

INVESTIGATION OF CHANGES IN RENAL FUNCTION PARAMETERS AND ORGAN HISTOLOGY OF DIABETIC RATS TREATED WITH *Pleurotus ostreatus* ETHANOL EXTRACTS

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ABSTRACT

This work aimed at investigating changes in renal function and organ histology in diabetic rats treated with ethanol extract of *Pleurotus ostreatus* (EEPO). The pharmacological model was 20% High Sucrose (HS) + 20% High Fat Diet (HFD) + 35mg/kg body weight (intraperitoneal) Streptozotocin (STZ) induced diabetic rat model, with the EEPO administered orally at 50, 150 and 300mg/kg b.w. A total of 54 rats were grouped into 6 of 9 animals each. Group 1 was normal control, groups 2-6 received high sucrose- high fat diet (HS-HFD) for 21 days, fasted overnight and thereafter intraperitoneally injected with 35 mg/kg b.w. of streptozotocin, allowed to stay for 72 hrs before treatment. Group 2 was diabetic control, groups 3, 4 and 5 received 50 mg/kg b.w., 150 mg/kg b.w and 300 mg/kg b.w. of EEPO respectively. Group 6 received 150 mg/kg b.w. of metformin HCL. After 72hrs of streptozotocin administration, the rats were fasted and blood and blood collected via tail cutting and their fasting blood glucose level were tested which confirmed hyperglycemia. Treatment was conducted once daily via intragastric gavage for 63 days. Three rats from each group were sacrificed after the 3rd, 6th and 9th week of treatment. Blood samples and kidneys were collected for biochemical and histopathological analysis respectively. After 3rd week of treatment, there was a marked significant ($p < 0.05$) reduction in the concentrations of urea, BUN, and creatinine in groups 3, 4, 5 and group 6 compared to group 2. After 6th week of treatment, there was significant reduction ($p < 0.05$) in the concentrations of urea, BUN and creatinine in treated groups 3, 4, 5 and 6 compared to the diabetic group (group 2). Also, after 9th week of treatment, the results showed a marked significant ($p < 0.05$) reduction in the concentrations of urea, BUN and creatinine in groups 3, 4, 5 and 6 compared to group 2. Marked elevated serum urea, BUN and creatinine concentration in the diabetic control, after treatment period was significantly ($p < 0.05$), time and dose dependently, reduced in treated diabetic rats. Kidney tissue section of the diabetic control showed congested blood vessels, degenerative glomeruli and tubular casts and these damages were reversed in groups 5 and 6 treated with 300mg/kg b.w. EEPO and 150mg/kg b.w. metformin HCL. EEPO has a nephroprotective effect against HS-HFD-streptozotocin induced diabetes.

Keywords: Nephroprotective effect; *Pleurotus ostreatus*; diabetes mellitus; high sucrose-high fat diet; streptozotocin.

INTRODUCTION

Diabetes mellitus is a well-known worldwide epidemic that is characterized by high blood glucose levels brought on by a malfunction in insulin action or secretion (Kumar et al., 2023). According to Goyal and Jialal. (2022), the disease is a metabolic systemic multi-factorial disease whereby the cells of the body have lost the ability to utilize or synthesize a sufficient amount of insulin commensurate with its needs. Among the symptoms of diabetes are osmotic diuresis, which causes an excessive loss of water from tissues, increased hunger and thirst, and a high blood lipid concentration. The disease has been described as a load of disturbance in the structural architecture and operation of biological systems, according to Jamaludin et al. (2016). One of the main contributing factors to the crisis in the metabolism of proteins, carbohydrates, and fats in the endocrine system is diabetes mellitus (Okoroh et al., 2021). Diabetes mellitus is chronic and so complicated that urgent medical attention is needed as well as numerous measures of associated risk reduction outside the control of glucose concentration (Fenta et al., 2023). The increasing prevalence of diabetes has made it a disease of public health concern in the global arena. In 2015, the prevalence of diabetes, globally was estimated to be 415 million people and by 2040, the expectation is that it will rise to 642 million and this rise will be more in low- and middle-income countries (Ogurtsova et al., 2015). Millions of lives have been impacted negatively in the areas of health, economy and socialization because of diabetes mellitus and this calls for a global concern to the scientific world (Lin et al., 2018). There is a clear correlation between diabetes mellitus and kidney disease. The kidney is a vital organ to monitor in the incidence of diabetes mellitus because its key function is in excretion which highlights filtration, secretion and re-absorption. The renal tissues undergo a lot of changes because of the resultant effect of diabetes mellitus (Pourghasem et al., 2015). Changes in the histological section of the kidney of rats

suffering from nephropathy linked diabetes resemble the human disease (Yamamoto et al., 1993). Diabetes mellitus is a main risk factor for chronic kidney disease (CKD), since people suffering from the disease have the vulnerability to developing renal complications. According to the WHO (2016), diabetes mellitus is a contributing factor to renal failure, stroke, impaired vision, and cardiovascular illnesses. Diabetic nephropathy is a rare form of kidney disease closely associated with diabetes that is one of the primary risk factors for the development of end-stage renal disease. The 12th most common cause of mortality in 2015 as reported by the Global Burden of Disease Study was kidney disease and it accounted for 1.1 million deaths worldwide. Chronic kidney disease along side with diabetes is one of the leading causes of death, suffering and loss of economy in the 21st century around the globe (Jager et al., 2019). Therefore, it is crucial to utilize efficient diabetes management techniques in order to maintain blood sugar levels and prevent probable kidney damage (Kumar et al., 2023).

Nowadays, the majority of anti-diabetic medications are used in conjunction with insulin to treat diabetes. These include biguanides, thiazolidinediones, and sulfonylurea. The impoverished find it difficult to obtain these expensive treatments. Synthetic medications come with adverse effects and are costly (Lee et al., 2012). It is therefore suggestive that medications for diabetes mellitus could be sourced from natural products of plant origin because they are cheap and easy to access with little or no side effect.

Pleurotus ostreatus is a member of the pleurotaceae family of mushrooms. The macrofungi is also called tree oyster mushroom or grey oyster mushroom and this name marks it out from the other species in the genus. *Pleurotus ostreatus* is a common, edible, and medicinal plant. The people of Germany cultivated this macrofungi just to sustain life during the world war. However,

Pleurotus ostretus is now widely grown and traded in order to generate income in addition to being consumed. The mushroom, which has a very firm texture when chewed and is generally known as *Eroatakata* in South-Eastern Nigeria; has excellent nutritional content, a wide range of therapeutic uses, and many other positive effects. For a long time, many people have utilized it as food and a way to treat illnesses across globe (Maria et al., 2015). *Pleurotus ostreatus* contains proteins, carbohydrates, dietary fibre, essential micronutrients and low fat content. The macrofungi exhibit significant antioxidant properties due to the presence of bioactive compounds such as flavonoids, phenolics, ergothioneine etc (Tiupova et al., 2023). *Pleurotus ostreatus* may help control blood glucose levels, hence useful in diabetes management (Patinan et al., 2023). Studies

MATERIALS AND METHODS

Preparation of High Fat Diet

High fat diet was prepared according to the formulation by Okoroh et al. (2013), as shown below ;

Table 1:High Sucrose –High Fat Diet (HS-HFD %)

Composition	Proportion (%)
Normal diet (Grower's marsh)	60.0
Sucrose	20.0
Lard	20.0
Total	100.0

*Diet was prepared daily to avoid microbial contamination and fed to the animals *ad libitum*, throughout the period of the experiment.

Collection of *Pleurotus ostreatus* and preparation of mushroom ethanol extract

Fruiting bodies of *Pleurotus ostreatus* (Figure 1) were collected from samples grown with organic supplements at the University of Port Harcourt Research Unit Demonstration Farm in Rivers State, Nigeria. Preparation of *Pleurotus ostreatus* extract had been reported in our previous studies and documented in

have shown that *P.ostretus* has anti-inflammatory, hepatoprotective and nephroprotective characteristics (Jayakumar et al., 2024).The purpose of this scientific study was toinvestigate the changes in the renal function parameters and organ histopathology in diabetic rats treated with organically cultivated *Pleurotus ostreatus* ethanol extracts . The therapeutic information obtained from this work could help educate the public on the health benefits of organically grown mushrooms in their surroundings and could open the door for the official health care systems of developed and developing countries to include mushrooms amongst the vital natural resources that could be employed in the treatment of degenerative diseases related to stress, like diabetes mellitus and its associated complications.

Okoroh et al. (Okoroh et al., 2021). Briefly, 300 ml of ethanol was used to macerate 100 g of ground-up macrofungi material. Three days (72 hrs) after, the mixture was filtered and the filtrate was concentrated using a rotary evaporator to obtain a concentrate which was further evaporated in a water bath at 55°C to obtain a dark brown paste, thereafter, designated as ethanol extract of *Pleurotus ostreatus*(EEPO) and kept in a refrigerator.



Figure 1: *Pleurotus ostreatus*

Experimental animals

For this study, a total of 54 female Wister albino rats with normal blood sugar were employed. The animals were bought from the Animal House of the Department of Biochemistry, Faculty of Science, University of Port Harcourt, Rivers State. They were housed in propylene cages at room temperature, with a 12 hour light/ 12 hour dark cycle, and adequate ventilation. The animals had enough food and water *ad libitum* throughout the experiment. Seven days before the experiment began, the animals were acclimatized to the laboratory environment.

Experimental design

A total 54 animals were divided into six (6) groups of nine (9) animals each. Before injection of streptozotocin, the animals were initially weighed using an electronic scale (TH 500) and their baseline fasting blood glucose level was measured using the Fine Test Auto-coding™ Premium Blood Glucose Monitoring System and Blood Glucose Strips via tail vein incision as documented in Okoroh et al. (Okoroh et al., 2021). Group 1 was designated normal control which was fed with guinea grower's mash diet while groups 2-6 received High Sucrose-High Fat Diet (HS-HFD) for 21 days, fasted overnight and induced with diabetes using a single intraperitoneal injection of streptozotocin (35mg/kg b.w). Group 2 served as diabetic control (HS-HFD-streptozotocin induced diabetic rats without treatment) while groups 3, 4 and 5 received 50 mg/kg b.w, 150 mg/kg b.w and 300 mg/kg b.w. EEPO respectively. Group 6 received 150 mg/kg b.w. of metformin HCL (standard drug). The treatment was done once daily by intragastric gavage for 63 days. Three rats from each group

were sacrificed after 3rd, 6th and 9th week of treatment. Blood samples and the kidneys were collected for biochemical and histological analyses respectively.

Evaluation of serum nephro-specific indices

Estimation of serum creatinine concentration

The serum creatinine concentration was determined using the method of Bartels and Bohmer (1972) as outlined in Randox kit (Randox Laboratories Ltd, Crumlin, England, UK). Creatinine combines with alkaline picrate reagent, chemically, to form picramate which produces an orange red colour and measured spectrophotometrically at 492 nm. Creatinine concentration is directly proportional to the amount of complex formed. About 0.1mL Randox standard creatinine solution was added to 1.0ml of Randox Alkaline picrate Reagent in a cuvette. This was followed by mixing of the contents and absorbance was read after half a minute, then in 2 min again, the absorbance was read again at 492nm using a spectrophotometer. This was done after using distilled water as blank to zero the spectrophotometer. The standard was then replaced with the sample and the analysis was repeated. Creatinine concentration was calculated as follows;

$$\text{Creatinine concentration in sample } (\mu\text{mol/L}) = \frac{\text{change in abs. of sample} \times \text{conc. of std. } (\mu\text{mol/L})}{\text{change in abs. of std.}}$$

Estimation of serum urea concentration and blood urea nitrogen (BUN)

Using Randox Test Kits (Randox Laboratories Ltd., Cruimlin, England, UK), the concentration of urea was determined according to the method of Bartels and

Bohmer ,(1972).The enzyme urease by the process of hydrolysis in the presence of water converts urea to ammonia. The NH_3 reacts with phenol and hypochlorite to yield a blue coloured compound called indophenol. Indophenols colour was measured photometrically.

Urea + water $\xrightarrow{\text{urease}}$ Ammonia +
Carbon(IV) oxide

Ammonia + hypochlorite + phenol $\longrightarrow$
Indophenol (blue compound)

The blank test tube contained 0.01mL distilled water and 0.10mL Radox reagent while the standard test tube contained 0.01 mL Radox standard urea solution and 0.10 mL Radox Reagent I. The test sample test tube contained 0.01mL serum and 0.10mL Radox Reagent I. This was followed by the mixing of the contents and incubation at 37°C for 10 min. About 2.50mL Radox phenol concentrate and 2.50mL Radox hypochlorite concentrate were added to each of the test tubes and their contents were mixed followed by incubation at 37°C for another 150min. Absorbance was read at 546nm against the blank using a spectrophotometer. Urea concentration was calculated as follows;

Urea concentration in sample
(mmol/L)=

$$\frac{\text{absorbance of sample} \times \text{conc.of standard} \left(\frac{\text{mmol}}{\text{L}}\right)}{\text{absorbance of standard.}}$$

Blood Urea Nitrogen (BUN) was calculated as follows:

BUN (mg/dl) = Urea concentration (mmol/l)
x 0.28

Histopathological examination of kidney tissues

Histopathological examination of the kidney tissues was carried out as described by Bancroft and Stevens (Bancroft & Stevens, 1977). For appropriate fixation, small fragments of kidney tissue were gathered and

placed in 10% formalin. After processing, the tissues were embedded in paraffin wax. Hematoxylin and eosin stain was applied after sections with a thickness of approximately 5 μm were cut, mounted, and examined. The slices were then examined under a light source (Nikon Opticphot-2, Tokyo, Japan) at magnifications of x200 and x400, respectively.

Statistical analysis

Using the SPSS/PC + program, data were statistically examined using a one-way analysis of variance (ANOVA). Fisher's Least Significance Difference (LSD) was used to perform multiple comparisons of mean differences. A p-value of less than 0.05 ($p < 0.05$) was considered significant.

RESULTS AND DISCUSSION

Results

Effect of *P. ostreatus* ethanol extract on renal function of diabetic rats after three weeks of treatment.

Table 2 shows the effect of EEPO on the renal function indices of HS-HFD-Streptozotocin induced diabetic rats after 3 weeks of treatment. There was a marked significant ($p < 0.05$) reduction in the concentrations of urea, BUN, and creatinine in group 1 (normal control), group 3 (HS-HFD-streptozotocin induced diabetic rats treated with 50 mg/kg b.w. of *P. ostreatus* extract), group 4 (HS-HFD-streptozotocin induced diabetic rats treated with 150 mg/kg b.w. of *P. ostreatus* extract), group 5 (HS-HFD-streptozotocin induced diabetic rats treated with 300 mg/kg b.w. of *P. ostreatus* extract) and group 6 (HS-HFD-streptozotocin induced diabetic rats treated with 150 mg/kg b.w. of metformin HCl) compared to group 2 (HS-HFD-streptozotocin induced diabetic rats without treatment). The reduction in the concentration of the renal function parameters in the treated groups (Groups 3,4,5 and 6) compared to the diabetic group (Group 2) was dose-dependent.

Table 2: Effect of *P. ostreatus* ethanol extract on renal function of diabetic rats after three weeks of treatment.

Treatment group	Urea Mmol/L	Blood urea nitrogen (BUN) mg/dL	Creatinine mg/dL
Group 1	6.90±0.76 ^b	19.33±0.84 ^b	1.27±1.85 ^b
Group 2	7.10±0.15 ^a	19.89±0.09 ^a	1.34±3.21 ^a
Group 3	6.85±0.53 ^c	19.19±0.46 ^c	1.27±1.21 ^b
Group 4	6.83±0.26 ^c	19.13±0.28 ^d	1.26±0.78 ^b
Group 5	6.81±0.06 ^c	19.08 ±0.10 ^e	1.24±1.34 ^c
Group 6	6.84±0.21 ^c	19.17±0.14 ^c	1.26± 0.20 ^b

Means ± SD (n = 9). Values in the same column with different superscript letters are significantly different at $p < 0.05$

Group 1: normal control

Group 2: diabetic control (HS-HFD-streptozotocin induced diabetic rats without treatment) Group 3: HS-HFD-streptozotocin-induced diabetic rats + 50 mg/kg b.w. of EEPO

Group 4: HS-HFD-streptozotocin-induced diabetic rats + 150 mg/kg b.w. of EEPO

Group 5: HS-HFD-streptozotocin-induced diabetic rats + 300 mg/kg b.w. of EEPO

Group 6: HS-HFD-streptozotocin induced diabetic rats + 150 mg/kg b.w. of metformin HCL.

Effect of *P. ostreatus* ethanol extract on renal function of diabetic rats after six weeks of treatment.

Table 3 shows the effect of EEPO on the serum renal function profile of HS-HFD-streptozotocin-induced diabetic rats after six weeks of treatment. There was a significant reduction ($p < 0.05$) in the concentrations of urea, BUN and creatinine in treated groups 3, 4, 5 and 6 compared to the diabetic group (group 2) whose values of urea, BUN and creatinine were higher. The concentrations of BUN and urea after 6 weeks of treatment in groups 3, 4, 5 and 6 as well as the normal reduced compared to the values after 3 weeks of treatment. However, there was no marked reduction in the concentration of creatinine, comparing the 3rd week and 6th week treatment.

Table 3: Effect of *P. ostreatus* ethanol extract on renal function of diabetic rats after six weeks of treatment.

Treatment group	Urea Mmol/L	Blood Urea nitrogen (BUN) mg/dL	Creatinine mg/dL
Group 1	6.63±0.06 ^c	18.57 ±0.04 ^d	1.29±0.79 ^b
Group 2	7.28±0.17 ^a	20.39 ±0.05 ^a	1.32±3.87 ^a
Group 3	6.81±0.15 ^b	19.08±0.21 ^c	1.27±3.06 ^c
Group 4	6.80±0.12 ^b	19.05±0.14 ^c	1.25±2.64 ^d
Group 5	6.79±0.35 ^b	19.03 ±0.23 ^c	1.23±0.35 ^e
Group 6	6.82±0.06 ^b	19.11 ±0.01 ^b	1.26 ±1.85 ^c

Means ± SD (n = 9). Values in the same column with different superscript letters are significantly different at $p < 0.05$

Group 1: normal control

Group 2: diabetic control (HS-HFD-streptozotocin induced diabetic rats without treatment) Group 3: HS-HFD-streptozotocin-induced diabetic rats + 50 mg/kg b.w. of EEPO

Group 4: HS-HFD-streptozotocin-induced diabetic rats + 150 mg/kg b.w. of EEPO

Group 5: HS-HFD-streptozotocin-induced diabetic rats + 300 mg/kg b.w. of EEPO

Group 6: HS-HFD-streptozotocin induced diabetic rats + 150 mg/kg b.w. of metformin HCL

Effect of *P. ostreatus* ethanol extract on renal function of diabetic rats after nine weeks of treatment.

Table 4 shows the effect of EEPO on the serum renal function profile of HS-HFD-streptozotocin induced diabetic rats after nine weeks of treatment. The results showed a marked significant ($p < 0.05$) reduction in the concentrations of urea, BUN and creatinine in the groups treated with *P.ostreatus* ethanol extracts at 50mg/kg b.w, 150mg/kg b.w., 300mg/kg b.w. (Groups 3, 4, and 5), and group treated with 150mg/kg b.w. of metformin (Group 6) compared to the diabetic group (Group 2). The reduction in concentrations of the renal function parameters was dose and time dependent. When the renal function parameters (urea and BUN concentrations) were examined in the 9th week and 6th week were compared, the values in the 9th week were found to be lower but there was no marked reduction in the creatinine concentration comparing the 9th and the 6th week. This same observation is consistent when comparing renal function values examined in the 3rd and 9th week.

Table 4: Effect of *P. ostreatus* ethanol extract on renal function of diabetic rats after nine weeks of treatment.

Treatment group	Urea Mmol/L	Blood urea nitrogen (BUN) mg/dL	Creatinine mg/dL
Group 1	6.64±0.06 ^e	18.59±0.05 ^e	1.26±0.15 ^b
Group 2	7.35±0.06 ^a	20.60 ±0.06 ^a	1.35±0.92 ^a
Group 3	6.77±0.10 ^b	18.97±0.12 ^b	1.25±2.82 ^b
Group 4	6.70±0.15 ^c	18.77 ±0.13 ^c	1.23±3.91 ^c
Group 5	6.68±0.06 ^d	18.72±0.06 ^d	1.24±2.02 ^b
Group 6	6.69±0.06 ^d	18.75±0.12 ^c	1.22±0.55 ^c

Means ± SD (n = 9). Values in the same column with different superscript letters are significantly different at $p < 0.05$

Group 1: normal control

Group 2: diabetic control (HS-HFD-streptozotocin induced diabetic rats without treatment) Group 3: HS-HFD-streptozotocin-induced diabetic rats + 50 mg/kg b.w. of EEPO

Group 4: HS-HFD-streptozotocin-induced diabetic rats + 150 mg/kg b.w. of EEPO

Group 5: HS-HFD-streptozotocin-induced diabetic rats + 300 mg/kg b.w. of EEPO

Group 6: HS-HFD-streptozotocin induced diabetic rats + 150 mg/kg b.w. of metformin HCL

Histopathological changes in the kidney of the rats.

Plate 1 showed the histological section of the kidney of group 1 (normal control group). The histologic features are shown to be normal compared to group 2 (diabetic group), which exhibited abnormal characteristics (Plate 2).

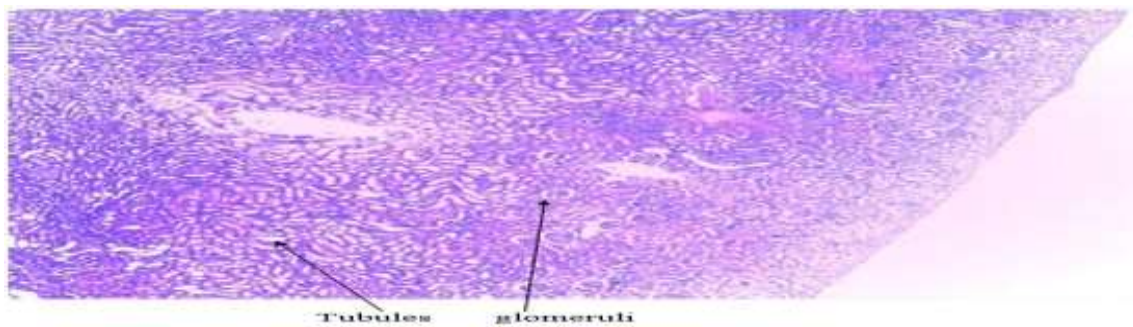


Plate 1: Histological section of the kidney of rats in group 1 (H &E X 100)

Plate 2 showed the histological section of the kidney of diabetic rats (Group 2). The result reveals some abnormalities such as tubular casts as well as degenerative changes in the glomeruli.

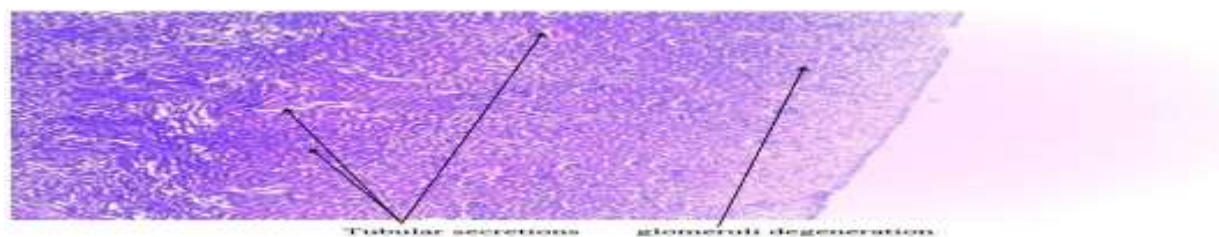


Plate 2: Histological section of the kidney of rats in group 2 (H&E X 100)

The histological section of the kidney of group 3 (diabetic rats treated with extracts at a dosage of 50mg/kg b.w.) are shown in Plate 3. There was no marked positive effect of the treatment at this dose level, on the architecture of the kidney as the organ section displayed abnormal features such as congested blood vessels, degenerating glomeruli and tubular casts.

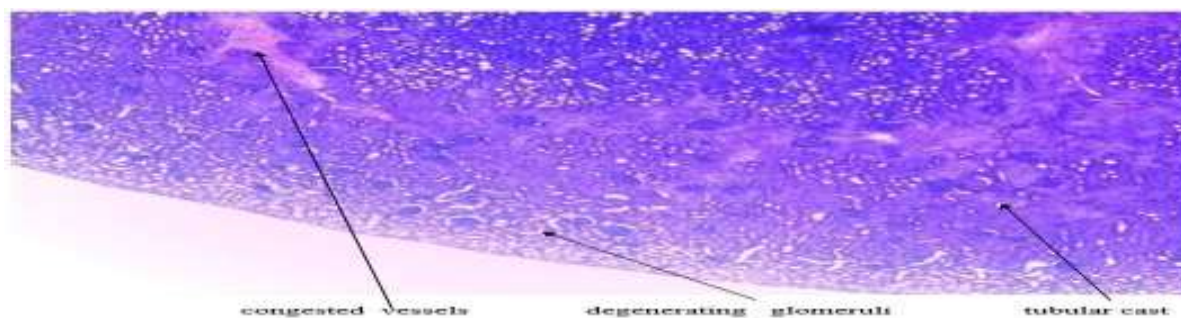


Plate 3: Histological section of the kidney of rats in Group 3 (Kidney H&E X 200).

The histological section of the kidney of group 4 (diabetic rats treated with extracts at dosage of 150mg/kg b.w.) are shown in Plate 4. There was no marked positive effect of the treatment at this dose level, on the architecture of the kidney as the organ tissue section displayed abnormal features such as congested blood vessels, degenerating glomeruli and tubular casts.

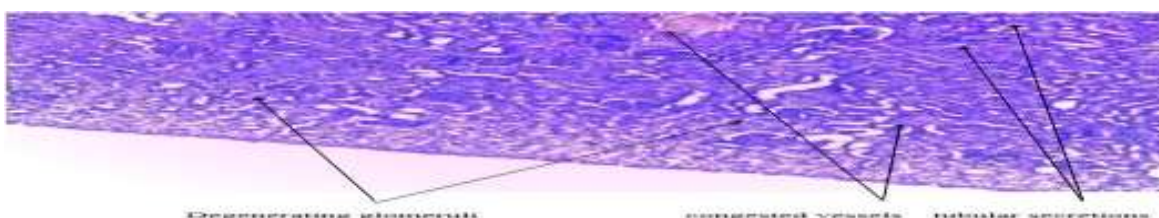


Plate 4: Histological section of the kidney of rats in group 4 (Kidney H &E X 200)

The histological section of the kidney of group 5 (diabetic rats treated with extracts at a dosage of 300mg/kg b.w.) is shown in Plate 5. There was marked positive effect of the treatment at this dose level, on the architecture of the kidney as the organ tissue section displayed no abnormal features such as congested blood vessels, degenerating glomeruli and tubular casts. The glomeruli and kidney tubules had no obvious changes in histology features. This may imply that the ethanol extract of *P.ostreatus* at a dose of 300mg/kg b.w. was able to restore the kidney after treatment of the diabetic rats in group 5.

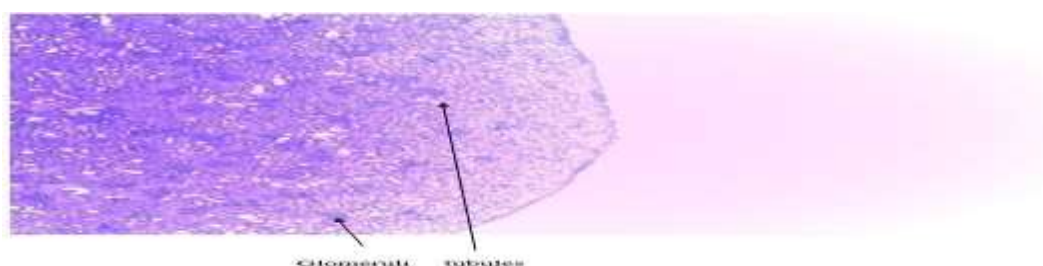


Plate 5: Histological section of the kidney of rats in group 5 (H & E X 100)

The histological section of the kidney of group 6 (diabetic rats treated with metformin HCl at a dosage of 300mg/kg b.w.) is shown in Plate 6. There was marked positive effect of the treatment at this dose level, on the architecture of the kidney as the organ tissue section displayed no abnormal features such as congested blood vessels, degenerating glomeruli and tubular casts. The glomeruli and kidney tubules had no obvious changes in histology features. This may imply that the metformin at a dose of 150mg/kg b.w. was able to restore the kidney after treatment of the diabetic rats in group 5.

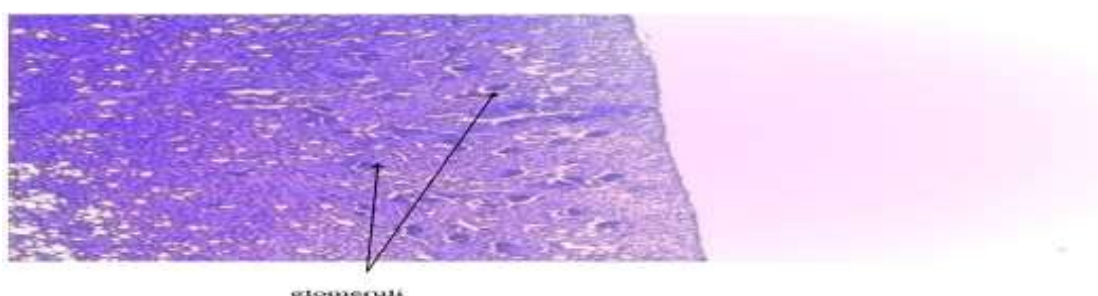


Plate 6: Histological section of the kidney of rats in group 6 (Kidney : H & E X 100)

DISCUSSION

Diabetes mellitus is a disease known to cause health complications and a high rate of mortality around the globe. Diabetes mellitus is a significant contributor to nephropathy and end-stage renal disease. Reduced glomerular filtration rate (GFR) and impaired renal function are the causes of excessive blood urea concentration and serum creatinine levels. Thus, the best biomarkers to track the development of diabetic nephropathy are urea and creatinine (Biri et al., 2021). Hyperglycemia due to diabetes is responsible for the damage of small blood vessels in the kidneys thereby impairing the kidney's ability to filter urea (by-product of protein

catabolism) and creatinine (by-product of muscle metabolism), bringing about an increase in the levels of urea, BUN and creatinine (Abu et al., 2023). Correlation between blood sugar level in fasting state, renal function parameters such as urea, BUN, creatinine etc and the course of diabetes mellitus can offer vital insights in the management and progression of diabetes (Sinha et al., 2024). This research was aimed at investigating changes in renal function parameters and organ histology of diabetic rats treated with *Pleurotus ostreatus* ethanol extracts (a natural medication) against metformin hydrochloride (a synthetic diabetic medication) as standard control of comparison. The marked elevated concentration of serum

urea, BUN and creatinine concentration in group 2 (diabetic control) in the 3rd, 6th and 9th week as revealed in our study could be associated with hyperglycaemia. (Abu et al., 2023), in their study also reported high levels of blood urea and creatinine caused by renal impairment associated with diabetes mellitus. In a study conducted by Abu et al. (2023), Induction of diabetes mellitus led to a significant increase in plasma urea concentration. However, there was marked reduction in the plasma concentrations of creatinine, urea and BUN in groups 3, 4, 5 and 6 in the 3rd, 6th and 9th week of treatment with *P.ostreatus* ethanol extract at various concentrations (Tables 1, 2 and 3). This could imply that the extract and metformin HCl had positive impact on renal function and histopathology of the diabetic rats. Renal function impairment linked to diabetes could be detected by measuring blood urea and serum creatinine. These clinical assays could help in the detection of any kidney impairment on time (Biri et al., 2021). Increased creatinine and urea concentrations of the diabetic rats clearly shows that there was kidney damage (Chutani & Pande, 2017). This study is similar to the work of [(Biri et al., 2021) which indicated increase in blood urea and serum creatinine in diabetic patients. The work of Anjaneyulu & Chopra, (2004) also showed increased urea and creatinine value in diabetic rats. The increase in urea, BUN and creatinine in the diabetic rats could be due to prolonged high blood sugar level which must have resulted to irreversible damage to the nephrons of the kidney. The maintenance of the fluid-electrolyte balance is the kidney's primary function, and it has been compromised. Since creatinine is an indirect indicator of GFR and a sign of decreased renal filtration capacity, an increase in serum creatinine and blood urea is caused by a decline in GFR (Sirivole, 2017). In this scientific study, it could be adduced that the lowering effect of the extract on the BUN, urea and creatinine concentrations was time and dose dependent meaning that it has the efficacy to improve kidney function. The

mechanism of action of *P.ostreatus* ethanol extract in the amelioration of diabetes mellitus could likely be due to its antioxidant, antihyperglycemic and anti-glycation properties which must have led to the deceleration in diabetic nephropathy progression in the diabetic rats. This result is in accordance with the protective effect of methanolic extract of *P.granatum* leaves in streptozotocin induced diabetic nephropathy (Mestry et al., 2017). Oluwole et al. (Akinola et al., 2011), also reported that ethanolic leaf extract of *A.indica* ameliorates hyperglycemia and diabetic nephropathy in rats. The ethanol extract of *P. sarmentosum* was reported to decrease the levels of BUN and creatinine in streptozotocin induced diabetic rats (Suwannasom et al., 2022). The kidney is another organ of interest in diabetes mellitus due to its major role in excretion which includes filtration, secretion and reabsorption. Numerous changes in the renal tissue are resultant effect of diabetes mellitus (Pourghasem et al., 2015). The commonly used animal model in the study of diabetes nephropathy is alloxan or streptozotocin induced diabetic rats (Yamamoto et al., 1993). Changes in the histological section of the kidney of rats suffering from nephropathy linked diabetes resemble the human disease (Yamamoto et al., 1993). The mechanism behind the tubular cast formation, glomerular degeneration, and congested blood vessels in the kidney section of the diabetic rats (plate 2) could be as a result of elevation of pressure in the glomerulus, oxidative stress, inflammation and cell signaling abnormality. All these factors brought about renal damage. Actually, when there is increase in blood glucose level, there will be hyperfiltration and the capillary pressure in the glomerulus increases there by damaging the tiny blood vessels and resultant degeneration of the glomerulus. Hyperglycemia in the rats may have caused oxidative stress as well as advanced glycation end products formation (AGES). Biomolecules such as lipids, nucleic acids and proteins are destroyed leading to inflammation and apoptosis. The increase in the levels of

cytokines and chemokines plus macrophage infiltration enhance infiltration and kidney tissue damage. Tubular casts are formed because of high levels of protein excretion and the damage of the tubular epithelial cells in the kidneys. The high blood glucose level for a long period in the diabetic rats led to the damage of the glomeruli, the glomerular base membrane became thickened. There was also mesangial cell proliferation and then glomerular sclerosis. The renal blood vessel dilation and congestion was due to diabetic nephropathy (QI et al., 2017; Calle & Hotler, 2020). Our results are in consonance with the previous studies conducted by Lin et al. (2018) and Alice et al. (2017). Treated diabetic rats (Groups 3 and group 4) also revealed glomeruli degeneration, tubular casts and congested blood vessels. This may imply that treatment with extract at these doses were ineffective. Glomerular degeneration has been reported as a serious negative change in diabetic nephropathy (An et al., 2015). Tubular hypertrophy, inflammation, and infiltration are highlighted (Najafian et al., 2011) as early histological changes in the kidney of diabetic subjects. Renal blood vessels are also affected by diabetes mellitus (Lee et al., 2012; Deen et al., 2001). However, another interesting indication in this study was that there were no obvious histological changes in the kidney of diabetic rats treated with extract at dose level of 300mg/kg and the kidney of diabetic rats treated with metformin at dose level of 150mg/kg respectively. This clearly showed that the extract compared to metformin, dose dependently, helped in reversing the pathological alteration in the kidney of the diabetic rats to normal. The reason for the nephroprotective effect of the extract may be due to the pharmacological activities of the mushroom against diabetes which could be linked to the presence of some secondary metabolites such as alkaloids, triterpenoids and steroids (Prince et al., 2020). According to Shahin et al. (2022), alkaloids, terpenoids, and other phytochemicals present in plants, which were detected in our previous study (Prince et al., 2020), are used to cure diabetic nephropathy. Alkaloids have been

investigated preclinically for their potential to slow the course of diabetic nephropathy including oxymatrine, magnoflorine, berberine, and trigonelline (Putra et al., 2023). Berberine, an alkaloid has been shown to prevent renal damage by preventing NF- κ B-induced renal inflammation and TGF- β /Smad3-mediated renal fibrosis (Sun et al., 2015). Terpenoids have been reported to prevent kidney damage associated with diabetes by lowering oxidative stress, apoptosis, and inflammation (Al hroob et al., 2018). These phytochemicals present in EEPO which have been reported in our previous study (Prince et al., 2020), might have utilized the foregoing mechanisms in protecting against renal damage associated with diabetes as found in our present study. The positive effect of *P.ostreatus* ethanol extracts and metformin on the histopathology of the diabetic rats (plate 5) in our study involved significant improvement of the histology of kidney via the reverse of the damages caused by hyperglycemia associated with diabetes in the rats. This includes insulin secretion recovery, restoration of damaged ducts in the nephron and the glomeruli. This may be due to the anti-hyperglycemic, anti-oxidative and anti-inflammatory activities of *P.ostreatus* attributed to the bioactive compounds in the macrofungi such as phenol, flavonoid, terpenes, polysaccharides etc (Devi et al., 2023). Phytochemical screening conducted on *P.ostreatus* in by Prince et al. (2020), revealed the presence of alkaloids, triterpenoids and steroids and these bioactives have the capacity to positively influence glucose catabolism, enhance the sensitivity of insulin and offer protection to the beta cells of the pancreas, they can tackle oxidative stress, and they can modulate pathways involved in inflammation signaling thereby reducing inflammation. The restoration effect of *P. ostreatus* and metformin on the histopathology of the kidney in our work is in consonant with the previous studies of Bashir et al. (2022), where *Acacia Senegal* and insulin significantly improved the renal ultrastructure in rats.

CONCLUSION

The findings in this study consequently imply that the nephrological abnormalities linked to high sucrose- high fat- streptozotocin induced diabetes in female Wister albino rats can be reversed by the crude ethanol extract of the fruiting bodies of organically cultivated *Pleurotus ostreatus*. The marked elevated concentration of serum urea, BUN and creatinine concentration in the diabetic rats, in the 3rd, 6th and 9th week was time and dose dependently reduced, considerably in diabetic rats treated with *P.ostreatus* ethanol extract. This was confirmed by the nephro-protective effect of *P.ostreatus* ethanol extract (300mg/kg b.w.) and metformin (150mg/kg b.w.) on the kidney histopathology of the treated diabetic rats as there was a significant improvement in the histology of the kidney via the reverse of the damages caused by hyperglycemia associated diabetes in the rats. It can be adduced that *P. ostreatus* crude ethanol extract may be used to treat renal function problems linked to diabetes mellitus.

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REFERENCES

- Abu, O.D, Imafidon, K.E. and Iribhogbe M.E. (2015). Biochemical effect of aqueous leaf extract of *Icacina trichanta* Oliv. On urea, creatinine and kidney oxidative status in CCl₄-induced wister rats. *Nigerian Journal Of Life Sciences*. 5 (1):85-89
- Abu, O.D., Awhin, E.P. and Iyare (2023). Assesment of renal function in diabetic wister rats treated with ethanol extract of *Cucumis sativus* fruit, *African Journal of Health, Science and Environment*, 4 (1): 101 -107.
- Al Hroob, A.M, Abukhalil, M.H., Alghonmeen, R.D. and Mahmoud, A.M. (2018). Ginger alleviates hyperglycemia-induced oxidative stress, inflammation and apoptosis and protects rats against diabetic nephropathy. *Biomedicine and Pharmacotherapy*, 106:381-9.
- Alicic, R.Z., Rooney, M.T. and Tuttle, K.R. (2017). Diabetic kidney disease : challenges, progress, and possibilities. *Clinical Journal of the American Society of Nephrology*, 12 (12) :2032-45.
- An ,Y., Xu, F. and Le, W. (2015) .Renal histologic changes and the outcome in patients with diabetic nephropathy. *Nephrology Dialysis Transplantation* , 30 (2) :257-266.
- Anjaneyulu, M., Chopra, K. (2004). Quercetin, an antioxidant bioflavonoid, attenuates diabetic nephropathy in rats. *Clinical Experimental Pharmacology and Physiology*, 31(4): 244-248.
- Bancroft, J.D., Stevens, A.(1977) Theory and practice of histological techniques. Edinburgh: Churchill Livingstone, 1977.
- Bartels, H. and Bohmer, M. (1972). *In vitro* determination of creatinine and urea. *Journal of Clinical Chemistry* , 2: 37-193.
- Bashir, S.O., Osman, O.M., Zakr, M.S.A., Aboonq, M.S., Mirdad, M.T., Khalil, K.I. and Morsy, M.D.(2022) . *Acasia Senegal* inhibits diabetic nephropathy ultrastructure alteration and renal biomarkers changes in rats, *International Journal of Morphorlogy* , 40 (3) : 808-816.
- Biri, S.R.K., Sankeerth, S.L.V., Sandhya, R.T., Rajkumar, G. and Vadlakonda, A. (2021). A study on evaluation of the blood urea and serum creatinine in diabetes mellitus patients. *International Journal of Clinical Biochemistry and Research* ,8 (4):285-288.

- Calle, P. and Hottter, G. (2020) . Macrophage phenotype and fibrosis in diabetic nephropathy. *International Journal of Molecular Sciences*, 21 (8):2806
- Chutani, A. and Pande, S. (2017). Correlation of serum creatinine and urea with glycemic index and duration of diabetes in Type 1 and Type 2 diabetes mellitus: A comparative study. *National Journal of Physiology, Pharmacy and Pharmacology*, 7 (9) :914-919.
- Deen, W.M., Lazzara, M.J. and Myers, B.D. (2001). Structural determinants of glomerular permeability. *American Journal of Renal physiology*, 281(4): F579-96.
- Devi, P.V., Islam, J., Narzary, P., Sharma, D. and Sultana, F.(2024). Bioactive compounds, nutraceutical values and its application in food product development of oyster mushroom. *Journal of Future Foods*, 4 (4) : 335-342.
- Fenta, E.T., Eshetu, H.B., Kebede, N., Bogale, E.K., Zewdie, A., Kassie, T.D., Anagaw, T.F., Mazengia, E.M. and Gelaw, S.S. (2023). Prevaence and predicators of chronic kidney disease among type2 diabetic patients worldwide, systematic review and meta-analysis. *Diabetology Metabolic Syndrome*, 15 :245.
- Goyal, R. and Jialal, I. (2022) Diabetes mellitus type :*statpearls Publishing*, Treasure Island (FL).
- Jager, K.J., Kovesdy, C., Langham, R., et al. (2019) A single number for advocacy and communication worldwide more than 850 million individuals have kidney diseases. *Kidney International*., 96: 1048-1050.
- Jamaludin, M., Nazratun, N., Zariyantey, A.H. and Budin, S.B. (2016). Mechanism of diabetes –induced liver damage:The role of oxidative stress and inflammation. *Sultan Qqboos University Medical Journal*, 16(2):e132-e141.
- Jayakumar, D., Santhapur, R. and Mclements, D.J.(2024) Preperation and characterization of plant protein-mushroom hybrids: Toward more healthy and sustainable foods. *Food Biophysics*, 19, 1077-1094.
- Kumar, M., Dev, S., Khalid, M.U., Siddenth, S.M., Noman, M, John, C. et al. (2023) The bidirectional link between diabetes and kidney disease ; Mechanisms and management. *Cureus*, 15(9): e45615.
- Lee, I.M., Shiroma, E.J., Lobelo, F., Puska, P., Blair, S.N.and Katzmarzyk, P.T. (2012). Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *The Lancet* ,380(9838): 219-29.
- Lin, Y.C., Chang, Y.H., Yang, S.Y., Wu, K.D. and Chu, T.S.(2018). Update of pathophysiology and management of diabetic kidney disease. Available at: *Journal of the Formosan Medical Association*, 117:662-675.
- Maria, E. , Talia, V. and O’ctavio, P. (2015) Edible Mushrooms: Improving Human Health and Promoting Quality life. *International Journal of Microbiology*: 14 pages.
- Mestry, S.N, Dhodi, J.B., Kumbhar, S.B. and Juvekar, A.R.(2017) Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by Punica granatum Linn. Leaves extract, *Journal of Traditional and Complementary Medicine* , 7 : 273-280 .
- Najafian, B., Alpers, C.E. and Fogo, A.B. (2011) Pathology of human diabetic nephropathy. *Diabetes and the kidney*, 170 : 36-47.
- Ogurtsova, K., da Rocha Fernandes, J.D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N.H., Cavan, D., Shaw, J.E. and Makaroff, L.E. (2017). IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Research and Clinical Practice*, 128: 40-50 .
- Okoroh, P.N., Onuoha, S., Uwakwe, A.A. and Ukegbu, C.Y. (2021). Effect of ethanol extract of the fruiting bodies of *P.ostreatus* on serum lipid profile and atherogenic indices of HS-HF-STZ induced diabetic rats. *Asian Journal of Research in Biochemistry*, 9 (2): 11-21.

- Oluwole, B.A, Laura Z., Olufunke O.D, Oluwafunmike, S.A., Luciana, D. and Ezekiel, A.C.M. (2011).Ameliorative effects of ethanolic leaf extract of *Azadirachta indica* on renal histologic alterations in streptozotocin-induced diabetic rats. *American Journal of Clinical Medicine*, 39 (5):903-16
- Patninan, K., Sermwittayawong, D., Nuinamwong, S., Khimmaktong, W., Noipha, K. and Nongporn, H.(2023). Purified polysaccharides extracted from gray oyster mushroom (*Pleurotus sajucaju* (Fr.) Sing.) stimulate glucose uptake in C2C12 myotubes through the activation of AMP-activated protein kinase (AMPK) and glucose transporter 1 (GLUT 1) proteins. *Sains Malaysiana.*, 52, 2815-2827.
- Pourghasem, M., Shafi, H. and Babazadeh. Z. (2015). Histological changes of kidney in diabetic nephropathy. *Carolina Journal of Interdisciplinary Medicine*, 6(3):120–127.
- Prince, O., Benjamin, A., Constance , N. *et al.* (2020). Proximate Nutrient Potential, Phytochemical Screening and Vitamin B-Composition of *Pleurotus ostreatus* Cultivated by Substrate Organic Supplementation Techniques. *Asian Journal of Research in Botany.*, 4(4):138-46.
- Putra, I.M., Fakhrudin, N., Nurrochmad, A. and Wahyuono, S.(2023). A review of medicinal plants with renoprotective activity in diabetic nephropathy animal models..*Life* , 13 (2): 560.
- Qi, C., Mao, X., Zhang, Z. and Wu H. (2017). Classification and differential diagnosis of diabetic nephropathy, *Journal of Diabetes Research.* , 8637138.
- Shahin, D.H.H., Sultana, R., Farooq, J.*et al.* (2022).Alotaibi AA. Insights into the uses of traditional plants for diabetes nephropathy: a review. *Current Issues in Molecular Biology*, 44: 2887–2902.
- Sinha ,A. and Bharty, R. (2024) . Exploring the correlation of fasting blood glucose, serum urea, serum creatinine, and diabetes duration in type 2 diabetes patients : A cross-sectional study, *Student’s Journal of Health Research Africa*, 5 (6), 3006-1059.
- Sirivole, M.R. and Eturi, S. (2017). A study on blood urea and serum creatinine in diabetes mellitus from sangareddy District, Telangana, India. *International Journal of Medical and Health Research* , 3(12):132-136.
- Sun, S.F., Zhao, T.T., Zhang, H.J.*et al.* (2015).Renoprotective effect of berberine on type 2 diabetic nephropathy in rats. *Clinical and Experimental Pharmacology and Physiology* ,42(6):662-70.
- Suwannason, N., Thepmalee, C., Khoothiam, K., and Thephinlap, C.(2022). Effect of ethanolic extract from *Piper sarmentosum* activity and complications in normal and streptozotocin-induced diabetic rats, *Journal of Applied Pharmaceutical Science*, 12 (04) : 071-079.
- Tiupova, A., Oledzki, R. and Harasym, J. (2025) . Physicochemical, functional and antioxidative characteristics of oyster mushrooms. *Appl. Sciences*, 15 (3) , 1655
- World Health Organization, WHO (2016). Global report on diabetes. Pp. 26-27. ISBN 978-92-4-156525-7.
- Yamamoto, T., Nakamura, T., Noble, N.A., Ruosiahti, E. and Border, W..A .(1993) Expression of transforming growth factor beta is elevated in human and experimental diabetic nephropathy. *Proceedings of the National Academy of Sciences of the United States of America*, 90:1814-1818.