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Hypoglycemic and hepatoprotective activity of *Phellinus fastuosus* on streptozotocin-induced diabetic rats and carbon tetrachloride-intoxicated rats, respectively

Hiralal Sonawane[1*†], Deepak Shelke[2†], Sagar Arya[3,4], Vikram Ghole[5], Bhaskar Behra[6] and Subhash Gaikwad[6]

Abstract

Background *Phellinus fastuosus* is a wood-eating medicinal fungus from Western Ghats of India. Therefore, we investigated hypoglycemic and hepatoprotective effects of *P. fastuosus* aqueous extract on streptozotocin-induced diabetic and carbon tetrachloride (CCl_4) induced hepatotoxicity in rats, respectively.

Result As compared to the diabetic control group, a 400 mg/kg dose had significant hypoglycemic effects, including a reduction in blood glucose (24.44%) and gain in body weight. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity reduced by 31.81% and 32.84%, respectively, were also noted, along with decreases in triglycerides (24.32%) and cholesterol (25.89%) levels. The albumin, bilirubin and creatinine levels were also significantly reduced after administration of *P. fastuosus* extract in diabetic rats. Administration of *P. fastuosus* extract showed a substantial decrease in the activity of ALT, AST, alkaline phosphatase (ALP), catalase (CAT) and superoxide dismutase (SOD) in addition a decrease in the level of lipid peroxidation (LPO) as compared to CCl_4-intoxicated rats. The cumulative effect of CCl_4 increased the erythrocyte membrane peroxidation, whereas *P. fastuosus* extract reduced the cholesterol and increased phospholipid, thus preventing the alteration of membrane fluidity as compared to CCl_4-intoxicated rats. FTIR and HR-LC-MS-based metabolic profiling revealed the presence of various functional groups and bioactive metabolites.

Conclusion The extract showed the hypoglycemic and hepatoprotective effects due to the presence of various bioactive metabolites. Exploration of therapeutic potential of *P. fastuosus* using bioassay-guided fractionation is needed.

Keywords *Phellinus fastuosus*, Hepatoprotective, Hypoglycemic, Activities, Bioactive metabolites

†Hiralal Sonawane and Deepak Shelke have contributed equally to this work.

*Correspondence:
Hiralal Sonawane
amolsbr@gmail.com
Full list of author information is available at the end of the article

Background

Medicinal mushrooms are higher fungi with additional qualities including low-fat content and a trans-isomer of unsaturated fatty acids, followed by high fiber, terpenes, phenolics, sterols, etc. [1]. Owing to this, they are widely used in pharmaceutical and nutraceutical industries, particularly for their bioactive potential such as anticancer, immunomodulatory, antibacterial, hypoglycemic and hepatoprotection [2, 3]. Among medicinal mushrooms, the *Phellinus* spp. possess great variability in chemical composition within individual species, hence, are widely used in traditional medication and exploited to evaluate their bioactive potential against cancer, cardiovascular diseases, and diabetes [4–6]. Though some species are explored for their bioactive potential, many of them still required scientific evaluation, for instance, *Phellinus fastuosus*, which also forms a part of folk medicine.

Diabetes mellitus is an endocrine metabolic disorder that disturbs the metabolism of proteins, carbohydrates, and fats. Worldwide more than 425 million individuals are affected by this chronic disorder, which can turn fatal in comorbid conditions [7]. Further, it is a major cause of blindness, kidney failure, cardiac arrest, and lower limb amputation. Numerous traditional remedies have been developed to treat diabetes. Among them, mushrooms are widely used as a natural source of biomolecules for treating ailments, including diabetes [7]. Studies have demonstrated that medicinal mushrooms particularly *Phellinus* spp. has beneficial effects with regards to diabetes. For instance, The *P. badius*, *P. linteus*, and *P. rimosus* showed hypoglycemic activity in experimental diabetic rat/mice models [6, 8, 9].

Liver is a large complex organ in the human body that is vital for detoxification, which is also involved in fat metabolism, protein synthesis and secretion of important enzymes. Drugs, toxic chemicals, or bacterial infections that might affect the liver's normal function are the most common causes of liver damage. Carbon tetrachloride (CCl_4) is a hepatotoxic agent widely used as a toxin to induce liver fibrosis in laboratory investigations [10]. Endoplasmic reticulum structural modifications result from the metabolic activation of CCl_4 by cytochrome P450-dependent mixed oxidases, which further results in the loss of enzymatic machinery, thus impairing normal functioning of liver. Mushroom extracts are reported to prevent CCl_4-induced hepatotoxicity in rats [11].

P. fastuosus is a wood-eating fungus (Fig. 1A), used in traditional formulations to treat ailments such as diabetes, diarrhea, and stomachache as well as to boost the immune system and promote health. It is found across continents, such as Asia and Australia, and is also studied for anti-cariogenic activity [12], plausible use in food preservation [13], and physiochemical characteristics [14]. However, there is no scientific evidence for its bioprospects, for instance to treat hypoglycemia as well as hepatotoxicity. Therefore, as supported by the traditional knowledge and scientific literature, here we seek to explore the hypoglycemic and hepatoprotective activity of *Phellinus fastuosus* mushroom from Western Ghats of India. The hypoglycemic and hepatoprotective activity of *P. fastuosus* aqueous extract was tested in streptozotocin (STZ) induced diabetic rats and carbon tetrachloride induced erythrocyte-/liver-damaged wistar rats, respectively. Based on the folklore of India's Western Ghats, the aqueous extract was created to resemble the conventional way of administration of medicine (Fig. 1B and C).

Methods

P. fastuosus (Kii 19) basidiocarps were gathered from Western Ghats of Maharashtra, India. The samples were authenticated (SPPU/Bot/42) from Mycology Laboratory, Savitribai Phule Pune University. Further the samples were washed, oven dried at 40 ± 2 °C and grounded using a commercial grinder (Restsch Ultra Centrifugal Mill and Sieving Machine, Germany) and stored in sterile reagent bottles (Borosil®, India).

Extract preparation

Hundred grams powder of *P. fastuosus* fruit body was suspended in distilled water at 90 °C for four hours and filtered with the help of Whatmann no. 1 paper. The procedure was carried out twice. Combining and filtering the filtrates via Whatmann no. 1 paper. The filtrate was concentrated in vacuum followed by addition of 90% ethanol (4:1 of filtrate) and stirred vigorously. The mixture was allowed to stand overnight at 4 °C. Next day, centrifuged the mixture at 10,000 rpm for 20 min. Subsequently, equal volume of 10% trichloroacetic acid was added and kept overnight. Next day, centrifuged the sample at 7000 rpm for 5 min and precipitate was wash with chilled 80% ethanol, dissolved in distilled water, and dialyzed for three days against running tap water and one day against distilled water, then centrifuge at 7000 rpm for 15 min. Obtained precipitate was lyophilized and used for bioactivity studies [11, 15].

Hypoglycemic study

Test animals and experimental conditions

Male wistar rats (NTC, Pune, India) of approximately 200 g housed at National Toxicology Center (NTC) were used for animal studies. The rats were housed in a room with stainless steel cages in a controlled environment. Throughout the trial, the rats were fed a commercial pellet meal and given unlimited access to water.

Diabetes was artificially induced by the administration of diabetogenic agent. After a week of

Fig. 1 *Phellinus fastuosus* **A** fruiting body, acquired from Devgad **B**, is located in the Western Ghats of India **C**. [Source: https://www.google.com/maps]

acclimatization, the rats had a 16-h fast. Streptozotocin (STZ, or a diabetogenic drug) was injected intraperitoneally at a dosage of 50 mg/kg body weight, diluted in a buffer solution of sodium citrate buffer (pH 4.5) from Sigma, India. The determination of fasting blood glucose was made two days after injecting STZ [16, 17]. When their fasting blood glucose level exceeded 300 mg/dL, the rats were deemed to have diabetes. Rats were divided into six groups, Normal control (NC); Diabetes control (DC); Standard Glibenclamide (STND) (10 mg/kg) [18]; STZ + doses of 100 mg/kg (Kii 19 100); STZ + doses of 200 mg/kg (Kii 19 200); STZ + doses of 400 mg/kg (Kii 19 400) [11, 15, 19]. A single oral dosage of extracts was administered to diabetic rats every day for 14 days. The animal study was conducted at National Toxicology Center (NTC), Pune, India and overall experimental protocol was approved by the institution animal ethical committee (IAEC 805).

Body and organ weight

The change in body weight was measured by calculating the initial and final body weight of the animals. The weight of body organs such as heart, liver, spleen, kidney, pancreas, and lungs were measured after sacrificing the animals.

Biochemical analysis

To estimate glucose level, the tail vein was pricked and followed by blood collection and monitoring using glucometer (Contour®TS, India). In heparinized tubes, blood was drawn, and the plasma was separated by centrifugation at 10,000 rpm for 10 min. An enzymatic colorimetric test kit (Merck Bioline, India) was used to assess the levels of triglycerides and cholesterol. Enzyme kits were used to measure the activity of alanine aminotransferase and aspartate aminotransferase (Merck Bioline, India) based on Reitman-Frankel

method [20]. Albumin, bilirubin, creatinine and urea levels were measured using diagnostic kits (Crest Biosystems, India).

Hepatoprotective study

Test animals and experimental conditions

Male wistar rats (150–200 g) were selected and maintained in environmentally controlled conditions. Prior to the experiment, the animals were kept in wire-meshed cages for a week to acclimatize and were given access to regular food and deionized distilled water. Hepatoprotective and erythrocyte protective activity.

Rats were divided into four groups, with ten in each group. Group I (served as control), which received olive oil p.o. three times a week for 14 days. Group II were administered silymarin 200 mg/kg. Group III received CCl_4 1 mL/kg body weight (BW), intraperitoneal (CCl_4 control), three times a week for 14 days. Group IV animals were administered with 400 mg/kg BW along with CCl_4 (Kii19 + CCl_4) for 14 days.

The rats were terminated via cervical dislocation 14 days into the experiment. Blood samples were drawn from the jugular vein, placed in heparinized tubes, and centrifuged for 15 min at 3000 rpm.

The packed cells were rinsed three times with physiological saline (0.9% w/v NaCl), suspended in cold distilled water (2–5 °C), then centrifuged at 7000 rpm for 30 min to lyse them. The particle that resulted represented the hemolysate, while the supernatant represented the erythrocyte membrane. Aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were tested in the plasma that was left over after the initial centrifugation in order to measure lipid peroxidation [20]. Using standardized kits, the enzyme levels were determined.

Catalase [21] and superoxide dismutase [22] activity were estimated using the hemolysate. Using a procedure previously described [23], the lipids from the erythrocyte membrane were extracted, and the amounts of cholesterol and phospholipids were measured [24, 25]. The ratio of cholesterol to phospholipids was then determined.

Histological examination

After the animal was sacrificed, the liver was taken out and washed in ice-cold saline. Before sectioning, the tissue samples were embedded in paraffin wax, treated with 10% formaldehyde, and dehydrated in a graduated series of ethanol. After cutting the paraffin slices, they were dewaxed and rehydrated. Light microscopy was used to examine the sections after they had been stained with hematoxylin and eosin [11].

Fourier transformed infrared spectroscopy (FTIR)

The aqueous extract of *P. fastuosus* was analyzed with FTIR. The FTIR spectra was obtained in the range of 4000–450 cm^{-1}using a Bruker, 3000 Hyperion Microscope with Vertex 80 FTIR System (Bruker, Germany).

HR-LC–MS-based metabolic profiling

The aqueous extract of the *P. fastuosus* was characterized using LC–MS at Indian Institute of Technology, Mumbai (IIT-Powai), India. TOF/Q-TOF mass spectrometer (Model—G6550A; Agilent Technologies) with dual AJS ESI as ion source was used. The details of are: Column—Hypersil GOLD C18 100 × 2.1 mm—3 µM, injection volume: 3 µl, flow rate: 0.3 ml/min, mobile phase A (0.1% formic acid) and mobile phase B (90% Acetonitrile + 10% H_2O + 0.1% formic acid), minimum range: 125 m/z and maximum range 1000 m/z, scan rate 1.00spectra/sec. The peaks were identified using inbuilt mass library.

Statistical analysis

Using the statistical package of the Social Science (SPSS) programmed, one-way analysis of variance (ANOVA) test was used to determine the results' statistical significance. Standard Deviation (SD) was used to represent all data as mean ± SD. A one-way analysis of variance and Duncan's multiple range tests were used to compare the group means. At $p < 0.05$, statistical differences were considered significant.

Results

Hypoglycemic activity

Phellinus fastuosus sample's hypoglycemic impact was tested for 14 days in diabetic rats induced by STZ.

Body and organ weight

Figure 2 depicts the effect of *P. fastuosus* administration on the body (Fig. 2A) and organ weight (Fig. 2B) in STZ-induced diabetic rats. When compared to diabetic groups, Kii- 19 400 showed a considerable gain in body weight. Further, body organ weight, i.e., heart, liver, kidney, pancreas and lungs significantly increased at a dose of 200 mg/kg, whereas when compared to the diabetic control group, the 400 mg/kg dosage revealed a substantial change in the weight of the liver and heart.

Blood glucose

Figure 3A shows the activity of *P. fastuosus* samples on the blood glucose level in STZ-induced diabetic rats over a period of 14 days. In the diabetic control group (STZ), the blood glucose level showed continuous increase throughout the experiment, finally reaching

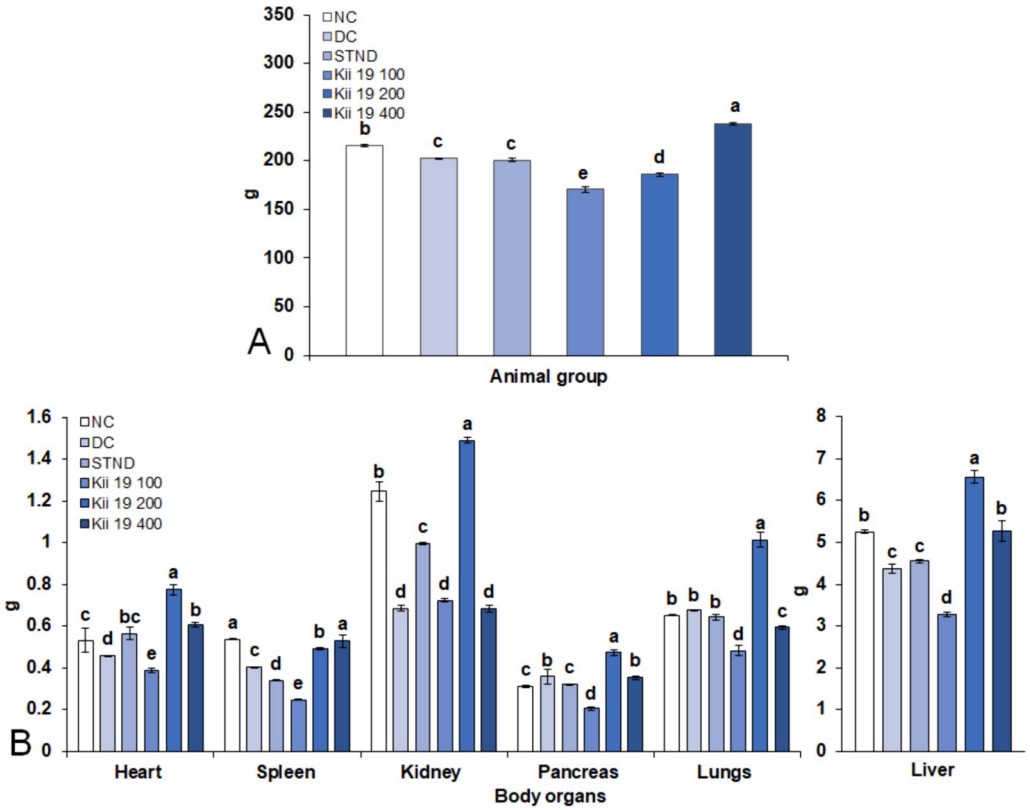

Fig. 2 Effect of *P. fastuosus* aqueous extract on **A** body, and **B** organ weight of extract fed and control rats after 14 days. Values are mean ± SD (n = 6), and small letters on the bars denote the significant difference between treatments at $p < 0.05$

a level of 454 mg/dL. In contrast, the administration of *P. fastuosus* extract significantly lowered the blood glucose level compared to that of the diabetic control group. Doses of 400 mg/kg of Kii 19 showed reduction in glucose level by 24.44%. Kii 19 shown a decrease in glucose level of 23.05% when compared to the diabetic control group at a dosage of 200 mg/kg.

Studies on lipid metabolism

The level of triglyceride was markedly reduced after the administration of *P. fastuosus* extract compared to diabetic control (Fig. 3B). Dose of 400 mg/kg of Kii 19 showed significant reduction of triglycerides level, i.e., 24.32% as compared to diabetes control group. As shown in Fig. 3B, the administration of *P. fastuosus* extract showed significant reduction in total cholesterol level. Kii 19 dose at 400 mg/kg reduced cholesterol by 25.89% as compared to diabetic control. Throughout the investigation, there was no discernible difference in the total cholesterol levels between the Kii 19 100 mg/kg and Kii 19 200 mg/kg groups.

Aspartate aminotransferase (AST) and Alanine aminotransferase level (ALT)

The plasma AST and ALT activity in the STZ group were higher than those in the NC group. Therefore, the remarkable reduction in levels of AST after oral administration of Kii 19 by 31.81% at a dose of 400 mg/kg which revealed a remedial role in liver function (Fig. 3C). The ALT activity was significantly reduced under the influence of *P. fastuosus* samples. The maximum reduction of 32.84% was observed in 400 mg/kg dose.

Bilirubin and Albumin

The level of bilirubin in diabetic rats were increased as compared to normal control rats (Fig. 3D). Administration of the *P. fastuosus* extracts signifciantly lowered the bilirubin level at a dose of 400 mg/kg by 25.13%. Albumin level was also significantly decreased in all groups as compared to diabetic control group. among all groups Kii-19 sample showed significant effect after 14 days.

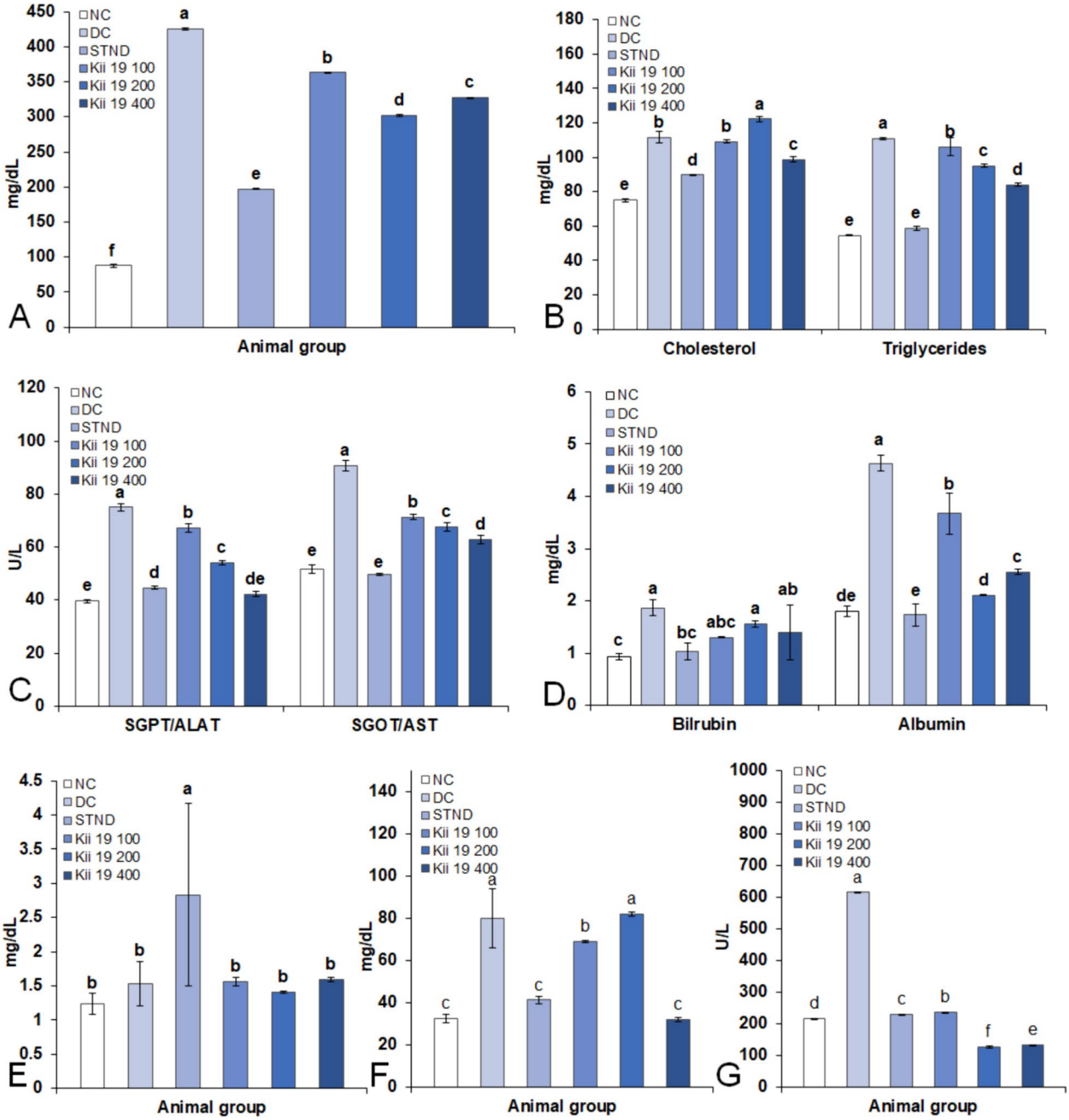

Fig. 3 Effect of *P. fastuosus* aqueous extract on the **A** blood glucose, **B** triglycerides and cholesterol, **C** Alanine aminotransferase (ALAT) and Aspartate aminotransferase (AST), **D** Bilirubin and albumin, **E** Creatinine, **F** Urea and, **G** Alkaline phosphatase in streptozotocin-induced diabetic rats for 14 days. Values are mean ± SD (n = 6), and small letters on the bars denote the significant difference between treatments at $p < 0.05$

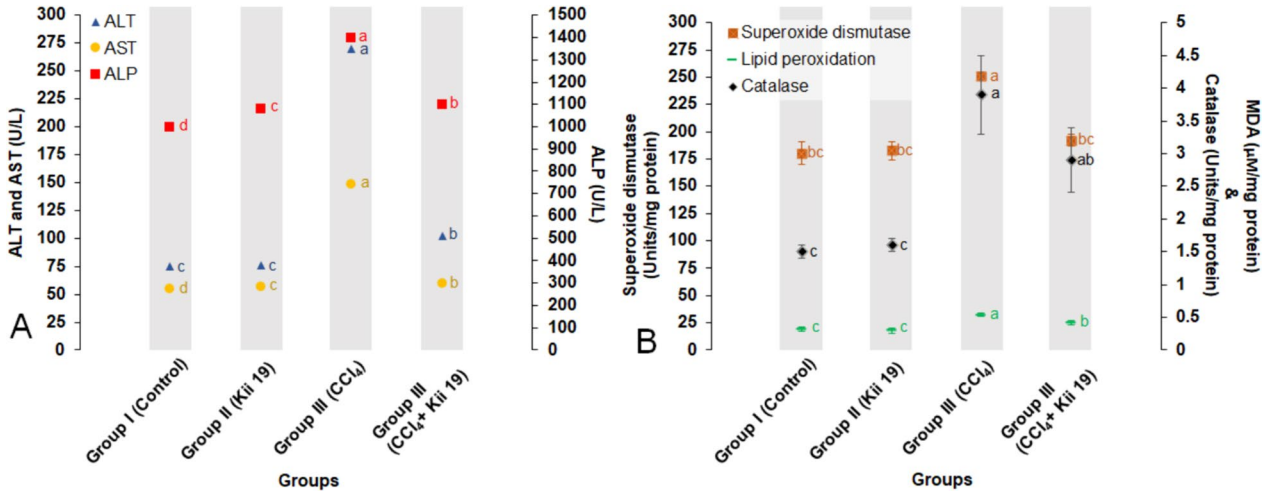

Fig. 4 Effect of *P. fastuosus* extract on **A** Plasma ALT, AST and ALP and **B** lipid peroxidation products and primary antioxidant enzymes of the erythrocytes of carbon tetrachloride-intoxicated rats in rats after 14 days of treatment. Values are means ± SD for ten rats per group. Means in the same column having different superscript are significantly different ($p < 0.05$)

Table 1 Effect of *P. fastuosus* extract on erythrocyte membrane lipids and cholesterol/phospholipid ratio of carbon tetrachloride-intoxicated rats

Groups	Cholesterol (mg/100 µL)	Phospholipid (mg/100 µL)	Cholesterol / Phospholipid
Group I (Control)	0.58 ± 0.01^{c}	1.12 ± 0.03^{a}	0.58 ± 0.02^{c}
Group II (Kii19)	0.58 ± 0.03^{c}	1.15 ± 0.05^{a}	0.58 ± 0.06^{c}
GroupIII (CCl_4)	0.74 ± 0.05^{a}	0.80 ± 0.02^{c}	0.92 ± 0.05^{a}
GroupIV (CCl_4 + Kii19)	0.62 ± 0.01^{b}	0.92 ± 0.01^{b}	0.70 ± 0.04^{b}

Values are means ± SD for ten rats per group. Means with different superscripts in the same column are substantially different ($p < 0.05$)

Creatinine, urea and alkaline phosphatase activity

Figure 3E and F shows that the significant decreased in creatinine level and urea in 400 mg/kg *P. fastuosus* extract fed rats compared to diabetic control.

Hepatoprotective and erythrocyte protective activity

After CCl_4 intoxication, the levels of plasma hepatic enzymes significantly increased. After administration of *P. fastuosus* extract to animals, significantly reduced the elevated levels plasma ALT, AST and ALP (Fig. 4A).

The increased lipid peroxidation products, superoxide dismutase and catalase activity, and decreases in membrane fluidity were evidence of carbon tetrachloride damage to erythrocytes. The accumulation of hydrogen peroxide caused by the elevated superoxide dismutase activity prompted an increase in catalase activity.

In comparison to the control group, the concentration of lipid peroxidation increased in the carbon tetrachloride-treated animals, indicating higher activity of lipid peroxidation. SOD activity increased significantly in carbon tetrachloride-treated animals compared to the normal group (Fig. 4B). CAT activity also increased significantly after administration of carbon tetrachloride (Fig. 4B). The accumulation of lipid peroxidation products in the plasma was dramatically reduced during treatments with the extracts. Superoxide dismutase and catalase activities significantly increased in the rats after carbon tetrachloride intoxication; however, these activities diminished when carbon tetrachloride was administered concurrently with the extracts (Fig. 4B).

Carbon tetrachloride intoxication increased the ratio of cholesterol to phospholipid, decreased membrane phospholipid, and increased membrane cholesterol (Table 1). The findings of this investigation show that the membranes are stiff. The administration of *P. fastuosus* extracts stopped alterations in membrane fluidity and lipid composition.

Histopathology

The histological examination (Fig. 5) reveals cellular damage compared to the CCl_4-intoxicated rats (Fig. 5C), for instance, administration of *P. fastuosus* extract (Fig. 5D) preserved the architecture of hepatocytes and sinusoidal spaces, which is similar to that of the controls (Fig. 5A and B), whereas CCl_4-intoxicated rats showed moderate parenchymal cell hypertrophy, dilatation of sinusoidal spaces (Fig. 5C).

Fourier transformed infrared spectroscopy

The FTIR spectra (Fig. 6) reported the range of 990–1200 cm^{-1}, indicating the presence of polysaccharide,

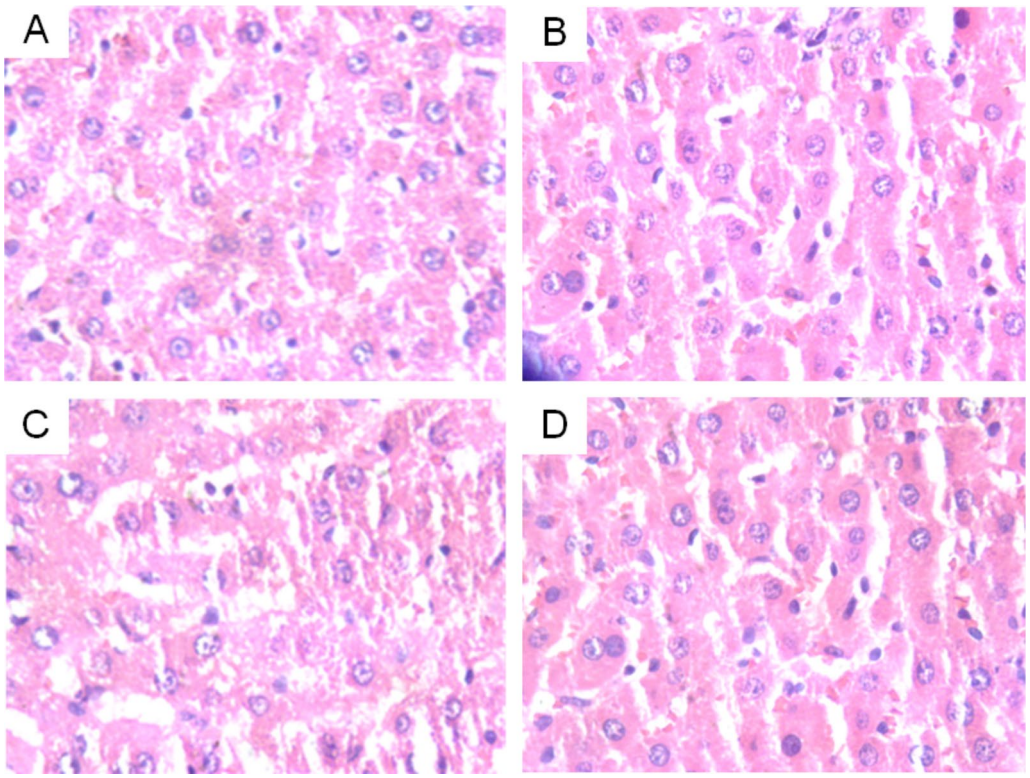

Fig. 5 The liver histopathological sections **A** control group; **B** animals treated with silymarin 200 mg/kg; **C** CCl_4 control; **D** Kii19 + CCl_4

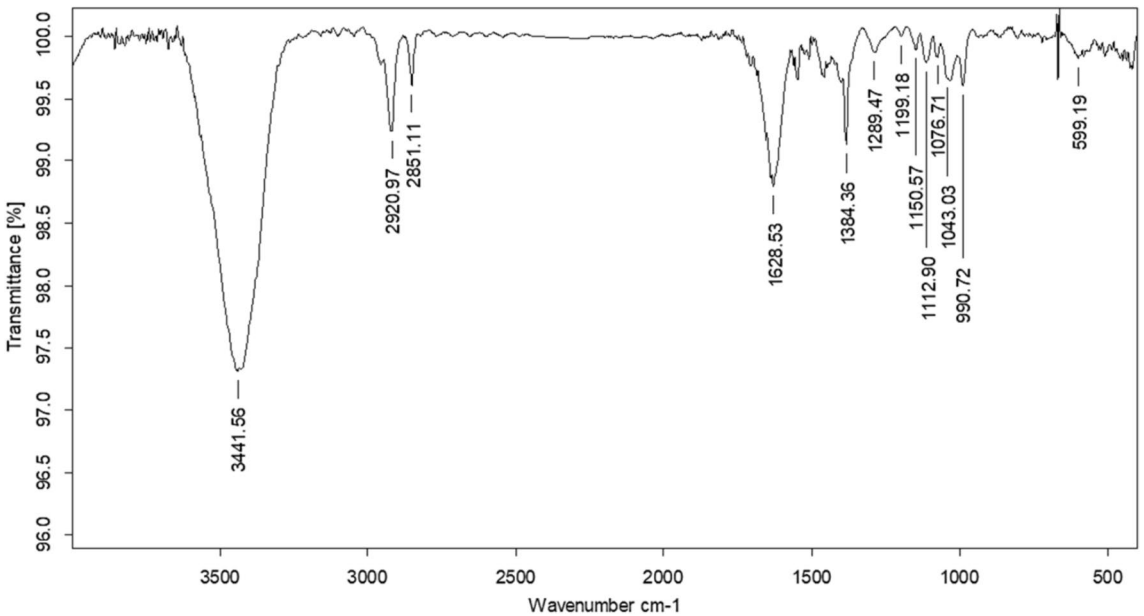

Fig. 6 FTIR study of *P. fastuosus* aqueous extract

while the peaks at 1150–1160 cm^{-1} indicate stretching of glycosidic bond. Furthermore, other peaks at 1384 cm^{-1} indicate presence of β-D glucan. The peak at 1628 cm^{-1} represents vibration of proteins. Other peaks at 2851, 2920 cm^{-1} are C–H stretching vibration and hydroxyl stretching vibration, respectively. However, the peaks for

Table 2 List of metabolites identified using HR-LC-MS-based metabolic profiling and their biological activities as mentioned in the literature

Metabolite	RT	Mass	*m/z*	Formula	Biological activity	References
TPPU	1.031	359.14	360.1484	C16H20F3N3O3	Antidepressant effect	[26]
N-isovalerylglycine	1.031	159.0886	160.0959	C7H13NO3	ROS inhibitor	[27]
2-Amino-2-Norbornanecarboxylic acid	1.112	155.0936	156.1008	C8H13NO2	Anti-obesity	[28]
Acronidine	5.6	311.1148	312.122	C18H17NO4	Antimicrobial and anticancer	[29]
2′-Hydroxyfurano[2″,3″:4′,3′]chalcone	5.631	264.0777	265.0849	C17H12O3	Antimicrobial	[30]
Pyridine-2-azo-pdimethylaniline	5.697	226.1219	249.1111	C13H14N4	Chromogenic reagent	[31]
Flaccidine	6.421	442.1269	465.1162	C23H22O9	Antimicrobial	[32]
Epoxyfumitremorgin C	7.327	393.1671	465.1163	C22H23 N3O4	Anticancer	[33]
Pongamoside A	7.942	440.1117	394.1744	C23H20O9	Antihyperlipidemic *Activity*	[34]
Dubamine	8.081	249.0777	250.085	C16H11NO2	Cytotoxicity	[35]
Dihydrodeoxystreptomycin	9.158	567.2865	568.2937	C21H41N7O11	Antifungal	[36]
Tetradecylamine	10.449	213.2447	214.2521	C14H31N	Hypolipidemic	[37]
1-Hexadecylamine	11.88	241.2759	242.2832	C16H35N	Antimicrobial activity	[38]
Docosanedioic acid	19.671	370.3067	371.314	C22 H42O4	Antibacterial activity	[39]
Vitamin E Succinate (tocopherol succinate)	21.325	530.3977	531.4055	C33H54O5	Antitumour activity	[40]
4-keto myristic acid	26.666	242.1866	265.1759	C14H26O3	Anxiolytic-Like Effects	[41]
1-Hexadecylamine	26.713	241.2759	242.2831	C16H35N	Anticancer activity	[42]

N–H stretching vibration were found between 3194 and 3448 cm^{-1}, whereas amide A (N–H stretching) was found at 3282 cm^{-1} and amide N–H stretch at 3441 cm^{-1}, respectively, indicating the presence of polysaccharide bound proteins.

HR-LC–MS-based metabolite profiling

The aqueous extract of the *P. fastuosus* subjected to HR-LC-MS profiling which showed the presence of bioactive compounds like acronidine, flaccidine, pongamoside A, epoxyfumitremorgin C, dihydrodeoxystreptomycin, tetradecylamine, hexadecylamine, docosanedioic acid, 4-keto myristic acid,meta 1-hexadecylamine, N-isovalerylglycine, 2-amino-2-norbornanecarboxylic acid, etc. with potential bioactivities were reported (Table 2). Many unknown compounds were present in the metabolite profiling of *P. fastuosus* extract shown in the chromatogram (Figure S1).

Discussion

Phellinus spp. (family: Hymenochaetaceae) gained attention due to their potent medicinal properties [6, 43, 44]. *P. fastuosus*, a member of this family is used as folk medicine, and is reported to be an important source of drosophilin A and drosophilin A methyl ether [45, 46]. Therefore present work demonstrates the hypoglycemic and hepatoprotective potential of *P. fastuosus* aqueous extract on Streptozotocin-induced diabetic and carbon tetrachloride-intoxicated rats, respectively.

Streptozotocin treatment affected the secretion of insulin by the pancreas through selective destruction of β-cells of islets of Langerhans. This induced hyperglycemia in rats leading to type-1 diabetes mellitus [6]. The significant increase in body weight and decrease in blood glucose level observed in *P. fastuosus* extract administered rats compared to diabetic control group demonstrates its glucose-lowering property. It can be hypothesized that the blood glucose-lowering effect of extract is due to increased glucose utilization in diabetic rats as a result of insulin secretion [47]. Consequently, the increase in glucose utilization positively correlated with digestion, which resulted in body weight gain. Similar hypoglycemic activity and increased in body weight was reported in rats administered with different medicinal mushroom extract [6, 15, 17].

Most drugs designed to treat diabetes mellitus focuses on controlling and lowering blood glucose levels [47]. Moreover, the various studied mushroom species prove their hypoglycemic property [15] and emerged as an ideal food for the prevention of hyperglycemia [47].

The increased in cholesterol and triglyceride level and AST and ALT in diabetic control group may be due to streptozotocin-induced liver and metabolic malfunction [47]. However, significant reduction in cholesterol and triglyceride level and AST and ALT level after extracting administration as compared to diabetic control group can be attributed to the reduction in fat emission by liver. Bilirubin and albumin levels serve as possible indicators of liver damage as well

as normal liver, gallbladder, and bile duct function [15]. There was not much variation recorded in bilirubin content but albumin level significantly decreased in extract administered rats. Elevated blood levels of urea and creatinine, which are important indicators of renal impairment, are brought on by diabetic hyperglycemia [48]. The result demonstrated that there is no significant variation in animal groups for creatinine level. However, after administration extract, significant decrease in the urea level was observed in comparison with diabetic control group, which it a clear indication of improved liver and kidney function. Further alkaline phosphatase is a membrane bound important enzyme mainly found in liver and bone as well as few other tissues. In diabetes, the level of alkaline phosphatase increases due to ruptured of cell membrane followed by leakage [49]. The *P. fastuosus* extract significantly reduced the alkaline phosphatase activity in a concentration dependent manner. The increase in organ weight such as kidney, heart, liver and lungs indicates that the *P. fastuosus* extract strongly promotes growth of organ tissue irrespective of hyperglycemic condition. This finding suggests that along with hypoglycemic effect there are few components which promote tissue growth, which may act via other protective mechanism such as hepatoprotection, antioxidation and anti-inflammation. However, change in oragn weight is one of the important and indirect diabetes diagnosis markers [47]. The findings presented for hypoglycemic activity of *P. fastuosus* are similar to the recent reports on *Phellinus* spp. including *P. baumii, P. pini* [50] and *P. linteus* [51].

ALT, AST and ALP are important liver enzymes that are responsible for efficient functioning of liver and also act as important markers for hepatocellular damage [52]. CCl_4 is one of the most commonly used hepatotoxins to experimentally induce liver damage [53]. Lipid peroxidative degradation of bio-membrane is one of the principal causes of hepatotoxicity caused by CCl_4 [54].

A popular herbal remedy for antiaging is *P. fastuosus* extract use [8]. Using CCl_4 to cause lipid peroxidation in rat plasma, we were able to demonstrate that *P. fastuosus* aqueous extract greatly reduced the levels of lipid peroxidation and further substantiated the extract's antilipid peroxidation function. The delivery of CCl_4 to the rats causes the liver and other tissues to produce free radicals such CCl_3, CClO, or C_2H_3O, which induce lipid peroxidation, cell death, and tissue damage, which result in severe liver disorders [55]. The degree of lipid peroxidation was represented in the levels of chain reactions that free radicals caused on the cell membrane to produce lipid peroxidation. This measure could offer an easy way to test for lipid peroxidation and damage to cellular membranes. The findings of this investigation showed that administration of *P. fastuosus* extract had an antilipid peroxidative effect and shielded red blood cells from damage brought on by carbon tetrachloride. According to the findings, *P. fastuosus* extract had an antilipid peroxidation impact on normal rats since it somewhat (not significant) lowered the levels of lipid peroxidation in non-toxication rats.

Low levels of lipid peroxidation products are present in tissues and cells under typical physiological conditions. More products from lipid peroxidation are produced as a result of cell damage when there is oxidative stress [55]. Superoxide dismutase, glutathione peroxidase, and catalase are examples of cellular antioxidant enzymes that typically fight oxidative stress [56]. Increases in lipid peroxidation products, superoxide dismutase and catalase activity, and decreases in membrane fluidity in this study's erythrocytes were evidence of carbon tetrachloride damage. The buildup of hydrogen peroxide caused by the elevated superoxide dismutase activity prompted an increase in catalase activity [55].

Superoxide, i.e., O_2^- is harmful to the body and can become one of the primer reasons to induce inflammation, aging and terminally to cancer [57]. SOD is an important enzyme that catalyzes the elimination of reactive oxygen species (ROS). SOD protects human erythrocyte membrane from ROS, which can elevate the fluidity of membranes by decreasing the cross-linking between the membrane proteins. Similarly, catalase are involved in the conversion of hydrogen peroxide to molecular oxygen and water molecules [55]. Due to CCl_4 cumulative impact, erythrocyte membrane peroxidation is increased, which may potentially result in hemolytic alterations [57]. The elevated level of CAT and SOD after the administration of extract provides reliable evidence to its protective action on the erythrocyte membrane.

The FTIR spectra peak at 1628 cm^{-1} represents vibration of proteins. The peaks at 2851, 2920 cm^{-1} are C–H stretching vibration and hydroxyl stretching vibration, respectively [58–60]. However, the peaks for N–H stretching vibration were found between 3194 and 3448 cm^{-1}, whereas amide A (N–H stretching) was found at 3282 cm^{-1} [61] and amide N–H stretch at 3441 cm^{-1}, respectively, indicating the presence of polysaccharide bound proteins [62]. The HR-LC-MS-based metabolite profiling of *P. fastuosus* aqueous extract reported various compounds like acronidine, pongamoside A, flaccidine, dubamine, dihydrodeoxystreptomycin, docosanedioic acid, etc. (Table 2) have bioactive potential [29, 32, 34–36, 39], which interns support the hypoglycemic and hepatoprotective activity of *P. fastuosus.*

Conclusion

The present study confirmed the hypoglycemic and hepatoprotective activity of *P. fastuosus* aqueous extract on streptozotocin-induced diabetic and carbon tetrachloride-intoxicated rats, respectively. Meanwhile, the metabolites identified through HR-LC-MS-based metabolic profiling of aqueous *P. fastuosus* extract support its bioactivities. The physio-biochemical and metabolic analysis indicate that *P. fastuosus* has the potential to provide hepatoprotective and hypoglycemic compounds; however, further bioassay-guided fractionation followed by molecular docking is warranted to identify the active ingredients for pharmaceutical applicability.

Abbreviations

AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
LPO	Lipid peroxidation
CAT	Catalase
SOD	Superoxide dismutase
CCl_4	Carbon tetrachloride
STZ	Streptozotocin
Kii 19	*P. fastuosus*
NC	Normal control
DC	Diabetes control
FTIR	Fourier transformed infrared spectroscopy
HR	High resolution
LC	Liquid chromatography
MS	Mass Spectroscopy

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-024-00654-w.

Additional file 1

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Author contributions

HS, DS and SA conducted the experiment, wrote original draft of manuscript and review the manuscript. VG, BB and SG design the study, supervised the work and finalized the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Animal Ethics Committee of National Toxicology Center (NTC), Pune, India. The consent to participate are not applicable for this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no any competing interest.

Author details

[1]PG Research Centre in Botany, PDEA's Prof. Ramkrishna More ACS College, Akurdi, Pune, Maharashtra 411044, India. [2]Department of Botany, Amrutesh-war Arts, Commerce and Science College, Vinzar, Velha, Pune 412213, India. [3]Center for Innovation, Research and Consultancy, Pune 411018, India. [4]Teri-Deakin Nanobiotechnology Centre, Gurgaon, Haryana 122001, India. [5]National Institute of Virology, Pashan, Pune, Maharashtra, India. [6]Agharkar Research Institue, Pune, India.

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