Saudi Journal of Biological Sciences 27 (2020) 913–924

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Evaluation of antidiabetic activity of *Pleurotus pulmonarius* against streptozotocin-nicotinamide induced diabetic wistar albino rats

P. Balaji [a,*], R. Madhanraj [a], K. Rameshkumar [b], V. Veeramanikandan [c], M. Eyini [d], A. Arun [e], Boobalan Thulasinathan [e], D.A. Al Farraj [f], M.S. Elshikh [f], A.M. Alokda [g], A.H. Mahmoud [h], J.-C. Tack [i], H.-J. Kim [i,1]

[a] *PG and Research Centre in Biotechnology, MGR College, Hosur, Tamilnadu, India*
[b] *PG and Research Department of Zoology, Vivekananda College, Madurai, Tamilnadu, India*
[c] *PG and Research Centre in Microbiology, MGR College, Hosur, Tamilnadu, India*
[d] *Centre for Research and PG Studies in Botany, Thiagarajar College, Madurai, Tamilnadu, India*
[e] *Bioenergy and Bioremediation Laboratory, Department of Microbiology, Alagappa University, Karaikudi 630003, Tamil Nadu, India*
[f] *Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia*
[g] *Department of Applied Medical Chemistry, Medical Research Institution, Alexandria university, Alexandria, Egypt*
[h] *Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia*
[i] *Department of Clinical Pharmacology, College of Medicine, Soonchunhyang University, Cheonan, Republic of Korea*

ARTICLE INFO

Article history:
Received 19 November 2019
Revised 20 January 2020
Accepted 22 January 2020
Available online 31 January 2020

Keywords:
Basidiomycetes
Hyperglycemia
Antioxidants
Anti-diabetic and *Pleurotus pulmonarius*

ABSTRACT

The current research aims to evaluate the antidiabetic properties of *Pleurotus pulmonarius*, an edible basidiomycetes mushroom fungi in diabetic induced wistar albino rats. Mycelial Hot Water Extracts (HWE) and Acetone Extracts (AE) of *Pleurotus pulmonarius* was orally administrated to STZ-NA induced (55 mg/kilogram body weight) diabetic wistar albino rats at a concentration of 200 and 400 mg/kg for 4 weeks. The outcomes revealed that the HWE of *Pleurotus pulmonarius* resulted in a significant ($p < 0.001$) reduction in blood glucose level. A noteworthy ($p < 0.001$) reduction in serum lipid profile and elevation in High-Density Lipoprotein Cholesterol (HDL-C) after administration with HWE, also demonstrating the protective effects of HWE in diabetes-related complications. Besides all antidiabetic parameters, pathological morphology of the pancreas, liver and kidney are regularised. This observation indicated that HWE of *Pleurotus pulmonarius* possessed higher antidiabetic activity than AE. Besides, HWE also promoted a significant control of alpha amylase enzyme in a concentration-dependent manner with a maximum activity of 99.23% inhibition at 1000 μg/ml. The outcomes of the present study indicated that the HWE possesses a potential antidiabetic activity both *in vitro* and *in vivo*. Thus, it can be used as a non-toxic complementary drug in the controlling of diabetes and related complications, thus providing scientific authentication of its use as an antidiabetic agent.

1. Introduction

In recent years, enormous reviews and publications have wedged the global researchers establishing the possibility of the utilization of mushrooms. They remain considered to possess many potent health-promoting agents with a variety of bioactivity (Al-Dhabi et al., 2015; Al-Dhabi and Arasu, 2016; Barathikannan et al., 2016 Bai et al., 2019; Cheng et al., 2018; Kothari et al., 2018; Rathore et al., 2019; Ruiz-Herrera and Ortiz-Castellanos, 2019; Yanan Sun et al., 2019). They are as dietary supplements for their hypoglycemic activities (Elsayed et al., 2014). Bioactive compounds are dynamically considered, and their prospective solicitations are sustained by novel proof on health welfares (Kozarski et al., 2015; Elango et al., 2017; Elangoet al., 2016a; Elangoet al., 2016b). It is prominent that the study on basidiomycetes mushroom fungi has facilitated to clarify the massive prospective of these fungi for improving of novel expertise, food, medications, and beauty products as the world of this scientific

* Corresponding author.
E-mail addresses: balaji_paulraj@yahoo.com (P. Balaji), hak3962@sch.ac.kr (H.-J. Kim).
[1] Co-Corresponding author.
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.01.027
1319-562X/

research anticipated (Glorybai et al., 2015; Fowsiya et al., 2016; Haritha et al., 2016; Reis et al., 2017; Gurusamy et al., 2019). Basidiomycetes mushroom fungi are essential dietary constituents in some cultures, used up in day-to-day food from the ancient times (Sokovic et al., 2016; Helan et al., 2016; Ilavenil et al., 2017). Edible mushrooms are found to be a valued nutritional basis for human beings due to their small quantum of calories and also for their health promoting effects (Marina Ramos, et al., 2019). Conventionally medicinal mushrooms are utilized as antidiabetic nutrition for over a period of several decades in many countries (Park et al., 2016a; Park et al., 2016b; Park et al., 2017; Gulati et al., 2019; Rajkumari et al., 2019).

Diabetes Mellitus (DM), metabolic syndrome that has become more and more prevalent and rampant throughout the world. Diabetes incorporates various disorders considered by the raised blood glucose levels (hyperglycemia) (ADA, 2019). Clinically, DM is linked with failure of beta cells to secrete adequate pancreatic hormone (Tabatabaei-Malazy et al., 2012). The researched identity of diabetes is chiefly noticed, recorded and marked by certain parameters which deal with the decreased secretion and resistance of insulin in target cell (Surendra et al., 2016a; Niaz et al., 2018; Roopan et al., 2019). In adult-onset diabetes that is noticeable by fating and postprandial sugar level and reduced hormonal secretion are closely connected and diagnosed with type 2 DM (Surendra et al., 2016b; Bule et al., 2019; Rahmani et al., 2018). Several impediments are made because of hyperglycemia, which is an outcome of late and inappropriate healing treatments of diabetes mellitus (Yu et al., 2011). Through over oxidation, free radicals are produced and that take up to oxidative anxiety, cell injury and finally various long-lasting illnesses because of the factors like insalubrious food varieties, the standard of living ecological factors and radiation. Reactive oxygen species (ROS) will be produced with an increased amount of blood glucose level, and this will result in cell membrane impairment and damage of β-cells (Aruna et al., 2013; Surendra et al., 2016c).

Research has revealed that mushrooms might assuage the damage of β-cells of the pancreas and increase the synthesis of insulin in STZ-NA diabetic animals compared to normal animals (De Silva et al., 2012). Plentiful experiments (both *in vitro* and *in vivo*) have revealed that eatable and therapeutic mushrooms own antioxidant-rich mycochemicals (Islam et al., 2019). Mushrooms could be taken up as a robust approaches of producing the agents for inhibiting oxidation and control of microorganisms (Özdal et al., 2019; Valsalam et al., 2019a; Valsalam et al., 2019b). Extractable metabolites from these mushrooms can be retailed as a pill for ailments hindrance (Han et al., 2015; Yildiz et al., 2015). Numerous biological activities have been reported from *Pleurotus,* such as antimicrobial, antioxidant, hypoglycemic and so on (Elkhateeb et al., 2018; Xu and Beelman, 2015).

For a reason, that of apparent efficiency, negligible side effects in medical practice and reasonably economical, natural drugs are extensively approved even after their biologically vigorous composites are mysterious. The current work is an effort to assess the antidiabetic potential of an edible basidiomycetes mushroom fungi, *Pleurotus pulmonarius*. This effort may be a milestone in producing a new medication from uncharted basidiomycetes sp., for the use of the human beings in a better way for the cure of the diabetes mellitus.

2. Materials and methods

2.1. Chemicals and standard drugs

Streptozotocin and Glibenclamide were obtained from Sigma–Aldrich, USA. All other chemical and solvents were procured from Hi-media Laboratories Pvt., Ltd., India.

2.2. Basidiomycetes mushroom sample preparation

Pleurotus pulmonarius, an edible basidiomycete mushroom fungus, was used in this study. Mycelial agar plugs (8 mm dia) taken from the growing edge of a seven days-old culture of *P. pulmonarius* were transferred aseptically to the sterile Potato Dextrose Broth (PDB). The flasks were then incubated in static condition for 28 days at 28 ± 2 °C for harvesting the mycelial biomass. The mycelia were separated from the media by vacuum filtration through Whatmann No. 1 filter paper. The biomass thus harvested was washed with sterile water, dried by using blotting paper, weighed and preserved for further studies. The dried mycelial biomass was ground to a fine powder by means of a mortar and pestle and was utilized for further extraction of organic compounds. Using soxhlet extraction apparatus with acetone and hot water the mycelial biomass were extracted. After the completion of extraction process, the solvent/water was evaporated by desiccation and used for further studies.

2.3. Animal management

Male Swiss albino mice weighing 18 – 25 g, and both sex of wistar albino rats (150 – 200 g weight) were used in the present study. The experimental animals were received from Kerala Veterinary and Animal Science University, Mannuthy, Thrissur, Kerala, India. Paddy husk was used as bedding for the animals which were placed for experimental procedure and then assigned to various control and test groups in propylene cages. Experimental rats were retained at 24 ± 2 °C temperature and 30–70% relative humidity with light:day (12:12) cycle. All experimental rats were permitted to freely access the water and feed with pelleted rat chow (M/s. Hindustan Lever Ltd, Mumbai, Maharashtra, India). The institutional animal ethical committee reviewed all the experimental procedures executed in the present study (688/PO/Re/S/02/CPCSEA; Proposal Number: NCP/IAEC/2017–2018/15) and were under the Institutional Ethical Guidelines.

2.4. Acute toxicity study

To execute the acute toxicity studies using experimental animals, the Organization of Economic and Cooperation Development (OECD-423) guidelines were followed. In the present study, the random sampling technique was involved in the case of Swiss albino mice (male). Four hours fasted animals allowed to freely access the water. Initially, Hot Water Extracts (HWE) and Acetone Extracts (AE) of *Pleurotus pulmonarius* at a concentration of 5 mg/kg body weight was orally administered and observed for any mortality. The observation continued for the first 24 hrs and after 72 hrs are over. The Mortality witnessed in any two of the three animals, the administered dose was considered to be lethal. The observation of mortality in an animal out of three animals tested, then the similar dose will be continued to confirm the lethal effect. If there is no mortality, then higher doses (50, 300, 2000 mg/kg) of the extracts were be used for further toxicity study. The general behaviours like sedative, hypnotics, convulsion, ptosis, analgesia, stupar reaction, motor activity, muscle relaxant, pilo erection, change in skin color, lacrimal secretion, and stool consistency were monitored during the study period (Ecobichon, 1997).

2.5. Investigational stimulation of diabetes in mice

Diabetes was experimentally induced through intra-peritoneal injection in twelve hours fasted experimental animals with Streptozotocin (STZ) (55 mg/kg body weight) dissolved in 100 mM citrate buffer (pH 4.5); followed by Nicotinamide (120 mg/kg) after 15 min. After 6 hr of STZ administration, a 10% glucose solution

was provided to all the rats to prevent hypoglycemic shock. On completion of 72 h testing and monitoring, rats above 200 mg/dL of blood glucose concentration noticed to be diabetic and this conceptualization could be used for further study.

2.6. Experimental design

The following group of studies were carried out, where Group I refers to be as control; Group II and III were considered to be the negative and positive control respectively.

Group I	:	0.1% Carboxy Methyl Cellulose Solution (1 mg/kg)
Group II	:	Streptozotocin (45 mg/kg., i.p) and Nicotinamide (120 mg/kg., i.p)
Group III	:	Glibenclamide (5 mg/kg)
Group IV	:	HWE of *P. pulmonarius* (200 mg/kg)
Group V	:	HWE of *P. pulmonarius* (400 mg/kg)
Group VI	:	AE of *P. pulmonarius* (200 mg/kg)
Group VII	:	AE of *P. pulmonarius* (400 mg/kg)

The levels of blood glucose were quantified from the experimental animals over a period of 28 days of drug administration at regular intervals. On 28th day, the animals went on fasting for a night, anesthetized with Pentobarbitone sodium anesthesia (60 mg/kg, i.p). Subsequently, the blood samples were collected in non-heparinized tubes, placed undisturbed for 30 min and processed to obtain serum. The lipid profiles, liver function and kidney function test were tested from the supernatant. All the animals were sacrificed with excess Pentobarbitone sodium. After that the pancreas, liver and kidney were separated out and retained in 10% formalin solution for histopathological studies.

2.7. Preparation of homogenate

Phosphate buffer (200 mM; pH 6.6) was used to homogenate the collected liver from the animals. The obtained homogenate was centrifuged and used for enzymatic and non-enzymatic tests.

2.8. Estimation of serum lipid profile

2.8.1. Total cholesterol

Total cholesterol (TC) of the samples were estimated calorimetrically. Free cholesterol and free fatty acids were formed by the hydrolyzation of cholesterol esters by cholesterol esterase. The pre-existing ones and the free cholesterol produced in the reaction got oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The formed H_2O_2, reacted with 4-aminoantipyrine and phenol in the presence of peroxidase to produce red coloured quinoneimine dye. Ten micro litre of sample was mixed with one ml of test reagent, incubated at 37 °C for 5 min. The cholesterol concentration was proportional to the intensity of the colour produced. The absorbance was measured at 510 nm against a blank. Cholesterol concentration was expressed as mg/dL (Roeschlau et al., 1974).

2.8.2. Triglycerols

Serum triacylglycerols (TG) were quantified by the formation of dihydroxyacetone phosphate and hydrogen peroxide from oxidation of glycerol 3 phosphate (Tietz, 1990). Ten micro litre of serum sample was mixed with one ml of test mixture and incubated at 37 °C. The optical density was measured at 510 nm against a blank and the content of triglycerides was expressed as mg/dl.

2.8.3. HDL cholesterol

The Very Low-Density Lipoprotein (VLDL) and Low-Density Lipoprotein (LDL) fractions of plasma samples were precipitated using phosphotungstic acid and then HDL in the supernatant was separated by centrifugation and measured for its cholesterol content. Two hundred micro litre of sample was mixed with 500 µl of precipitation reagent and centrifuged at 4000 rpm for 10 min. The cholesterol extracted in supernatant was estimated as described by Lopes-Virella et al. (1977).

HDL Cholesterol = ΔAsample / ΔAstandard × concentration of standard.

2.8.4. LDL & VLDL cholesterol

LDL and VLDL were calculated according to the formula of Friedewald et al. (1972).

VLDL cholesterol = Triglycerides / 5

LDL = TC – (HDL + VLDL)

The values were expressed as mg/dl

2.9. Determination of blood urea

The method of Natelson et al. (1951) was followed to estimate blood urea. Three glass tubes were marked as B, T, and S. In glass tube B and T, 0.02 ml of H_2O and 0.02 ml of blood was pipetted respectively. Tube S was pipetted with 0.02 ml standard urea solution (0.04%). Hundred microliter of diacetyl monoxime solution and 5 ml of thiosemicarbazide was incorporated into all the glass-tubes. Reagents were vortexed and kept in a hot bath and then cooled. The absorbance was read at 500 nm and the content of urea was expressed as mg/dl.

2.10. Determination of serum creatinine

The serum creatinine was estimated by method (Slot, 1965). Labeled three test-tubes as B, T and S. into B, pipetted, 2 ml of water, into T, 2 ml serum and 4 ml of water, into S, three ml of H_2O and one ml of creatinine standard (4 mg/dl). 2 ml of ammonium sulphate and in all tubes, 2 ml of sodium tungstate was added. Three ml of supernatant was removed after centrifugation from each test tube. 1 ml of distilled water and picric acid was added to the test tubes B, T and S. Optical density was read at 520 nm and serum creatinine was calculated as mg/dl.

2.11. Determination of serum insulin

Serum insulin was assessed following radioimmunoassay technique (Keilacker et al., 1987). The insulin existing in the test serum competes with the radio-labeled insulin for its antibody when mixed to a tube comprising a stable volume of antibody and a constant volume of the insulin (I^{125}-labelled). The quantity of radio-labeled insulin destined to the antibody was in reverse related to the aggregate of insulin in the serum sample. Gamma scintillation counter was used to measure the bound radiolabeled insulin.

2.12. Determination of Malondialdehyde (MDA)

The estimation is carried out by following Nieshus and Samuelsson (1986). Fivefold dilution of serum is made and to this 1 ml of Trichloroacetic acid, 2-thiobarbituric acid and hydrochloric acid in 1:1:1 ratio was added and centrifuged. The optical density of the pink color formed in the supernatant considered to be directly proportional to the concentration of serum MDA was read at 535 nm. The values were indicated as nano moles of MDA/min/mg protein.

2.13. Estimation of lipid hydroperoxides

Around 100 μl of homogenized tissue was treated with 900 μl of Fox reagent and incubated for 30 min. The optical density was measured after the colour development at 560 nm using a spectrophotometer. The concentration of lipid hydroperoxides were indicated as nano moles/mg protein (Nieshus and Samuelsson, 1986).

2.14. Determination of enzymatic Anti-Oxidants

2.14.1. Superoxide dismutase (SOD)

The strength of SOD was quantified by the hindrance of auto catalyzed adrenochrome development in the tissue homogenate at 480 nm following the method of Misra and Fridovich (1972). SOD was indicated as units/mg tissue protein.

2.14.2. Catalase (CAT)

The potency of catalase was assessed by the procedure of Aebi, 1984. One unit of catalase enzyme action was specified as the quantity of enzymes causing the degradation of micro mol H_2O_2/mg protein/min at 25 °C.

2.14.3. Peroxides (Px)

Peroxidase was estimated by a subsequent modification in optical density at 460 nm by the addition of oxygen to O-dianisidine in the occurrence of enzyme source and hydrogen peroxide. Unit of enzyme activity defined as μmol O-dianisidine/min at 37 °C (Lobarzewski and Ginalska, 1995).

2.14.4. Glutathione peroxidase (GPx)

GPx was measured by the procedure of Paglia and Valentine (1967) and expressed as μg of GSH utilized/min/ mg of protein.

2.14.5. Glutathione reductase (GSSH)

GSSH was quantified by the procedure of Racker (1955). The activity was expressed by the amount of Nicotinamide adenine dinucleotide phosphate hydrogen consumed in the translation of oxidized glutathione (GSSG) to reduced glutathione (GSH). Glutathione reductase was stated as moles of NADPH oxidized/ min/mg at 30° C.

2.15. Determination of non-enzymatic antioxidant

2.15.1. Reduced glutathione (GSH)

The quantification protocol was centred on the response of GSH with dithionitrobenzoic acid (DTNB) to yield a molecule that is absorbed in 412 nm (Beutler et al., 1986). Concisely, 500 μl of supernatant after centrifugation was reserved and mixed with 2 ml of 300 mM disodium hydrogen phosphate solution. To the reaction mixture, 200 μl DTNB was added. The absorbance of the reaction mixture was noted immediately and expressed in micro mol GSH/min/mg protein.

2.15.2. Vitamin E

Tissues were homogenized in 0.54 M KCl solution and the mixture containing 1.5 ml of tissue homogenate, 1 ml absolute alcohol and 0.5 ml of 25% ascorbic acid as an antioxidant was incubated. One milliliter of 10 N KOH was added and incubated further in an ice-bath and 4 ml hexane was added to extract and mixed by vortexing for a min. The hexane layer was unglued and the resulting residue was dissolved in 20–50 μl of pure chloroform and spotted on silica gel plates. The alpha tocopherol standard was also spotted, keeping 2 cm distance using benzene : ethyl acetate (2:1) as the running solvent system. Reference plates were flood with 0.001% florescent rhodamine dye dissolved in methanol. From the test silica gel plate, an area corresponding to R_f of the alpha tocopherol standard was scraped and eluted. Elute was added with 0.2% bathophenanthroline, followed by $FeCl_3$ and 0.001 M *ortho*-phosphoric acid. The optical density was measured at 536 nm against distilled water.

$$\text{Conc. of Vitamin E} = \frac{\text{Ab. of the test} - \text{Ab. of blank}}{\text{Ab. of Std} - \text{Ab of blank}} \text{x Conc. Std}$$

The alpha tocopherol concentration in the sample was represented as μg/mg protein (Desai, 1984).

2.15.3. Vitamin C

Vitamin C was estimated following the procedure of Omaye et al. (1979). To 500 μl of sample, 1 ml of 5% TCA was added. The mixture was centrifuged at 4 °C at 14,000 × g for 20 mins. The supernatant (270 μl) was added with 0.08 ml of 85% phosphoric acid, 1.37 ml of 0.5% dipyridyl and 0.028 ml of 1% $FeCl_3$. The sample mixture was thoroughly mixed for 60 min for the development of ferrous–dipyridyl chromophore. Samples were centrifuged and optical density was measured at 525 nm.

2.16. Statistical analysis

The experimental data were represented as mean ± SEM. The results were evaluated by using the one-way analysis of variance (One Way ANOVA) using GraphPad version 5. P values < 0.05 were considered to be significant.

2.17. Histopathological analysis

The experimental mice were forfeited under light ether anesthesia, by decapitation. The pertinent organ pancreas, liver and kidneys were cut apart out and collected in ten percent formalin solution and straightaway sealed with by the paraffin technique. 5μ thickness slices were taken and stained with hematoxylin and eosin (H and E) for histopathological examination.

3. Results and discussion

3.1. Oral acute toxicity

The results of the acute toxicity study of HWE and AE showed no mortality even after 72 hrs at 2000 mg/kg. The AE showed mile sedation at 2000 mg/kg but however, HWE didn't alter any of the general behavior. No fatality or noxious reactions were found during and after the treatment period with both extracts.

3.2. Anti-hyperglycaemic effect

HWE and AE of *P. pulmonarius* was studied for its hypoglycaemic effect against diabetic rats (Table 1). Diabetes in experimental animals was recognised by the raise in the Blood Glucose Levels (BGL) more than 200 mg/dl later to the administration of STZ.

BGL in Group III, Group IV and Group V reduced to normal levels after receiving treatment for 28 days. Glibenclamide, used as reference control expressively ($P < 0.001$) decreased the BGL from the fourth day onwards. On the 28th day, the blood sugar was found to be (106.60 ± 8.43 mg/dl) but was still a little higher compared with that of Group I (Normal Control). The 200 mg of HWE moderately decreased the blood sugar level (116.54 ± 3.35 mg/dl) from the 4th day onwards and the effect was maintained until the end of the treatment against the reference control (106.60 ± 8.43 mg/dl). The 400 mg/kg of HWE was significant ($P < 0.001$), with 97.19 ± 5.52 mg/dl as that of the reference control (106.60 ± 8.43 mg/dl).

Table 1
Effect of different extracts of *Pleurotus pulmonarius* on Blood Sugar Levels in STZ induced diabetic rats.

Drug Treatment	Mean Blood Sugar Level (mg/dl)					
	Before STZ	After STZ	4th Day	7th Day	14th Day	28th Day
Control (0.1% CMC)	103.66 ± 6.65	100.30 ± 7.76	104.54 ± 7.33	98.71 ± 6.88	97.54 ± 6.92	103.83 ± 8.72
Diabetic Control	99.53 ± 5.31	223.59 ± 9.35	221.62 ± 12.60	229.34 ± 9.52	220.51 ± 5.83	227.58 ± 8.34
Reference Control	103.16 ± 7.41	217.32 ± 8.71	210.44 ± 11.96	182.05 ± 8.62**	127.28 ± 8.50***	106.60 ± 8.43***
HWE (200 mg/kg)	90.30 ± 4.20	218.00 ± 8.13	203.27 ± 3.91	195.47 ± 3.65	163.11 ± 4.06**	116.54 ± 3.35***
HWE (400 mg/kg)	114.60 ± 4.80	215.26 ± 6.30	210.00 ± 5.24	161.09 ± 3.51**	136.54 ± 6.67***	97.19 ± 5.52***
AE (200 mg/kg)	96.53 ± 6.21	215.47 ± 7.80	219.43 ± 6.24	218.34 ± 7.07	203.31 ± 6.14	197.45 ± 7.75
AE (400 mg/kg)	88.32 ± 4.44	226.24 ± 5.83	215.48 ± 4.06	206.82 ± 5.11	196.27 ± 3.20	187.43 ± 5.77*

Values are in mean ± SEM (n = 6),
* P < 0.05
** P < 0.01
*** P < 0.001 Vs Diabetic Control

Table 2
Effect of different extracts of *Pleurotus pulmonarius* on insulin level in STZ induced diabetic rats.

Drug Treatment	Serum Insulin Level (μU/ml)
Control	25.66 ± 1.37
0.1% CMC	
Diabetic Control	6.05. ± 0.25
Reference Control	17.45 ± 0.95***
Glibenclamide (5 mg/kg)	
HWE (200 mg/kg)	11.24 ± 0.82*
HWE (400 mg/kg)	16.70 ± 0.96**
AE (200 mg/kg)	8.48 ± 0.14
AE (400 mg/kg)	14.53 ± 0.48*

Values are in mean ± SEM (n = 6),
* P < 0.05
** P < 0.01
*** P < 0.001 Vs Diabetic Control

Furthermore, the animals in Group VI and Group VII showed higher BGL related with the Group I ($p < 0.05$). AE at 200 mg/kg displayed a slight decrease in BGL (197.45 ± 7.75 mg/dl) and 400 mg/kg of AE showed a slightly more decrease in blood sugar (187.43 ± 5.77 mg/dl) level from 14th and 28th day of treatment. Among the two different extracts, HWE showed good hypoglycemic activity against STZ induced diabetes in rats, which displayed substantial ($P < 0.001$) decrease in BGL from 7th to 28th day of treatment.

It was apparent, from Table 2 that diabetic animals exhibited a drop in insulin concentration. The diabetic animals with commercial drug treatment revealed a extraordinarily increased insulin level related with those in the diabetic control group (Pari and Latha, 2005). Besides, the insulin content of those animals administered with HWE was significantly ($P < 0.01$) lesser in Group I and Group III, but were greater than Group II. The hopeful mode of action whereby HWE facilitated its hypoglycaemic effect possibly will be by increasing insulin sensitivity that was equivalent to be stated in earlier reports (Wang et al., 2017a,b; Zhan et al., 2014).

3.3. Lipid profile

Uncommon fatty acid metabolism in diabetes, may cause dyslipidemia and a sequence of heart diseases and other connected difficulties (Ktari et al., 2013). Diabetic dyslipidemia is typically characterized by high difference in serum lipid profiles (Oluwatosin et al., 2012). Selected reports also showed that HDL-C played an indispensable role in cholesterol transference and reduces the risk of cardiovascular ailments. Hence, the abnormalities of fatty acid levels in the serum of all experimental groups were investigated.

Table 3 shown a noteworthy upsurge in serum lipid levels and expressively decreased in HDL-Cholesterol level as matched to normal control. During the study period, commercial drug, HWE and AE let the decrease in TC, TG, LDL-C and VLDL-C levels (significance of $P < 0.01$) of diabetic rats. Whereas HDL-C levels improved related with Group I ($p < 0.001$). The outcomes exhibited that commercial drug, HWE and AE could drop the threat of heart diseases by tempering the lipid abnormalities in experimental animals. Notably, HWE (400 mg/kg) showed the lower lipid profile concentrations and the higher HDL-C concentration matched with the AE (400 mg/kg) which exemplifies that the HWE of *P. pulmonarius* showed the more positive effect on lipid profiles than AE.

3.4. Renal functions

Diabetic nephropathy (DN) is a severe difficulty of diabetes, categorised by increased size of kidney cells, reduced kidney function and increased urinary albumin evacuation (Murussi et al., 2008; Abe et al., 2011). DN is one of the main reasons of long-lasting kid-

Table 3
Effect of different extracts of *Pleurotus pulmonarius* on Lipid Levels in STZ induced diabetic rats.

Drug Treatment	Lipid Profiles (mg/dl)				
	Total Cholesterol	Triglyceride	HDL - Cholesterol	LDL - Cholesterol	VLDL Cholesterol
Control (0.1% CMC)	117.63 ± 8.52	65.21 ± 3.64	40.27 ± 2.95	41.34 ± 2.55	16.97 ± 1.08
Diabetic Control	163.24 ± 8.04	121.75 ± 7.62	21.54 ± 1.86	79.33 ± 6.24	34.40 ± 2.76
Reference Control	124.40 ± 10.63**	69.27 ± 5.88***	38.65 ± 2.54***	46.30 ± 2.52***	18.39 ± 1.12***
HWE (200 mg/kg)	138.10 ± 7.51**	78.14 ± 5.31**	28.28 ± 1.11**	57.44 ± 2.31**	25.78 ± 1.1**
HWE (400 mg/kg)	125.00 ± 8.44**	71.59 ± 4.43**	34.33 ± 1.11***	46.10 ± 1.47***	19.1 ± 1.05***
AE (200 mg/kg)	139.46 ± 5.11*	82.41 ± 3.41**	24.39 ± 1.33*	64.52 ± 2.31*	28.26 ± 1.02*
AE (400 mg/kg)	130.13 ± 5.71**	76.63 ± 5.19**	31.69 ± 1.80***	54.32 ± 1.01**	22.11 ± 0.99***

Values are in mean ± SEM (n = 6),
* P < 0.05
** P < 0.01,
*** P < 0.001 Vs Diabetic Control

ney dysfunction and end-stage kidney disease universal, particularly in developed nations and is consequently creating a budding world-wide menace (Ziyadeh and Sharma, 2003).

The typical display of the progress of DN in rats is due to the increased level of kidney function test. Similarly, discharge of protein and albumin from urine are the indication of glomerular dysfunction (Xu et al., 2012). Therefore renal function was measured by Serum Creatinine (SC) and Blood Urea Nitrogen (BUN). The experimental results of renal function associated factors were shown in Table 4. SC and BUN in diabetic animals considerably improved related with Group I ($P < 0.001$), indicating an injured renal function in these rats. Treatment with glibenclamide / HWE and AE (both lower and higher concentration) considerably ($P < 0.001$) declined the elevation of SC and BUN, while BUN concentration in HWE (400 mg/kg) was remarkably lower than the reference control as well.

Table 4
Effect of different extracts of *Pleurotus pulmonarius* on Kidney functions in STZ induced diabetic rats.

Drug Treatment	Kidney Function Test	
	BUN (mg/dl)	Creatinine (mg/dl)
Control (0.1% CMC)	17.35 ± 0.96	0.48 ± 0.02
Diabetic Control	39.63 ± 2.63	1.84 ± 0.01
Reference Control	19.66 ± 1.04***	0.63 ± 0.03***
HWE (200 mg/kg)	21.43 ± 1.54***	0.76 ± 0.02***
HWE (400 mg/kg)	19.47 ± 1.22***	0.68 ± 0.03***
AE (200 mg/kg)	25.11 ± 0.05**	0.73 ± 0.03**
AE (400 mg/kg)	20.76 ± 0.11***	0.63 ± 0.02***

Values are in mean ± SEM (n = 6),
*P < 0.05
** P < 0.01
*** P < 0.001 Vs Diabetic Control

3.5. Malondialdehyde (MDA) and lipid hydroperoxides (LH)

Malondialdehyde, the terminal produce of lipid peroxidation conceivably utilized as an evidence of cellular damage. Increased levels of MDA testify severe oxidative impairment (Zhang *et al.*, 2018). As shown in Table 5, MDA and LH status significantly raised in the diabetic rats than Group I ($P < 0.01$).

Over 28 days of treatment, conversely, the MDA and LH level was significantly ($P < 0.01$) abridged in the diabetic rats. The decreased MDA in rats treated with glibenclamide, HWE and AE perhaps due to the improved actions of free radicals scavenging enzymes (Zhang et al., 2016).

3.6. Enzymatic antioxidants

Oxidative stress, by virtue of an unevenness among the hunting of free radicals *in vivo*, possibly will hint to an amplified reactive oxygen species (ROS) or a reduction of free radical scavenging defense (Ullah et al., 2016). There are a number of articles resolved that this stress contest a vital role in diabetes and related problems (Pan et al., 2017).

The free radical scavenging enzymes are considered as the primary defence agent against ROS and can defend beside injury (Zhang et al., 2016). As revealed in Table 6, anti-oxidative enzyme actions in diabetic animals were suggestively lesser ($P < 0.01$) than normal rats. Past publications had shown that STZ possibly will produce ROS, signifying that free radical scavenging treatment may be favourable for considering the oxidative damage of diabetes mellitus (Eliza et al., 2010).

Table 5
Effect of different extracts of *Pleurotus pulmonarius* on liver MDA and LH in STZ induced diabetic rats.

Drug Treatment	MDA (nmoles/min/mg protein)	LH (nmoles/min/mg protein)
Control (0.1% CMC)	0.78 ± 0.03	0.64 ± 0.04
Diabetic Control	1.32 ± 0.02[a]	1.08 ± 0.02[a]
Reference Control	0.99 ± 0.01[b]	0.87 ± 0.01[b]
HWE (200 mg/kg)	1.11 ± 0.04[b]	0.96 ± 0.01[b]
HWE (400 mg/kg)	0.91 ± 0.01[b]	0.79 ± 0.01[b]
AE (200 mg/kg)	1.27 ± 0.01[b]	0.99 ± 0.03[b]
AE (400 mg/kg)	1.0 ± 0.03[c]	0.93 ± 0.04[c]

Values are mean ± SEM; n = 6 in each group
[a] P < 0.01 when compared to normal control
[b] P < 0.001
[c] P < 0.05, when compared to diabetic control (one way ANOVA followed by Dunnett's 't' test).

Table 7
Effect of different extracts of *Pleurotus pulmonarius* on liver non-enzymatic antioxidants in STZ induced diabetic rats.

Drug Treatment	GSH (nmoles/min/mg protein)	Vitamin C (µg/mg protein)	Vitamin E (µg/mg protein)
Control (0.1% CMC)	12.4 ± 0.9	4.8 ± 0.03	7.1 ± 0.02
Diabetic Control	9.6 ± 0.8[a]	3.4 ± 0.02[a]	5.8 ± 0.03[a]
Reference Control	11.8 ± 0.7[b]	4.5 ± 0.04[b]	6.9 ± 0.02[b]
HWE (200 mg/kg)	9.2 ± 0.4[b]	2.2 ± 0.02[b]	5.9 ± 0.03[b]
HWE (400 mg/kg)	9.8 ± 0.5[b]	2.1 ± 0.01[b]	5.1 ± 0.02[b]
AE (200 mg/kg)	9.5 ± 0.7[b]	2.6 ± 0.01[c]	5.2 ± 0.02[b]
AE (400 mg/kg)	10.1 ± 0.5[b]	3.0 ± 0.01[b]	5.6 ± 0.02[b]

Values are mean ± SEM; n = 6 in each group;
[a] P < 0.01 when compared to normal control;
[b] P < 0.001
[c] P < 0.05, when compared to diabetic control (one way ANOVA followed by Dunnett's 't' test).

Table 6
Effect of different extracts of *Pleurotus pulmonarius* on liver enzymatic antioxidants in STZ induced diabetic rats.

Drug Treatment	CAT (µmoles/min/mg protein)	SOD (nmoles/min/mg protein)	GSSH (nmoles/min/mg protein)	Px (nmoles/mg protein)	GPx (nmoles/mg protein)
Control (0.1% CMC)	42.1 ± 1.8	5.1 ± 0.06	34.8 ± 1.2	7.6 ± 0.02	10.2 ± 0.03
Diabetic Control	30.6 ± 1.4[a]	3.6 ± 0.04[a]	21.9 ± 0.8[a]	6.2 ± 0.03[a]	7.8 ± 0.02[a]
Reference Control	41.3 ± 2.1[b]	4.8 ± 0.05[b]	32.6 ± 0.9[b]	7.3 ± 0.02[b]	9.7 ± 0.06[b]
HWE (200 mg/kg)	35.1 ± 0.8[b]	3.5 ± 0.02[b]	24.2 ± 0.4[b]	5.4 ± 0.01[c]	7.2 ± 0.01[b]
HWE (400 mg/kg)	38.5 ± 0.8[b]	2.8 ± 0.06[c]	27.0 ± 0.6[b]	5.5 ± 0.04[b]	7.5 ± 0.02[b]
AE (200 mg/kg)	36.1 ± 1.2[b]	3.7 ± 0.02[b]	26.8 ± 0.9[b]	5.5 ± 0.01[b]	7.5 ± 0.03[b]
AE (400 mg/kg)	39.1 ± 1.0[b]	3.3 ± 0.04[b]	29.7 ± 1.1[b]	6.4 ± 0.03[b]	8.3 ± 0.06[b]

Values are mean ± SEM; n = 6 in each group;
[a] P < 0.01 when compared to normal control;
[b] P < 0.001, [c]P < 0.05, when compared to diabetic control (one way ANOVA followed by Dunnett's 't' test).

After 4 weeks of action with standard drug, there was not any significant rise in the expression of CAT, SOD, GSSH, Px and GPx ($p < 0.01$). The outcomes recommens that glibenclamide haven't modulated the enzyme activities in diabetic rats, similar to those observed in previous studies (Liu et al., 2017). Contrarily, the treatment of HWE and AE significantly improved the actions of enzymes in diabetic rats matched with diabetic control groups. This demonstrated that HWE and AE could improve the oxidative stress of experimental animals by commendably improvement in actions of antioxidant enzymes.

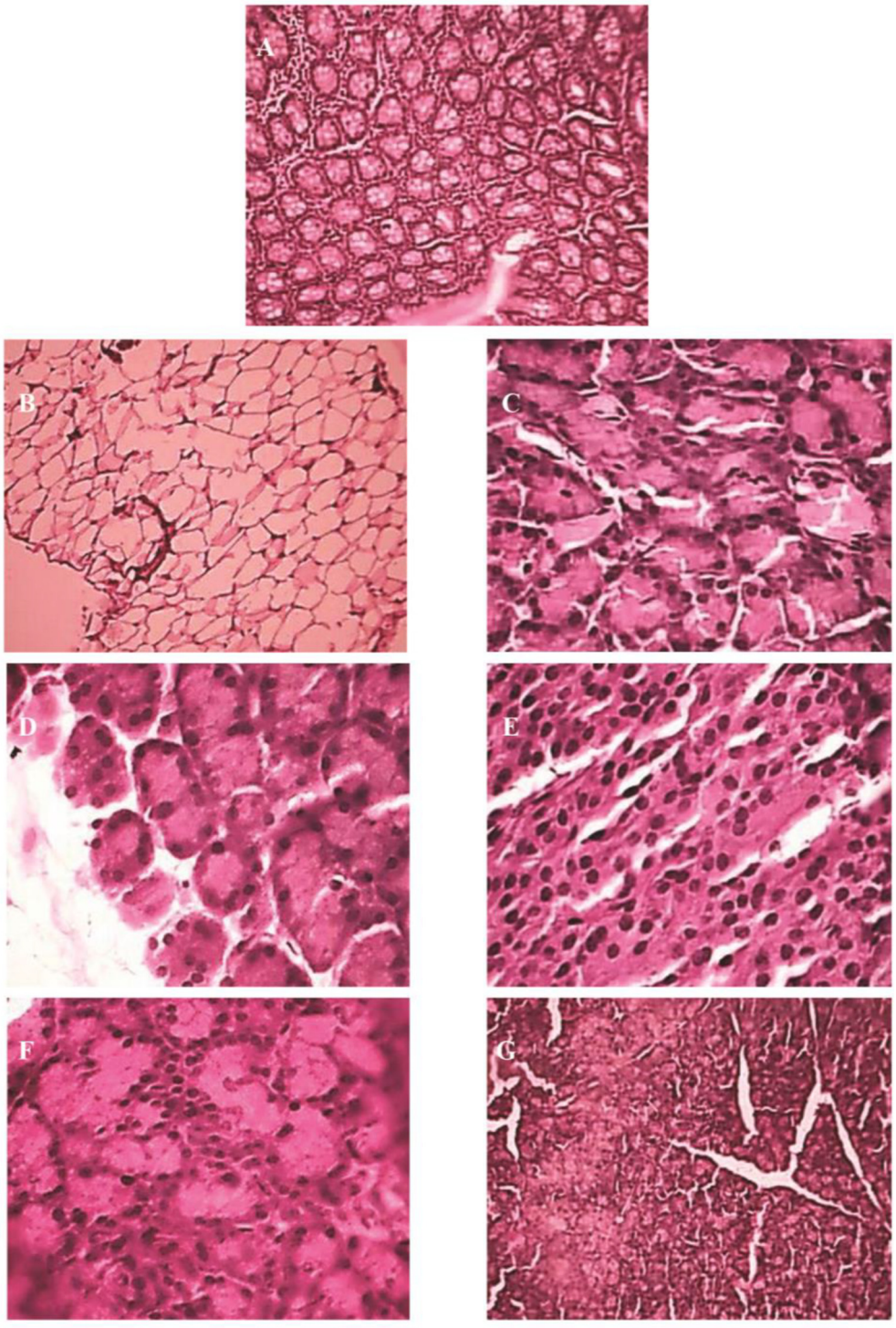
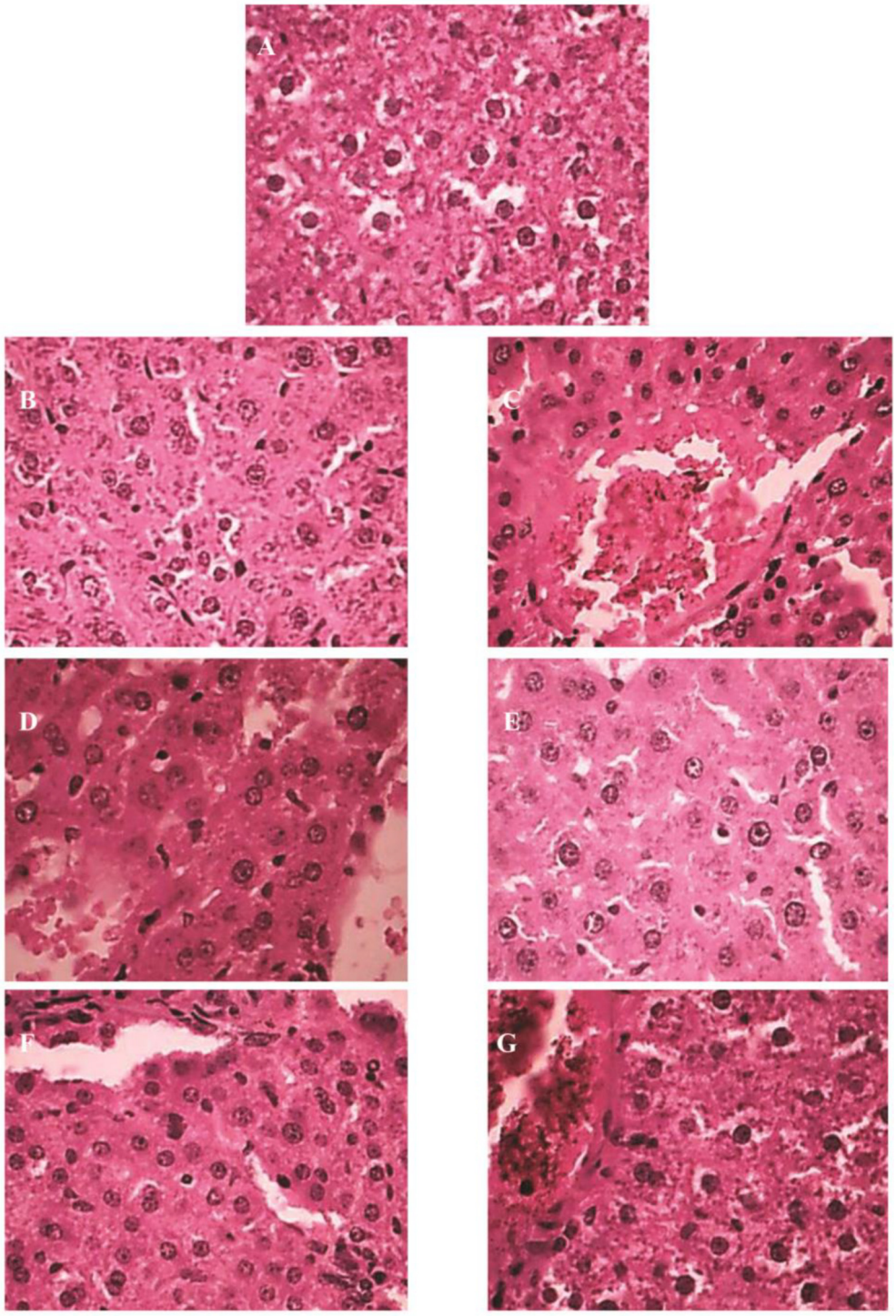


Fig. 1. Histological study of Pancreas of streptozotocin-induced wistar albino rats with different mycelial extracts of *P. pulmonarius.*

3.7. Non-enzymatic antioxidant

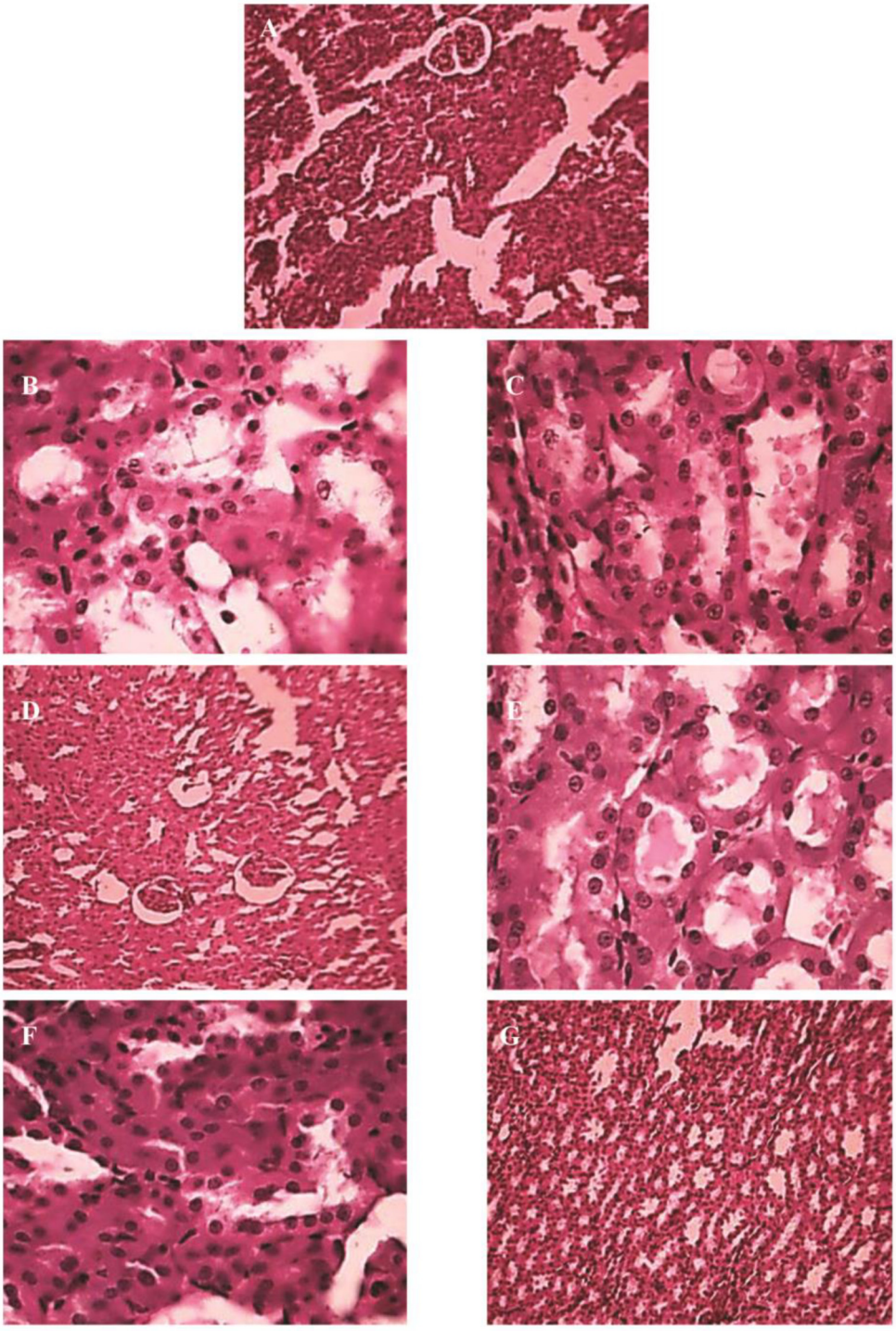
A substantial reduction in the level of vitamin C, vitamin E, and GSH were detected in diabetic groups when compared with control group. This drop perhaps a significant fact for cell impairment as a result of adverse free radicals generation. HWE and AE tend to fetch these concentrations into close to standard (Table 7). A notable drop in the concentration of GSH was perceived in diabetes-induced rats was raised to a normal level after the treatment. It is confirmed that diabetics consume low concentration of non-enzymatic free radical scavengers and their add-on may help in diabetes cure.



A: Control; **B:** Diabetic Control; **C:** Glibenclamide;
D: Hot Water Extract (200 mg); **E:** Hot Water Extract (400 mg)
F: Acetone Extract (200 mg); **G:** Acetone Extract (400 mg)

Fig. 2. Histological study of Liver of streptozotocin-induced wistar albino rats with different mycelial extracts of *P. pulmonarius.*

Mostly, these antioxidants are vital to avoid the peroxidation of membrane lipids (Opara, 2002). Reduced glutathione, an antioxidant required for cell defense, such as reclamation of ROS, and elimination of poisons (Brown et al., 2004), decrease of tissue reduced glutathione content established evidently states the higher use by the hepatic cells (Furfaro et al., 2012). In past reports, it was demonstrated that these concentration drops in experimental animals (Sayed, 2012). It is also detected that the action with glibenclamide, HWE and AE suggestively ($P < 0.01$) elevated non-enzymatic antioxidant levels in experimental animals.



A: Control; **B:** Diabetic Control; **C:** Glibenclamide;
D: Hot Water Extract (200 mg); **E:** Hot Water Extract (400 mg)
F: Acetone Extract (200 mg); **G:** Acetone Extract (400 mg)

Fig. 3. Histological study of Kidney of streptozotocin-induced wistar albino rats with different mycelial extracts of *P. pulmonarius*.

3.8. Histopathological analysis

Histopathological investigations of pancreas, liver and kidney of animals were revealed in Fig. 1, Fig. 2 and Fig. 3, respectively. Scientific reviews described that diabetic animals exhibited severe deterioration in the liver (Wang et al., 2017a,b).

In the current work, a microscopic examination of the pancreatic sections of the STZ-induced diabetes rats showed that there were pancreatic acini with diminished islet cells, peripancreatic adipose tissue with swelling and overfilled blood vessels in STZ-induced diabetes rats (Fig. 1B). Liver tissue was observed with slight modification in structural design, periportal inflammation, broadening of sinusoids, and normal kupfer cell action (Fig. 2B), while kidney tissues were witnessed with hypercellular glomeruli, thick-walled jammed blood vessels, overfilled proximal convoluted tubules and distal convoluted tubules (Fig. 3B).

In difference, control animals had an ordered hepatic design with usual pancreatic acini with enlarged ducts, slight stratification, thick-walled overfilled blood vessels and rare islet cells (Fig. 1A). Normal liver structural design with the usual portal triad and slight central vein jamming were exhibited by liver tissue (Fig. 2A). Kidney tissue with regular glomeruli with usual proximal convoluted tubules and distal convoluted tubules with normal vascularity were perceived (Fig. 3A). As shown in Fig. 1C, 2C and 3C, the pancreas, liver and kidney respectively in animals treated with standard drug almost recuperated to normal.

HWE, AE and glibenclamide therapy enriched the pancreas architecture similar to the control with no alteration. 400 mg of HWE exhibited hyperplasia of pancreatic acini, islet cell pool, overfilled blood vessels and thick-walled ducts (Fig. 1E); 400 mg of AE exhibited hyperplasia of pancreatic acini with overfilled blood vessels and diminished islet cell pool (Fig. 1G).

The treatment of HWE (400 mg) significantly marked improvement in the liver histopathology exhibited by liver tissue with minimal congestion of central vein, dilated sinusoids, periportal inflammation, kupfer cell activity, the proliferation of bile ducts and mild ballooning degeneration of the hepatocytes with minimal necrosis. There was no liver cell necrosis witnessed in a higher concentration of hot water extract (Fig. 2E), Notable changes in liver structural design, central vein jamming and periportal inflammation were observed in 400 mg AE (Fig. 2G). Histopathological irregularities no longer existed in liver tissue treated with HWE signifying the incredible effect of HWE on enhancing liver defects.

Besides, diabetes is reflected by a series of renal structure irregularity, including membrane thickening (Kaur et al., 2016). As revealed in Fig. 1A, animals in normal control had ample renal architecture. However, the architecture in diabetes animals showed various pathological injuries. 400 mg HWE exhibited hypercellular glomeruli, thinned out cortex, more significant than before perineural adipose tissue, overfilled blood vessels in the interstitium with inflammatory cells composed of lymphoplasmacytes (Fig. 3E), whereas 400 mg AE displayed kidney with limited jammed hypercellular glomeruli, normal proximal convoluted tubules and distal convoluted tubules with negligible inflammation of the interstitium (Fig. 3G). 400 mg of HWE had the greatest influence on the defense of the liver beside damages in diabetic animals.

Earlier studies specified that the histological damages in diabetes were mostly validated by the reduction of abnormal amount of lipids and unusual hyperglycemia (Shao et al., 2014; Lien et al., 2008), demonstrating that administration of the HWE and AE upturned these morphological changes in the pancreas, liver and kidney of diabetic rats.

4. Conclusion

In conclusion, our study revealed that the HWE and AE revealed antihyperglycaemic, antihyperlipidemic, and defensive effects in diabetic rats by reducing BGL, decreasing lipid profiles, enhancing the status of antioxidants and decreasing injuries in pancreas, liver and kidney. Notably, the HWE exhibited better effects. The outcomes delivered cites for the consumption of the HWE and AE extracted from mycelia of *P. pulmonarius* in relating the inhibition and relief of hyperglycemia. These findings raise the spirits in studying this fungal strain further for its potential biological applications. However, more experimental examination is required to demarcate the mode of action in the defensive effect of these extracts on diabetic animals.

Acknowledgment

The authors extend their appreciation to the management of MGR College, Hosur, Tamilnadu, India for their support in carrying out the research. The authors thank Alagappa University for the support for the performance of the work and RUSA – Phase 2.0 grant [Letter No. F.24-51/2014-U, Policy (TN Multi-Gen), Dept. of Edn. Govt. of India, Dt. 09.10.2018] for funding. The authors extend their appreciation to The Researchers supporting project number (RSP-2019/108) King Saud University, Riyadh, Saudi Arabia. The authors Hak-Jae Kim thank the support received from Soochunhyang University research fund for this research work.

Conflict of interests

The authors have no conflicts of interest to declare.

References

Abe, H., Matsubara, T., Arai, H., Doi, T., 2011. Role of Smad1 in diabetic nephropathy: molecular mechanisms and implications as a diagnostic marker. Histol. Histopathol. 26, 531–541.

Aebi, H., 1984. Isolation, purification, characterization, and assay of antioxygenic enzymes. Meth. Enzymol. 105, 121–126.

Al-Dhabi, N.A., Arasu, M.V., Rejiniemon, T.S., 2015. In vitro antibacterial, antifungal, antibiofilm, antioxidant, and anticancer properties of isosteviol isolated from endangered medicinal plant *Pittosporum tetraspermum*. Evidence-Based Complementary and Alternative Medicine.

Al-Dhabi, N.A., Arasu, M.V., 2016. Quantification of phytochemicals from commercial *Spirulina* products and their antioxidant activities. Evidence-Based Complementary and Alternative Medicine.

American Diabetes Association (ADA), 2019. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes, Diabetes Care, 42: S13–S28

Aruna, S.M., Yadav, D.B., Chandrashekar, A.K., 2013. Antioxidant and *in vivo* antihyperglycemic activity of *Muntingia calabura* leaves extracts. Der Pharmacia Lettre. 5 (3), 427–435.

Bai, J., Ren, Y., Li, Y., Fan, M., Qian, H., Wang, L., Rao, Z., 2019. Physiological functionalities and mechanisms of β-glucans. Trends Food Sci. Technol. 88 (February), 57–66.

Barathikannan, K., Venkatadri, B., Khusro, A., Al-Dhabi, N.A., Agastian, P., Arasu, M. V., Choi, H.S., Kim, Y.O., 2016. Chemical analysis of Punica granatum fruit peel and its in vitro and in vivo biological properties. BMC Complimentary and Alternative Medicine 16, 264.

Beutler, E., Duron, O., Kelly, B.M., 1986. Improved method for the determination of blood glutathione. J. Lab Clin. Med. 61, 882–888.

Brown, L.A., Harris, F.L., Ping, X.D., Gauthier, T.W., 2004. Chronic ethanol ingestion and the risk of acute lung injury: a role for glutathione availability. Alcohol 33, 191–197.

Bule, M., Abdurahman, A., Nikfar, S., Abdollahi, M., Amini, M., 2019. Antidiabetic effect of quercetin: a systematic review and meta-analysis of animal studies. Food Chem. Toxicol. 125, 494–502.

Cheng, L., Wang, Y., He, X., Wei, X., 2018. Preparation, structural characterization and bioactivities of Se-containing polysaccharide: a review. Int. J. Biol. Macromol. 120, 82–92.

De Silva, D.D., Rapior, S., Hyde, K.D., 2012. Medicinal mushrooms in prevention and control of diabetes mellitus. Fungal Divers. 56, 1–29.

Desai, I.D., 1984. Vitamin E analysis methods for animal tissues. Methods Enzymol. 105, 138–147.

Ecobichon, D.J., 1997. The Basis of Toxicology Testing. CRC Press, New York, pp. 43–86.
Elango, G., Roopan, S.M., Al-Dhabi, N.A., Arasu, M.V., Damodharan, K.I., Elumalai, K., 2017. *Cocos nucifera* coir-mediated green synthesis of Pd NPs and its investigation against larvae and agricultural pest. Artificial Cells, Nanomedicine, and Biotechnology.
Elango, G., Roopan, S.M., Al-Dhabi, N.A., Arasu, M.V., Dhamodaran, K.I., Elumalai, K., 2016a. Coir mediated instant synthesis of Ni-Pd nanoparticles and its significance over larvicidal, pesticidal and ovicidal activities. J. Mol. Liq. 223, 1249–1255.
Elango, G., Roopan, S.M., Dhamodaran, K.I., Elumalai, K., Al-Dhabi, N.A., Arasu, M. V., 2016b. Spectroscopic investigation of biosynthesized nickel nanoparticles and its larvicidal, pesticidal activities. J. Photochem. Photobiol., B 162, 162–167.
Eliza, J., Daisy, P., Ignacimuthu, S., 2010. Antioxidant activity of costunolide and eremanthin isolated from Costus speciosus (Koen ex Retz) Sm. Chem-Bio. Interact. 188, 467–472.
Elkhateeb, W.A., Zaghlol, G.M., El-Garawani, I.M., Ahmed, E.F., Rateb, M.E., Moneim, A.E., 2018. *Ganoderma applanatum* secondary metabolites induced apoptosis through different pathways: in vivo and in vitro anticancer studies. Biomed Pharmacother. 101, 264–277.
Elsayed, E.A., El Enshasy, H., Wadaan, M.A., Aziz, R., 2014. Mushrooms: a potential natural source of anti-inflammatory compounds for medical applications. Mediators Inflamm. 2014, 805–841.
Fowsiya, J., Madhumitha, G., Al-Dhabi, N.A., Arasu, M.V., 2016. Photocatalytic degradation of Congo red using Carissa edulis extract capped zinc oxide nanoparticles. J. Photochem. Photobiol., B 162, 395–401.
Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18, 499–502.
Furfaro, A.L., Nitti, M., Marengo, B., Domenicotti, C., Cottalasso, D., Marinari, U.M., 2012. Impaired synthesis contributes to diabetes-induced decrease in liver glutathione. Int J Mol Med 29, 899–905.
Glorybai, L., Barathi, K.K., Arasu, M.V., Al-Dhabi, N.A., Agastian, P., 2015. Some biological activities of Epaltes divaricata L. - an in vitro study. Ann. Clin. Microbiol. Antimicrobials 14, 18.
Gurusamy, S., Kulanthaisamy, M.R., Hari, D.G., Veleeswaran, A., Thulasinathan, B., Muthuramalingam, J.B., Balasubramani, R., Chang, S.W., Arasu, M.V., Al-Dhabi, N.A., Selvaraj, A., Alagarsamy, A., 2019. Environmental friendly synthesis of TiO2-ZnO nanocomposite catalyst and silver nanomaterials for the enhanced production of biodiesel from Ulva lactuca seaweed and potential antimicrobial properties against the microbial pathogens. J. Photochem. Photobiol., B 193, 118–130.
Han, S.R., Kim, K.H., Lim, K.O., Oh, T.J., 2015. Biological activity analysis of different solvent extracts from *Pleurotus ostreatus*. Indian Journal of Science and Technology 8, 1–8.
Haritha, E., Roopan, S.M., Madhavi, G., Elango, G., Al-Dhabi, N.A., Arasu, M.V., 2016. Green chemical approach towards the synthesis of SnO_2 NPs in argument with photocatalytic degradation of diazo dye and its kinetic studies. J. Photochem. Photobiol., B 162, 441–447.
Helan, V., Prince, J. Joseph, Al-Dhabi, Naif Abdullah, Arasu, Mariadhas Valan, Ayeshamariam, A., Madhumitha, G., Roopan, Selvaraj Mohana, Jayachandran, M., 2016. Neem leaves mediated preparation of NiO nanoparticles and its magnetization, coercivity and antibacterial analysis. Results Phys. 6, 712–718. https://doi.org/10.1016/j.rinp.2016.10.005.
Ilavenil, S., Kim, D.H., Srigopalram, S., Kuppusamy, P., Arasu, M.V., Lee, K.D., Lee, J.C., Song, Y.H., Jeong, Y.I., Choi, K.C., 2017. Ferulic acid in Lolium multiflorum inhibits adipogenesis in 3T3-L1 cells and reduced high-fat-diet-induced obesity in Swiss albino mice via regulating p38MAPK and p44/42 signal pathways. J. Funct. Foods 37, 293–302.
Kaur, N., Kishore, L., Singh, R., 2016. Antidiabetic effect of new chromane isolated from *Dillenia indica* L. leaves in streptozotocin-induced diabetic rats. J. Funct. Foods. 22, 547–555.
Keilacker, H., Besch, W., Woltanski, K.P., Diaz-Alonso, J.M., Kohnert, K.D., Ziegler, M., 1987. Measurement of insulin in human sera using a new RIA Kit. 2. Determination of free and total insulin–correlations to insulin antibody levels. Exp. Clin. Endocrinol. 90 (3), 271–277.
Kothari, D., Patel, S., Kim, S.K., 2018. Anticancer and other therapeutic relevance of mushroom polysaccharides: a holistic appraisal. Biomed. Pharmacother. 105 (April), 377–394.
Kozarski, M., Klaus, A., Jakovljevic, D., Todorovic, N., Vunduk, J., Petrovic, P., Niksic, M., Vrvic, M.M., van Griensven, L., 2015. Antioxidants of Edible Mushrooms, Molecules (Basel, Switzerland). 20, 19489-19525.
Ktari, N., Mnafgui, K., Nasri, R., Hamden, K., Bkhairia, I., Hadj, A.B., Boudaouara, T., Elfeki, A., Nasri, M., 2013. Hypoglycemic and hypolipidemic effects of protein hydrolysates from zebra blenny (*Salaria basilisca*) in alloxan-induced diabetic rats. Food. Funct. 4, 1691–1699.
Lien, A.P.H., Hua, H., Chuong, P.H., 2008. Free radicals, antioxidants in disease and health. Int. Biomed. Sci. 4, 89–96.
Liu, Y.T., You, Y.X., Li, Y.W., Zhang, L., Yin, L.L., Shen, Y.B., Li, C., Chen, H., Chen, S.J., Hu, B., Chen, D.W., 2017. The characterization, selenylation and antidiabetic activity of mycelial polysaccharides from *Catathelasma ventricosum*. Carbohyd. Polym. 174, 72–81.
Lobarzewski, J., Ginalska, G., 1995. Industrial use of soluble or immobilized plant peroxidases. Plant Peroxidase Newsletter 6, 3–7.
Lopes-Virella, M.F., Stone, P., Ellis, S., Colwell, J.A., 1977. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin. Chem. 23, 882–884.
Ramos, Marina, Burgos, Nuria, Barnard, Almero, Evans, Gareth, Preece, James, GrazAndrea, Michael, Ruthes, Caroline, Jiménez-Quero, Amparo, Martínez-Abad, Antonio, Vilaplana, Francisco, Ngoc, Long Pham, Brouwer, Abraham, der Burg, Bartvan, Garrigós, Maríadel Carmen, Jiménez, Alfonso, 2019. *Agaricus bisporus* and its by-products as a source of valuable extracts and bioactive compounds. Food Chem. 292, 176–187.
Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247 (10), 3170–3175.
Murussi, M., Murussi, N., Campagnolo, N., Sliveiro, S.P., 2008. Early detection of diabetic nephropathy. Arq. Bras. Endocrinol. Metabol. 52, 442–451.
Natelson, S., Scott, M.L., Beffa, C., 1951. A rapid method for the estimation of urea in biological fluids. Am. J. Clin Pathol. 21, 275–281.
Niaz, K., Maqbool, F., Khan, F., Hassan, F.I., Momtaz, S., Abdollahi, M., 2018. Comparative occurrence of diabetes in canine, feline, and few wild animals and their association with pancreatic diseases and ketoacidosis with therapeutic approach. Veterinary World 11 (4), 410.
Nieshus, W.G., Samuelsson, B., 1986. Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur. J. Biochem. 6, 126–130.
Oluwatosin, A., Massoud, A., Mehran, H.R., Parvin, P., Ali, M.M., 2012. Methanolic extract of African mistletoe (Viscum album) improves carbohydrate metabolism and hyperlipidemia in streptozotocin-induced diabetic rats. Asian. Pac. J. Trop. Me. 5, 427–433.
Omaye, S.T., Turabull, J.D., Sanberlich, H.E., 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol. 62, 1–11.
Opara, E.C., 2002. Oxidative stress, micronutrients, diabetes mellitus, and its complications. J. R. Soc. Promot. Health 22, 28–34.
Özdal, M., Gülmez, Ö., Gür Özdal, Ö., Algur, Ö.F., 2019. Antibacterial and Antioxidant Activity of Mycelial Extracts of Different *Pleurotus* Species. Food and Health. 5 (1), 12–18.
Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70 (1), 158–169.
Pan, Y.X., Wang, C., Chen, Z.Q., Li, W.W., Yuan, G.Q., Chen, H.X., 2017. Physicochemical properties and antidiabetic effects of a polysaccharide from corn silk in high-fat diet and streptozotocin-induced diabetic mice. Carbohyd. Polym. 164, 370–378.
Pari, L., Latha, M., 2005. Antidiabetic effect of Scoparia dulcis: effect on lipid peroxidation in streptozotocin diabetes. Gen. Physiol. Biophys. 24, 13–26.
Park, C.H., Baskar, T.B., Park, S.-Y., Kim, S.-J., Arasu, M.V., Al-Dhabi, N.A., Kim, J.K., Park, S.U., 2016a. Metabolic profiling and antioxidant assay of metabolites from three radish cultivars (*Raphanus sativus*). Molecules 21, 157.
Park, Y.J., Baskar, T.B., Yeo, S.K., Arasu, M.V., Al-Dhabi, N.A., Lim, S.S., Park, S.U., 2016b. Composition of volatile compounds and *in vitro* antimicrobial activity of nine *Mentha* spp. SpringerPlus. 5, 1628.
Park, Y.J., Park, S.-Y., Arasu, M.V., Al-Dhabi, N.A., Ahn, H.-G., Kim, J.K., Park, S.U., 2017. Accumulation of carotenoids and metabolic profiling in different cultivars of Tagetes flowers. Molecules 22, 313.
Racker, E., 1955. Glutathione reductase from bakers' yeast and beef liver. J. Biol. Chem. 217 (2), 855–865.
Rahmani, S., Khalili, N.P., Khan, F., Hassani, S., Ghafour-Boroujerdi, E., Abdollahi, M., 2018. Bisphenol A: what lies beneath its induced diabetes and the epigenetic modulation? Life Sci. 214, 136–144.
Rajkumari, J., Maria, Magdalane C., Siddhardha, B., Madhavan, J., Ramalingam, G., Al-Dhabi, N.A., Arasu, M.V., Ghilan, A.K.M., Duraipandiayan, V., Kaviyarasu, K., 2019. Synthesis of titanium oxide nanoparticles using Aloe barbadensis mill andevaluation of its antibiofilm potential against Pseudomonas aeruginosa PAO1. J. Photochem. Photobiol., B.
Rathore, H., Prasad, S., Kapri, M., Tiwari, A., Sharma, S., 2019. Medicinal importance of mushroom mycelium: Mechanisms and applications. J. Funct. Foods 56 (February), 182–193.
Reis, F.S., Martins, A., Vasconcelos, M.H., Morales, P., Ferreira, I.C.F.R., 2017. Functional foods based on extracts or compounds derived from mushrooms. Trends Food Sci. Technol. 66, 48–62.
Roeschlau, P., Bernt, E., Gruber, W.A., 1974. Enzymatic determination of total cholesterol in serum. Clin. Biochem. 12, 226–228.
Roopan, S.M., Priya, D.D., Shanavas, S., Acevedo, R., Al-Dhabi, N.A., Arasu, M.V., 2019. CuO/C nanocomposite: Synthesis and optimization using sucrose as carbon source and its antifungal activity. Mater. Sci. Eng., C 101, 404–414.
Ruiz-Herrera, J., Ortiz-Castellanos, L., 2019. Cell wall glucans of fungi. A review. The Cell Surface 5, (February) 100022.
Sayed, A.A., 2012. Ferulsinaic acid modulates SOD, GSH, and antioxidant enzymes in diabetic kidney. Evid. Based Complement. Alternat. Med. 58, 1–9.
Shao, M.L., Lu, X.M., Cong, W.T., Xing, X., Tan, Y., Li, Y.Q., Li, X.K., Jin, L.T., Wang, X.J., Dong, J.C., Jin, S.Z., Zhang, C., Cai, L., 2014. Multiple low-dose radiation prevents type 2 diabetes-induced renal damage through attenuation of dyslipidemia and insulin resistance and subsequent renal inflammation and oxidative stress. PLoS ONE 9, e92574.
Slot, C., 1965. Plasma creatinine determination: a new and specific Jaffe reaction method. Scand. J. Clin. Invest. 17, 381.

Sokovic, M., Ciric, A., Glamoclija, J., Stojkovic, D., 2016. The bioactive properties of mushrooms. In: Ferreira, I.C.F.R., Morales, P., Barros, L. (Eds.), Wild Plants, Mushrooms and Nuts: Functional Food Properties and Applications. John Wiley & Sons Ltd, Chichester, UK, pp. 83–122.

Sun, Y., Zhang, M., Fang, Z., 2019. Efficient physical extraction of active constituents from edible fungi and their potential bioactivities: a review. Trends in Food Science and Technology, (February), 0–1.

Surendra, T.V., Roopan, S.M., Al-Dhabi, N.A., Arasu, M.V., Sarkar, G., Suthindhiran, K., 2016a. Vegetable peel waste for the production of ZnO nanoparticles and its toxicological efficiency, antifungal, hemolytic, and antibacterial activities. Nanoscale Res. Lett. 11, 546.

Surendra, T.V., Roopan, S.M., Arasu, M.V., Al-Dhabi, N.A., Rayalu, G.M., 2016b. RSM optimized Moringa oleifera peel extract for green synthesis of M. oleifera capped palladium nanoparticles with antibacterial and hemolytic property. J. Photochem. Photobiol., B 162, 550–557.

Surendra, T.V., Roopan, S.M., Arasu, M.V., Al-Dhabi, N.A., Sridharan, M., 2016c. Phenolic compounds in drumstick peel for the evaluation of antibacterial, hemolytic and photocatalytic activities. J. Photochem. Photobiol., B 161, 463–471.

Tabatabaei-Malazy, O., Larijani, B., Abdollahi, M., 2012. A systematic review of in vitro studies conducted on effect of herbal products on secretion of insulin from Langerhans islets. J Pharm Pharm Sci 15 (3), 447–466.

Islam, Tahidul, Ganesan, Kumar, Baojun, Xu., 2019. New Insight into Mycochemical Profiles and Antioxidant Potential of Edible and Medicinal Mushrooms: A Review. International Journal of Medicinal Mushrooms. 21 (3), 237–251.

Tietz, N.W., 1990. Clinical guide to laboratory tests. W.B. Saunders Company, Philadelphia.

Ullah, A., Khan, A., Khan, I., 2016. Diabetes mellitus and oxidative stress––a concise Review. Saudi. Pharm. J. 24, 547–553.

Valsalam, S., Agastian, P., Arasu, M.V., Al-Dhabi, N.A., Ghilan, A.K.M., Kaviyarasu, K., Ravindran, B., Chang, S.W., Arokiyaraj, S., 2019. Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of Tropaeolum majus L. and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties. J. Photochem. Photobiol., B 191, 65–74.

Valsalam, S., Agastian, P., Esmail, G.A., Ghilan, A.K.M., Al-Dhabi, N.A., Arasu, M.V., 2019.. Biosynthesis of silver and gold nanoparticles using Musa acuminata colla flower and its pharmaceutical activity against bacteria and anticancer efficacy. J. Photochem. Photobiol., B. 10.1016/j.jphotobiol.2019.111670.

Gulati, Vandana, Singh, Mansi Dass, Gulati, Pankaj, 2019. Role of mushrooms in gestational diabetes mellitus. AIMS Medical Science. 6 (1), 49–66.

Wang, C., Chen, Z.Q., Pan, Y.X., Gao, X.D., Chen, H.X., 2017a. Anti-diabetic effects of Inonotus obliquus polysaccharides-chromium (III) complex in type 2 diabetic mice and its sub-acute toxicity evaluation in normal mice. Food. Chem. Toxicol. 108, 498–509.

Wang, J., Wang, C., Li, S.Q., Li, W.W., Yuan, G.Q., Pan, Y.X., Chen, H.X., 2017b. Anti-diabetic effects of Inonotus obliquus polysaccharides in streptozotocin-induced type 2 diabetic mice and potential mechanism via PI3K-Akt signal pathway. Biomed. Pharmacother. 95, 1669–1677.

Xu, J., Li, Z., Cao, M., Zhang, H., Sun, J., Zhao, J., Zhou, Q., Wu, Z., Yang, L., 2012. Synergetic effect of *Andrographis paniculata* polysaccharide on diabetic nephropathy with andrographolide. Int. J. Biol. Macromol. 51, 738–742.

Xu, T., Beelman, R.B., 2015. The bioactive compounds in medicinal mushrooms have potential protective effects against neurodegenerative diseases. Adv Food Technol Nutr Sci Open J. 1, 62–66.

Yildiz, O., Can, Z., Laghari, A.Q., Şahin, H., Malkoç, M., 2015. Wild edible mushrooms as a natural source of phenolics and antioxidants. J. Food Biochem. 39, 148–154.

Yu, Z., Yin, Y., Zhao, W., Yu, Y., Liu, B., Liu, J., 2011. Novel peptides derived from egg white protein inhibiting alpha-glucosidase. Food Chem. 129, 1376–1382.

Zhan, Y., Ren, C.J., Lu, G.B., Cui, W.Z., Mu, Z.M., Gao, H.J., Wang, Y.W., 2014. Purification, characterization and anti-diabetic activity of a polysaccharide from mulberry leaf. Regul. Toxicol. Pharm. 70, 687–695.

Zhang, J.F., Fan, S.R., Mao, Y.G., Ji, Y., Jin, L.Q., Lu, J.X., Chen, X.M., 2016. Cardiovascular protective effect of polysaccharide from *Ophiopogon japonicas* in diabetic rats. Int. J. Biol. Macromol. 82, 505–513.

Ziyadeh, F.N., Sharma, K., 2003. Overview: combating diabetic nephropathy. J. Am. Soc. Nephrol. 14, 1355–1357.