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RESEARCH

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The possible effect of crude phenolic-rich extract from mushroom (*Pleurotus ostreatus*) on infertility induced by streptozotocin in rats fed a high-fat diet as a model of type 2 diabetes

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Abstract

Background Phenolic compounds (PC) are found in various foods that are part of the human diet, such as mushrooms. The identification of PC is crucial due to their bioactive properties and their role in disease prevention. This study aimed to assess the antioxidant and antidiabetic properties of the phenolic component extract (PCE) derived from the *Pleurotus ostreatus* mushroom. The extract was separated using high-performance liquid chromatography (HLPC). The PCE was then tested on diabetic rats fed a high-fat diet (HFD). Also, this study was an attempt to treat infertility caused by type 2 diabetes in male rats.

Methods Streptozotocin (STZ) was administered to Wistar rats after a 4-week HFD as a model of type 2 diabetes (D2). Animals were given phenols orally on a high-fat diet for four weeks after being diagnosed with diabetes.

Results PCE constituents were 48.02% carbohydrates, 21.15% protein, 6.16% fiber, 2.06% fat, and 6.93% ash. The evaluation of the PCE was carried out by assessing its retention rates and subsequently comparing the UV data to standards.

Total phenols and flavonoids in the crude extract were 23.04 mg/g gallic acid equivalent and 8.14 mg/g quercetin equivalent, respectively. Catechin was found as the significant PC (121.53 µg/mL), while gallic acid was the second PC (11.64 µg/ml) in the PCE of mushrooms. PCE showed high radical scavenging activity on DPPH. With dosage and incubation duration in dark-dependent conditions, it was discovered to be 93.23% at a 500 µg/ml concentration after 120 min in the dark. PCE reduces blood glucose levels while elevating insulin levels and antioxidant activity indicators. Our results show that phenols and their bioactive components can change glucose metabolism in STZ-induced diabetic HFD rats (D2).

Conclusions This study supports PCE's therapeutic potential in treating hyperglycemia, increasing insulin secretion, and addressing diabetes-induced infertility.

Keywords Diabetes, Mushroom phenols, High-fat diet, Antioxidants, Infertility

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Background

Mushrooms are categorized as macro-fungi because they have spore-bearing structures and a fleshy fruiting body (Chowdhury et al. 2015). The popularity of mushrooms has steadily increased due to their acknowledged nutritional value and potential health advantages, increasing their production and consumption (Sharifi-Rad et al.

2020). Recent research shows a significant increase in the use of mushrooms in the nutraceutical, cosmeceutical, and pharmaceutical sectors. Over the years, there has been growing attention toward the utilization of mushrooms as a potential medicine or treatment for many hazardous disorders on a global scale (Usman et al. 2021). *Pleurotus ostreatus* is a notable example of a dietary mushroom with significant therapeutic characteristics and promising pharmacological potential. A sizable body of research that has surfaced in recent decades has supported the efficacy of these therapies in improving health outcomes and addressing particular medical issues.

Furthermore, *P. ostreatus* mushrooms are widely recognized for their conventional medicinal attributes and therapeutic capabilities. These properties involve antioxidative, anti-inflammatory, anticarcinogenic, antimicrobial, antibacterial, antifungal, antidiabetic, antiangiogenic, immune-modulatory, hepatoprotective, hypoglycemic, antiviral, antitumor, anti-hypercholesterolemic, antihypertensive, liver-protective, general fitness-promoting, anti-obesity, anti-asthmatic, anti-ulcer, and anti-atherosclerotic effects. Mushrooms are also utilized as functional foods (Gupta et al. 2018; Ferdousi et al. 2019).

Diabetes mellitus type 1 (DMT1) accounts for only around 7–10% of all DM cases globally, whereas diabetes mellitus type 2 (DMT2) accounts for more than 90% of all DM cases (Moore et al. 2023). According to the World Health Organization (WHO), about 80% of the population in developing countries relies on plants and herbal products as their primary form of therapy (Organization 2013).

Diabetes stands as a formidable metabolic disorder, wreaking prolonged havoc on multiple organs. Within its ominous array of complications, a particularly significant repercussion is the degenerative impact it inflicts on the reproductive system, giving rise to issues such as infertility and disrupted gonadal function (Barsiah et al. 2019).

Mushrooms possess a significant amount of bioactive constituents, including phenolic compounds, which contribute to their nutritional and therapeutic importance. They contain phytochemicals with significant antibacterial, antioxidant, anticancer, and anti-inflammatory activities (Mishra et al. 2018). Antioxidant-rich foods reduce oxidative damage, increasing free radical production (Ayyappan et al. 2016). Because they have unpaired electrons, free radicals are unstable and exceedingly reactive; they generate oxidative stress, which causes DNA damage, carcinogenesis, oxidation of macromolecules, and cellular deterioration (Juan et al. 2021). Mushrooms contain phenolic compounds that have antioxidant properties. These compounds are effective in scavenging radicals, inhibiting free radicals, and acting as phytonutrients (Jeena et al. 2016). This diverse group of chemicals includes flavonoids, phenolic acids, stilbenes, lignin, and tannins, all characterized by having at least one aromatic ring with attached hydroxy groups (Lin et al. 2018).

The application of phenolic compounds from food or supplements for preventing chronic illnesses has developed significantly, and the efficiency of such techniques in humans primarily relies on their bioavailability and metabolism (Dias et al. 2021). Although food and nutritional variables have received considerable attention, the influence of disease states like obesity or diabetes on absorption, metabolism, and final efficacy is something to consider (Dias et al. 2021). These characteristics must be addressed to design successful methods for preventing chronic illness using bioactive phenolic compounds. Mushrooms include secondary metabolites such as phenolic compounds, polyketides, terpenes, and steroids (Devi et al. 2020). In addition, a mushroom phenolic compound that is not mutagenic has been discovered to be a vital antioxidant and synergist (Chandra et al. 2020). Therefore, this investigation aimed to assess the antioxidative properties and antidiabetic potential of phenols isolated from *Pleurotus ostreatus*.

Methods

The procedure for collecting samples and extracting

The fungal specimen of *P. ostreatus* was procured from the Microbiological Culture Collection Center (Cairo Mircen) and utilized throughout the duration of the investigation. Air-dried powder form of a mushroom specimen 200 g was accurately weighed and set into a conical flask 5 L, and then, 2 L distilled water was added for the preparation of water extracts. The sample was extracted for 24 h and then centrifuged by a centrifuge (Sigma-Laborzentrifugen, 2K15) at 5000 rpm for 10 min; the extraction was repeated two times. The supernatant (phenolic extract) was transferred into new flask and stored at – 20 °C.

Chemical analysis

The protein, fiber, fat, and ash concentrations were assessed according to the official methods of the Association of Official Analytical Collaboration (AOAC). This study used the phenol–sulfuric acid method to measure the amount of sugar in mushroom phenol extract. A glucose standard was used as a standard.

Determination of total phenolic content

The total phenolic compounds (TPCs) were quantified via the Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), following the methodology outlined by Singleton et al. (1999). One milliliter of the sample was

mixed with five milliliters of Folin–Ciocalteu reagent, which had been mixed with water in a specific way (one-tenth of a volume for every volume of water), and four milliliters of a 75-g-per-liter sodium carbonate solution. The tubes underwent vortex mixing for 15 s and were left undisturbed for 30 min at a temperature of 40 °C to facilitate the development of color. The absorbance measurement was taken at a wavelength of 765 nm via a UV/VIS spectrophotometer (UV-1700 Pharmaspec, Shimadzu). The standard curve (20–200 µg/ml) was established using gallic acid, and the quantification of the samples' reduction of Folin–Ciocalteu reagent was documented in milligrams of gallic acid equivalents (GAE) per gram of extract.

The quantification of the total flavonoid content

The colorimetric analysis of aluminum chloride (Sigma-Aldrich, St. Louis, MO, USA) by Li et al. (2008) was employed to determine flavonoids. The dehydrated extract was reconstituted in distilled water at a concentration of (mg/ml). A solution of 1 ml of dissolved extract was combined with 3 ml of methanol in a test tube. The blend consisted of 0.2 ml of aluminum chloride solution with a concentration of 10%, 0.2 ml of potassium acetate solution (Sigma-Aldrich, St. Louis, MO, USA) with a concentration of 1 M, and 5.6 ml of distilled water. After that, the mixtures were allowed to stand at room temperature for half an hour. A UV–VIS spectrophotometer (UV-1700 Pharmaspec, Shimadzu) was used to measure the absorbance of the reaction mixture at a wavelength of 415 nm. A calibration curve was produced by producing solutions of quercetin (Sigma-Aldrich, St. Louis, MO, USA) in distilled water at different concentrations (mg/ml), from which the content was calculated by extrapolating. Milligrammes per milliliter of extract was the unit of measurement for flavonoids.

High-performance liquid (HPLC) analyses

The extract was screened using liquid chromatography (LC), explicitly using tools from the Kyoto, Japan-based Shimadzu Corporation. The separation process was successfully conducted using a reverse-phase C18 column possessing dimensions of 250 × 4.6 mm and a particle size of 5 microns while maintaining a temperature of 24 °C. In the better chromatographic method, the mobile phase comprised two solvents: solvent A, which was methanol, and solvent B, which was 0.5% acetic acid in water. The emission profile exhibited the following characteristics: In the first observation, after 10 min, 10% of substance A was found in substance B. In the second observation, after 28.6 min, 60% of substance A was found in substance B. Finally, in the third observation, after 30 min, 10% of substance A was found in substance B. A linear pattern was observed in all gradients. A volume of 20 µl was used for the injection, and a flow rate of 1 ml per minute was noted. The measurement of absorption was taken at a wavelength of 290 nm. The UV–visible detector was employed for the detection process. The eluted components were identified using the system controller CMB-20A/20Alite to compare their retention time to a standard. The samples' phenolic chemicals were identified by comparing their retention times to those of standards from Himedia and Sigma-Aldrich that are available in the market. Their UV–visible spectra characterized them.

Radical scavenging behavior test using DPPH

The radical scavenging activity (RSA) percentage of the phenolic extraction process was determined by Zeković et al. (2010). Different concentrations from phenolic mushroom extract (100–500 µg/ml) were combined with methanol (96%) and a solution of DPPH at a concentration of 63 µmol/L. After different periods (30, 60, 90, and 120 min), at room temperature, the absorbency was measured at a wavelength of 517 nm, and the samples were looked at by using a calculation method that involved finding the DPPH scavenging behavior, which is the relative decrease in absorbance seen in the samples that were looked at. The DPPH scavenging activity was determined by applying the subsequent equation: the equation for calculating DPPH scavenging activity is expressed as:

$$100 \times (Ac - As)/Ac$$

where Ac- A = absorbance, C = control "Absorbance of control".

As- A = Absorbance, S = Sample "Absorbance of the sample."

Scavenging test for hydrogen peroxide

A 2 mM/l hydrogen peroxide solution was produced using a standard phosphate buffer at a pH of 7.4. Various PCE concentrations (100 to 500 µg/ml) were introduced into a 0.6 ml hydrogen peroxide solution in distilled water. After 10 min, the absorbance at 230 nm wavelength was measured using a blank solution made of phosphate buffer that did not contain hydrogen peroxide. The scavenging activity percentage was assessed at various concentrations of PCE, and the IC50 value was obtained according to Oktay et al. (2003).

Power reduction

The investigation of reducing power involved studying the transition of Fe3 + to Fe2 + in the presence of the fractions, as reported in the study conducted by Fejes et al. (2000). The concentration of Fe2 + can be quantified by

assessing the development of Perl's Prussian blue at a wavelength of 700 nm, as demonstrated by Meir et al. (1995). A volume of 1 ml of the fraction with a concentration range of 100–500 µg/ml, along with 2.5 ml of phosphate buffer at a pH of 6.6 and 2.5 ml of a solution containing 1% potassium ferricyanide, were subjected to incubation at a temperature of 50 °C for 30 min. Following this, 2.5 ml of a solution containing 10% trichloroacetic acid was added to the combination, which was subsequently subjected to centrifugation at a force of 3000×g for 10 min. About 2.5 ml (ml) of the supernatant was mixed with 2.5 ml of water to make it less concentrated, and then, it was mixed with 0.5 ml of freshly made ferric chloride solution that was 0.1% concentrated. The measurement of absorbance was taken at a wavelength of 700 nm. The experiments were performed three times, and the resulting graph was generated using the mean value of the three measurements**.**

In vivo *study*

Experimental design

The Wistar rats used in this study were collected from the Egyptian Organization for Biological Commodities and Vaccines (VACCERA) laboratory animal farm in Helwan, Cairo, Egypt. The thirty-two male WRs that comprised the sample had a typical weight of 170 gm and were eight weeks old. The rats were housed in an animal care facility and kept on a conventional rat diet while following a 12-h light–dark cycle.

The rats were divided into three groups using a random allocation method:

- Group I (Normal) comprised normal rats fed the standard AIN-93 M diet (XTADM001) (Table 1) (AIN, $n=8$).
- Group II consisted of normal rats treated with phenolic extracts ($n=8$).
- Group III (HFD) consisted of rats fed a high-fat diet (45% of energy from fat-XTHF45) (Table 2) for four weeks ($n=16$).

Daily food consumption and body weight were monitored every seven days.

After the four weeks, the rats in the HFD group were intraperitoneally delivered a singular dosage of 40 mg per kilogram of body weight of STZ (Sigma Chemical Co., St. Louis, MO, USA), dissolved in a 0.1 M-citrate buffer with a pH of 4.4. This was done for rats in Group IIIa (DHFD) and Group IIIb (DHFD treated with phenolic extracts orally at 100 mg/kg) daily for four additional weeks (each subgroup contains eight rats). Group IIIa received only the citrate buffer injection daily four additional weeks. The diagnosis of diabetes was established after 72 h post-STZ injection by monitoring blood glucose levels. Rats with blood glucose levels equivalent to or exceeding 200 mg/dl were selected for further analysis.

Following four weeks of treatment and an overnight fast, the rats were anesthetized using intraperitoneal sodium thiopental injection (60 mg/kg of body weight). Blood samples were collected from the heart's aorta into polyethylene terephthalate tubes with plasma coagulant (Monroe-REF-454246, USA). These samples were solidified at room temperature for 20 min, followed by centrifugation at 3000xg. Subsequently, serum samples were separated and stored in aliquots at − 70 °C for subsequent biochemical evaluation.

Table 1 AIN-93 M ingredients (XTADM001)

Ingredients	g/kg
Casein	140.00
L-Cystine	1.89
Corn Starch	495.69
Maltodextrin	125.00
Sucrose	100.00
Soybean oil	40.00
Cellulose	50.00
Mineral Mix S10022M	35.00
Vitamin Mix V10037	10.00
Choline bitartrate	2.50
TBHQ, antioxidant	0.01

Table 2 Rodent diet with 45% calories from fat (XTHF45)

Ingredients	g/kg
Casein	200.00
L-Cystine	3.00
Corn starch	72.80
Maltodextrin	100.00
Sucrose	172.80
Lard	177.50
Soybean oil	25.00
Cellulose	50.00
Calcium carbonate	5.50
Dicalcium phosphate	13.00
Potassium citrate, 1H2O	16.50
Mineral Mix S10026	10.00
Vitamin Mix V10001	10.00
Choline bitartrate	2.00
Red food color	0.05

All experimental protocols were approved by local ethical guidelines (Ethical Committee for Animal Care and Use, Egypt). The study protocol was approved by the members of the Research Ethics Committee in the National Center for Radiation Research and Technology (REC-NCRRT) in Egypt (number 16A/22).

Biochemical measurements

A glucose detection kit (Egypt-Chem) was used for glucose oxidase assays, which were done according to the standard procedure for finding fasting blood sugar levels (mg/dl) (Trinder 1969). Insulin levels were determined through radioimmunoassay following the protocol outlined by Clark (1999). Plasma samples were used for measuring triglycerides, high-density lipoprotein cholesterol (HDL-C), total cholesterol, and low-density lipoprotein cholesterol (LDL-C). Low-density lipoprotein cholesterol (VLDL-C) was determined using triglyceride/5 = VLDL-C (mg/100 mL).

Homeostatic model assessment

The variables of interest in this study are insulin resistance, sensitivity, and beta-cell function, which are assessed using the homeostasis model assessment (HOMA) method. Precisely, we measured HOMA-IR to evaluate insulin resistance, HOMA-IS to determine insulin sensitivity, and HOMA-β cell function to examine beta-cell role following the methods presented by Hsing et al. (2003) and Park et al. (Park et al. 2009) as follows.

$$\text{HOMA} - \text{IR} : \text{Computed using the equation} : \text{HOMA} - \text{IR} = \text{fasting insulin} \times \text{fasting glucose}/405.$$

$$\text{HOMA} - \text{IS} : \text{Computed using the equation} : \text{HOMA} - \text{IS} = 10,000/(\text{fasting insulin} \times \text{fasting glucose}).$$

$$\text{HOMA} - \beta \text{ Cell Function} : \text{Determined through the equation} : \text{HOMA} - \beta \text{ cell function} = 20 \times \text{fasting insulin}/\text{fasting glucose} - 3.5.$$

Testicular tissue homogenization

The testicular tissue samples were homogenized in a pH 7.4 saline solution at a weight/volume ratio of 1:10. A homogenizer from IKA® in Staufen, Germany, named Ultra-Turrax T-25, was used for this purpose. Centrifugation at 10,000 × g for 15 min was used to obtain the supernatant following homogenization. After that, it was carefully frozen at a controlled temperature of -80 °C until the analysis involving the oxidative stress marker.

Oxidative stress marker in the plasma and testes

The thiobarbituric acid reactive substances (TBARS) assay was employed to determine oxidative stress status as developed by Ohkawa et al. (1979). Superoxide dismutase (SOD) activity was measured using the technique described by Sun et al. (1988); a technique was used. Nitric oxide (NO) levels were quantified by incubating the sample with the Griess reagent (Sigma-Aldrich, St. Louis, MO, USA) for 10 min at ambient temperature, shielded from light, and then measuring the absorbance at 540 nm, following the method outlined by Green et al. (1982). The data are given in nanograms of NO produced per milligram of protein.

Superoxide radicals are made when xanthine and xanthine oxidase work together. They react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to make a red formazan dye.

According to Aebi (1974), catalase (CAT) activity was measured by incubating the tissue homogenate with a hydrogen peroxide (H2O2) substrate. The enzymatic reaction was then stopped by adding 1 ml of ammonium molybdate (Sigma-Aldrich, St. Louis, MO, USA), which allowed for the determination of CAT's enzymatic activity.

Glutathione peroxidase (GPx) activity was employed using the method described in Wingler et al. 1999. One milligrams of protein was taken from the homogenate and mixed with 1.6 ml of 50 mM phosphate buffer (pH 7.0). This buffer comprised 1 IU/mL glutathione reductase, 0.2 mM NADPH, and one mM GSH. This mixture was incubated for five minutes at room temperature and then treated with 100 µl of 0.25 mM H2O2. Initial and final (after 5 min) absorbance readings at 340 nm were recorded using a spectrophotometer and analyzed within the homogenized testicular tissues.

Sperm analysis

On a transparent glass slide, a droplet of epididymal content was gathered into a droplet of sodium citrate solution (3%), and the plate was slightly heated. According to Semaida et al. (2022), sperm concentration was determined using a hemocytometer, and among the essential parameters for evaluating epididymal sperm quality are sperm counts, motility, progressive motility, vitality, and morphology. A hemocytometer was used to look at 5 µl of the sperm sample, and an OMAX light microscope (model M8311Z, Washington, USA) was used to count the number of normal sperm cells.

The analysis of a 10 µl sperm sample was done to determine the motility of the sperm and its increasing motility.

Viable sperm were counted and mixed with 10 µl of Eosin-Y stain solution (0.5% in saline) on clean glass slides. We obtained a thin film and air-dried it after gently mixing and incubating it for 2 min at room temperature. We examined the paste under a light microscope at 400× magnification. Eosin-Y is a supravital stain that can penetrate the damaged membranes of dead or dying sperm cells and turn pink or red. Live sperm cells with intact membranes remain unstained and appear white or transparent. We counted and classified a minimum of 200 sperm cells as either live (unstained) or dead (stained pink or red). We calculated the percentage of live (viable) sperm cells as follows: The percentage of live (viable) sperm cells was calculated as follows:

$$\text{Sperm Vitality (\%)} = (\text{Number of live sperm cells} / \text{Total number of sperm cells counted}) \times 100$$

Radioimmunoassay for sex hormones

Hormones FSH (follicle-stimulating) (IRMA-KIP0841), luteinizing hormone (LH) (KIP1311), and testosterone were quantified using Radioimmunoassay (RIA-CT-KIP1709) techniques. Commercial kits were employed for these assays from DIAsource Immunoassays in Louvain-la-Neuve, Belgium. The measurements were taken on a Gamma Counter RIA Star Model.

Western blot analysis for nitrotyrosine detection

Testicular tissue samples were collected from male rats. The testes were dissected, and the tissue was homogenized in ice-cold lysis modified Radio-Immunoprecipitation Assay (RIPA) buffer and (50 mM Tris HCl pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) with protease inhibitors, supplemented with protease inhibitors (1 mM PMSF, 1 µg/mL aprotinin, 1 µg/mL leupeptin). The homogenates were centrifuged at 12,000× g for 10 min at 4 °C.

The preparation of protein extracts was conducted using TriFast reagent (Peqlab, VWR) in accordance with the procedure provided by the manufacturer. The determination of protein concentrations was conducted with the Bradford assay. The protein samples, each weighing 30 µg, were subjected to 12% SDS-PAGE separation and subsequently deposited onto Hybond nitrocellulose membranes manufactured by GE Healthcare. The membranes were placed in Tris-buffered saline with 0.1% Tween-20 (TBST) and covered with 5% nonfat dry milk for 1 h at room temperature. Subsequently, the membranes were subjected to overnight incubation at a temperature of 4 °C using a primary antibody to nitrotyrosine (ABIN1385080, obtained from antibodies-online.com) that had been diluted at a ratio of 1:400 in blocking buffer. After washing 3 times with TBST for 10 min each, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody diluted in blocking buffer for 1 h at room temperature. The membranes were washed 3 more times with TBST and developed using enhanced chemiluminescence substrate (ECL). The loading control employed in this study was β-actin. Gel documentation system (Geldoc-it, UVP, England) was applied for data analysis using Totallab analysis software, ww.totallab.com, (Ver.1.0.1).

Histological examination

Autopsy-derived testicular samples were fixed in Bouin's fluid for 24 h. Following fixation, they were rinsed in tap water and subjected to dehydration through a series of alcohol dilutions (methyl, ethyl, and absolute ethyl). Subsequently, the specimens were cleared in xylene and embedded in paraffin. Paraffin-embedded tissue blocks were created by allowing the paraffin-wax mixture to solidify at 56 °C in a hot air oven for 24 h. These tissue blocks were then sectioned to a thickness of 4 microns (µm) using a Sledge Microtome (Leica et al., USA). The resulting tissue slices were obtained and placed onto glass slides, deparaffinized and subjected to staining using the hematoxylin and eosin stain. The stained sections were then examined using light microscopy for further analysis (Maina et al. 2008).

Statistical analysis

The data are presented as mean values ± standard errors. Statistical comparisons among the different groups were conducted utilizing a one-way analysis of variance (ANOVA), followed by Tukey's test for post hoc analysis by using Graph Pad Prism Software (V. 10.2.0, GraphPad Software Inc., San Diego, CA, USA). A significance level of $P < 0.05$ was established for determining the significance of the observed differences between groups.

Results

The results demonstrated that the mushroom *P. ostreatus* had a high concentration of carbohydrates and protein, accounting for 48.02% and 21.15% of its composition, respectively. Conversely, it displayed relatively low fiber and fat levels, constituting 6.16% and 2.06% of its dry weight, respectively. Additionally, the mushroom included 6.93% of ash in dry weight.

Quantitative analysis of phenolic and flavonoid content

Total phenolic compounds were 23.04 mg/g gallic acid equivalent in the crude extract of *P. ostreatus*, while total flavonoids were 8.14 mg/g quercetin equivalent. The levels of total phenolic and flavonoids in mushrooms were found to be relatively low compared to previously reported data from the literature on Pleurotus species, which recorded a value of 45.6 mg/g.

Phenolic compound detection by HPLC

Phenolic substances fall under several subclasses and have various structural variations. We found catechin (121.53 µg/ ml), gallic acid (11.64 µg/ml), syringic acid (2.6 µg/mL), quercetin (2.35 µg/mL), caffeic acid (1.46 µg/ml), coumaric acid (0.96 µg/ ml), and cinnamic acid (0.82 µg/ ml) can be observed in the sample at retention times 7.82, 4.55, 9.45, 15.36, 9.21, 11.34 and 16.98 min, respectively. The compounds from PC were discovered (Fig. 1) by comparing their chromatographic characteristics with those of standard substances.

DPPH radical scavenging test

The ability of the phenolic extract to scavenge DPPH radicals is compared to that of ascorbic acid. With an IC50 of less than 20 µg/mL at 30 min, ascorbic acid demonstrated high activity. RSA changes of phenolic extract at varying concentrations (100-500) and at different durations of incubation in dark (30–120) min are presented in Fig. 2. The extract's capacity for radical scavenging activities on DPPH showed high activity with dose and incubation time in dark-dependent and is found to be 93.23% at a specific concentration level (500 µg/ml) after 120 min in dark and has IC50 (100 µg/ml at 90 min; 200 µg/ml between 60 and 90 min; 300 µg/ml between 60 and 90 min; 400 µg/ml at 60 min and 500 µg/ml at 30 min). The research indicates that ascorbic acid standard has higher antioxidant activity than mushroom phenolic extract.

Hydrogen peroxide scavenging assay

PCE of *P. ostreatus* showed high H2O2 scavenging activity and was discovered to be 58.0% at the concentration of 500 µg/ml with IC50 between 300- 400 µg/ml. With increasing doses, PCE's radical scavenging action on H2O2 showed high activity.

The power reduction

Table 3 presents the reductive capacities of PCE utilizing various concentrations. The reducing power of the phenolic extract exhibited a positive correlation with its antioxidant activity, as observed through a rise in reducing power with higher quantities of the phenolic extract. Similarly, the scavenging activity of the phenolic extract also showed a positive relationship with its antioxidant potential.

The acquired results from the reduction of Fe (III) ions demonstrate a positive correlation between the reductive activity of phenol and the concentration levels when utilizing the K3Fe (CN) 6 reduction procedure. The findings show that phenolics exhibited a substantial capacity for reduction, as indicated by its highest measured reducing power of 0.9439, determined using absorbance analysis at a concentration of 500 µg/ml PCE.

Biochemical estimations

In rats with DFHD, the levels of glucose and insulin in the blood were significantly higher (F (3, 28) = 174, 292.88%, $p < 0.05$). Conversely, in rats without DFHD, the levels of glucose and insulin in the blood were significantly lower (F (3, 28) = 132.95, 61.60%, $p < 0.05$) (Table 4). The DHFD/ethanol extract of phenol resulted in a significant decrease in blood glucose levels ($P < 0.05$) and an increase in insulin levels ($P < 0.05$) compared to the untreated rats. The HOMA-β and IS indices in the DFHD group were significantly lower (34% and 90.59%, respectively) than in normal rats ($P < 0.05$) (Table 4). HOMA IR was considerably elevated in the DHFD group compared to the

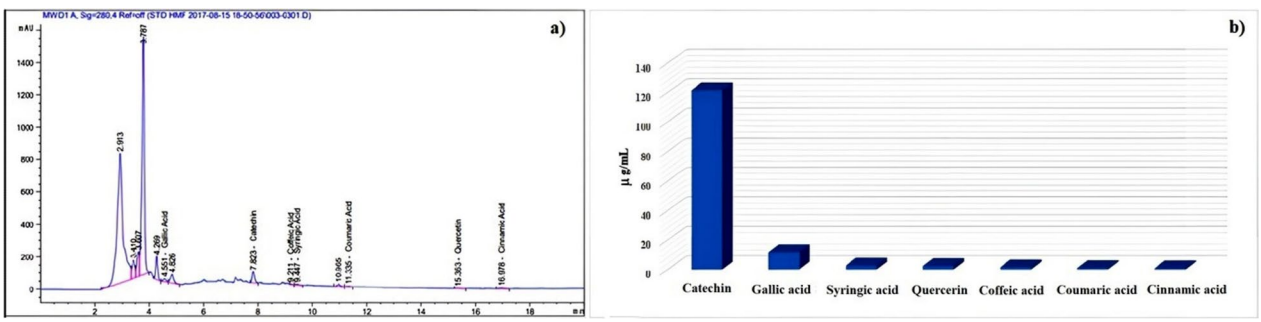

Fig. 1 The high-performance liquid chromatography (HPLC) chromatogram obtained at a wavelength of 290 nm for the *P. ostreatus* extract. The observed peaks in the chromatogram correspond to phenolic chemicals

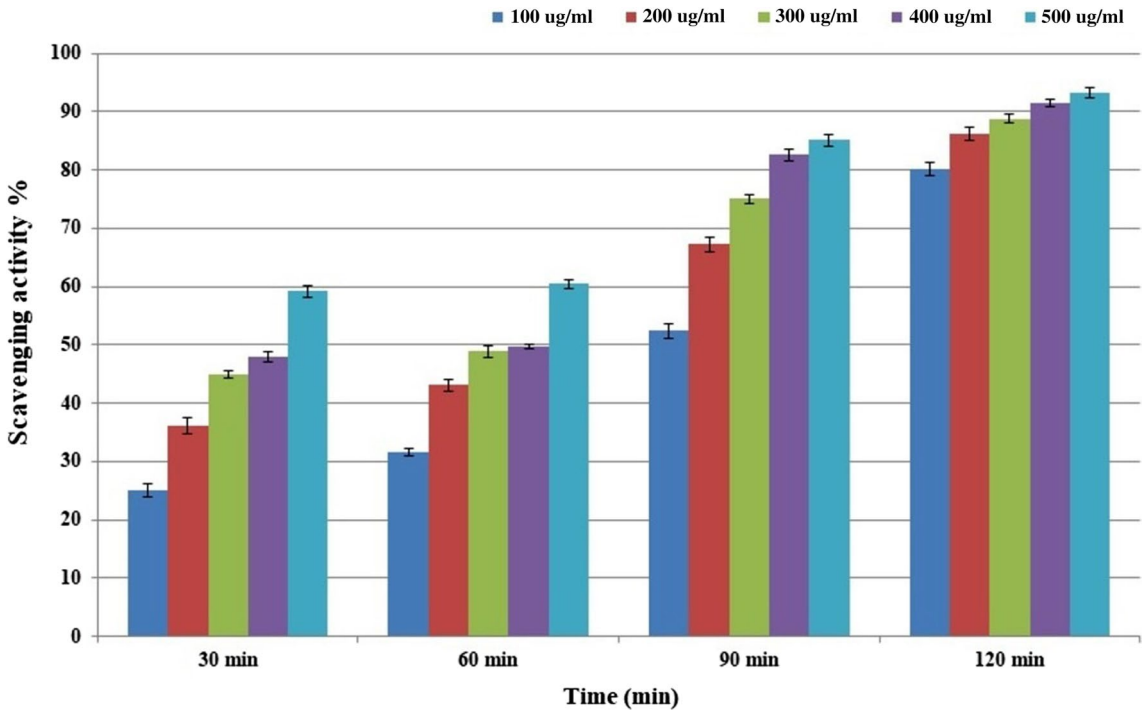

Fig. 2 DPPH scavenging activity of phenolic extract from *P. ostreatus*

Table 3 Hydrogen peroxide scavenging activity of phenolic extract from *P. ostreatus*

Phenol extract concentrations (µg/ml)	Radical scavenging activity (mean ± std error)
100	25.06 ± 0.01
200	42.49 ± 0.02
300	48.96 ± 0.01
400	54.55 ± 0.02
500	58.00 ± 0.02

control ones (50.61%) (Table 4). Phenols extracted from mushrooms reduced HOMA β, IS, and IR ($p < 0.05$) in the DFHD-caused infertility rats (Group I).

Effects of treatments on body and testicular weights

There were notable disparities in body and testicular weights among different treatment groups ($P < 0.05$, Tukey's test) (Fig. 3a, b**,** respectively). Dietary intake in the HFD control group exhibited a higher value of body weight (BW) than in regular groups, with AIN93 dietary intake ($P < 0.05$ Tukey's test) (Fig. 3a**).** There were no notable variations in testicular gain weight between the control, AIN 93, and HFD groups. Streptozotocin strongly decreased BW and testicular weight ($p < 0.05$) in the DHFD group compared to the standard group (Fig. 3a, b**,** respectively). The body and testicular weights of DHFD rats went up significantly ($p < 0.05$) when phenols (50 mg/kg BW) were given to them compared to the rats that were not treated.

Table 4 Effect of treatment with polysaccharide on glucose, insulin, and HOMA-β, HOMA-IR, and HOMA-IS concentrations in control, and STZ-HFD induced infertility in rats

Groups	Group I	Group II	Group IIIA	Group Iii B	F (Mean ± Std Error)	*P*-value
Glucose (mg/dl)	80.17 ± 5.12^{b}	$79.90.04 \pm 8.11^{b}$	316.23 ± 10.25^{a}	83.75 ± 4.83^{b}	201.20*	0.001
Insulin (µIU/ml)	41.31 ± 2.84^{a}	40.44 ± 1.78^{a}	16.02 ± 1.66^{b}	38.10 ± 1.11^{a}	22.65*	0.001
HOMA-B-(pg/ml)	0.30 ± 0.004^{b}	0.31 ± 0.004^{b}	0.21 ± 0.007^{a}	0.32 ± 0.006^{b}	96.50*	0.001
HOMA-IR	8.18 ± 0.14^{b}	7.90 ± 0.15^{b}	12.35 ± 0.40^{a}	7.86 ± 0.13^{b}	85.81*	0.001
HOMA-IS	10.62 ± 0.004^{b}	10.46 ± 0.004^{b}	1.0 ± 0.007^{a}	9.44 ± 0.006^{b}	604.30*	0.001

Results are the mean ± SEM. Values in the same row with the different superscript are significantly at $P < 0.05$ (one-way ANOVA and then Tukey's post hoc comparison

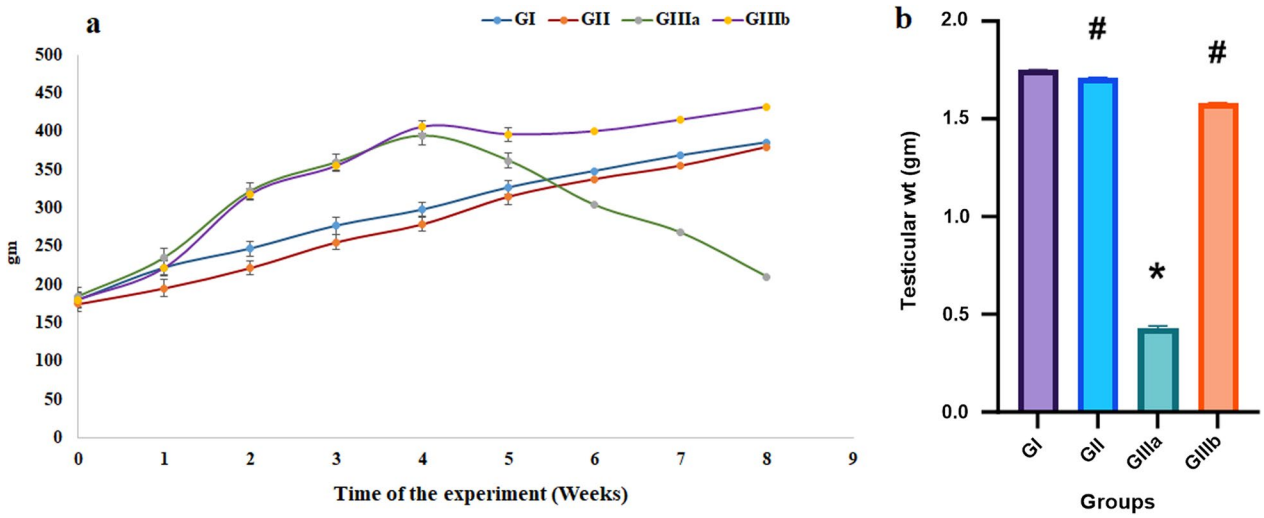

Fig. 3 Differences in body weight (1a) and testicular weight (1b) across treatment groups. GI: normal rats, GII: normal rats treated with phenolic extracts. GIIIa (DHFD) and GIIIb (DHFD treated with phenolic extracts orally at 100 mg/kg) daily for four weeks

Lipid profiles

Compared to normal rats, DFHD rats had higher levels of serum total cholesterol (F (3, 28) = 208.11, 212.60%, $p < 0.05$), triglyceride (F (3, 28) = 118.28, 321.54%, $p < 0.05$), LDL-cholesterol (F(3, 28) = 121.68, 91.06%, $p < 0.05$), and VLDL (F(3, 28) = 116.29, 321.37%, $p < 0.05$) while lower levels of HDL-cholesterol were seen (Fig. 4a). Phenols derived from mushrooms shown a noteworthy effect in reducing serum lipid profiles, specifically total cholesterol, LDL-cholesterol (as depicted in Fig. 4b), and triglycerides ($p < 0.05$) (Fig. 4c), TCh (Fig. 4d), VLDL-cholesterol (Fig. 4e). Additionally, there was a notable increase in HDL levels ($p < 0.05$).

The results are illustrated in Fig. 4a. There was an apparent change in the shape and function of spermatozoa when exposed to phenol extract at a concentration

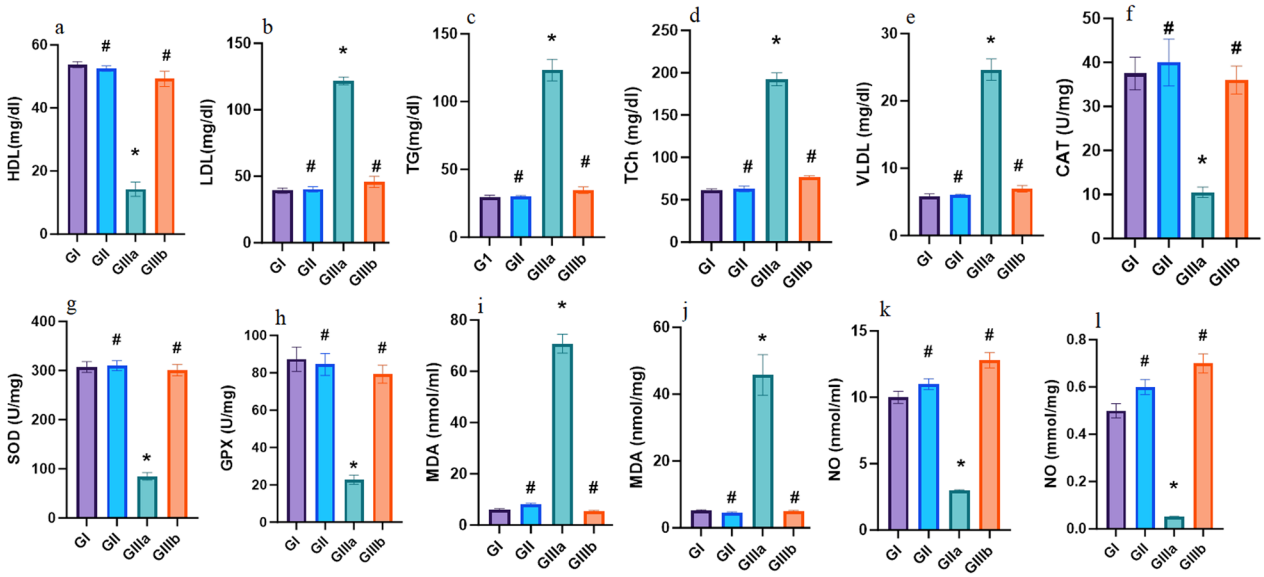

Fig. 4 Impact of phenols extract on **a** LDH, **b** LDL, **c** TG, **d** TCh, **e** VLDL, **f** CAT, **g** SOD, **h** GPX, **i** MDA in plasma, **j** MDA in testes, **k** NO in plasma, **l** NO in testes. Results are the mean ± SEM. Values in the same row with the different superscript are significantly at $P < 0.05$ (one-way ANOVA and then Tukey's post hoc comparison. *$P < 0.05$ DHFD vs. Control group, and vs CPE groups; #$P < 0.05$ DHFD group vs CPE + DHFD group. GI: normal rats, GII: normal rats treated with phenolic extracts. GIIIa (DHFD) and GIIIb (DHFD treated with phenolic extracts orally at 100 mg/kg) daily for four weeks

of 50 mg/kg. One dose of STZ (40 mg/kg-IP) lowered the quality of all the sperm that was tested, including the number of sperm (F (3,28) = 320.60, 89.53%), their motility (F (3,28) = 549.52, 94.2%), their progressive motility (317.35, 98.13%), their vitality and morphology (F (3,28) = 477.97, 83.42%), and 174.71, 86.11%, respectively).

Effect of treatments on antioxidants in plasma and testes

The findings revealed that streptozotocin significantly reduced the activities of CAT, SOD, and GSH-PX in the diabetic group compared to the normal group (Fig. 4f–h). The decreases seen are linked to a rise in lipid peroxidation in the plasma, and the MDA measurement shows that the testes in the diabetic group are affected by infertility (F (3, 28) = 314.38, 89.20, respectively; increases for both were up to 500%, $p < 0.05$. Antioxidant status is restored with phenol extract in the diabetic animals that received treatment with 50 mg/kg BW. Once that is done, giving rats phenol extract stops the increase in lipid peroxidation by lowering the activity of malondialdehyde (MDA) in their blood and testicular tissue (Fig. 4i, j). Similarly, streptozotocin considerably decreased the amounts of nitric oxide (NO) in both the plasma and the testes. Nitric oxide (NO) levels in both plasma and testicular tissue were higher in the diabetic group, along with the decrease. The statistical values F (3, 28) = 127.02 for plasma and 102.64 for testicular tissue, respectively, show that this increase in NO is associated with induced infertility (Fig. 4k, l). A treatment with phenol extracts also lowered the level of lipid peroxidation by reducing NO activity in the testicular and plasma tissues of diabetic rats.

Effect of treatments on sex hormones

The STZ dose used on FHD rats significantly decreased FSH levels (F (3, 12, 50%) = 8.781; $P = 0.002$), LH levels (F (3, 12, 68.42%) = 7.853; $P = 0.004$), and testosterone levels (F (3, 12, 59.33%) = 27.484; $p < 0.001$). Remarkably, the administration of phenol extract therapy for a duration of four weeks effectively halted the decline in hormone levels ($p < 0.05$) in comparison with the rats on a high-fat diet (DHFD) (Fig. 5a, b).

Sperm quality

The findings are depicted in Fig. 5d–h. The assessment of sperm quality encompasses various factors, such as the sperm count (SP count), SP motility, SP progressiveness, and the primary morphological and vital characteristics of spermatozoa. Notably, the phenols extract exhibited a

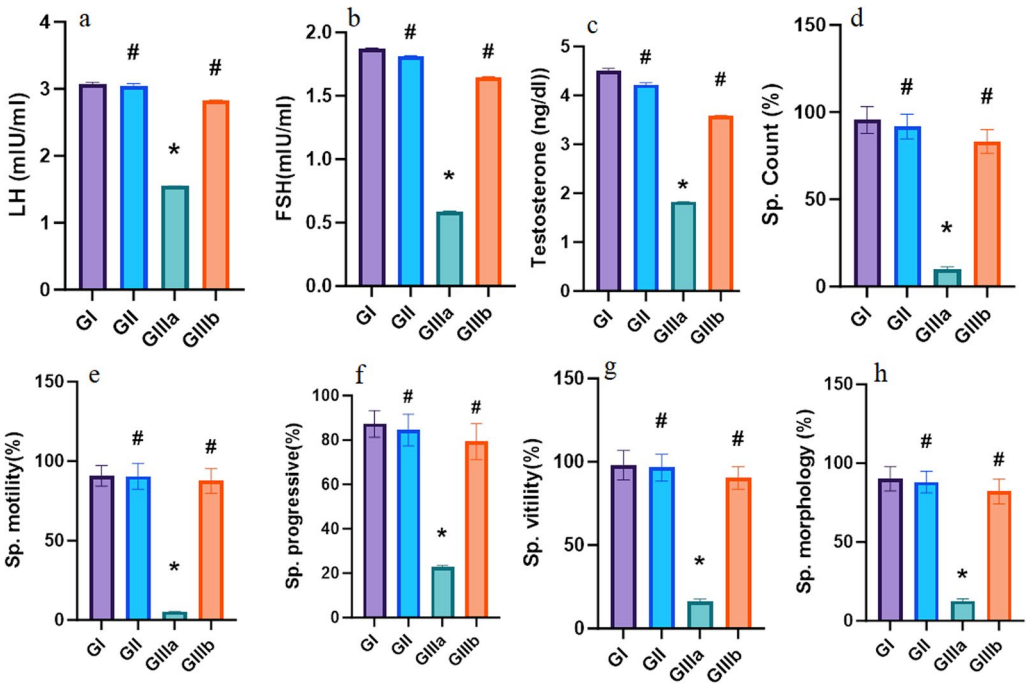

Fig. 5 Effect of PCE on sex hormone levels in diabetic rats **a** LH, **b** FSH, **c** testosterone, and sperm quality indices **d** sperm count, **e** sperm motility, **f** sperm progressive, **g** sperm vitality, **h** sperm morphology. Results are the mean ± SEM. Values in the same row with the different superscript are significantly at $P < 0.05$ (one-way ANOVA and then Tukey's post hoc comparison. *$P < 0.05$ DHFD vs. Control group, and vs CPE groups; #$P < 0.05$ DHFD group vs CPE + DHFD group. GI: normal rats, GII: normal rats treated with phenolic extracts. GIIIa (DHFD) and GIIIb (DHFD treated with phenolic extracts orally at 100 mg/kg) daily for four weeks

significant impact on these attributes when administered at a dose of 50 mg/kg. Overall, the administration of a single dosage of STZ (40 mg/kg-IP) negatively affected all the examined measures of sperm quality, such as sperm count, motility, progressive motility, vitality, and morphology. Additionally, it resulted in infertility in rats fed a high-fat diet (HFD). Nevertheless, a notable and statistically significant improvement ($p < 0.05$) was observed in all the aforementioned variables as a result of the various treatments involving the phenols extract.

Western blot analysis for nitrotyrosine detection

Western blot analysis was employed to investigate the presence of nitrotyrosine modifications in testicular proteins. Nitrotyrosine serves as a specific marker for tyrosine nitration, indicative of oxidative and nitrosative stress.

Western blot analysis showed that nitrotyrosine levels were significantly increased in the DHFD group compared to control normal tissue (Fig. 6a, b). Densitometric analysis revealed that nitrotyrosine levels were 6.10% in the control group and 18.40% in the disease group, representing a 3.01-fold increase ($p < 0.05$) (Fig. 6b–d). Treatment with PCE reduced nitrotyrosine levels to 9.02%, corresponding to a 2.04-fold decrease compared to the untreated group ($p < 0.05$).

Histopathological findings

No changes were seen in the histology or pathology of the seminiferous tubules, and the structure looked normal. There were many sperm in the tubular lumen in both control and normal rats that were given phenol extract (Fig. 7a, b, respectively). In DHFD rats, eosinophilic transudate built up in the interstitial stroma of Leydig cells between the seminiferous tubules. Also, four weeks after the STZ-HFD treatment, the examination showed changes related to degeneration and a noticeable rise in thickness in some seminiferous tubules (Fig. 7c). Furthermore, the tubular lumens were observed to be devoid of spermatozoa. Using phenol extract for four weeks restored normal seminiferous tubule lining with many spermatogenic cells in the animals used in the study (Fig. 7d).

Discussion

While previous studies, such as that by Ajani (2022), have investigated the effects of ethanol extracts of *Pleurotus ostreatus* on sperm quality and hemo-biochemical parameters in male Wistar rats, our study extends this knowledge into phenolic compound extracts (PCE) from the same mushroom species. In particular, our study examines the dual role of PCE in diabetes and type 2 diabetes-induced infertility in male rats.

Individuals in modern culture show a higher level of concern about their eating habits and fitness (Tagde et al. 2021). Currently, oyster mushrooms are highly regarded as a food option, valued not only for their sensory attributes such as texture and flavor but also for their chemical composition, nutritional profile, potential therapeutic benefits, and health benefits (Roy et al. 2015). In today's times, *P. ostreatus* has emerged as the preeminent and widely consumed mushroom owing to its palatability,

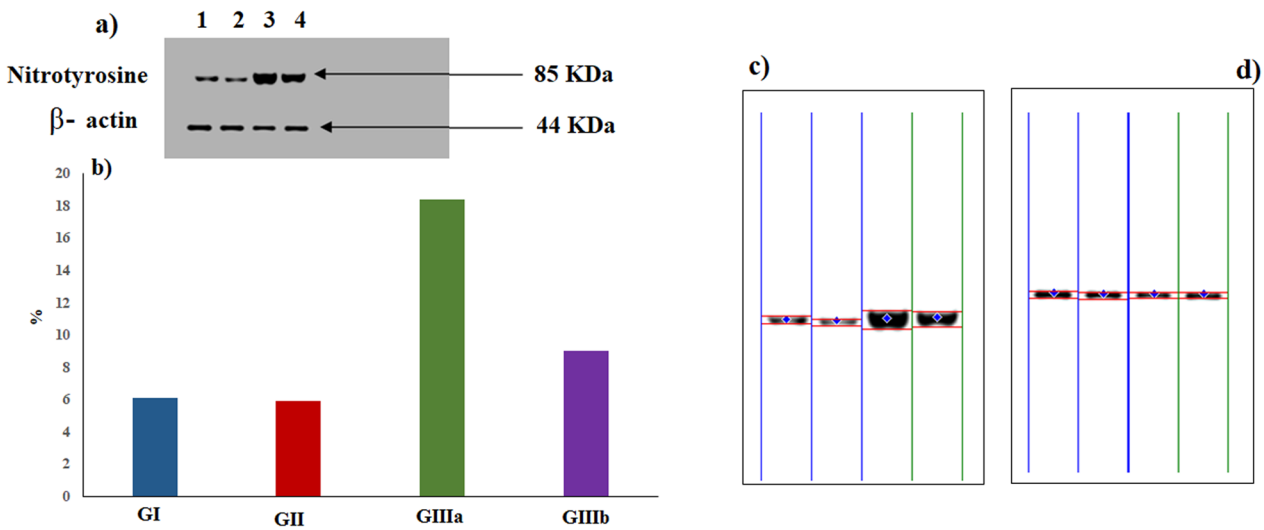

Fig. 6 CPE inhibits the nitrotyrosine activation. **a** Western blot analysis for the determination of protein expression in rat testes tissues. **b**, **c** Computerized analysis of Nitrotyrosine protein expression level for samples. **d** Computerized analysis of β- actin protein expression level for samples. **P* < 0.05 DHFD vs. control group, and vs CPE groups; #*P* < 0.05 DHFD group vs CPE + DHFD group

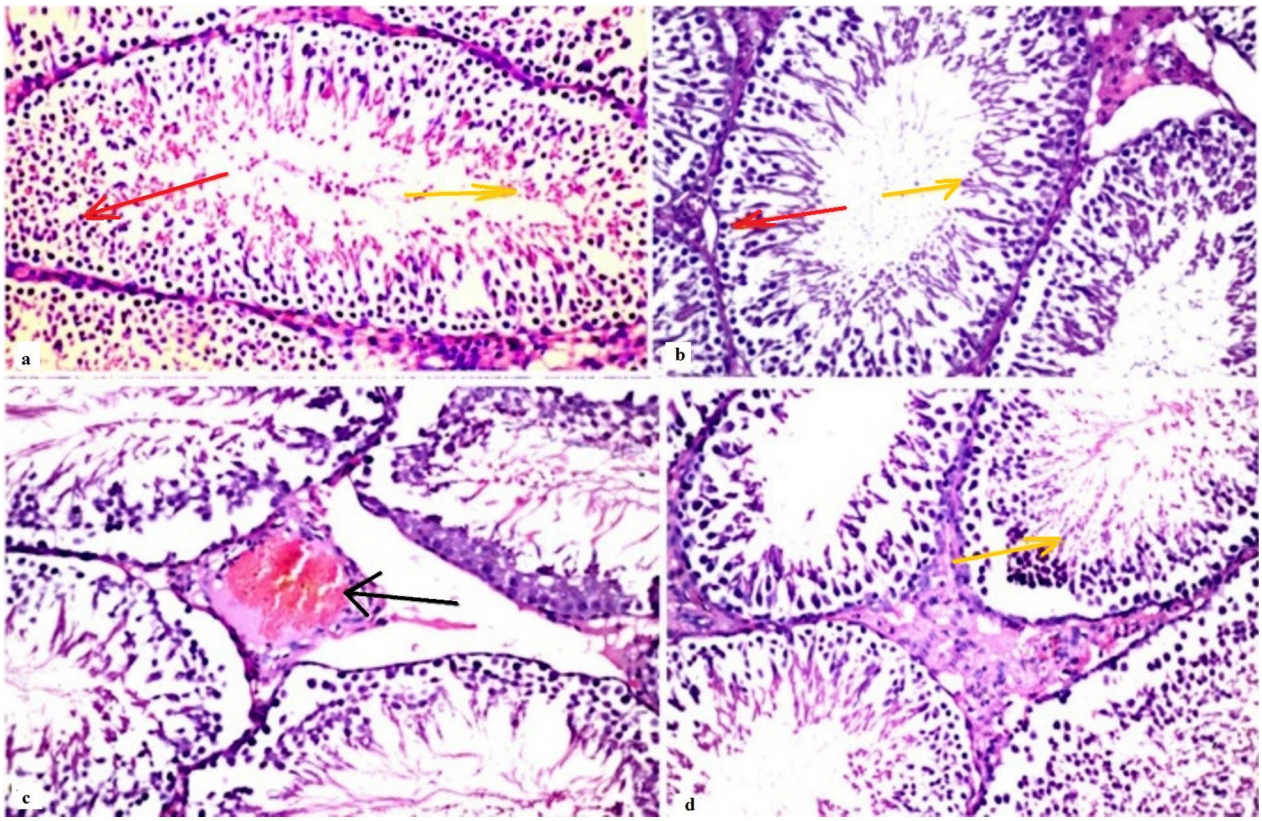

Fig. 7 Histopathological findings in testes: **a** Histopathological section of testes in control rats. The **red arrow** points to the seminiferous tubules. The **yellow arrow** points to the lumen of the seminiferous tubule, where mature spermatozoa are released. H E (40X). **b** Histopathological section of testes in normal rats with phenols extract treatment. The **red arrow** points to the seminiferous tubules, which are responsible for spermatogenesis. In normal rats, these tubules show an organized structure with various stages of spermatogenic cells, including spermatogonia, spermatocytes, spermatids, and mature spermatozoa. The **yellow arrow** is pointing to the lumen of the seminiferous tubule. In normal conditions, this lumen is clear and contains mature spermatozoa. **c** Histopathological section of testes in DHFD rats The **black arrows** are pointing to a region that appears to show significant cellular damage. **d** Histopathological section of testes in DHFD rats with PCE treatment. The **yellow arrow** points to an area that appears to show healthy seminiferous tubules with various stages of spermatogenesis visible

distinctive flavor profile, substantial nutritional content, and notable therapeutic qualities (Akyüz and Kirbağ 2010). These days, *P. ostreatus* has gained significant recognition as a valuable and functional component of food, mainly owing to its cholesterol-free nature and its low content of calories, carbs, fat, and sodium (Sahoo et al. 2022).

The major constituents of mushroom PCE analyses include quantifying total protein, total carbohydrate, total fat, crude fiber, and ash content. *P. ostreatus* mushrooms are a well-known food source of dietary fiber and carbs (Manikandan 2020). According to Deepalakshmi and Mirunalini (Deepalakshmi and Mirunalini 2014), using edible oyster mushrooms is becoming acknowledged within academic circles as an up-and-coming option for exploring novel protein sources. The dietary oyster mushrooms often exhibit high crude protein levels (Gupta et al. 2018). Manikandan (2020) demonstrated that the amount of protein in mushrooms varied from 12 to 35%, based on the specific species. When compared to carbs and protein, according to the report, the fat content of *P. ostreatus* ranges from 0.2 g to 8 g per 100 g of dried fruit bodies, and this agrees with our finding.

Recent investigations showed that mushrooms enriched with phenolic extract exhibit significant potential as antioxidants. The fruiting body of mushrooms is best known for being a powerful antioxidant. *Auricula auricula's* ferric reducing power was observed to be dependent on the concentration. At 4 mg/mL, *A. auricula* had a reducing power of 0.403 nm, the results indicate that A. auricula has a greater potential for hydrogen donation, implying that it possesses significant reducing power (Packialakshmi et al. 2017).

According to Dziezak (1986), the antioxidant effects observed in mushroom extracts can be attributed to phenolic compounds, which function as decomposers of peroxidase, metal inactivators, oxygen scavengers, or free radical

inhibitors. Phenolic compounds contain various chemical substances, such as phenolic acids, oxidized polyphenols, hydroxybenzoic acids, flavonoids, tannins, hydroxycinnamic acids, stilbenes, and lignans (D'Archivio et al. 2010).

Multiple studies have been conducted to investigate the phenolic acid contents of *P. ostreatus*. Acids such as cinnamic acid, protocatechuic acid, p-hydroxybenzoic acid, and p-coumaric acid have been found in multiple investigations (Taofiq et al. 2015; Aly et al. 2023).

Obesity, diabetes, and associated repercussions are on the rise all across the world. Obesity and uncontrolled diabetes both have a high risk of reproductive problems (Thong et al. 2020). Even in the absence of the obese phenotype, a high dietary fat intake may provoke metabolic and reproductive problems. As a result, it is critical to remember that HFD consumption might affect fertility. Obesity increases the risk of insulin resistance, type 2 diabetes, vascular disease, endocrine problems, and impaired fertility (Craig et al. 2017). Other studies imply that the development of obesity as a consequence of HFD causes reproductive issues. Thus, the dramatic rise in glucose in the rats resulting from decreased insulin led to insufficiency and infertility (He and Li 2020).

The combination of obesity and diabetes has been shown to hurt various aspects of sperm quality as well as hormone levels and testicular health, as observed in the study by Alkafafy et al. (2021). These results are similar to previous studies using obese and diabetic rat models. When obesity and diabetes happened together, there were fewer sperm, more immobile and non-viable sperm, and lower levels of LH and testosterone. This consistency in findings across studies underscores the adverse consequences of the interaction between obesity and diabetes on male reproductive health. Studies have confirmed positive relationships between testosterone levels and total fat and polyunsaturated fatty acid consumption when either total energy or carbohydrates replace these rich ingredients (Wrzosek et al. 2020). ROS triggers by diabetes lowers the activity of Leydig cells, which in turn lowers testosterone biosynthesis (Basque et al. 2022). As indicated in this study, the decline in testosterone levels can have implications for germinal cell mitotic division. The lower levels of testosterone may be linked to the changes seen in the seminiferous tubules, such as the narrowing of the tubes and the loss of epithelial height.

Our findings of considerably lower insulin levels in the DFHD-treated group compared to the control group imply that STZ-induced pancreatic injury is causing decreased insulin output. This idea is backed further by the fact that the HOMA- index score is lowest in the DFHD-treated group. Our findings confirm previous research (Inácio et al. 2018) indicating that the beneficial effect of phenols on insulin resistance is most likely due to improved sensitivity to insulin receptors and pancreatic -cells. The oxidative stress that STZ causes in the testicular environment may negatively affect several sperm health-related factors.

This study reported an elevation in levels of LDL, TG, VLDL, and total cholesterol in rats with STZ-induced diabetes that were subjected to a high-fat diet. Notably, LDL cholesterol is derived from VLDL cholesterol and transports cholesterol from the liver to peripheral organs. The current study's elevation of VLDL levels following STZ injection suggests a heightened synthesis of LDL-cholesterol.

STZ-induced pancreatic beta-cell damage and subsequent insulin deficiency can lead to insulin resistance and impaired endothelial function. These conditions can reduce endothelial nitric oxide synthase (eNOS) activity, leading to decreased NO production (Shamsaldeen et al. 2020). The study's results revealed notable alterations in NO levels following the administration of STZ in the diabetic rat model. This finding aligns with previous research indicating a potential link between diabetes-induced oxidative stress and impaired NO synthesis. Therefore, the observed changes in NO levels may signify an imbalance in endothelial function, possibly contributing to the vascular complications associated with diabetes. On the other hand, the administration of PCE concurrently with STZ may have modulated the observed changes in NO levels. Previous studies have suggested that certain phenolic compounds possess antioxidant properties, potentially mitigating oxidative stress-induced damage and, consequently, impacting NO synthesis positively (Pisoschi et al. 2021), (Rudrapal et al. 2022).

Given the coexistence of oxidative stress and diminished antioxidant state in diabetes, which might exacerbate the harmful impact of free radicals, it is plausible to hypothesize that PCE may possess a protective influence in diabetes, albeit partially, by mitigating localized oxidative and nitrative harm. We found that the high-fat and STZ treatment significantly increased nitrotyrosine expression 3.01-fold compared to the control, indicating increased oxidative stress and protein damage. However, PCE administration in the high-fat and STZ regimen substantially attenuated the nitrotyrosine down-regulation, decreasing it to 2.04-fold relative to untreated group.

These results align with previous reports that STZ treatment exacerbates oxidative stress and increases 3-nitrotyrosine formation in animal models (Yagishita et al. 2014). The reduction in nitrotyrosine with phenolic compounds is also consistent with studies indicating antioxidant and anti-nitrosative effects of phenolics (Gu et al. 2022). This result is likely related to the ability of phenolics to enhance nitric oxide (NO) production and availability, as we observed increased NO levels with

phenolic treatment. NO can scavenge reactive oxygen species and prevent oxidative damage to proteins.

The significant mitigating effect of PCE on nitrotyrosine levels suggests it conferred additional antioxidant benefits beyond phenolics alone in this diabetic model. PCE has been shown to boost antioxidant capacity and suppress lipid peroxidation and protein oxidation (Zeb 2020). By attenuating oxidative damage to proteins, PCE treatment may help prevent downstream structural and functional protein impairments related to nitrotyrosine modifications in diabetes.

Previous research also showed that lipid profiles were disturbed after streptozotocin injections; our current findings align with that Beloucif et al. (2021). This study's overarching goal is to learn more about the effects of phenol treatment on lipid profiles and the likelihood of infertility linked to STZ-induced diabetes in high-fat diet rats. The present research employing STZ-HFD rats shows that phenol administration raised SOD, CAT, and GSH-Px levels and decreased MDA in testicular and plasma samples.

Moreover, in obese diabetic rats, we observed structural damage to the testes and decreased sperm function (Morgante et al. 2011). Excessive dietary cholesterol consumption and improved cholesterol transport into the testis may be responsible for this occurrence. Similarly, a high-cholesterol diet may affect testosterone production by causing histological abnormalities in the testicular Leydig cells (Liu et al. 2020). Given that androstenedione serves as a precursor to testosterone, it is plausible that obesity could contribute to hormonal imbalances. The potential mechanisms by which phenols could enhance male fertility may involve their positive effects on the testicular tissues.

Conclusions

This study demonstrates the potential therapeutic effects of the phenolic component extract (PCE) derived from the Pleurotus ostreatus mushroom. It suggests that PCE may be beneficial in managing elevated blood glucose levels, enhancing insulin synthesis, and correcting diabetes-induced infertility in a rat model of type 2 diabetes. *Pleurotus ostreatus*, a widely grown edible fungus, has gained fame due to its high concentration of bioactive substances, particularly phenolic compounds with significant antioxidant capabilities.

In rats subjected to a high-fat diet, the phenols obtained from *P. ostreatus* can decrease testicular lipid peroxidation (MDA) levels and reinstate antioxidant equilibrium. This study demonstrates that this mushroom has the potential to serve as a natural reservoir of medicinal ingredients for treating infertility caused by obesity and oxidative damage. Pharmaceutical companies are interested in researching phenolic compounds from *P. ostreatus* because they are healthy and may be able to treat metabolic disorders and obesity-related male infertility naturally. However, more research is needed to clarify and evaluate its long-term safety and effectiveness and its safe use in clinical treatments.

Abbreviations

AIN-93M	American Institute of Nutrition (AIN) committee in 1993 improving the AIN-76A standard diet
AOAC	Association of Official Analytical Chemist
D2	Diabetes type-2
DFHD	Diabetes high-fat diet
DMT1	Diabetes mellitus type 1
DMT2	Diabetes mellitus type 2
DNA	Deoxyribonucleic Acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FSH	Follicle-stimulating hormone
GPx	Glutathione peroxidase
H_2O_2	Hydrogen peroxide
HDL-C	High-density lipoprotein cholesterol
HFD	High-fat diet
HOMA	Homeostatic Model Assessment
HOMA-IR	Homeostatic Model Assessment-Insulin resistance index
HOMA-IS	Homeostatic Model Assessment-insulin sensitivity
HOMA-β	The homeostasis model assessment of β-cell function
HPLC	Liquid chromatography with high performance
LDL-C	Low-density lipoprotein cholesterol
LH	Luteinizing hormone
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
PCE	Phenolic component extract
STZ	Streptozotocin
TPCs	The total phenolic compounds
UV-VIS	Spectrophotometer
VLDL-C	Very low-density lipoprotein cholesterol

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Author contributions

G I and S M conceptualized and gathered and gathered the data with regard to this work, methodology, data analyses, writing, and helped in review. AH and M G conceptualized and gathered the data with regard to this work, methodology, writing the original draft, review, and editing. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published. The raw data supporting the conclusion of this article will be made available by the authors without undue reservation.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the members of the Research Ethics Committee in the National Center for Radiation Research and Technology (REC-NCRRT) in Egypt (number 16A/22).

Consent for publications

Not applicable.

Competing interests
The author declares no competing interests.

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