Biomass from *Fusarium Venenatum* and Preliminary Characterization of Bioactives: Exploration of Hypolipidemic Potential

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**Abstract**

*Fusarium venenatum* derived mycoprotein (biomass) marketed under the trade name Quorn has been designated as a source of first class protein with low cholesterol. Our previous study, demonstrates the significant anti-hyperlipidemic potential of biomass in acute Triton X-100 induced hyperlipidemic model in rats and anti-oxidant activity by DPPH assay. The biomass was produced through cost effective fermentation process in Vogel’s mineral medium using sucrose as the carbon source optimized using Central Composite Response Surface Design (CCRSD). In continuation to our previous reported work, in the current study the hypolipidemic potential of biomass was studied in the chronic high fat diet induced hyperlipidemic model in rats. The high fat diet fed rats showed significant increase in plasma lipid levels [total cholesterol (TC), triglycerides (TG), very low density lipoproteins (VLDL), low density lipoproteins (LDL)] with decreased high density lipoproteins (HDL) levels. Biomass (100, 200 and 400 mg/kg) and simvastatin (10 mg/kg) administered orally reduced the elevated serum lipids (TC, TG, VLDL, LDL), restored the decreased HDL and improved the atherogenic index (p<0.01). Also the treatment with biomass decreased the liver enzymes levels (SGOT, SGPT) comparable to standard treated group. The biomass treatment also improved histoarchitecture of hepatocytes in hyperlipidemic rats. The acute oral toxicity study carried out as per OECD guidelines demonstrated the safety of the biomass. LC-MS analysis of methanolic extract of biomass showed two major peaks (Compound 1: m/e 209.10 and Compound 2: m/e 329.25). Spectral matching with NIST libraries indicated that compound 2 may be structurally similar to pregnenolone, a naturally occurring steroid. Also, compound 1 may be 4- aminothiophenol, N,S-diacety (m/e 209.10). These probable bioactives, exhibits varied biological activities including for the treatment of inflammation, and its related disorders as suggested by literature reports. In conclusion, the study proves the hypolipidemic potential of biomass derived from *Fusarium* venenatum.
**venenatum** in preclinical acute and chronic animal models. Further investigations to isolate the bioactives and elucidate the probable mechanisms and role in triggering hypolipidemic activity need to be undertaken.

**Keywords:** *Fusarium venenatum*; LC-MS analysis; High Fat Diet; Antihyperlipidemic activity.

**Abbreviations:** CCRSD: Central Composite Response Surface Design; VLDL: Very Low Density Lipoproteins; HDL: High Density Lipoproteins; LDL: Low Density Lipoproteins; NTC: National Toxicological Centre; GRAS: Generally Recognized as Safe; HFD: High Fat Diet; TC: Total Cholesterol; TG: Triglycerides; CMC: Carboxy Methyl Cellulose; NIST: National Institute of Standards and Technology

**Introduction**

The world is facing problems of malnutrition, mainly protein-energy malnutrition and micronutrient deficiencies, which continues to be a major health burden in developing countries [1]. It is globally the most important risk factor for illness and death, with hundreds of millions of pregnant women and young children particularly affected. Apart from marasmus and kwashiorkor (the two forms of protein-energy malnutrition), deficiencies in iron, iodine, vitamin A and zinc are the main manifestations of malnutrition. The Indian population is mainly dependent on cereals and pulses as the major sources of protein. The growing concern over the eventual food crisis triggered due to environmental and ecological reasons has laid a major research emphasis on use of single cell proteins [2,3].

Since many years *Fusarium venenatum* has been reported as an alternative protein source. It has been used for human consumption in England for over a decade under the trade name Quorn [4,5]. This protein product has high fibre content, carbohydrates essential amino acids, and is designated as a source of first class protein with low cholesterol. It is an excellent meat alternative. As it is rich in zinc, it is useful in vegetarian diet where zinc levels can be limited but is essential as an enzyme activator [6,7]. The mycoprotein has been reported to reduce blood lipid levels [8,9] with satiety and satiation properties [10,11].

In our previous study, we have reported the cost effective production of biomass from *Fusarium venenatum* using sucrose as the carbon source grown in Vogel’s mineral medium. Optimization of selected carbon source and seed size using Central Composite Response Surface Design (CCRSD) indicated that that sucrose (1.64 g/100ml) and seed size (10% v/v) were optimal in maximizing biomass (0.5602 g/100 ml, p<0.0001) and protein yield (49.99%, p<0.01) of *Fusarium venenatum* [12]. The study also demonstrated the efficacy of the biomass to lower the elevated blood lipid levels in Triton X-100 induced hyperlipidemic model in rats. In continuation to our previous reported work, in the current study attempt has been made to further evaluate the anti-hyperlipidemic potential of biomass in animals using chronic model. Quantitation of the carbohydrate and steroid content in biomass was performed, as previously suggested by LC-MS study of the acetonitrile extract of the biomass.

**Materials and Methods**

**Drugs and Chemicals**

Cholesterol, cholic acid (Himedia Laboratories Pvt. Ltd., Mumbai, India), Simvastatin (Lupin Ltd., Mumbai, India) were used in the study. All other chemicals employed were of analytical grade and obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. Diagnostic reagents kits (Bio Lab Diagnostics (I) Pvt. Ltd., Mumbai, India) were used for the biochemical assays.

**Fugal Strain and Other Materials**

*Fusarium venenatum* (MTCC 9910; ATCC PTA-2684) as active culture slants, were obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. The culture was sub-cultured and maintained on potato sucrose agar (200 g potatoes, 20 g sucrose, 20 g agar and distilled water 1000ml at pH 6.5).

**Inoculums, Medium Preparation and Production of Biomass**

The inoculum, media preparation and production of biomass were carried out as per our previously reported procedure. The inoculum of *Fusarium venenatum* were prepared in Vogel mineral medium in Erlenmeyer flask, incubated with continuous shaking on a rotary shaker at 25-27°C at 200 rpm for 48 hrs to ferment, followed by RNA reduction treatment with subsequent separation by filtration and washings, and dried to obtain biomass [13].
Preparation of Biomass Extracts

The biomass obtained was subjected to ultrasonication assisted solvent extraction. To approximately 5 g of wet biomass, methanol (30 ml) was added and then probe sonicated (approx. 11-13 cycles of 1 min duration each, with a rest time of 1 min between cycles, total sonication time of approximately 25 min) by applying 150 W. The extract was then filtered through five layers of muslin cloth, and then concentrated using rotary evaporator to obtain a concentrated extract. The concentrated extract was reconstituted in methanol to yield test sample solutions for further LC-MS studies.

Qualitative Evaluation of Biomass

The preliminary phytochemical evaluation of the biomass was carried out using standard procedures [14].

Quantification of Carbohydrate Content of Biomass Extracts Preparation of D-Glucose Standards and Calibration Curve

100 mg of glucose AR was weighed accurately and transferred to a 100 ml volumetric flask. The volume was made up to the mark with 100 ml by distilled water to obtain a standard of concentration 1 mg/ml. Suitable aliquots (0.2-1.0 ml) of standard were withdrawn in test tubes, volume made up to 1.0 ml with distilled water. To each, 4.0 ml of anthrone reagent was added, heated for eight minutes in a boiling water bath, and cooled rapidly. The resulting green to dark green colour was read at 630 nm against a suitably prepared blank on a UV spectrophotometer (Shimadzu-1700). The standard graph was drawn by taking the concentration of glucose on X axis and spectrophotometer reading on Y axis.

Preparation of Biomass Sample and Quantitation

An accurately weighed amount of biomass (100 mg) was transferred to a boiling tube, hydrolysed with 5.0 ml of HCl (2.5 N) in a boiling water bath for three hours, then cooled to room temperature and neutralized with solid sodium carbonate until effervescence ceases. The volume was then made up to 100 ml with distilled water. The resulting solution was centrifuged (100 rpm) and the supernatant was collected. From it, 0.2 to 1.0 ml was taken for analysis. The sample solution was also treated with anthrone reagent, absorbance recorded at 630 nm. The concentration of carbohydrate equivalent to glucose in the sample was computed against the prepared calibration curve [15].

Preparation of Cholesterol Standards and Calibration Curve

100 mg of cholesterol AR was weighed accurately and dissolved in 100 ml glacial acetic acid transferred to a 100 ml volumetric flask. The volume was made up to the mark with glacial acetic acid to obtain a standard of concentration 1 mg/ml. 0.1 ml and 0.2 ml of triple acid extract was taken and a set of standards (0.5 to 2.5 ml) were taken and made up to 5 ml with ferric chloride diluting reagent [15]. A blank was prepared simultaneously by taking 5.0 ml diluting reagent. Then add 4.0 ml of concentrated sulphuric acid to each tube. After 30 minutes incubation, intensity of the colour developed was read at 540 nm against a suitably prepared blank on a UV spectrophotometer (Shimadzu-1700). The standard graph was drawn by taking the concentration of cholesterol on X axis and spectrophotometer reading on Y axis.

LC Separation and Preliminary Characterization of Compounds in Biomass Extract

Literature reports suggest that various fungal biomass may contain bioactive compounds exhibiting broad spectrum of biological activity [16]. Our previous study suggested the presence of a sterol glycoside in the acetonitrile extract of biomass established through LC-MS investigations. The study also demonstrated the antioxidant potential of both the acetonitrile and methanolic extracts through DPPH assay which suggest the possibility of presence of bioactives. In the current study, an attempt has been made to characterize the compounds in the methanolic extract of biomass by LC and LC-MS studies.

HPLC Analysis

A Shimadzu HPLC system equipped with a manual injection valve (Rhenodyne injector) with a 20 μl loop, PDA detector and integrated software of LC-Solution was
used for the analysis. An isocratic elution was performed on a Neosphere C18 R column (250 mm x 4.6 mm, 5μm particle size). The mobile phase consisted of two different solvents, solvent A and solvent B. Solvent A was acetonitrile and solvent B was methanol where the proportion of solvent A and B were in the ratio 50:50 % v/v. The mobile phase components were sonicated in water bath sonicator and filtered through 0.45 micron membrane filter under vacuum. Separation was effected by isocratic elution using acetonitrile: methanol (50:50 % v/v) as the mobile phase. The mobile phase flow rate was set to 1 mL/min and the injection volume was 20 μL. UV detection was performed at 295 nm. The total runtime was 20 min. The number of chromatographic peaks eluting out in the set run time were noted. Using these chromatographic conditions, the retention time of unknown compounds were determined.

**LC-MS Analysis**

The LC-MS studies of the methanolic extract of biomass was performed on a Shimadzu LC-MS8040 system equipped with Quaternary pump HPLC with a PDA detector and coupled with Modes of analysis: Q3 Scan, Multiple reaction monitoring (MRM), Mass Spectrometer System was used for the analysis. The LC conditions were similar to the previously optimized chromatographic conditions, only the column used here was Acclaim TM 120 (C18 5μm 120 A° 4.6 X 250 mm) Mass Conditions: Ionization mode: Positive Electro spray Ionization Ion source voltage: 2500V, Source Temperature: 200°C, Nebulizer: 0.3 Bar, Dry Gas Flow: 4.0 l/min. A scanning range of 50-1200 m/z was used for the mass acquisition.

**Acute Oral Toxicity Study Animals**

Acute oral toxicity study of biomass produced under the modified experimental conditions was performed on 05 healthy Swiss albino mice (males and females), 25-30g body weight, aged 8 to 10 weeks obtained from National Toxico logical Centre (NTC), Pune, India. The mice were distributed into two groups. The experimental protocol was approved by the Institutional Animal Ethical Committee as per protocol number DYPISR/IACE/16-17/P-21 and conducted according to the Indian National Science Academy Guidelines (INSA) for the use and care of experimental animals [17].

**Acute Toxicity Assay**

The mice were housed individually in polypropylene cages and marked on the tail for individual identification. Animals were maintained on a 12-h light/dark cycle, at room temperature (25 ± 2°C) with constant humidity. They were acclimatized to laboratory conditions for a week prior to experimentation. Animals had access to drinking water and food provided ad libitum throughout the experiment. The GRAS (generally recognized as safe) notifications (GRAS) of Quorn product suggest that the safety assessment of the mycoprotein has been carried out extensively in experimental animals and in human clinical subjects [18]. As per the expert panel recommendations, the mycoprotein is safe and suitable for human consumption. AS per the GRAS notification and according to the procedures outlined in the OECD guidelines (OECD), the acute oral toxicity of biomass was evaluated in mice [19]. A single dose of biomass (2000 mg/kg) was administered to test mice by the oral route as suspension in 1% w/v carboxy methyl cellulose (CMC). The mice were closely monitored for any toxicity indications within the first six hours after the treatment period, and further daily for a period of 14 days. During the study period, the weight of the animals were recorded and visually observed for signs of mortality, convulsion, muscle spasm, lacrimation, diarrhoea, salivation, respiration, skin colour, visual placing response, mucus membrane changes in behavioral pattern, physical appearance, injury, pain and signs of illness. At the end of the treatment period, the mice were sacrificed. Vital organs such as heart, kidneys, liver, lung and spleen were isolated and examined for any lesions.

**Evaluation of Antihyperlipidemic Potential of Biomass in Chronic Animal Model**

Male Wistar rats (body weight 150-200 g), aged 3.0-4.0 months, were used in the study. Animals were obtained from the NTC, Pune, India, and were housed in animal place at room temperature being maintained at 25 ± 2°C. Animals were fed on a standard pellet diet and kept under normal light/dark cycle. Animals were given free access to food and water. The experimental protocol (protocol number DYPISR/IACE/16-17/P-21) approved by the Institutional Animal Ethical Committee was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

**High Fat Diet Induced Hyperlipidemia in Rats**

Hyperlipidemia was induced in male Wister rats (150-200 g) by maintaining them on a high fat diet HFD (Table 1) for a period of 45 days. The rats were randomly divided into 5 groups (6 rats/group) and received the following treatment: Group I- Normal control (NC) maintained on normal diet throughout 45 days + 1% w/v CMC (1 mL/100 g, body weight, po) for 15 days (from 31st - 45th day of treatment period). Group II- HFD control group maintained on high fat diet throughout 45 days + 1% w/v CMC po for 15 days (from 31st - 45th day of treatment period). Group III- Standard group, maintained...
on HFD throughout 45 days + Simvastatin (10 mg/kg, po.)
for 15 days (from 31st - 45th day of treatment period);
Group IV, V and VI- Test 1, Test 2 and Test 3, maintained
on HFD throughout 45 days + biomass 100 mg/kg, po,
200 mg/kg, po and 400 mg/kg, po respectively for 15
days (from 31st - 45th day of treatment period). At the end
of the treatment period, animals were fasted for 12 h and
the blood was collected by cardiac puncture under light ether anesthesia and then sacrificed. The liver was
excised promptly, washed in cold normal saline and the
liver tissue was preserved in 10% formalin for further
histopathological studies. Serum was separated by
centrifugation and further analysis was done by using
Elitech Selectra automated biochemistry analyzer. The
serum lipid and enzyme levels were estimated using
biomedical kits (Total cholesterol, HDL, SGOT and SGPT:
Pathozyme Diagnostics, Mumbai, India; Triglycerides:
GPO-PAP-kit, Biolab Diagnostics Pvt. Ltd., Mumbai, India;
ALP: Merk Diagnostics, Pune, India) [20-22].

| S. No | Content         | Per 100 g of food pellet |
|-------|-----------------|-------------------------|
| 1     | Sodium chloride | 0.5                     |
| 2     | Cholic acid     | 0.5                     |
| 3     | Cholesterol     | 1                       |
| 4     | Egg yolk        | 80                      |
| 5     | Gram flour      | 20                      |
| 6     | Coconut oil     | 20                      |
| 7     | Corn flour      | 20                      |
| 8     | Wheat flour     | 30                      |

Table1: Composition (%) of high fat diet used for inducing hyperlipidemia in rats.

Measurement of Body Weight

The body weights of rats were recorded at every 15
days interval during the experimental period.

Determination of Serum Lipid Profile and Biomarkers

TC, TG and HDL in serum were assayed using the
readymade diagnostic kits. VLDL-TC and LDL-TC values
were calculated using formula, (VLDL-TC = TG/5), LDL-TC
= TC - (HDL-TC+ VLDL-TC). Atherogenic index was
calculated using formula, Atherogenic index = TC / HDL-
TC). The serum aspartate amino transferase (AST) and
alanine amino transferase (ALT) were estimated and
expressed as IU/L[22].

Histopathological Studies

All the animals were sacrificed by using CO₂
asphyxiation, cut open and observed grossly for
abnormality, if any Liver from all the animals were
collected and fixed in neutral buffered formalin (10%v/v).
The tissues were trimmed longitudinally and processed in
ascending grades of alcohol, xylene and finally embedded
in paraffin wax. Paraffin wax embedded tissue blocks
were cut into sections at 3-6 µm thickness with rotary
microtome (Make-Leica, Model no-RM 2125). All the
slides of liver tissues were stained with Hematoxylin &
Eosin (H & E) stain. The prepared slides were examined
under microscope to note histopathological lesions, if any.
Severity of the observed lesions were recorded as
minimal (<1%), mild (1-25%), moderate (26-50%),
moderately severe (51-75%), severe (76-100%) and
distribution was recorded as focal, multifocal and diffuse.

Statistical Analysis

The results were expressed as mean ± S.E.M. values
using statistical analysis software, GraphPad InStat,
version 3.01. Statistical evaluation was performed using
one-way ANOVA followed by Dunnett’s multiple
comparison tests. P value <0.05 was considered
statistically significant and were expressed as *p<0.05,
**p<0.01.

Results

Production of Biomass from Fusarium venenatum

The biomass was produced from Fusarium venenatum
on Vogel’s mineral media using sucrose as carbon source
(1.64 g/100 mL) and seed size (10% v/v). Under the
previously optimized conditions, optimal biomass yield of
0.50 g/100 ml with 50.32% protein yield was obtained.

Quantitative Evaluation of Biomass Content of Carbohydrate

The total carbohydrate content of biomass extract was
estimated by the method of Hedge and Hofreiter, 1962. In
this procedure, carbohydrate is first hydrolysed into
simple sugars using dilute hydrochloric acid (glucose is
dehydrated to hydroxymethyl furfural). These compounds
react with anthrone to obtain a green coloured product
with absorption maximum at 630 nm. The concentration
of carbohydrate present in the biomass sample was found
to be 0.50% w/w as obtained against the standard
calibration graph constructed by measuring the
absorbance of D-glucose standards at 630 nm (Figure 1).
Quantification of Steroid Content of Biomass Extract

The steroid content in biomass was determined by Zak's method. Here steroids, in the presence of concentrated sulphuric acid react with ferric chloride to give a pink colour read at 540 nm on a UV-visible spectrophotometer. The intensity of the color developed is directly proportional to the amount of steroids present in sample. The concentration of steroid in biomass sample was obtained from the calibration curve of standard cholesterol as 6.973μg/ml (0.60% w/w, Figure 2)

LC and LC-MS Analysis of Methanolic Biomass Extract

The RP-HPLC separation on C-18 column employing acetonitrile (pH adjusted to 6.0 with triethylamine) and methanol (50:50 v/v) with spectral scanning at 295 nm showed partial separation and presence of two chromatographic peaks at retention time of 2.30 min and 3.24 minutes respectively (Figure 3a). The LC-MS recording was performed under similar chromatographic conditions using positive ESI mode.

The mass spectral recording of compound 1 showed a peak at m/z 209.10amu, which might be the molecular ion peak indicating molecular mass of 209.22 (Figure 3b). The spectral matching with library of compounds available in National Institute of Standards and Technology (NIST) database indicated that it may be S-(3-acetamidophenyl) ethanethioate which is widely used as an intermediate to synthesize various classes of compounds [23]. The mass spectra of compound 2 (Figure 3c), showed a peak at m/z 329.25, which on spectral matching with NIST database suggest the presence of pregn-5,16-diene-20-one oxime, 3beta-hydroxy). This is supported by the quantitative testing that suggests presence of 0.6% w/w of steroid in the biomass. In our previous study, the LC-MS analysis of the ACN extract also showed presence of a steroidal glycoside at m/z 701.49. It is postulated that the glycoside linked to the steroidal moiety through the glycosidic bond may have undergone hydrolysis in the alcoholic medium to yield the steroidal structure (Figure 4).
Acute Oral Toxicity Study

The toxic effect of methanolic extract of biomass on appearance and general behavioural pattern of mice were recorded. No toxic clinical symptoms or mortality were observed in any of the test animals, up to 14 days after the administration of methanolic extract at single dose level of 2000 mg/kg body weight po. The behavioural pattern of animals was observed up to 14 h after administration of biomass. The animals did not display any significant changes in behavior, skin effects, breathing, impairment in food intake and water consumption, postural abnormalities and hair loss, lacrimation, salivation, skin colour, or muscle spasm. The animals did not exhibit any significant changes in body weight post treatment with biomass. At the end of the experiment period, histopathology analysis of isolated organs revealed no apparent changes in the liver, kidney and heart in both control and treated mice. The microscopic structures of the organs (Figure 5) showed no observable differences between the control and test group. No major alteration in cell structure or any unfavorable effects were observed when compared with the control organs.

High Fat Diet Induced Hyperlipidemia in Rats

The rats maintained on HFD showed significant increase in body weight as compared to the normal group fed with standard diet (Table 2). Hyperlipidemic rats treated with biomass and standard simvastatin showed significant lowering of body weight. At the end of the 45 days treatment period, the plasma total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels of standard simvastatin and test biomass treated groups of animals is as shown in (figure 6a). The results confirmed that high fat diet successfully induced hyperlipidemia among experimental rats. The lipid profile of hyperlipidemic control rats was significantly higher (**p<0.01) than corresponding normal control rats. The HFD rats receiving biomass 100, 200 and 400 mg/kg/day respectively for 15 days, showed highly significant reduction in TC, TG, LDL-TC, VLDL-TC, artherogenic index with increased plasma HDL-TC levels (**p<0.01) (Figures 6a-6c).
Figure 6a: Effect of mycoprotein on lipid levels

Figure 6b: Effect of mycoprotein on SGPT, SGOT and ALP

Figure 6c: Effect of mycoprotein on LDL/HDL and atherogenic index
Table 2: Effect of mycoprotein on body weight in high fat diet induced hyperlipidemic rats. [Values are mean ± SEM of 6 animals.

Effect of Mycoprotein on AST and ALP

As shown in (Figure 6b) the levels of ALP, SGOT and SGPT in high fat diet treated group were significantly higher (p<0.01) when compared to normal and test treated groups. Under normal conditions, the enzymes aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT) are predominantly contained within liver cells. However, if the liver is injured or damaged, the liver cells spill these enzymes into the blood. This elevates the enzyme blood levels, signaling liver disease. It was observed that the mycoprotein-treated groups (100, 200, 400 mg/kg) and standard simvastatin (10 mg/kg) significantly decreased the levels of ALP, SGOT and SGPT.

Effect of Mycoprotein on LDL/HDL Ratio and Atherogenic Index

It was found that the high fat diet (HFD) treated group elevated the level of LDL/HDL ratio and atherogenic index compared to normal and mycoprotein-treated groups (Figure 6c). However, the standard and biomass treated groups demonstrated significant decrease in level of LDL/HDL ratio and atherogenic index. The results indicate that the biomass treatment may help to decrease the cardiac risk indicator Atherogenic Index due to decrease in cholesterol level with increase in HDL level.

Histopathological Studies

In HFD induced hyperlipidemic model, a significant fatty infiltration, granular degeneration and increased mononuclear cell infiltration within lobules was observed in the liver sections of HFD control rats as compared to normal rats.

Microscopic examination of liver of normal control group did not reveal any lesion of pathological significance. Microscopically, liver of treated rats revealed various lesion of pathological significance. Multifocal mild per vascular lymphocytic infiltration, multifocal moderate fatty change of hepatocytes in liver of Group 2 animals fed with HFD. Animals of Group 4 (Test 1, receiving biomass 100 mg/kg, po) and Group 5 (Test 2, receiving biomass 200 mg/kg, po) respectively showed focal moderate and mild cytoplasmic vacuolation of hepatocytes and hepatocellular swelling in the liver. In comparison, no abnormality of pathological significance was detected in Group 3 (Test 3, receiving biomass 400 mg/kg, po) and Group 6 (Standard treated) respectively.

Patho-morphological observation in present report suggests that treatment of High fat diet produce inflammatory and degenerative lesions in liver. Treatment of mycoprotein at 400 mg/kg and standard simvastatin reverse the effect of HFD. Whereas, partial mitigation is noted in rats treated with mycoprotein at 100 and 200 mg/kg body weight (Figure 7).
Discussion

India is one of the developing countries in the world which might face a major problem of malnutrition due to protein deficiency. This can lead to several health problems ranging from indigestion, fatigue to physiological changes in skin, hair texture and decline in immunity. As all hormones and enzymes are protein-based, the implications of this deficiency can be serious and life threatening. Absolute deficiency of protein may lead to an excessive intake of carbohydrates, thereby leading to degeneration accompanied with obesity, heart illnesses and diabetes. These finding make it necessary to investigate suitable alternative protein sources. Proteins extracted from yeast, fungi and bacteria are currently available in market as nutritional supplements. During the 1960s and 1970s, rapid developments in production and applications of microbial protein occurred. Extensive research was conducted on a wide range of microorganisms as possible alternate protein sources, motivated by large increase in the price of conventional animal feed [24]. Mycoprotein derived from Fusarium venenatum and marketed under the trade name Quorn is a widely used protein source. This product protein, designated as a source of first class protein has high fibre content, contains all nine essential amino acids, with zero cholesterol and is an excellent meat alternative [25,26]. This protein derived from fungi has been reported to possess satiety and satiation properties and exhibit blood lipid lowering effect. Turnbull WH, et al. in a study conducted in healthy individuals, has reported that the mycoprotein exerts beneficial effects on blood lipids [9]. The studies also hypothese that the mycopropein may contain bioactive that may decrease cholesterol synthesis via inhibition of the HMG Co-A reductase enzyme which catalyzes the rate limiting step in cholesterol synthesis [24,3]. However the literature reports did contain any research being conducted to study its potential for therapeutic application in treatment of hyperlipidemic. Based on these findings and in continuation to our previous studies that suggest that the mycoprotein can reduce elevated blood lipid levels in the acute Triton 100x induced hyperlipidemic model in rats, it was decided to study the effect of the biomass using the chronic high fat diet induced hyperlipidemic model in rats.

Chronic hyperlipidemia leads to atherosclerosis which is implicated in the pathophysiology of coronary artery disease and myocardial ischemia. High fat diet induced hyperlipidemia in rats is an established and documented animal model to evaluate effects of anti-hyperlipidemic drugs. Chronic hyperlipidemia finally alters morphological and hemodynamic functions of vital organs including the heart and liver [27]. The liver plays a major role in regulating the cholesterol and plasma LDL metabolism through the secretion of cholesterol and bile acids. Consumption of high fat diet increases cholesterol input in liver and hepatic synthesis of VLDL and LDL-cholesterol, resulting in increased accumulation of cholesterol in liver [28,29].

In the present study, treatment with mycoprotein and standard simvastatin reduced increased body weight of hyperlipidemic rats. HFD caused a significant rise of serum TC, TG, LDL-TC, VLDL-TC and concomitant decrease in serum HDL-TC. Treatment of HFD fed rats with mycoprotein (100, 200 and400 mg/kg) and Simvastatin (10 mg/kg), significantly decreased the elevated TC, TG, LDL-TC, VLDL-TC levels and restored the decreased HDL-TC in serum. The treatment with mycoprotein at 400 mg/kg dose was most effective and brought the elevated lipid level back to normal values. The atherogenic index is an important and sensitive indicator to determine the cardiac risk as stated in the NCEP guidelines [30]. Treatment with mycoprotein significantly reduced the atherogenic index, thereby demonstrating its promising anti-hyperlipidemic activity.

Jeon, et al. [31] has reported that the intake of high fat diet leads to abnormal increase in lipid and lipoprotein levels. This may promote increased lipid peroxidation leading to oxidative damage due to the formation or generation of excessive free radicals in the liver. The formation of these highly reactive free radicals may cause oxidative stress there by accelerating the progress of atherogenesis. In the present study, LC-MS separation of the methanolic extract of biomass led to the preliminary identification of two compounds, S-(3-acetamidophenyl) ethanethioate and pregna-5,16-diene-20-one oxime, 3beta-hydroxy [31].

A study of the literature also indicates that pregnenolone is a naturally occurring steroid, and is a precursor to other hormones, including cortisone, estrogen, testosterone, and progesterone [32,33]. These pregnenolone derivatives have been synthesized, and evaluated for diverse biological activities, such as anti-inflammatory, hypotensive, hypocholesterolemic, and diuretic activities [34,35]. S-(3-acetamidophenyl) ethanethioate identified by spectral matching using NIST database contains two fragments, the acetamidophenyl and ethanethioate moieties that show promising biological activities. A study carried out by Desai, et al. [36] indicates the eﬃcacy of (E)-5-[Hbenzo[d]imidazol-2-yl 2-benzyliden] hydrazinyl) ethanethioate derivatives against different strains of bacteria, fungi and...
mycobacteria [36]. A newer series of chalcone derivatives S-4-(isonicotinamido)-5-(phenoxy)methyl - 4H-1,2,4 triazole-3-yl-3-(4-chlorophenyl) propanethioate were found to be promising antitubercular [37]. S-hexyl (heptyl) ethanethioate derivatives were designed and synthesized as anti tumor agents against MCF-7 cell lines [38]. These studies substantiate the potential of the ethanethioate moiety. Meng-yuan Li, et al. in a recent study has demonstrated the efficacy of 6-[[2-((3-acetamidophenyl) amino) pyrimidin-4-yl) oxy]-N-phenyl-1-naphthamide, targeting the VEGF/VEGF receptor (VEGFR) pathway for cancer treatment. Molecules containing the 3-acetamidophenyl fragment are also being investigated for the treatment of inflammation, diabetes and related disorders [39,40].

The decreased lipid peroxidation may be one such avenue that can reduce the chances of atherosclerosis development due to hyperlipidemia. The bioactives (s) present in mycoprotein, may have resulted in hypolipidemic effect. However, the other probable mechanism (s) such as effect on cholesterol synthesis, metabolism and its excretion, by the phytochemical (s), especially sterols need to be investigated [41-44].

In conclusion, the present study demonstrate that the administration of mycoprotein elicited promising anti-hyperlipidemic activity, that may be attributed to the bioactive (s) present, which has reversed the genesis of hyperlipidemia, manifested due to oxidative stress on cellular system. This needs further isolation of the bioactives and further investigation of their anti-hyperlipidemic activity in order to confirm the precise role of biomass in triggering hypolipidemic activity.

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Compliance with ethical standards
Competing Interests The authors declare that there are no competing interests.

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