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# RENAL PROTECTIVE EFFECT OF *PLEUROTUS OSTREATUS* AND *LENTINUS SUBNUDUS* IN STREPTOZOTOCIN- NICOTINAMIDE INDUCED DIABETIC NEPHROPATHY IN RATS

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## ABSTRACT

Diabetic nephropathy is defined clinically as the gradual development of renal impairment in the setting of hyperglycemia. This illness is becoming the leading cause of renal failure in many nations, eventually leading to death. Microalbuminuria (MA) is the most common early sign of diabetic nephropathy (DN), the major cause of chronic kidney disease and end-stage renal disease. Diabetic nephropathy development may be slowed by early detection of MA and diabetes therapy. According to the International Diabetic Federation, the prevalence of diabetes in individuals aged 20 to 79 years old was 8.8 percent in 2015, indicating that the illness afflicted nearly 440 million people globally. It is expected that by 2035, the overall population would have risen to roughly 550 million people. The goal of this study, however, is to look at how oyster mushrooms (*Pleurotus ostreatus*) and shiitake mushrooms (*Lentinus subnudus*) lower blood glucose levels in diabetic rats induced by streptozotocin-Nicotinamide. A 55 mg/kilogram body weight combination of *Pleurotus ostreatus* and *Lentinus subnudus* (POS) was administered orally to diabetic wistar rats with STZ-NA induced diabetes. The current study's goal is to create the POS combination, standardize its Pharmacognostic and physio-chemical properties, and assess its acute and sub-acute toxicity. The Pharmacognostic, physio-chemical examination was carried out using standard procedures. The OECD criteria Nos. 407 and 423 were strictly followed for both the acute and sub-toxicity investigations. A single dosage administered to rats resulted in negligible morbidity in the acute toxicity study. During the sub-acute toxicity research, the treated rat's body weight changed when compared to the control rat. At a dosage of 1000 mg/kg, morphological changes were also found in the pancreas, lungs, kidney, and stomach of the treated rat. Toxicity investigations revealed that the POS extract is safe, however at the maximum dose (1000mg/kg body weight), mild toxicity indications developed in the liver and kidney. The current research sought to determine whether or not POS had anti-diabetic potential, both in vitro and in vivo. As a result, it is conceivable to utilize it as a nontoxic additional therapy in the management of diabetic nephropathy and related complications, and this provides experimental proof for the drug's use in diabetes treatment.

**Keywords:** Diabetic nephropathy, Microalbuminuria, *Pleurotus ostreatus*, *Lentinus subnudus*, OECD

**Introduction :-**

Type 1 diabetes affects about 30% of people, while type 2 diabetes affects 40% of people. which is long term consequences of DM Alicic et al [1]. End-stage renal disease (ESRD) is typically brought on by diabetic nephropathy (DN)Parving et al [2]. High arterial blood pressure, rapid glomerular filtration loss, and a high risk of cardiovascular morbidity and mortality, is persistent albuminuria are signs of DN Hovind et al [3]. Recent epidemiological studies have highlighted the distinctive variety of these disorders' natural histories, leading to the adoption of the phrase diabetic kidney disease to refer to any sort of renal injury that occurs in diabetes patients Doshi and Friedman [4]. In both (DM) type 1 and type, DN is defined as chronically increased albuminuria of more than 300mg/24hr or an albumin/creatinine ratio of more than 300 mg/g creatinine, verified in at least two out of three samples, and the lack of any other signs of renal impairment Ritz et al [5]. In the US, approx. 54.4% of patients with DM (Type 1) may require kidney replacement. 2 According to Furthermore, the number of dialysis patients with DM in Taiwan reported to enhanced from 3778 in 2000 (11.9 % of prevalent dialysis patients) to 23,139 in 2012 (34.2 % of prevalent dialysis patients) Lin et al [6]. In about one-third of diabetic patients, DN develops after a latency phase that can last many years. The personalized medicine approach recommends screening people for DN or checking for microalbuminuria so that resources with more intensive treatment and early preventive actions can be made available Susztak et al [7]. There is severe glomerulopathy during microalbuminuria, yet a large proportion of people with microalbuminuria can regress to normoalbuminuria. Perkins et al [8]. Diagnosis of DN is additionally hampered by the fact that many individuals with DN do not exhibit the typical DN pattern. Chen et al [9]. Mushrooms are a dietary staple in many countries throughout the world. Protein and chitin, as well as phenolic acid, flavonoids, and alkaloids, are predominant in insoluble carbohydrates. Agunloye et al [10]. In today's eating habits, foods high in sugar, food additives, and calories are consumed at a higher rate. On the other hand, consumers have begun to recognize the link between nutrition and health. As a result, global food consumption patterns suggest a consumer preference for foods that are rich in nutritional value and offer health benefits. Leonard et al [11]. Because of its substantial concentration of useful chemicals and capacity to produce essential amino acids Pleurotus ostreatus is one of the most well-known edible mushrooms species Valverde et al [12]. According to earlier studies, Pleurotus ostreatus fermentation increased the protein content and bio accessibility of P. vulgaris and A. sativa plants as well as the levels of antioxidant and antinutrient compounds. Numerous studies have revealed that the fruiting body of Pleurotus ostreatus serves as a functional component in the creation of finished foods. We are aware of no concurrent use of Pleurotus ostreatus as a biotransformation agent or component in the manufacture of functional meal. Espinosa-Páez et al [13]. Bioactive constituents of Pleurotus ostreatus (P.ostreatus) are steroids, alkaloids, terpenoids, lectins, and nucleotides phenols These compounds have all been extracted, identified, and have shown promising biological effects. However, the findings are generally dispersed Lindequist et al [14]. Many individuals consume cereal-based meals or inhabit mineral depleted soil, which can result in a diet deficient in essential minerals. Johns and Eyzaguirre [15]. It can, however, alter numerous activities such as sleep, metabolism, and body temperature, leading to a range of illnesses C.A. McClung [16]. Proteins from Pleurotus ostreatus have high levels of glutamic, aspartic, and lysine as well as vitamin C and folic acid, according to their amino acid profiles. The

mushroom growth causes biochemical changes in the substrates Sopanrao et al [17]. It has a distinctive flavour and aroma and is low in fat while being abundant in protein, fiber, carbs, minerals, and vitamins. Equal nutritional value is provided by both naturally occurring and commercially manufactured mushrooms Kalmıs et al [18]. On the other hand, Lentinus subnudus which is a Nigerian edible mushroom, having a dietary component in many countries of the world. In Nigeria, Mushroom hunting is always a delightful activity; these fungi are utilized for therapeutic purposes, and they also serve as a competitive, rewarding, and profitable venture, especially as a food source Gbolagade et al. [19]. According to Yang et al., Many mushrooms have been documented in terms of their nutritional benefits and flavour components. Yang et al [20]. Nearly majority of the world's population relies on natural goods to preserve their health since these remedies are not only inexpensive but also free of any adverse repercussions. This is because higher plants and fungi are rich sources of bioactive chemicals, which may be used in the creation of innovative medication Jagtap et al [21]. Both types of mushrooms have medicinal qualities such hepatoprotective, anti-inflammatory, anti-tumor, and anti-diabetic activity. 1.2 Physiology and Pathology of DN DN is characterized by tubular hypertrophy followed by intestinal fibrosis as well as tubular atropy, with arteriolar hyalinosis. In chronic cases, endothelial cell fenestration, decreased podocyte number, and infiltration of macrophages and T lymphocytes are also present. Weil et al [22].

Management of DN:

The risk of cardiovascular morbidity among diabetes patients with microalbuminuria is 7- 40 times greater compared to an age-matched general population with normoalbuminuric diabetes. Treatment for diabetic nephropathy should take into account all the factors that increase the patient's risk for cardiovascular disease as well as measures to delay the progression of renal impairment.

Hypertension

Because of the well-established merits of lowering blood glucose levels on both the course of renal failure and cumulative cardiovascular disease mortality, blood pressure monitoring and management has become an integral part of diabetes therapy. In investigations of people with DM (Type 1), the significance of antihypertensive medications in maintaining kidney function was initially identified. In a cohort DM (Type1) of patients with overt nephropathy, Mogensen lowers mean blood pressure from 163/103 to 144/95 mmHg and monthly GFR decline from 1.23 to 0.49 ml/min. Mogensen et al [23]. Blood pressure lowering decreases or stabilizes AER in both DM (type 1 and type 2) with microalbuminuria, delaying the onset of overt nephropathy. Melbourne et al [24].

Blood pressure target

Although the precise BP below which there are no more advantages is unknown, the British Hypertension society recommends initiating medications in DM patients with a BP of > 140/90 mmHg with a targeted BP of 140/80 mmHg , or 125/75 mmHg in individuals with (Type 1) with > 1g/day of proteinuria Ramxay et al [25].The Joint National Organization on the Detection, Evaluation, and Treatment of High Blood Pressure in the United States has suggested that patients with diabetes keep their blood pressure below 130/85mmHg JNC et al [26].

ACE Inhibitors

Other common hypertension medications have been shown to be helpful, but ACE inhibitors are well recognized for having extra antihypertensive characteristics and Reno protective instances Viberti et al [27]. First-line treatment for both DM (Type 1 and Type 2) , as well as for non-hypertensive diabetic patients, includes the use of ACE inhibitors. The dose of these drugs is gradually increased until the AER is back to normal. Lovell HG [28].Various other medications may also be useful as alternative to ACE Inhibitors like Angiotensin 2 blocker but their broad studies in different situations is not known and can be used only in patients who can tolerate ACE inhibitor, so standard medications can be used as per guidelines Reichard et al [28].

Glycemic control

Despite the lack of evidence that it impacts the course of nephropathy in these individuals, adequate glycemic management reduces the incidence of microalbuminuria and overt diabetic nephropathy in diabetes patients with microalbuminuria Holman et al [30]. This is due to the potential benefits in both renal disease and cardiovascular disease as well as other factors Perkovic et al [31] Both the American and British criteria place a strong emphasis on attaining and maintaining strict management of blood glucose, with a target HbA1c of <7%. Duckworth et al [32].

Low-protein diet

2 meta-analyses, dietary protein was shown to delay the advancement of DN patients with type 1 diabetes Pedrini et al [33]. Because we do not yet know how individuals will behave to protein restrictions or whether they will have an impact on general practice long-term prospective studies are being carried out to deal with all of these problems Waugh and Robertson [34].

Lipid control

In primary renal disease, dyslipidemia is a risk factor for the development and progression of renal impairment Maschio et al [35]. Lipid reduction has demonstrated to be beneficial in diabetic people with established cardiovascular disease. According to the Scandinavian Simvastatin Survival Research and the Cholesterol and Recurrent Events Study, the effectiveness of statins in reducing coronary events was equivalent to, if not greater than, the overall group in two significant secondary prevention trials Haffner SM [36].

Collection & Preparation of samples.

Fresh mycelial biomass Pleurotus ostreatus and Lentinus subnudus were procured from Green roots farm. The samples were ground to a fine powdered and utilized for further extraction of organic compounds.

Preparation of the mushroom extract and its qualitative analysis:

Sample was placed on a grinder to obtained fine powder. The Soxhlet apparatus was used in order to accomplish the task of powder extraction. 20g of powder were put into the Soxhlet apparatus, and they extracted it using 500 mL of a mixture of hexane, ethanol, and water that was poured in gradation at a temperature that was kept between 45-50 °C. Additionally, the extracted substance was subjected to concentration. Finally, the combination of both samples were obtained and named as POS. The use of standard techniques allowed for the identification of a wide variety of phytochemical constituents that were found in the extracts. Further The POS was kept in dessicator for examination of its oral toxicity and its pharmacological research against DN Jayakumar et al [37].

Physicochemical evaluation

LOD/Moisture content:

The LOD was determined using a hot air oven. The material was weighed at 2 grams and placed in a petridish of known weight. The petridish holding the known weight sample was then dried in an oven at 105 C for 60 minutes, followed by 30 minutes, and the moisture content was determined using the formula provided below Manzi et al [38].

Moisture Content = (Fw - Pw) × 100/ W

Fw = final constant weight of sample & petridish

Pw = pre-weight of petridish

W= initial weight of the sample.

Estimation of Ash content:

Total ash:

About Two (g)powder was weighed in a clean, dried, first weighed silica crucible. The crucibles were transferred to muffle furnace then set the temperature at 550˚C. The furnace was incinerated until carbon free white ash was formed. Later the crucible was carefully placed in desiccators and weighed after cooling . Total ash percentage was estimated as follows:-

Acid-insoluble Ash:

The ash that had been gathered above was heated with 25 parts of 1M HCl before being filtered through ashless filter paper. Hot water was used to rinse away the filter paper's insoluble material before it was burnt to a uniform weight. The insoluble material was moved from the filter paper to the original crucible, dried on a heating plate, and burned to a consistent weight (W4). Prior to being reweighed, the residue was chilled in a desiccator for 30 minutes. W1 represents the weight of the empty silica crucible, W2 the sample's weight in the crucible prior to ignition, W3 the sample's weight in the crucible post ignition, and W4 the constant weight after addition.

Acid insoluble ash value (%) = (Fwll - Fwl) × 100/W

Fwll = Final constant weight of the crucible with total ash

Fwl = final constant weight of crucible with acid insoluble ash

W = Initial weight of the powdered plant material.

4.3.2.3 Water – soluble Ash of POS:

25 ml of water was added to the crucible, which contained complete ash, and was then heated for 5 minutes before filtering using ashless filter paper. The filter paper was covered with the insoluble substance, washed with hot water, and then burnt for 15 minutes at 500 °C in a crucible. After chilling in a desiccator for 30 minutes, the residue was cleaned once again. The % water soluble ash formula is:

Water soluble ash value (%) = (W7-W6 mg/g) × 100

Phytochemicals Estimations

Estimation of Total Phenol Content (TPC)

The sample's stock solution (1 mg/ml) was made. A appropriate amount of the sample was collected from the stock solution and placed in a 25 ml volumetric flask.In the volumetric flask, about 10 ml of distilled water and 1.5 ml of the folin's reagent were added. The mixture for the reaction was let to stand for 5 minutes. The volumetric flask was filled with 4 ml of about 20% Na2CO3 after which the capacity was made up. For 30 minutes, the mixture was held motionless. Various concentrations of C7H6O5 were used to create a calibration curve (10g Gallic acid in 100 ml methanol). At 765 nm, spectrophotometric absorbance was measured. TPC in the unprocessed Sample was evaluated using the methodology below Ainsworth and Gillespie [39].

**Animals**

Six Wistar rats weighing between 100 and 150 g were housed in polyethylene kennels beneath realistic conditions (25±2°C) ambient temperature, 45%-55% relative humidity, and a 12-hour light/12-hour dark cycle). The animals had five days to become acquainted to their environment and the testing methods before the trials began. The National Institutes of Health's (NIH) "Guide for the Care and Use of Laboratory Animals" and internal guidelines approved by the Indian government's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA/AC/09/1273) were both followed in all of the experiments.

Toxicity studies

Organization for Economic Co-operation and Development (OECD)-423:

Acute oral toxicity study The acute (oral) toxicity research followed the guidelines for the acute toxicity class technique, according to Organization for Economic Co-operation and Development. (OECD)-423. Three animals of each sex were used throughout each stage in line with these norms, with females being preferred because of their higher sensitivity. Determine the dosage for the following stage based on the animals' death and/or moribund state. Thus, two to four processes must be completed in order to get a conclusive determination regarding acute toxicity (OECD-423)

Animal requirements:

To investigate the drug's oral toxicity, albino wistar rats of either sex weighing 100-150g were utilized to assess oral toxicity of drug (POS)

Preparation of dose:

Animals were chosen based on the criteria, and they were then placed in the proper cages in accordance with the established classifications. Wistar rats were given the chance to get acclimated to the lab environment before the five days of testing started. They were made to fast overnight but were given water before the dose.

Method:

The rat was divided into 3 groups (n=3) animals. The rat fasted overnight and the drug was administered orally. The rat received doses of 500, 1000, and 2000 mg/kg of the POS, and he animal was monitored for mortality and clinical signs for 30 min, 4 hours, 24 hours, 1 week, and 2 weeks for changes in the skin, BW, fur, eyes, and mucous membranes, as well as for tremors, salivation, diarrhoea, sleep, and coma, as well as any obvious signs of illness or

treatment response. The rat was kept under strict observation for a maximum of 14 days, and the number of rats that perished during the course of the study was noted.

Repeated dose 28-dose days' sub-acute oral toxicity of POS: -

After desired data gained on LD50 of the POS by acute toxicity studies the repeated dose of the POS was assessed to analyse its toxic. The possible side effects include the effects on the immune, endocrine and nervous system was observed. The multiple dose administration and its effects was studies are given. Results indicate relationship of the dose and response; NOAEL is also subjected. Wistar rats were housed in the typical setting, both male and female, under normal and healthy conditions. The cycle of light and dark (12:12h) and the temperature (222°C) were both maintained constant. The test animals received the pellet diet in addition to water and lithium. Both the male and female Wistar rats were housed in separate cages. Throughout this trial, housing for male and female Wistar rats was managed separately. The women used were not pregnant or nulliparous.

Preparation of doses: -

Animals were chosen based on the needs and housed in the appropriate cages for the different groups. The Wistar rats were given five days to get used to the lab environment before the trials started. The experiments might then start after that. The previously chosen groups of experimental animals were regularly administered oral doses of the medication under test in increasing concentrations. One animal from each experimental group is selected to receive the dose for a total of 28 days at each dosage level. For each dosage level, male and female Wistar rats weighing (15020) kg apiece were chosen. The selection of each dosage level was considered for any existing toxicity

Method

The maximum dose was chosen for the induction of toxic consequences rather than with the intention of causing extreme suffering or death. The dose for repeated dose toxicity was chosen from the observed results of the POS on acute oral toxicity and was 250 mg/kg, 500 mg/kg, 750 mg/kg, or 1000 mg/kg. The following parameters, which were evaluated in accordance with the protocol, were noticed in the animal.

Haematological Parameters:

At the conclusion of the experiment, the rat was put to death. Blood samples were then taken through the retro-orbital sinus, put in tubes containing K2EDTA, and had their Hematological parameters assessed at the Shri Mata Prasad Veterinary Diagnostic Laboratory in Lucknow. The Hematological parameters include Red blood cell (RBC) count, haemoglobin (Hb) levels, lymphocytes (LY), monocytes (MO), granulocytes (GR), and platelet count (PLT)

Serum Biochemical Parameters

The serum separated was analysed at Shri Mata prasad veterinary hospital and trauma centre Lucknow. The following enzymes were measured, BUN, creatinine (kidney) , AST , ALT , ALP( Liver) Natelson et al [40].

Oral glucose tolerance test (OGTT)

To perform the oral glucose tolerance test, all animals were fasted for 16 hours with just water (OGTT). We then separated them into six groups using a random selection. Following that, the rats were administered POS (100 mg/kg, 200 mg/kg, and 300 mg/kg), a vehicle, and metformin (250 mg) orally in 200 ml doses. After 30 minutes, glucose at a rate of 2 g/kg was

administered. Blood was drawn after snipping the tail vein at 0, 30, 60, 90, and 120 minutes, and blood glucose levels were analyzed with a glucometer. Median and colleagues [77].Group I; control, Group II; Vehicle, Group III standard (Metformin treated) Group IV, V and VI as test control and treated with POS (100 mg/kg), POS (200mg/kg) and POS (300 mg/kg) respectively. After respective treatments of 30 min the glucose 2g/kg was feed and blood was withdrawn tail vein at predefined time intervals (0, 30, 60, 90 and 120 min) to record blood glucose level using glucometer

Induction of diabetic nephropathy by using STZ-Nicotinamide (STZ-NA) to the animals

Male Wistar rats were given a single I.P. of STZ (55 mg/kg body weight) dissolved in ice cold 0.1 M citrate buffer after fasting overnight, followed by Nicotinamide (120 mg/kg) after 15 minutes. This method was used to create diabetes in rats. The control group was given a citrate buffer as a vehicle. After a fortnight, rats' blood was obtained and their glucose levels were recorded. The hyperglycemic rats (blood glucose level more than 300 mg/dl) were chosen and employed in the investigation Parveen et al [41].

Experiment designed on STZ-NA rats

After generating diabetic nephropathy, the mice were separated into seven groups and marked:Group 1: healthy Control, Group 2: Diabetic control, Group 3: Vehicle control, Group 4: standard control (metformin treatment), Group V, VI and VII as diabetic test control and treated with POS 100 mg/kg, 200 mg/kg, 300 mg/kg respectively An intragastric tube was used to give the POS and metformin for 21 days. Following the cervical dislocation that was used to euthanize all of the rats on the 21st day, blood was drawn from the retro-orbital plexus, serum was separated, and it was then subjected to a battery of lipid-related biochemical tests (including total cholesterol, HDL, LDL, VLDL, and triglycerides).

Determination of Body weight (BW)

The body weight was monitored every day during the research period Hu et al [42].

Determination of blood glucose level:

Blood glucose was determined by collecting the blood from tail was measured by using glucometer P. Trinder [43].

Estimation of Lipid profile:

Triglyceride, high-density lipoprotein cholesterol, and total cholesterol concentrations in serum Very-low-density lipoprotein cholesterol and low-density lipoprotein cholesterol

Result:-

Collection, Authentication and processing of samples

Fresh mycelial biomass Pleurotus ostreatus and Lentinus subnudus were obtained by Green root farm (Kharade village, shahapur, Dist. Thane). Sample were authenticated from Christ Church College Dr. Naveen Kumar (Botanist). Material was air dried under shade and powdered manually using mortar and pestle. Size of powdered material was further reduced with the help of an electronic grinder. Powdered materials of different parts were stored in different containers for further use.

Preparation of the POS extract and its qualitative Analysis:

Extraction was done through Soxhlet Apparatus by gradient solvent method. The whole extraction runs for 5 cycles. Each time extract was poured in pre-weighed Petridish and evaporated on a rotavapor. After complete evaporation of solvent, Petridish was kept in an oven at temperature up to 40ºC for 30 minutes. Petridish was weighed again. Finally, the obtained extract was named as POS and then kept in a desiccator for further qualitative analysis.

Physicochemical evaluation:

LOD/Moisture content:

Moisture content of sample

| S.No | Drug | %w/w |
|---|---|---|
| 1 | *L. subnudus* | 95±1.6 |
| 2 | *P. ostreatus* | 87±1.2 |

An excessive amount of moisture can cause the enzymatic breakdown of constituents, the growth of fungi and bacteria, as well as alterations in the biochemical makeup of plant medications. The formulation that contains less moisture ought to be more stable for a greater amount of time. According to the literature review, the moisture content of *L. subnudus* was 92% **Gbolagade et al [44]** and *P. ostreatus* was 85% **Yehia RS [45].** The current research revealed that *L. subnudus* had a moisture level of 95% and *P. ostreatus* had a moisture content of 87%. As a result, the moisture content readings of both species lie within the allowable limits. And the results are depicted in Table no 5.1. and Fig.5.1

**Determination of Ash content**

**Table 5.2 Ash value of Mushroom extract**

| S.No | Drug | Physiochemical Properties | w/w |
|---|---|---|---|
| 1 | *L. subnudus* | Acid Soluble Ash | 1.79±0.01 |
| | | Water Soluble | 2.4±0.08 |
| | | Total Ash | 2.29±0.01 |
| 2 | *P. ostreatus* | Acid Soluble Ash | 4.4±0.08 |
| | | Water Soluble | 2.3±0.08 |
| | | Total Ash | 4.4±0.12 |

The quality control parameter includes loss on drying, extractive values and ash values as per the Ayurvedic Pharmacopoeia were evaluated for dried crude drug. The ash value is the most important parameter for sample to ensure quality control. As per the literature review the total ash value of *Lentinus subnudus* was 5.9 **Gbolagade et al [44]** and *Pleurotus ostreatus* was found to be 6.1 **kikuchi et al [46]**. The total

ash value of *L. Subnudus* was found to be 2.29±0.01 and *P. ostreatus* was found to be 4.4±0.12, which is consistent with earlier research.

**Phytochemicals total estimations:**

**Estimation TPC and TFC**

**Table 5.3 Estimation of TPC and TFC**

| **S.NO** | **PHYTOCHEMICAL** | ***PO*** | ***LS*** | ***POS*** |
|---|---|---|---|---|
| 1. | TPC | 4.89±0.02 | 5.89±0.02 | 10.55±0.017 |
| 2. | TFC | 3.71±0.011 | 2.89±0.02 | 6.27±0.03 |

**Toxicity study**

**Acute toxicity Study:**

**Effect on Behavioral Parameter Table 5.7 Behavioral parameters**

| | **Skin & fur** | **Mucous membrane** | **Lacrimation & salivation** | **Idleness** | **Piloerection** | **Enuresis** | **Defecation** | **Shiver and turbulence** | **Lifespan** |
|---|---|---|---|---|---|---|---|---|---|
| **30 min** | | | | | | | | | |
| **C1** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 1** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 2** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 3** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 4** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **4 hrs** | | | | | | | | | |
| **C1** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 1** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 2** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 3** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 4** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **24 hrs** | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **C1** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 1** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 2** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 3** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 4** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **1 week** | | | | | | | | | |
| **C1** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 1** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 2** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 3** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 4** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **2 weeks** | | | | | | | | | |
| **C1** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 1** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 2** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 3** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |
| **Group 4** | **N** | **N** | **N** | **N** | **N** | **-** | **N** | **-** | **-** |

Where**, N=Normal**, **None = Not observed** C1 =control (Saline) Group 1 =Experimental (POS) N=Normal

According to literature review No sign of toxicity was observed in the wellness parameters during the14-day observation period **Deepalakshmi et al [47].** The acute toxicity effect of POS ethanolic extract was assessed according to OECD guidelines 423, with the doses 500, 1000 ,2000 mg/kg. There was no treatment related adverse symptoms or death recorded after the administration of POS at a dose of 500, 1000, 2000 mg/kg. The behavior of the treated groups with the POS ethanolic extract and control group was first examined for 30 min, 4 hours, 24 hours, 1 week, 2 weeks. The findings revealed no change in behavior, skin effect, mucous membrane, Salivation and lacrimation, idleness, piloerection, enuresis, defecation, shiver and turbulence or lifespan.

**Effect on Body Weight**

| S. No | Groups | 0 Day | $7^{th}$ Day | $14^{th}$ Day |
|---|---|---|---|---|
| 1 | Group 1(control) | 162±0.94 | 160±1.41 | 160±0.47 |

| | | | | |
|---|---|---|---|---|
| 2 | Group 2(500mg/kg) | 165±2.05 | 160±4.71 | 150±4.72 |
| 3 | Group 3(1000 mg/kg) | 160±1.69 | 155±2.16 | 150±6.2 |
| 4. | Group 4 (2000 mg/kg) | 165±2.05 | 150±4.9 | 135±6.2 |

**Effect on body weight:**

In the present study, on 0day, 7day, 14 day of the research, the rats weight was assessed and recorded in Table No. 5.9. and figure no. 5.7 show the weight changes that were calculated and recorded. when compared to the control rats, rats treated with POS had a lower body weight.

**LD 50 determination: -**

| S. No | Dose | Dose Difference (DD) | No of animals | No. of dead animals | Mean mortality (MM) | DDxMM |
|---|---|---|---|---|---|---|
| 1. | 500 mg/kg | 0 | 3 | 0 | 0 | 0 |
| 2. | 1000 mg/kg | 500 | 3 | 0 | 0.5 | 250 |
| 3. | 2000 mg/kg | 1000 | 3 | 1 | 0.5 | 500 |

LD50 =1750

Where DD=Dose difference and MM=Mean mortality

J. W. Trevan devised the LD50 test, generally known as the median lethal dosage test, to estimate the amount of a test sample that would result in a 50% fatality rate in a certain species of animal. It is generally the initial test performed on any substance before further toxicity testing are performed. It is used to assess the potential toxicity of substances to humans. Although death is the primary goal, non-lethal acute effects may arise as toxicity indicators depending on the substance being evaluated.

To establish the unfavorable consequences that may arise as a result of accidental or purposeful short-term exposure, substances' acute toxic potential must be examined**. Erhirhie EO et al [ 48]** The toxicity status of the test substance can be determined based on the results of an acute toxicity test. AS per OECD guidelines with LD50 > 5 mg/kg are categorized as being highly dangerous, whereas compounds with an LD50 of more than 15,000 mg/kg are considered to be quite innocuous.

**Sub-Acute toxicity body weight**

**Effect on body weight**

**Weight variation**

| S.no | Group | Bodyweight | | | |
|---|---|---|---|---|---|
| | | Day 0 | Day 7 | Day 14 | Day 21 |
| 1. | Group1 (Control) | 175±2.35 | 170±2.36 | 170±2.35 | 170±1.24 |
| 2. | Group2( 50 mg /kg) | 175±2.35 | 170±2.05 | 170±2.49 | 170±4.71 |
| 3. | Group 3 (500 mg/kg) | 166±0.47 | 160±0.94 | 155±1.41 | 150±4.71 |
| 4. | Group 4(750 mg/kg) | 165±0.47 | 155±2.61 | 150±2.35 | 150±2.35 |
| 5. | Group 5(1000 mg/kg) | 165±0.46 | 150±1.24 | 147±2.49 | 135±4.08 |

According to prior research, weight gain in P. ostreatus-treated mice demonstrated that the administered Pleurotus ostreatus had no detrimental effects on animal growth **Deepa Lakshmi et al [49].** The effect of POS administration on body weight in the current study indicates small reductions in body weight from 0 to 21 days, which is consistent with prior research.

**Hematological Parameters:**

**Table 5.11: Hematological parameter**

| | **Control** | **250 mg /kg** | **500 mg /kg** | **750 mg /kg** | **1000 mg /kg** |
|---|---|---|---|---|---|
| Hb (g/L) | 12.7± 1.72 | 12.8±1.36 | 13.8±0.41 | 13.9±0.49 | 15.8±0.98 |
| RBC (milli/mm3) | 5.4±0.74 | 4.7±0.57 | 8.24± 0.82 | 8.05±0.81 | 7.8±0.30 |
| PCV (L/L) | 38.4±0.91 | 39.8±1.12 | 45.4 ±1.68 | 44.2±1.35 | 45±0.82 |
| MCV (fl) | 21.4±0.78 | 64.8±1.32 | 62.7±1.55 | 60.8±0.72 | 65.6±1.23 |
| MCHC(g/l) | 30± 0.97 | 30.5±1.27 | 37.8±0.77 | 35.2 ±0.30 | 32.4±1.25 |

| | | | | | |
|---|---|---|---|---|---|
| MCH (Induction pg) | 17.4±1.02 | 25.8±1.31 | 23.6±1.22 | 22.4 ±1.23 | 24.1±0.77 |
| RDW CV (%) | 0.112±0.001 | 0.127±0.002 | 0.125±0.001 | 0.114±0.001 | 0.126±0.002 |
| Neutrophiles | 4.2±0.060 | 5.14±0.47 | 6.48±0.48 | 8.42±0.48 | 8.86±1.37 |
| Lymphocytes | 1.03±0.01 | 1.6±0.12 | 2.8±0.12 | 1.36±0.01 | 1.47±0.036 |
| Eosinophiles | 0.3±0.12 | 0.14±0.01 | 0.16±0.20 | 0.21±0.01 | 0.24±0.01 |
| Monocytes | 0.2±0.08 | 0.36±0.01 | 0.15±0.12 | 0.19±0.012 | 0.26±0.02 |

The current investigation found no differences in Hematological parameters between the treatment and control groups. The effects of subacute administration of POS on Hematological parameters presented in Table no. 5.11. Most Hematological parameters, like haemoglobin, Total RBCs, neutrophils, monocytes, lymphocytes in treated rat shows no differences from the control group.

**Serum biochemical Parameters**

**Liver enzyme parameter**:

| | **control** | **250 mg /kg** | **500 mg /kg** | **750 mg /kg** | **1000 mg /kg** |
|---|---|---|---|---|---|
| AST | 74.82±1.76 | 76.18 ± 1.27 | 78.65 ± 1.70 | 80.34 ±3.33 | 82.26±4.11 |
| ALT | 24.08±1.63 | 24.92±3.39 | 26.54±1.21 | 28.18±4.14 | 28.56±4.54 |
| ALP | 85.16±1.65 | 87.32±2.06 | 88.35±3.30 | 89.24±2.09 | 91.32 ±3.30 |

In ALP concentration was lower in POS-treated rats than in normocholesterolemic rats in the earlier research **Alam et al [50]**. Table no. 5.12 and Figure 5.11 show the effect of subacute toxicity injection of POS on liver parameters in the current investigation. There were variations in serum AST, ALT, and ALP levels between the treatment and control groups.

**Kidney Parameters: -**

**Effect of POS on Kidney**

| | **Group 1** | **Group 2 (250 mg /kg)** | **Group 3 (500 mg /kg)** | **Group 4 (750mg /kg)** | **Group 5 (1000 mg /kg)** |
|---|---|---|---|---|---|
| Blood urea nitrogen (BUN) (mg/dl) | 23.8± 0.47 | 24.3 ±0.47 | 25.5±0.63 | 25.8 ±2.18 | 26.8±0.3 |

| | | | | | |
|---|---|---|---|---|---|
| Creatinine | 1.4± 0.04 | 1.5±0.04 | 1.4±0.04 | 1.6±0.43 | 1.5±0.08 |

In the present study the effect of subacute administration of POS on Kidney Parameters are presented in table. The POS had no effect on serum electrolytes. The kidney function parameters like Blood urea nitrogen, creatinine, did not reveal any changes in treated group as compared to control group as depicted in Table no. 5.13 and fig. 5.12

**Oral glucose tolerance test (OGTT): Table 5.14 Oral glucose tolerance test**

| | | Time(min) | | | | |
|---|---|---|---|---|---|---|
| **S. No** | **Groups** | **0** | **30** | **60** | **90** | **120** |
| 1 | Control | 99±0.81 | 102±1.24 | 121±1.69 | 115±1.69 | 102±2.44 |
| 2 | Vehicle | 88.2±0.89 | 114.2±0.97 | 122.3±0.89 | 107.7±0.85 | 98±0.81 |
| 3 | Metformin | 86.7±0.84 | 95.7±1.7 | 86.4±0.91 | 81.7±0.89 | 72.5±0.85 |
| 4 | POS (100mg/kg) | 84.6±0.73 | 107.2±1.6 | 99.2±0.85 | 96.4±1.9 | 85.4±1.7 |
| 5 | POS (200mg/kg) | 89.6±0.58 | 88±0.81 | 75.2±0.93 | 73.6±0.85 | 66.3±0.89 |
| 6 | POS (300mg/kg) | 89±0.82 | 97.4±0.89 | 91.7±0.84 | 90±1.2 | 77.2±1.6 |

The Oral glucose tolerance test was implemented to determine the body's capability to utilize glucose, the principal energy source. Because of this test, we were able to determine the drug's dosage as well as its possible hypoglycemic effect. When compared to the vehicle control group, POS (200 mg/kg) and POS (300 mg/kg) were revealed to have significant blood glucose lowering capabilities. However, toxicity tests of individual components were considered before conducting comparison research in a STZ-NA induced diabetes model in rats.

**Experimental Design on STZ-NA Induced DN in rats:**

**Effect of POS on kidney**

**Effect of POS on Nephron function in STZ- NA induced DN rats**.

| S. No | Drug Intervention | Nephron Function Test |
|---|---|---|

| | | Blood Urea Nitrogen (BUN) (mg/dl) | Serum creatinine (SC) (mg/dl) |
|---|---|---|---|
| 1 | Control | 18.32±1.17 | 0.49±0.02 |
| 2 | Diabetic control | 42.65±1.25 | 1.94±0.01 |
| 3 | vehicle | 35.42±1.63 | 0.82±0.01 |
| 4 | Metformin | 25.42±1.23 | 0.75±0.31 |
| 5 | POS (100mg/kg) | 23.47±0.47 | 0.68±0.08 |
| 6. | POS (200mg/kg) | 19.47±1.26 | 0.59±0.01 |
| 7. | POS (300mg/kg) | 21.42±1.69 | 0.64±0.01 |

In previous study BUN and creatinine was found to be (20.76 ± 0.11) and (0.63 ± 0.02) **Ziyadeh and Kumar [51].** Nephron function was examined by SC and BUN. In present study, treatment with POS 200 mg/kg lowered the BUN and creatinine level than control as well as standard. BUN and creatinine were found to be (19.47±1.26) and (0.59±0.02) and results are shown in Table no.5.15 and Fig.5.14.

**Effect on body weight:**

**Table 5.16 weight variation**

| S.No | Groups | Body weight |
|---|---|---|
| 1 | Control | 195.2±1.89 |
| 2 | Diabetic Control | 140.2±1.73 |
| 3 | vehicle | 150.2±1.63 |
| 4 | Metformin | 190.5±0.04 |
| 5 | POS (100 mg/kg) | 178.4±3.13 |
| 6 | POS (200mg/kg) | 189.3±0.50 |
| 7 | POS (300mg/kg) | 195.5±1.66 |

DN induction leads to structural proteins degradation of, resulting to reduction in the body weight (BW). Low level of insulin also reduces synthesis of other proteins leading to lower BW. The BW of diabetic treated rat was found to be (140.2±1.73), while treated with POS 200 mg/kg, body weight was found to be (189.3±0.50) as compared to normal group.

**Effect on Blood Glucose Level Blood glucose level**

| | | Days | | | | | |
|---|---|---|---|---|---|---|---|
| **S.NO** | **Groups** | **O** | **$4^{th}$** | **$8^{th}$** | **$12^{th}$** | **$16^{th}$** | **$21^{th}$** |
| 1 | Control | 94±2.86 | 98±2.35 | 102±2.49 | 98±2.16 | 92±2.82 | 94±1.69 |
| 2 | Diabetic Control | 101±4.64 | 323±13.36 | 324±11.43 | 325±15.19 | 327±14.8 | 331±11.08 |
| 3 | vehicle | 103±2.05 | 338±3.68 | 327±8.05 | 329±4.78 | 327±2.05 | 298±8.80 |
| 4 | Metformin | 94±9.46 | 339±0.81 | 232±3.09 | 156±2.86 | 128±5.71 | 88±2.86 |
| 5 | POS (100mg/kg) | 82.5±1.47 | 312±3.26 | 213±4.32 | 163±2.16 | 143±1.69 | 98±3.74 |
| 6 | POS (200mg/kg) | 95±7.31 | 273±3.29 | 212±8.34 | 142±6.34 | 118±3.39 | 82±2.86 |
| 7 | POS (300mg/kg) | 98±2.62 | 325±10.49 | 226±5.73 | 152±2.62 | 127±4.92 | 88±3.29 |

Streptozotocin is a glucosamine-nitrosourea derived from Streptomyces achromogenes that is used to induce diabetes. By destroying pancreatic cells, STZ produces hypo-insulinemia and hyperglycemia. This study found that on the fourth day, there was already a rise in blood glucose levels. Animals with hyperglycemia-induced symptoms were kept for further study. Marking was done on five animals from each group at random. Blood glucose levels began to fall dramatically on day 12 and continued to fall until the experiment's conclusion.

**Estimation of Lipid profile:**

In the present investigation, POS and a positive control (metformin) were administered to diabetic control rats for 21 days before the animals were subjected to changes in blood glucose levels and blood biochemical testing to evaluate their lipid profile. To investigate the effect of intervention on lipid profile level, total cholesterol, triglyceride, LDL, and VLDL were amplified, and the level of HDL was much lower in the diabetic control rat. POS 200 mg/kg dosage was more efficacious than 100 mg/kg dose in decreasing cholesterol in diabetic control rats.

**Effect of treatments on the fasting plasma glucose and serum lipid levels in different treated animals.**

| **S.No** | **Biochemical Parameters** | **Contr ol** | **Diabetic Control** | **vehicle** | **Metformin** | **POS (100mg/kg** | **POS (200mg/kg)** | **POS (300mg/kg)** |
|---|---|---|---|---|---|---|---|---|

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | Fasting plasma glucose (mg/dL) | 86.07 ±2.3 | 276.70 ± 4.3 | 86.07 ± 2.3 | 96.52 ± 1.2 | 131.38 ± 4.6 | 87.14 ± 1.9 | 111.3 ± 3.2 |
| 2 | Total cholesterol (mg/dL | 80.4±1.21 | 128.27±0.81 | 120.3±0.49 | 89.36±0.91 | 109.67±0.82 | 87.91±0.84 | 95.64±0.58 |
| 3 | Triglycerides (mg/dL) | 70.2±2.20 | 137.74±0.82 | 130.7±0.94 | 77.07±2.61 | 119.64±0.82 | 76.28±2.15 | 96.28±0.82 |
| 4 | HDL cholesterol (mg/dL) | 60.3±1.28 | 201.25±0.70 | 190.2±3.46 | 53.14±0.82 | 60.47±0.62 | 46.38±0.83 | 49.58±0.82 |
| 5 | LDL cholesterol (mg/dL) | 60.3±1.28 | 82.28±0.81 | 76.7±1.07 | 22.71±0.81 | 58.27±0.82 | 26.71±0.82 | 42.25±0.83 |
| 6 | VLDL cholesterol (mg/dL) | 18.6±0.53 | 32.64±0.83 | 30.5±0.58 | 16.02±0.92 | 24.46±0.47 | 15.3±0.87 | 22.7±0.09 |

**Total Cholesterol:**

The animals administered streptozotocin (STZ-NA) had higher total cholesterol levels than the control rat. In previous study, Total cholesterol of diabetic control and Highest concentration of POS was found to be (148±0.33) and (104±0.4) **Pierre et al [52]**. While in current study, Metformin (5 mg/kg) significantly ($P<0.001$) decreased the total cholesterol (89.36±0.91) enhanced by STZ (128.27±0.81). The low dose (100mg/kg) of POS decreased the total cholesterol (109.67±0.82) as compared to diabetic control (128.27±0.81).The moderate dose (200mg/kg) significantly ($P<0.01$) of POS drastically decrease the total cholesterol (87.91±0.84) as compared to diabetic control (128.27±0.81). and Higher dose (300mg/kg) of POS decrease the total cholesterol (95.64±0.58) as compared to diabetic control (128.27±0.81).

**Triglycerides:**

The animals fed streptozotocin-NA had higher triglyceride levels than the control animals. In a prior study, discovered that the quantity of Triglycerides was (148±1.12) and that the greatest dose of POS was found to be (106±1.03).**Pierre et al [52]**. while in current study, Metformin (5 mg/kg) When compared to the normal control group, the animals treated with STZ-NA had higher

triglycerides. Metformin (5 mg/kg) significantly (P<0.001) decreased the triglycerides (97.07±0.94) enhanced by streptozotocin (STZ) (137.74±0.82). The low dose (100 mg/kg) of POS showed more significant (P<0.01) decrease in triglycerides by (119.64±0.82) as compared to diabetic control (137.74±0.82). The moderate dose (200mg/kg) of POS drastically decrease the triglyceride (76.28±2.15) as compared to diabetic control (137.74±0.82) and Higher dose (300mg/kg) of POS significantly decreased the triglyceride (96.28±0.82) as compared to diabetic control (137.74±0.82).

**HDL cholesterol:**

Streptozotocin-NA treatment increased HDL cholesterol levels in mice compared to controls. In a prior study, the quantity of HDL cholesterol detected was (37.4±5.2), while the greatest dose of POS observed was (59.4±8.8) **Pierre et al [52]**. while in current study, Metformin (5 mg/kg) significantly (P<0.001) reduced the HDL cholesterol (53.14±0.82) enhanced by streptozotocin (201.25±0.70). The low dose (100 mg/kg) of POS showed more significant (P<0.01) decrease in HDL Cholesterol by (60.47±0.62) as compared to diabetic control ((201.25±0.70). The moderate dose (200mg/kg) of POS drastically decrease the HDL (46.38±0.83) as compared to diabetic control ((201.25±0.70) and Higher dose (200mg/kg) of POS significantly (P<0.01) decreased the HDL (49.58±0.82) as compared to diabetic control ((201.25±0.70).

**LDL and VLDL Cholesterol:**

STZ-NA treated mice showed greater LDL cholesterol levels than untreated animals. In previous study, the level of LDL was found to be (88.19±0.84) and highest dose of POS was found to be (31.92±2.4) **Pierre et al [52]**. while in current research revealed, Metformin (5 mg/kg) significantly (P<0.001) decreased the LDL cholesterol (22.71±0.81) enhanced by streptozotocin (STZ) (82.28±0.81). The low dose (100 mg/kg) of POS showed more significant (P<0.01) decrease in LDL Cholesterol by (58.27±0.82) as compared to diabetic control (82.28±0.81). The moderate dose (200mg/kg) of POS drastically decrease the LDL (26.74±0.82) as compared to diabetic control (82.28±0.81) and Higher dose (300mg/kg) of POS significantly (P<0.01) decreased the LDL (42.25±0.83 as compared to diabetic control (82.28±0.81).

Metformin (5 mg/kg) significantly (P<0.001) decreased the VLDL cholesterol (19.02±0.83) enhanced by streptozotocin (STZ) (32.64±0.83). In previous study, the level of LDL was found to be (30.46±0.19) and highest dose of POS was found to be (23.39±0.27) **Pierre et al [52]**. while in current study, The low dose (100mg/kg) of POS showed more significant (P<0.01) decrease in VLDL Cholesterol by (24.46±0.47) as compared to diabetic control (32.64±0.83). The moderate dose (200mg/kg) of POS drastically decrease the VLDL (15.3±0.87) as compared to diabetic control (32.64±0.83). Higher dose (300mg/kg) of POS significantly (P<0.01) decreased the VLDL (22.7±0.09) as compared to diabetic control (32.64±0.83).

**CONCLUSION**

Diabetes and its consequences are among the world's main public health issues. According to the findings of our inquiry and evaluation, the produced POS has a substantial impact in the treatment of diabetic nephropathy in STZ-NA caused experimental wistar rats. Because of the reduction in FBG of diabetic rats given POS, the selected mushrooms have an antihyperglycemic effect. Surprisingly, the antihyperglycemic impact of the chosen mushrooms might be attributed to a reduction in -amylase activity in diabetic rats mediated by bioactive chemicals discovered in POS.

According to our findings, the PO, LS, and POS demonstrated therapeutic effects for diabetic rats, including antihyperglycemic, antihyperlipidemic, and protective capabilities. These results included a drop in lipid profiles, a decrease in blood creatinine and BUN, an improvement in antioxidant activity, and a decrease in pancreatic, stomach, liver, and kidney damage. Furthermore, POS outperformed PO and LS in terms of performance.

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