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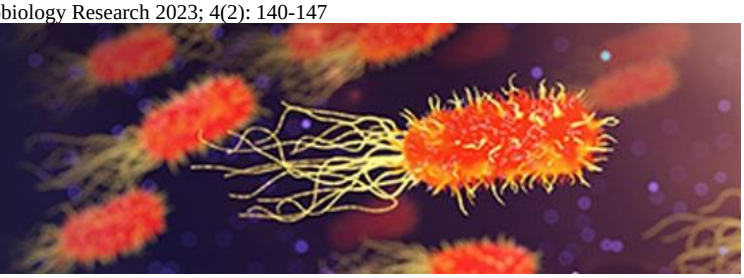

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Effects of probiotic *Lactobacillus* species on the kidney and liver tissues of type 2 diabetic mice

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Abstract
Type 2 Diabetes mellitus (T2DM) is a metabolic disease which causes severe damage to the liver and kidneys. This study aims to assess the effect of probiotic *Lactobacillus* species on the liver and kidney tissues of T2DM mice. Streptozotocin-induced diabetic male mice were treated with probiotic *Lactobacillus* species (1 x 10^9 CFU/mL) while the non-diabetic control group was given placebo for the treatment period. After four weeks of treatment, the mice were made to fast, after which they were weighed and anesthetized. Their liver and kindey samples were removed, weighed and used for histological analsis. The liver and kidney index was also calculated. The mice treated with *Lactobacillus* had liver index ranging from 4.12±0.2% – 4.69±0.3% which was significantly (p<0.05) lower than the liver index of the diabetic control group (5.41±0.2%). On the other hand, the kidney index of the *Lactobacillus* treated mice ranged from 1.31±0.2% to 1.51±0.1% which was significantly (p<0.05) lower than the kidney index of the diabetic control group (1.68±0.1%). The findings of this study show that probiotic *Lactobacillus* species were able to reverse glomerulosclerosis and glomerulonephritis in the kidneys, and focal necrosis in the liver of the T2DM mice. Thus, probiotic *Lactobacillus* species were able to improve liver and kidney disorders in T2DM mice.

Keywords: *Lactobacillus*, kidney, liver, probiotics, type 2 diabetes

1. Introduction
Diabetes mellitus is a metabolic disease that impairs the ability of the body to process blood glucose, which leads to elevation in blood glucose levels. Type 2 Diabetes mellitus (T2DM) causes severe damage to the liver and kidneys, especially when it is left untreated. It has been reported that T2DM causes inflammatory necrosis as well as fibrosis in the liver, which occurs as a result of the abnormalities in lipid metabolism [1]. Diabetic nephropathy is common in T2DM and causes segmental sclerosis of the kidney's glomeruli. The segmental sclerosis of the glomeruli, otherwise known as glomerulosclerosis, is the scarring of the glomeruli which results in the loss of protein in the urine. Probiotics are live microorganisms which provide health benefits to the consumer. The use of probiotic *Lactobacillus* species has been reported to improve kidney function [2]. They have also been reported to improve liver function by regulating liver metabolism and improving liver enzyme levels in T2DM patients [3]. This study aims to assess the most efficient probiotic *Lactobacillus* species for the treatment of liver and kidney disorders in T2DM mice.

2. Materials and Methods
2.1 Collection of Samples and Identification of *Lactobacillus* Species
The following strains of *Lactobacillus: L. casei* CP006690.1, *L. delbrueckii* NR029106.1, *L. fermentum* CP082359.1 and *L. plantarum* NR117813.1 identified in a previous work [4] were used for this study.

2.2 Experimental Setup
Twenty-four (24) male mice (BALB/c) of about 6 – 8 weeks old were purchased and kept in a room with environmental conditions as described by a previous study [3]. The mice were given free access to standard rodent diet and water throughout the experiment and were allowed to acclimatise to laboratory conditions for 7 days. Randomly, all mice were divided into two groups namely, the non-diabetic (ND) control group which had four mice and the type 2 diabetes mellitus (T2DM) group which had twenty mice.

The mice in the T2DM group were injected intraperitoneally with streptozotocin (STZ) at 40 mg/kg body weight, dissolved in 50mM sodium citrate buffer (pH 4.5) for five days. The ND control group received sodium citrate buffer for five days. A day after injection, hyperglycaemia was monitored using a glucometer and confirmed with fasting blood glucose (FBG) levels greater than 126 mg/dL (7.0 mmol/L).

The T2DM induced mice were then randomly divided into four groups containing four mice each. The groups were: the D Control group (diabetic control group), *L. plantarum* group, *L. delbrueckii* group, *L. fermentum* group, and the *L. casei* group. The mice in the ND control group and D control group were orally gavaged with 0.2 normal saline daily for 30 days. On the other hand, the mice in the *L. plantarum*, *L. delbrueckii*, *L. fermentum*, and *L. casei* groups were orally gavaged with *L. plantarum*, *L. delbrueckii*, *L. fermentum* and *L. casei* respectively by giving 1mL of cell suspension (10^9 CFU/mL) daily for 30 days.

2.3 Tissue Sample Collection and Histological Evaluation

At the end of the experimental period, mice were made to fast for 12 hours, after which they were weighed. Following this, mice were anesthetized using sevoflurane and their liver and kidney tissue samples were removed, rinsed, weighed and fixed in formalin for preservation prior to histological analysis. The kidney and liver tissue samples were sectioned, stained and viewed under a light microscope for histological evaluation [5]. Liver and kidney index were calculated using the formula:

$$\text{LIVER INDEX} = \frac{\text{Liver weight}}{\text{Mice weight}} \text{ x } 100$$

$$\text{KIDNEY INDEX} = \frac{\text{Kidney weight}}{\text{Mice weight}} \text{ x } 100$$

2.4 Statistical Analysis

All data in this study were expressed as mean ± standard deviation. Statistically significant difference at $p<0.05$ was computed using two-way analysis of variance (ANOVA). Turkey's pairwise comparison was used to separate the means. All graphics were constructed using SPSS 16.0.

3. Results

3.1 Liver Weight & Liver Index of the Mice after *Lactobacillus* Treatment

The liver weight and index of the mice after five days of T2DM inducement, and thirty days of *Lactobacillus* treatment is presented in Table 1. The diabetic control group had the highest liver weight and index (1.49±0.3g and 5.41±0.2% respectively), which was significantly ($p<0.05$) higher than the non-diabetic control group which had the lowest liver weight and index (1.29±0.3g and 3.54±0.2% respectively). No significant difference ($p > 0.05$) was recorded in the liver weight and index of the *L. casei*, *L. delbrueckii*, and *L. fermentum* groups. However, the liver weight and index of the mice treated with *L. plantarum*, was significantly lower ($p<0.05$) than the other *Lactobacillus* groups. In addition, the liver weight and index of the mice treated with *Lactobacillus* were significantly ($p<0.05$) lower than that of the diabetic control group.

Table 1: Variations in Liver Weight & Index of the Mice After *Lactobacillus* Treatment

Treatment groups	Liver Weight (g)	Liver Index (%)
D Control	1.49±0.3 ^{d}	5.41±0.2 ^{d}
L. casei	1.42±0.2 ^{c}	4.69±0.3 ^{c}
L. delbrueckii	1.42±0.5 ^{c}	4.77±0.3 ^{c}
L. fermentum	1.42±0.4 ^{c}	4.73±0.4 ^{c}
L. plantarum	1.35±0.3 ^{b}	4.12±0.2 ^{b}
ND Control	1.29±0.3 ^{a}	3.54±0.2 ^{a}

*Means with similar superscript along the column show no significant difference ($P>0.05$)

3.2 Kidney Weight & Kidney Index of the Mice after *Lactobacillus* Treatment

The kidney weight and index of the mice after five days of T2DM inducement, and thirty days of *Lactobacillus* treatment is presented in Table 2. The diabetic control and *L. casei* groups had the highest kidney weight (0.46±0.3g and 0.46±0.4g respectively), while the non-diabetic control group had the lowest kidney weight (0.41±0.1g). This difference was statistically significant ($p<0.05$). There was no significant difference ($p > 0.05$) in the kidney weight of the mice in the *L. plantarum* group, when compared to that of the *L. fermentum* and *L. delbrueckii* groups. However, the kidney weight of the *L. plantarum* group was significantly lower ($p<0.05$) than that of the *L. casei* and diabetic control groups. There was no significant difference ($p > 0.05$) in the kidney weight of the *L. plantarum* group when compared to the non-diabetic control group.

The diabetic control group had the highest kidney weight index (1.68±0.1%), while the non-diabetic control group had the lowest kidney weight index (1.14±0.2%). This difference was statistically significant ($p<0.05$). The kidney weight index of the *L. plantarum* group was significantly lower ($p<0.05$) than the *L. casei*, *L. delbrueckii*, and *L. fermentum* groups. The mice in the *L. plantarum* group had a significantly lower ($p<0.05$) kidney weight index when compared to the diabetic control group, and a significantly higher ($p<0.05$) kidney weight index when compared to the non-diabetic control group.

Table 2: Variations in Kidney Weight & Index of the Mice After *Lactobacillus* Treatment

Treatment groups	Kidney Weight (g)	Kidney Index (%)
D Control	0.46±0.3 ^{c}	1.68±0.1 ^{d}
L. casei	0.46±0.4 ^{c}	1.51±0.1 ^{c}
L. delbrueckii	0.45±0.4 ^{bc}	1.50±0.2 ^{c}
L. fermentum	0.45±0.3 ^{bc}	1.49±0.4 ^{c}
L. plantarum	0.43±0.3 ^{ab}	1.31±0.2 ^{b}
ND Control	0.41±0.1 ^{a}	1.14±0.2 ^{a}

*Means with similar superscript along the column show no significant difference ($P>0.05$)

3.3 Histopathology of the Kidney and Liver Tissues of the Mice

The histopathology analysis results of the kidney and liver tissues of the six mice groups are shown in Figure 1 to 12. The histopathology of the six mice groups all revealed normal tubules in their kidney tissue sections. However, the mice groups presented varying glomeruli conditions. Normal glomeruli were observed in the non-diabetic control group as well as the *L. plantarum* group (Fig 1 & 9

respectively). The diabetic control and *L. delbrueckii* groups both presented segmental sclerosis of the glomeruli (Fig 3 & 7 respectively). However, the *L. casei* and *L. fermentum* groups both presented mesangial proliferation of the glomeruli (Fig 5 & 11 respectively).

The six mice groups all presented varying conditions in their liver tissue. The non-diabetic control, *L. plantarum*, and *L. fermentum* groups all showed normal central veins and hepatocytes in their liver tissue sections (Fig 2, 10 & 12 respectively). However, the *L. casei* and *L. delbrueckii* groups presented congested central veins and fatty infiltration of their hepatocytes (Fig 6 & 8 respectively). For the diabetic control group, the histopathology of the liver tissue also revealed congested central vein. In addition, there was focal necrosis of the hepatocytes in the diabetic control group (Fig 4).

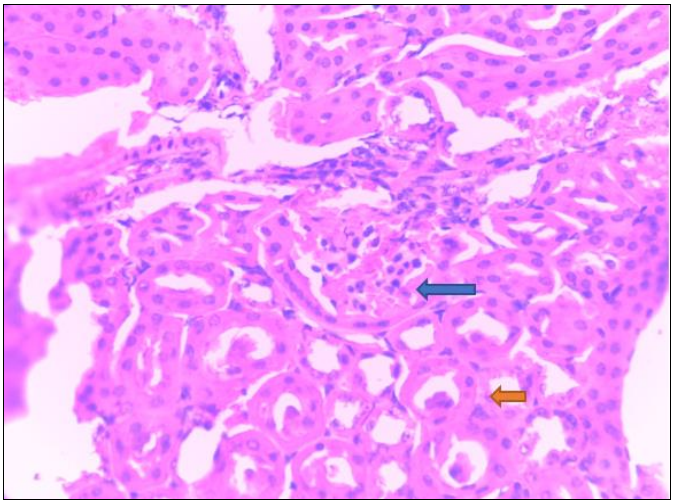

Fig 1: Kidney tissue section of the non-diabetic control mice showing normal glomeruli (blue) and normal tubules (red)

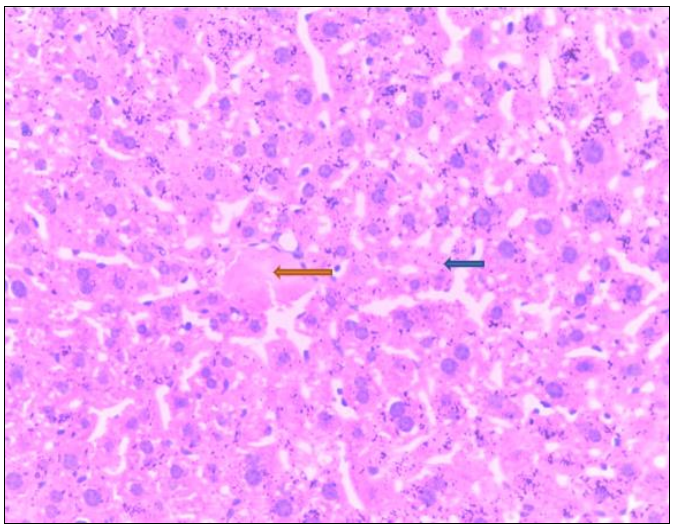

Fig 2: Liver tissue section of the non-diabetic control mice showing normal central vein (red) and normal hepatocytes (blue)

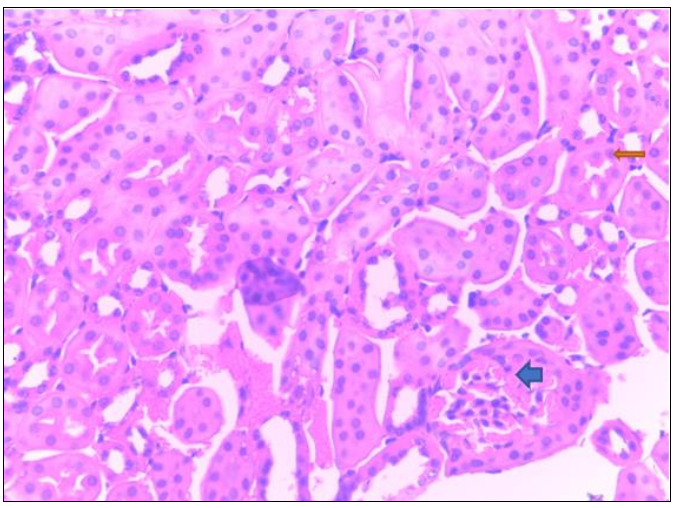

Fig 3: Kidney tissue section of the diabetic control mice showing segmental sclerosis of the glomeruli (blue) and normal tubules (red)

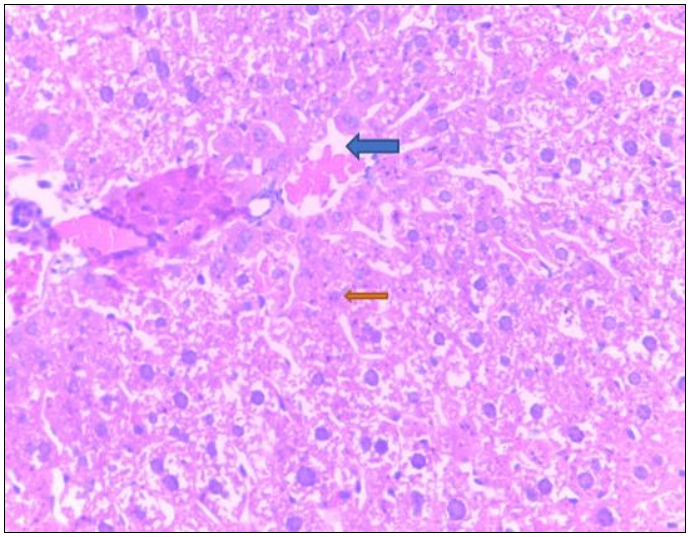

Fig 4: Liver tissue section of the diabetic control mice showing focal necrosis (red) of hepatocytes and congested central vein (blue)

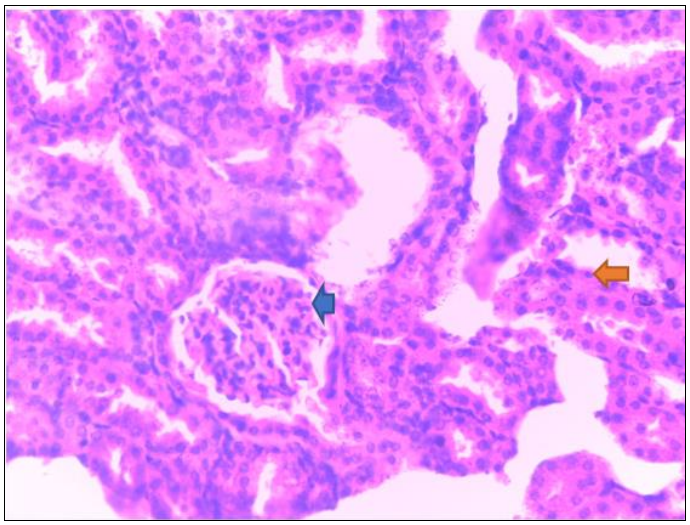

Fig 5: Kidney tissue section of the *Lactobacillus casei* mice showing mesangial proliferation of the glomeruli (blue) and normal tubules (red)

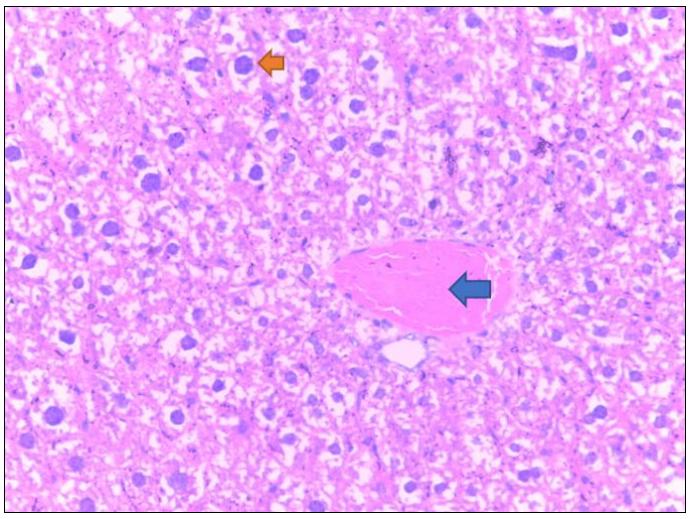

Fig 6: Liver tissue section of the *Lactobacillus casei* mice showing congested central veins (blue) and fatty infiltration (red) of hepatocytes (fatty liver)

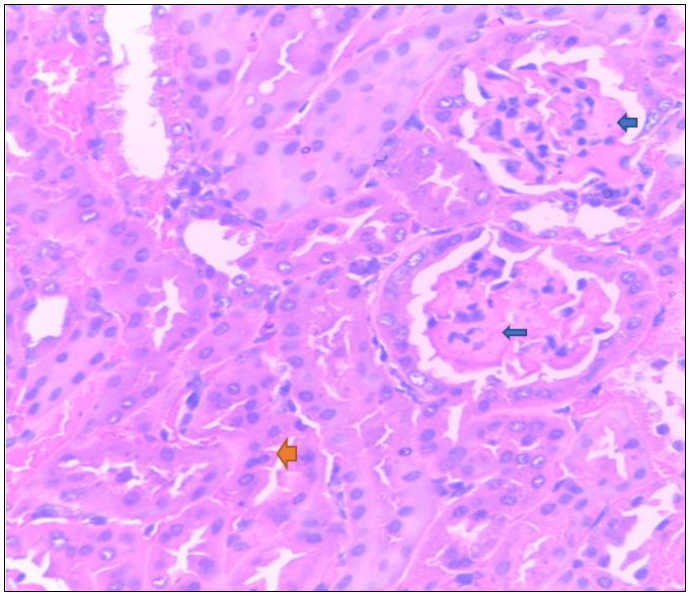

Fig 7: Kidney tissue section of the *Lactobacillus delbrueckii* mice showing segmental sclerosis of the glomeruli (blue) and normal tubules (red)

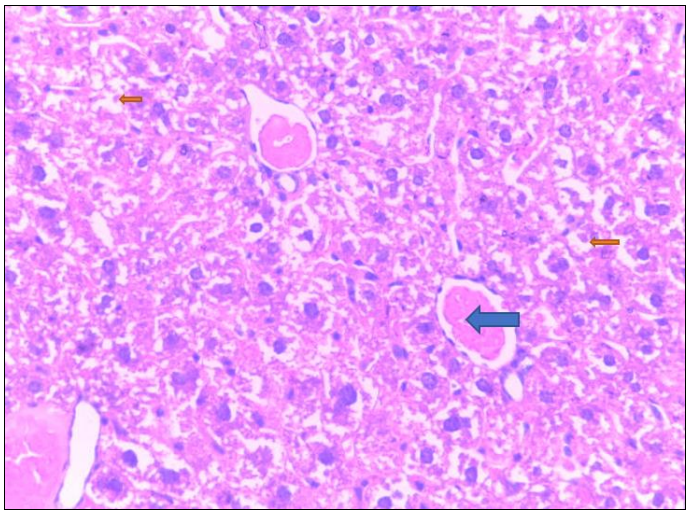

Fig 8: Liver tissue section of the *Lactobacillus delbrueckii* mice showing congested central veins (blue) and fatty infiltration (red) of hepatocytes (fatty liver)

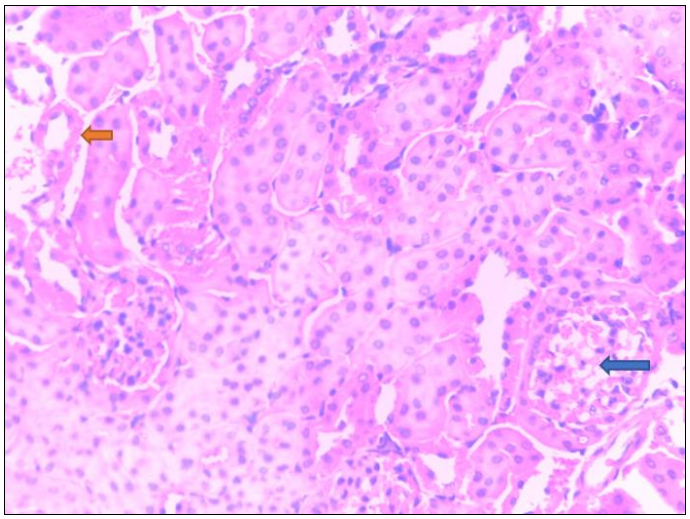

Fig 9: Kidney tissue section of the *Lactobacillus plantarum* mice showing normal glomeruli (blue) and normal tubules (red)

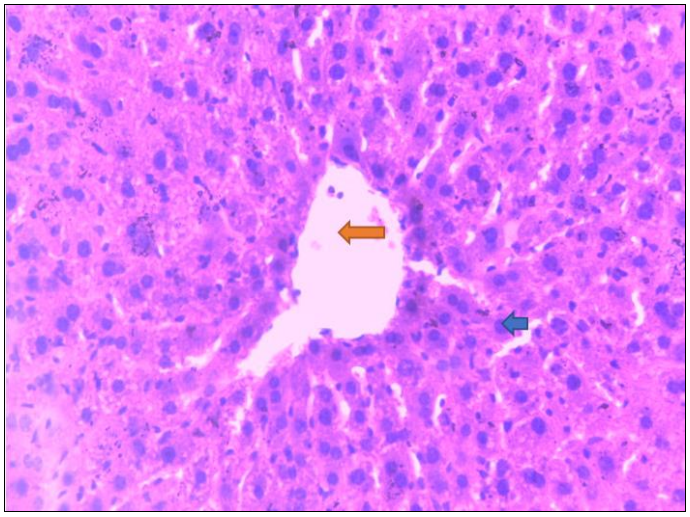

Fig. 10: Liver tissue section of the *Lactobacillus plantarum* mice showing normal central vein (red) and normal hepatocytes (blue)

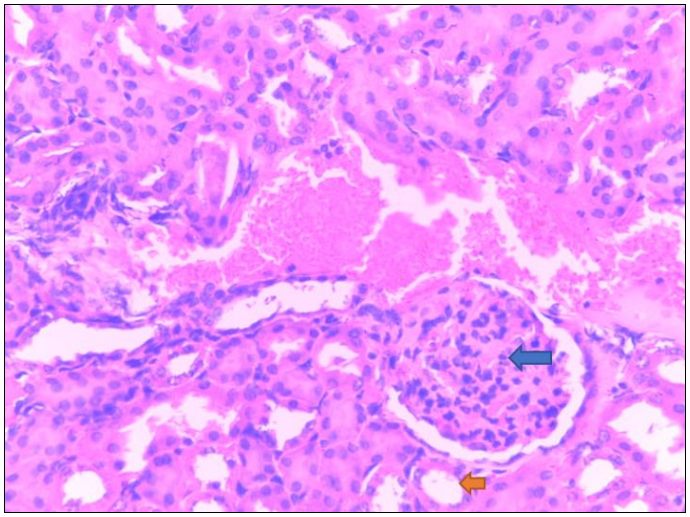

Fig 11: Kidney tissue section of the *Lactobacillus fermentum* mice showing mesangial proliferation in the glomeruli (blue) and normal tubules (red)

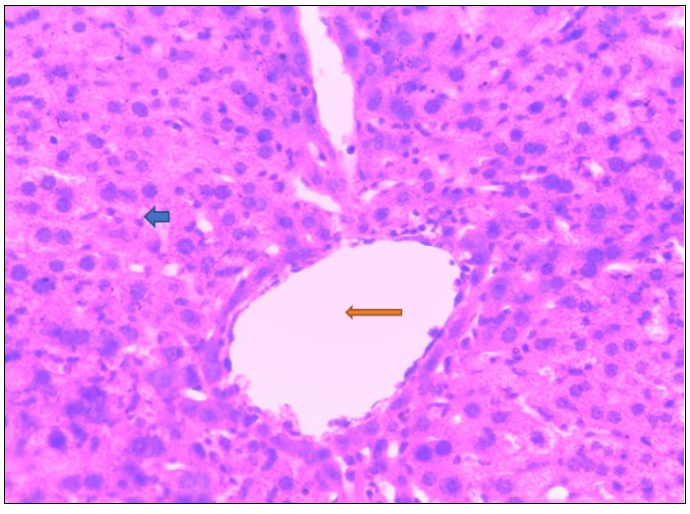

Fig 12: Liver tissue section of the *Lactobacillus fermentum* mice showing normal central vein (red) and normal hepatocytes (blue)

4. Discussion

4.1 Liver Weight Index

With untreated diabetes, the liver undergoes functional abnormalities as it is insulin-sensitive [6]. The liver weight increases while the body weight decreases, which yields a high liver to body weight ratio or liver weight index. The enlarged liver, otherwise known as hypertrophy is caused by hepatocyte enlargement. Liver hypertrophy has been reported to be associated with non-alcoholic fatty liver disease in type 2 diabetes patients, as it is linked to abnormal liver function [7]. The results of this study are in agreement with a previous study [8] which also reported increased liver to body weight ratio in diabetic mice. The result of this study also agrees with another study [5], which

also reported reduction in the liver index of T2DM mice treated with *Lactobacillus brevis*.

4.2 Kidney Weight Index

The results of this study show that the kidney index of the mice treated with *Lactobacillus* was significantly ($p<0.05$) lower than that of the diabetic control mice group. The increase in the kidney index of the diabetic mice reported in this study is in agreement with a study which concluded that there is an association between T2DM and kidney to body weight ratio [9]. Likewise, another study also reported an increase in the kidney to body weight ratio of T2DM induced mice [8]. Kidney enlargement, also known as kidney hypertrophy, has been reported to occur as a result of diabetic nephropathy [10]. Diabetic nephropathy is a complication of T2DM which is characterised by the destruction of the glomeruli as a result of glucose metabolism complications [11]. The results of this study shows that *Lactobacillus* species are able to improve kidney hypertrophy by reversing the increase in kidney to body weight ratio which occurred in T2DM induced mice.

4.3 Histopathology of the Liver Tissue of the Mice

The findings of this study show that *L. plantarum* and *L. fermentum* were able to reverse focal necrosis, fatty infiltration of the hepatocytes as well as congestion of the central vein, which is in agreement with other studies [8, 12, 13]. Congested central veins in the liver are also known as congestive hepatomegaly and occurs as a result of non-alcoholic fatty liver disease (NAFLD) which is due to type 2 diabetes mellitus [14]. It is characterised by enlargement of the liver, as well as backed up blood in the hepatic veins. The hepatic veins aid in the drainage of blood from the liver. When they back up, the liver becomes enlarged and congested. Congestive hepatomegaly also occurs due to congestive heart failure, which is common in T2DM patients [14, 15]. Focal necrosis is the death of a large hepatocyte group within a lobule, and is an indicator of liver injury [16]. Hepatic steatosis (fatty liver disease) and liver damage have been reported to be closely associated with hyperglycaemia [17]. The fatty infiltration of the hepatocytes is the accumulation of excess lipids such as triglycerides, in the hepatocytes. Fatty infiltration of the liver is also known as hepatosteatosis or hepatic steatosis, and is an indicator of non-alcoholic fatty liver disease (NAFLD) which is common in T2DM patients [18]. Probiotics improve the health of the liver by increasing insulin sensitivity, decreasing the concentration of endotoxins, reducing total cholesterol levels as well as improving gut dysbiosis [19].

4.4 Histopathology of the Kidney Tissue of the Mice

The findings of this study indicate that *L. plantarum* is able to reverse glomerulosclerosis and mesangial proliferative glomerulonephritis which occurs in T2DM mice. The ability of *Lactobacillus* species to improve glomerular injury and kidney damage was also reported by other studies [20, 21]. Segmental sclerosis of the glomeruli is also known as glomerulosclerosis, and is the scarring of the glomeruli which occurs as a result of diabetic nephropathy [10]. Diabetic nephropathy is common in both type 1 and type 2 diabetes mellitus (T1DM and T2DM) [11]. The glomerulus are the filtering units of the kidneys and they aid in keeping fluids within the blood vessels. Thus, their scarring results in the loss of proteins in the urine, also known as proteinuria. The mesangial proliferation of the glomeruli is an increase in the number of mesangial cells within the glomerulus [10]. Mesangial proliferation of the glomeruli, also known as mesangial proliferative glomerulonephritis, occurs due to injured glomeruli and is common in T2DM patients [11].

5. Conclusion

The results of this study showed that probiotic *Lactobacillus* species are able to improve liver function in type 2 diabetes induced mice. Among the four *Lactobacillus* species, *L. plantarum* improved liver function to the highest degree. The species, *L. plantarum* and *L. fermentum* were able to reverse congestive hepatomegaly in the T2DM mice. The results of this study shows that *Lactobacillus* species are able to improve kidney hypertrophy. The *Lactobacillus* species, *L. casei* and *L. delbrueckii* were able to reverse focal necrosis in T2DM mice. On the other hand, *L. plantarum* and *L. fermentum* were able to reverse focal necrosis, fatty infiltration of the hepatocytes as well as congestion of the central vein. Thus, it is recommended that these *Lactobacillus* species, especially *L. plantarum* be used for the treatment of T2DM in mice.

6. Conflict of Interest

Not available

7. Financial Support

Not available

8. References

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