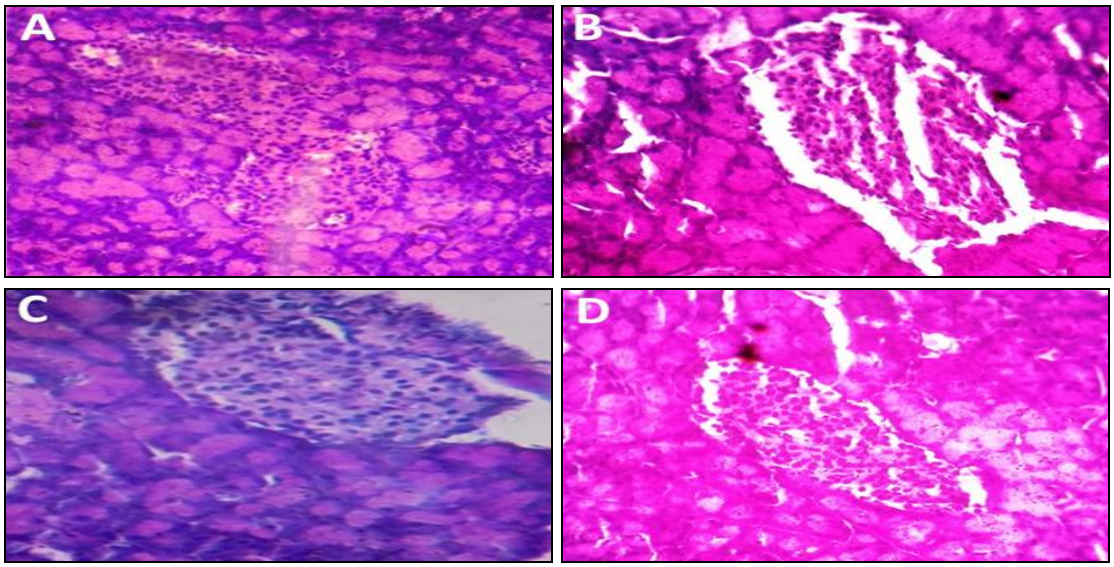

FIG. 3: EFFECT ON HISTOLOGICAL CHANGES OF PANCREAS IN HIGH FRUCTOSE DIET INDUCED DIABETIC RATS. H&E STAINING OF PANCREAS WAS PERFORMED FOLLOWING 8 WEEKS OF ADMINISTRATION, MAGNIFICATIONS, X100 (A) PANCREATIC SECTIONS OF RATS IN THE NORMAL CONTROL GROUP (NCG); (B) PANCREATIC SECTIONS OF RATS IN THE HIGH 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 PARACASEI* MCC3195 AND HIGH FRUCTOSE TREATED GROUP (LPHFG)

Fig. 4 shows the results of histology analysis of the livers of the four experimental groups. **Fig. 4A** shows the normal histology in NCG group with a small number of cells undergoing a degenerative process. **Fig. 4B** shows 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 micro vesicular cytoplasm undergoing steatosis process.

Fig. 4C shows the histological structure of rat liver in MTHFG group. Liver of metformin treated rat shows adequate protection from HFD-induced changes in the normal structure of hepatocytes. Fig (4D) shows the histological structure of rat pancreas in LPHFG group. Liver of diabetic rat treated with Probiotic *Lactobacillus paracasei* MCC3195 shows maximum restoration of hepatocytes and minimal necrosis with normal appearance of hepatic cells.

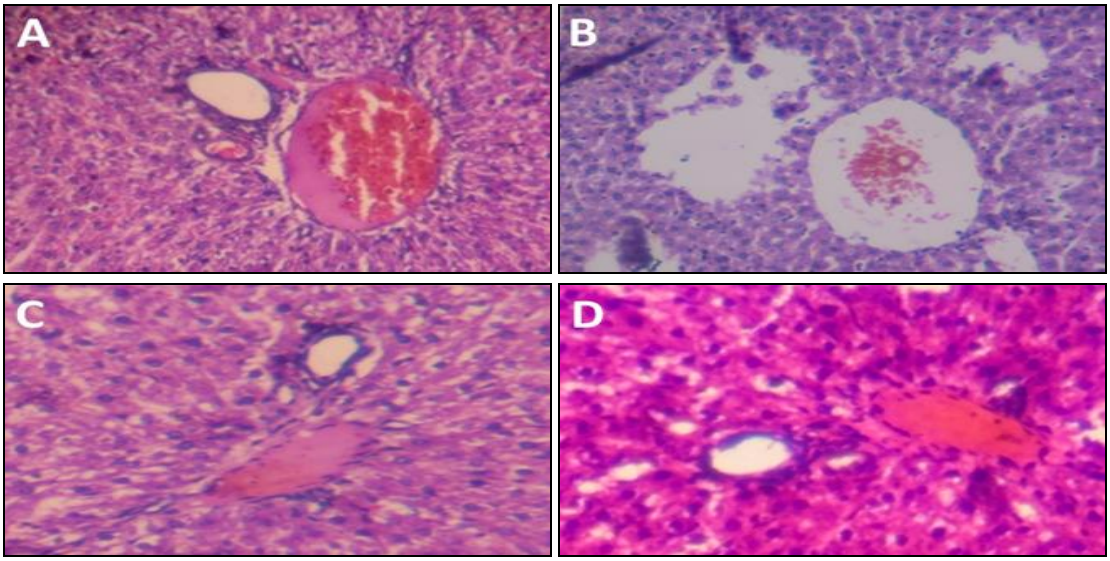

FIG. 4: EFFECT ON HISTOLOGICAL CHANGES OF LIVERS IN HIGH FRUCTOSE DIET INDUCED DIABETIC RATS. H&E STAINING OF PANCREAS WAS PERFORMED FOLLOWING 8 WEEKS OF ADMINISTRATION, MAGNIFICATIONS, X100 (A) LIVER SECTIONS OF RATS IN THE NORMAL CONTROL GROUP (NCG); (B) LIVER SECTIONS OF RATS IN THE HIGH FRUCTOSE 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 PARACASEI* MCC3195 AND HIGH FRUCTOSE TREATED GROUP (LPHFG).

DISCUSSION: Probiotics are known to confer the various health benefits to host when consumed in adequate amount. Various clinical conditions can be prevented and treated by lactic acid bacteria who serve as probiotics [20]. Several recent studies show that *Lactobacilli* group exerts beneficial effect in type 2 diabetes mellitus [21, 22]. *L. acidophilus* and *L. casei* contained in dahi significantly reduce the high fructose induced diabetic symptoms in wistar rats [23]. In the present study we analyze the antidiabetic and anti-inflammatory effect of *Lactobacilli paracasei* MCC3195 in wistar rats for 8 week of experimental period. We have found that *Lactobacilli paracasei* MCC3195 administration prevents and delays the onset of high fructose induced type 2 diabetes in wistar rats. Body weights in various experimental groups were significantly ($P < 0.05$) influenced by time, diet and dose of drug and bacteria. Excessive body weight gain was prevented by *L. paracasei* MCC3195 (LPHFG) and metformin (MTHFG) administration as compared to HFCG rats, they also prevents the symptoms of T2DM. Body weight gain in these groups was near to NCG group values at 8 week. However, few studies confirm that *L. casei* prevents the body weight gain in T2DM mice induced by high fat diet and streptozotocin [24, 25].

Results of OGTT reveals that *L. paracasei* MCC3195 improves the glucose tolerance in LPHFG group while diabetic rats of HFCG group shows impaired glucose tolerance at 4 week. *Lactobacillus paracasei* subsp. *Paracasei* may protect the impaired glucose tolerance in high fat and high fructose diet induced rats through the possible mechanism of improving intestinal

microbiota, preserves their gut integrity and lowers the LPS-induced inflammation [26].

Elevated Fasting blood glucose is one of the important measures of T2DM condition [27]. In our study, FBG was significantly increased in HFCG animals during 8 week of experimental period which is the indicative of onset of T2DM while *L. paracasei* MCC3195 (LPHFG) and metformin (MTHFG) administration in rats prevents the onset of T2DM and significantly decreased the FBG than that of HFCG group. FBG in these groups was near to NCG group values. This outcome reveals the antidiabetic potential of *L. paracasei* MCC3195 in rats. *Lactobacillus gasseri* BNR17 has been reported to reduces the blood glucose level in db/db mice with T2DM [28]. Consumption of probiotics in the adequate amount improves the glucose metabolism within the 8 week of experimental period [29].

HbA1c is one of the very common measures of glycemia in T2DM. HbA1c indicates the no. of glucose molecules binds with the heamoglobin in RBC which provide average blood glucose level of individual of about preceding two-three months. This glycated heamoglobin is considered as important marker to test T2DM [30]. HbA1c was significantly increased in HFCG animals during 8 week experiment which is may be due to increase in blood glucose level of rats while *L. paracasei* MCC3195 (LPHFG) and metformin (MTHFG) administration in rats prevents the rising of HbA1c indicates reduction of blood glucose level for a larger period. *L. paracasei* MCC3195 found to significantly ($P < 0.05$) reduce the HbA1c level in high fructose induced rats. Yadav *et al.*, reported that dahi containing *L. acidophilus* and *L. casei* significantly reduce the HbA1c in high fructose induced type 2 diabetic rats [31].

Insulin is a major hormone that plays a key role in glucose metabolism. Insulin resistance developed due to presence of high amount of lipid in fat deposit tissue namely adipose tissue and their constant release in blood plasma after metabolism. Insulin receptor become resist of insulin due to this condition. In this study high fructose diet induced insulin insensitivity significantly improved by *L. paracasei* MCC3195 and metformin in LPHFG group and MTHFG group respectively. Probiotics such as *L. rhamnosus*, *L. acidophilus* and *Bifidobacterium bifidum* also reported to improve insulin resistance in high fat diet induced mice [32].

The liver plays an important role in managing blood glucose homeostasis, by converting high amount of blood glucose into glycogen [33]. In the present study, Glycogen level were increased in HFCG group due to high fructose diet. Usually hepatic glycogen is favorable to body but extra sugar releases bad cholesterol which may lead to diabetic dyslipidemia. Administration of *L. paracasei* MCC3195 decrease the glycogen accumulation in LPHFG group as compared to HFCG group.

Dyslipidemia is very common condition in type 2 diabetic patient it can be major source of cardiovascular disease in diabetic individuals [34]. In this study we have found that the concentration of serum TC, TG, LDL-C and VLDL-C was significantly ($P < 0.05$) high and HDL-C concentration was significantly ($P < 0.05$) low in HFCG animals as compared to NCG animals. However, significant ($P < 0.05$) decrease 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 LPHFG animals after *L. paracasei* administration than that of HFCG group. Moreover, there was a significant ($P < 0.05$) decrease in TC, TG, LDL-C, VLDL-C and a non significant increase in serum HDL-C was found in MTHFG as compared to HFCG animals. Various mechanisms are involved in lowering the cholesterol level by probiotics such as reduction in cholesterol assimilation and absorption by intestine [35].

Higher amount of blood glucose may lead to Caridiovascular disease and impair the antioxidant system in type 2 diabetic condition. Oxidative stress may induce cellular damage and increases the diabetic complication. Lipid peroxidation and their by product such as malondialdehyde (MDA) increased in type 2 diabetes mellitus (T2DM) [36]. In the present study Administration of *L. paracasei* MCC3195 significantly decreased the LPO level ($P < 0.05$) in LPHFG group as compared to HFCG group. The concentration of GSH, SOD and CAT were significantly increased ($P < 0.05$) in LPHFG group as compared to HFCG group which shows

the excellent antioxidative property of *L. paracasei* MCC3195 in pancreas.

Inflammatory markers such as IL-6 and IL-10 are the major factor in the development of T2DM. IL-6 is a pro inflammatory cytokine and their elevated level is significantly linked with higher risk of type 2 diabetes [37]. We have found the significant ($P < 0.05$) increase in the level of IL-6 in diabetic rats as compared normal rats. However, there was significant ($P < 0.05$) decreased of IL-6 level in the LPHFG group after administration of *L. paracasei* MCC3195. IL-6 level was also decreased in MTHFG group after administration of metformin. *L. paracasei* MCC3195 and metformin both are involved in the lowering of IL-6 level. IL-10 belongs to an anti-inflammatory cytokine that are involved in the lower production of inflammatory cytokine and inhibition of antigen presentation to MHC cells [38].

We have found the significant ($P < 0.05$) decrease in the level of IL-10 in diabetic rats as compared normal rats. However, there was significant ($P < 0.05$) increase in the level of IL-10 level in the LPHFG group after administration of *L. paracasei* MCC3195. IL-10 level was also increased in MTHFG group after administration of metformin. *L. paracasei* MCC3195 and metformin both are involved in the increase of IL-10 level. IL-10 increases the insulin sensitivity in skeletal muscle and involved in the treatment of type 2 diabetes induced by high fat diet [39]. It has been suggested that the antidiabetic effect of *L. paracasei* MCC3195 may be a character of its immunoregulatory attribute. Histological analysis of pancreas and liver also reveals the protective effect of islet of langerhans and hepatic cell respectively by *L. paracasei* MCC3195 in type 2 diabetic rats.

CONCLUSION: The results of this study demonstrates that *Lactobacillus paracasei* MCC3195 administration in high fructose induced type 2 diabetic Wistar rats significantly protect and improves the glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidema, oxidative stress and ameliorates the IL-6 and IL-10 levels. Histopathological analysis of pancreas and liver confirms the protective effect *L. paracasei* MCC3195 in type 2 diabetic rats.

Lactobacillus paracasei MCC3195 exerts the antidiabetic, antioxidative and anti-inflammatory effect in high fructose fed type 2 diabetic rats and protects the diabetes induced complication. These results supported that *Lactobacillus paracasei* MCC3195 can be used for the prevention and delay the onset of T2DM. It can also be used as functional food to manage T2DM. Few more studies are required to determine these activities in other diabetic model for the clinical application of this bacterium in the management of type 2 diabetes.

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