In Vitro Anti-Obesity Effects of Medicinal Mushroom Extracts on Adipocyte Lipid Metabolism, Inflammation, and Oxidative Stress: A Nutraceutical Approach

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Abstract: ***This study evaluated the in vitro anti-obesity potential of medicinal mushroom extracts from Chaga (Inonotus obliquus), Fuscoporia gilva, and Bracket fungi using adipocyte-related assays. Antioxidant activity was assessed using DPPH radical scavenging; anti-inflammatory potential by a protein denaturation assay; cytotoxicity by an MTT assay; and lipid accumulation by triglyceride quantification in differentiated adipocyte-like cells. Combination extracts demonstrated stronger antioxidant and anti-inflammatory activity than several individual extracts, suggesting possible synergistic effects. Treatment also reduced triglyceride accumulation in mature adipocyte-like cells while maintaining acceptable cell viability at tested concentrations. These findings indicate that medicinal mushroom extracts may possess anti-obesity-related bioactivity in vitro and warrant further in vivo validation for nutraceutical development.***

Keywords: Obesity; Adipocytes; *Inonotus obliquus*; *Fuscoporia gilva*; Medicinal mushrooms; Nutraceuticals; Lipid accumulation; Oxidative stress; Anti-inflammatory activity.

1. Introduction

Obesity is a condition characterized by a body mass index (BMI) of 30 kg/m2 or higher, in which the accumulation of body fat in adipose tissue can result in adverse health consequences. The complications of obesity, such as diabetes, cardiovascular diseases (CVD), pulmonary diseases, obstructive sleep apnea, cancer, and osteoarthritis, are typically the result of a genetic predisposition, a sedentary lifestyle, and a high food intake. Therefore, obesity is a metabolic syndrome indicative of an imbalance between energy intake and expenditure [1]. Adipose tissue is a substantial organ that plays a role in the body's energy balance. An unusual progression in adipose tissue is induced by high-fat accumulation, which is a contributing factor to obesity in humans [2]. In most cases, obesity results from a combination of physical inactivity and excess nutrient intake. Furthermore, obesity is caused by genetic factors, pharmacological treatments, psychological problems, and endocrine abnormalities [3]. According to additional research, hormonal imbalances, insufficient sleep, variations in temperature, smoking, cravings, medication use (such as antipsychotic drugs), later pregnancy, genetic risk factors, and high body mass index are some of the possible causes of increased obesity levels [4, 5]. The worldwide prevalence of obesity rose more than threefold from 1980 to 2014. Nearly 40% of individuals aged 18 and older were classified as overweight. Over 41 million children under 5 years were classified as overweight or obese in Asia. It is anticipated that by 2030, there will be approximately 1.12 billion people classified as obese globally [6].

Medicinal mushrooms are fungal organisms that are regarded as healthful nutritional supplements and nutraceuticals. While this may appear straightforward, the category of medicinal mushrooms includes a broader range than merely mushrooms. This occurs because a mushroom represents merely one stage or component of a fungal organism [7]. Medicinal mushrooms possess over 120 properties, including antitumor, antihypercholesterolemic, antiviral, antibacterial, immunomodulatory, antioxidant, free-radical-scavenging, cardiovascular, antiparasitic, antifungal, detoxification, hepatoprotective, and antidiabetic [8].

Inonotus obliquus is the common name for the sterile canker that develops on birch trees (Betula spp.), such as paper birch (Betula papyrifera), as a response to infection by the pathogenic fungus *obliquus*. *Inonotus obliquus* is characterized by a black, cracked outer layer containing melanin [9] and a rusty brown, streaked inner tissue [10, 11]. The actual fertile fruiting body of I. obliquus, by contrast, forms under detached bark on dead or fallen trees as a grayish resupinate mass up to 3-4 nm long and 40-50 cm wide, with downward-cascading pores that release spores [12]. *Inonotus obliquus* is commonly found in the northern circumpolar region, as *Inonotus* parasitizes birch. Over decades, *I. obliquus* mycelia spread within the host tree, degrading cellulose, hemicellulose and lignin, causing heartwood decay. *Inonotus obliquus* contains approximately 10% fungal mycelium, with the remainder consisting of decayed wood tissue [13]. These fungi are primarily observed at higher latitudes, where extremely low temperatures slow their growth [14]. Owing to this, infected trees can grow for 30-80 years without signs of decline, reaching diameters of more than 50cm in old trees [15]. Since the 16th century, *I. obliquus* has been used as folk medicine in Siberia, Russia, and other occidental countries. It has been used to treat diseases such as gastrointestinal cancer, cardiovascular diseases, and diabetes with minimal toxicity [16]. People in Siberia used the fungus in traditional medicine to treat helminthic infections, tuberculosis, and liver diseases [17]. *Inonotus obliquus*, made into tea or concentrates, is also widely consumed in Russia and Korea for its health-promoting properties [18].

(a)

(b)

(c)

Figure 1: (a) *Inonotus obliquus* (Chaga), (b) *Bracket Mushroom*, (c) *Fuscoporia gilva* (Top part and Bottom part)

Fuscoporia gilva, commonly known as the oak conk (Aabe Arora, David,1986) [19]. It is a species of fungal plant pathogen that infects several hosts [20]. This mushroom typically grows in rows of horizontal platforms that develop over several years and sometimes "smear" onto the wood. There are 5-8 pores per square millimeter. The flesh is tough and corky. The spore print is yellow [21]. In traditional Chinese medicine, it is used to treat stomachaches and cancer; polysaccharides isolated from lab-grown *Fuscoporia gilva* have been shown to inhibit melanoma growth in a mouse model [22].

Bracket mushroom, also known as polypore or shelf fungi. Bracket fungi grow on trees, usually when their hosts are weakened or dead. Bracket fungi sporocarps are lightweight, with a hard, bracket- or shelf-like shape and a woody texture. They play an important ecological role in decomposing wood by breaking down cellulose and, in some cases, also lignin [23]. Bracket mushrooms contain Glucan and chitin; they form a basket-like scaffold around the plasma membrane linked by hydrogen bonds for structural support and protection of cell walls [24]. A common European species is *Fomes fomentarius*, also known as tinder fungus. This bracket fungus has reportedly been used for making leather-like materials, for instance, for protective clothing. Furthermore, it has a long history of medical uses as an antiseptic and a wound dressing [25].

Obesity is a major global health concern, often associated with chronic diseases such as type 2 diabetes, cardiovascular disorders, and metabolic syndrome. Adipocytes, or fat cells, play a crucial role in obesity by regulating lipid storage, insulin sensitivity, and inflammation. Targeting adipogenesis (the formation of fat cells) and lipid metabolism in adipocytes is a promising approach to anti-obesity therapy. Medicinal mushrooms are rich in secondary metabolites that exert antioxidant, anti-inflammatory, and metabolic regulatory effects. These compounds can modulate adipocyte differentiation, suppress lipid accumulation, activate energy-metabolism pathways such as AMPK (AMP-activated protein kinase), and inhibit adipogenic transcription factors such as PPAR-γ and C/EBP-α [26]. Chaga mushroom, a parasitic fungus mainly found on birch trees, is known for its high antioxidant content and traditional medicinal use in Russia and Asia. Recent studies have shown that *Inonotus obliquus* extracts significantly suppress adipocyte differentiation and reduce triglyceride accumulation in 3T3-L1 preadipocyte models. The ethanolic extracts of I. obliquus inhibit PPAR-γ and C/EBP-α expression, which are essential regulators of adipogenesis [2]. Furthermore, Chaga polysaccharides have been shown to activate AMPK, promoting lipolysis and mitochondrial biogenesis [27].

Fuscoporia gilva, a wood-decaying mushroom, has been less explored but is gaining attention for its antioxidant and cytotoxic properties. While direct studies on anti-obesity effects are limited, recent metabolomic profiling has revealed that phenolic and triterpenoid compounds from *F. gilva* possess anti-adipogenic potential [28]. These compounds may interfere with lipid droplet formation and oxidative stress in adipocytes. Preliminary studies suggest that extracts from *F. gilva* inhibit lipid accumulation and promote apoptosis in hypertrophic adipocytes, possibly by modulating oxidative stress and activating mitochondrial pathways. Bracket fungi (e.g., Ganoderma, Trametes, Phellinus) are known for producing bioactive compounds with anti-obesity effects. Although the term "bracket mushrooms" is broad, several species in this group have been studied: Ganoderma lucidum extracts have demonstrated potent anti-obesity effects by modulating gut microbiota, inhibiting fat accumulation, and reducing inflammatory cytokines in high-fat diet-induced mice [29]. Trametes versicolor, another bracket fungus, produces protein-bound polysaccharide (PSK) that may exert metabolic regulatory effects, although specific studies on adipocytes are limited. Polypore extracts have also been shown to inhibit pancreatic lipase activity, a key enzyme in fat digestion, thereby reducing fat absorption and deposition [30]. The existing literature supports the potential of *Inonotus obliquus, Fuscoporia gilva* and *Bracket mushrooms* as promising natural agents for obesity management. These mushrooms act by modulating adipogenic signaling pathways, reducing lipid accumulation, and enhancing metabolic activity in adipocytes. While *Inonotus obliquus* is the most studied among them, more focused in vitro and in vivo studies on *Fuscoporia gilva* and other bracket fungi are necessary to validate their anti-obesity potential.

The objective of the present study is to examine the Anti-obesity effects of medicinal mushroom extracts on adipocytes: a nutraceutical approach.

2. Materials and Methods

2.1 Materials

L929 (NCTC clone 929) cell lines were bought from the National Centre for Cell Science (NCCS) in Pune, India. 5 FU (Invivogen, USA) DMSO (Dimethyl sulfoxide) was purchased from SRL (Sisco Research Laboratories Pvt. Ltd.). 1x PBS (phosphate-buffered saline) was purchased from HiMedia (Mumbai, India). RPMI media (Roswell Park Memorial Institute), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) purchased from Thermo Fisher Scientific India PVT.LTD. FBS (Fetal bovine serum) was purchased from Gibco (Bangalore, India). Ascorbic acid tablet, Aspirin tablet, Egg albumin, Lysis buffer.

2.2 Sample Collection

Inonotus obliquus (chaga mushroom), *Fuscoporia Gilva* (Oak conk mushroom), and *Fomes Fomentarius* (Bracket mushroom) mushrooms were collected from the Deccan Plateau Zone near Horsley Hills Reserve Forest, Madanapalle, Andhra Pradesh, India.

2.3 Preparation of mushroom extract

a) Water extract

Inonotus obliquus / *Fuscoporia gilva* / *Bracket* mushrooms were washed with distilled water twice, allowed to dry, and then chopped into small pieces. Then, the mushroom was ground into a fine powder using a mixer, and 30 g was weighed and mixed with distilled water at a 1:2 ratio. Allowed the mixture to boil over high heat for 20 minutes, then over low heat. Upon cooling, it was filtered through a strainer and again through Whatman's No. 1 filter paper for fine filtration.

b) Methanol extract

Inonotus obliquus / *Fuscoporia gilva* / *Bracket* mushrooms were washed with distilled water twice, allowed to dry, and chopped into small pieces. Then, the mushroom was made into fine powder using a mixer and weighed about 30 grams. To this, 100 ml of 70% methanol was added. Then it was kept in a water bath at 70 °C for 1 hour and allowed to cool at room temperature. It was filtered through a strainer and finally through Whatman's No. 1 filter paper for fine filtration. The resultant methanol extract was evaporated on a hot plate at 70 °C. The dried extract was reconstituted with water and stored in a refrigerator for further use [31].

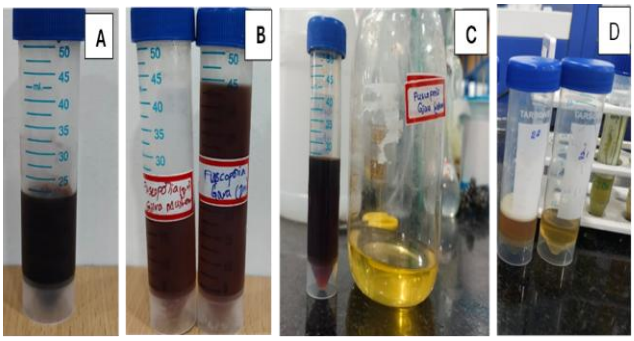

Figure 2.1: Extracts, (A) *Inonotus obliquus*, (B) *Fuscoporia gilva*, (C) *Inonotus obliquus & Fuscoporia gilva*, (D) *Bracket Mushroom*

2.4 Antioxidant Activity– DPPH Radical Scavenging Assay

DPPH solution was prepared by dissolving 3.9 mg in 100 mL of methanol. Plant extracts were equilibrated to room temperature, and 1 mL of extract was mixed with 1 mL DPPH. Absorbance was recorded at 517 nm using a UV-Vis spectrophotometer. Citric acid served as the reference standard [32].

Radical scavenging activity (RSA%) was calculated as:
% Of antioxidant activity= [(OD Control – OD Sample) ÷OD Control] × 100

Both methanol and water mushroom extracts (chaga, *Fuscoporia gilva* were taken in this ratio of A:B (1:1); A:C (1:1); B:C (1:1); A:B:C (33:33:33). Taken (100 µl +100 µl) samples for the ratio of A:B; A:C; B:C; and (66 µl+66 µl+66 µl) for the ratio of A:B:C make up it for 200 µl.

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2.5 Anti-Inflammatory Activity Protein Denaturation Assay

A 5% egg albumin solution was prepared in 1× PBS. Test samples (2 mL of extract) were mixed with 2.6 mL of PBS and 0.4 mL of egg albumin. Aspirin (1 mg/mL in 70% ethanol) was used as the positive control. Samples were incubated at 37°C (15 min) followed by 70°C (5 min) in a water bath, cooled, and the absorbance was measured at 660 nm. Percentage inhibition of protein denaturation was calculated as a measure of anti-inflammatory potential [33]. A combination of three mushroom extracts (*Inonotus obliquus, Fuscoporia gilva, and Bracket*) was taken in distilled water and methanol. In the ratio of A:B (1:1); A:C (1:1); B:C (1:1); A:B:C (33:33:33). Taken (100 µl +100 µl) samples for the ratio of A:B; A:C; B:C; and (66 µl+66 µl+66 µl) for the ratio of A:B:C make up it for 200 µl.

2.6 Cell Culture

L929 (mouse fibroblast, normal) cell lines were revived from liquid nitrogen and maintained in RPMI 1640 medium supplemented with 10% FBS and 1% antibiotic at 37°C in a 5% CO2 incubator. For subculturing, confluent cells were washed with PBS, detached with trypsin-EDTA, neutralized with complete medium, centrifuged at 1300 rpm for 17 min, and reseeded.

2.6.1 Differentiation of L929 Cell Lines into Adipocytes

A gelatin solution was prepared by weighing 150 mg of gelatin, mixing it with 30 mL of distilled water, and stirring it on a hot plate. 100 µL of gelatin solution was added to the wells of the 96-well plate, then the plate was placed in UV light in the cabinet-2 airflow chamber overnight. After 24 hours, excess gelatin was removed from the plate, and the L929 cells were seeded onto it as described in section 2.6. and incubated at 37°C in a 5% CO2 incubator for 1 week. With continued culture, the cells underwent progressive differentiation and lipid droplet accumulation, and by day 5, displayed the characteristic morphological features of mature adipocytes (MA-L929). These cells appeared enlarged and transitioned from an oval to a rounded morphology, with numerous small lipid droplets distributed throughout the cytoplasm, particularly in the perinuclear region [34].

2.7 MTT Assay

Adipocytes/L929 cells were seeded in 96-well plates, incubated for 24 hours, and treated with varying concentrations of methanol and aqueous extracts. 5-FU (100 µM) served as the positive drug control. After 24-hour incubation, 50 µL of MTT solution (dissolved in 1× PBS) was added, and the mixture was incubated for 1 hour at 37°C. The media was removed and replaced with DMSO to dissolve the formazan crystals. Absorbance was read at 540 nm. Percentage cell proliferation and cytotoxicity were calculated relative to untreated controls [35]. Samples were diluted in a 1:1:1 ratio of each mushroom extract either water or Methanol were mixed and used in treating MA-L929 (mature adipocytes). One-way ANOVA was used for measuring statistical significance. $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***) were considered significant. N=3 (biological/technical replicates). All the graphs were plotted using PRISM software. Standard error mean (SEM) was represented as error bars on the graphs.

2.8 Preparation of Mushroom Extracts and Determination of Cellular Triglyceride Content

Aqueous and methanolic extracts of the selected mushroom samples were prepared using standard extraction procedures. Prior to cell treatment, methanolic extracts were concentrated by complete evaporation of the solvent under reduced pressure/ambient conditions and subsequently reconstituted in serum-free culture medium to the original extraction volume. Aqueous extracts were used directly after filtration. All extracts were sterilized by membrane filtration before use in cell culture experiments.

MA-L929 cells were cultured under standard conditions and exposed to mushroom extracts for 24 h. Treatment groups included: (i) neat (undiluted) aqueous and methanolic extracts of each individual mushroom, (ii) extracts diluted 1:2 with serum-free culture medium, and (iii) a combination of all three mushroom extracts administered either neat or diluted 1:2 and 1:5 in serum-free medium. Untreated cells maintained in serum-free medium served as controls.

Following treatment, cellular triglyceride content was determined using a commercial Triglyceride Assay Kit (ERBA Diagnostics, India) according to the manufacturer's instructions. Briefly, cells were harvested and lysed following the treatment period, and 100 µL of each sample was transferred into assay tubes. Subsequently, 500 µL of triglyceride reagent was added and mixed thoroughly. The triglyceride standard supplied with the kit was processed in parallel according to the manufacturer's protocol. The reaction mixtures were incubated at room temperature for 10 min to allow complete enzymatic color development.

The absorbance of the resulting colored complex was measured at 520 nm using a colorimeter or an automated clinical chemistry analyzer equipped with a green filter. Triglyceride concentrations were calculated relative to the supplied standard according to the manufacturer's instructions. Results were expressed as triglyceride concentration or as a percentage relative to the untreated control group. All experiments were performed in triplicate, and data were reported as mean ± standard deviation [36].

$$\{\text{Triglyceride (mg/dL}\} = (\text{Absorbance of Test} / \text{Absorbance of Standard}) \times 100$$

3. Results

3.1 Antioxidant Activity

The antioxidant activity of individual mushroom extracts was evaluated using the DPPH assay. Among all samples (**Fig. 3.1.a**), Chaga (W) and Fuscoporia gilva (W) showed the highest radical-scavenging activity, indicating significant antioxidant potential. *Bracke*t (W) and *Fuscoporia gilva* (M) exhibited moderate activity, while Chaga (M) showed the lowest antioxidant effect. Overall, water extracts exhibited greater activity than methanol extracts, suggesting that water is a more effective solvent for extracting antioxidant compounds from these mushrooms.

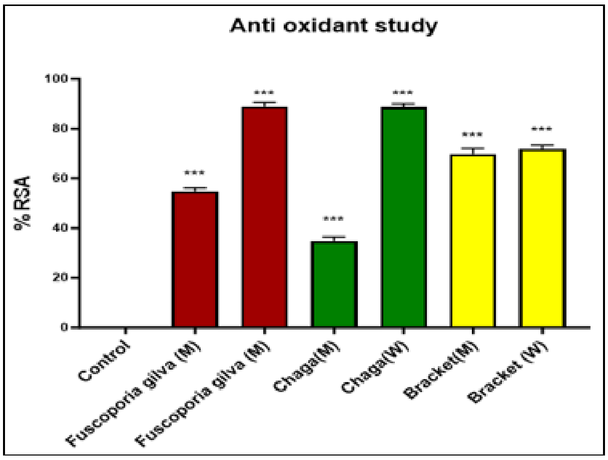

Figure 3.1 (a): Antioxidant activity of Methanolic and Water extracts of mushrooms

Figure 3.1b demonstrates that combinations of Chaga, *Fuscoporia gilva*, and *Bracket* combinations (1:1 or 1:1:1), especially in methanol extracts, significantly enhance antioxidant activity.

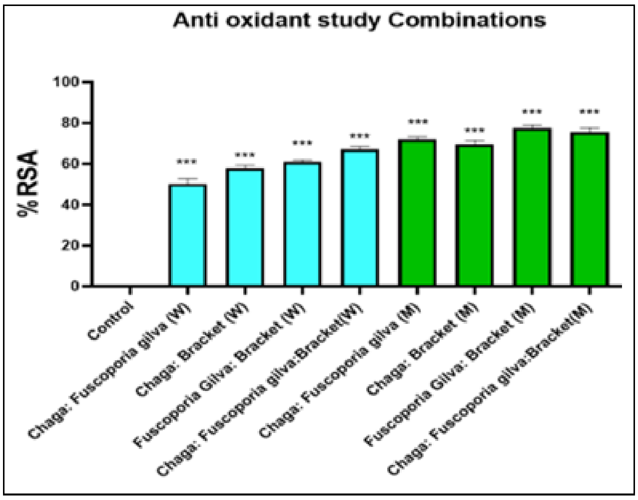

Figure 3.1.b: Antioxidant activity of Methanolic and Water extracts of mushrooms in various combinations

3.2 Anti-inflammatory activity

Bracket mushroom extracts in both water and methanol exhibited the highest significant anti-inflammatory activity (Fig. 3.2.a), with nearly 100% inhibition of protein denaturation and significant suppression compared with all other groups, including the control. Methanol extracts of *Fuscoporia gilva* and Chaga mushrooms show moderate activity, while water extracts exhibit minimal activity.

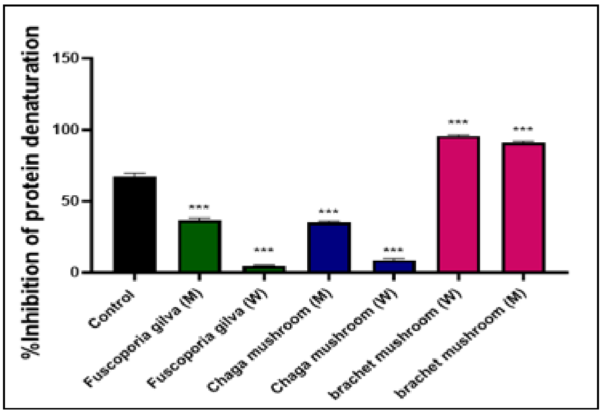

Figure 3.2 (a): Anti-inflammatory activity of Methanolic and Water extracts of mushroom combinations.

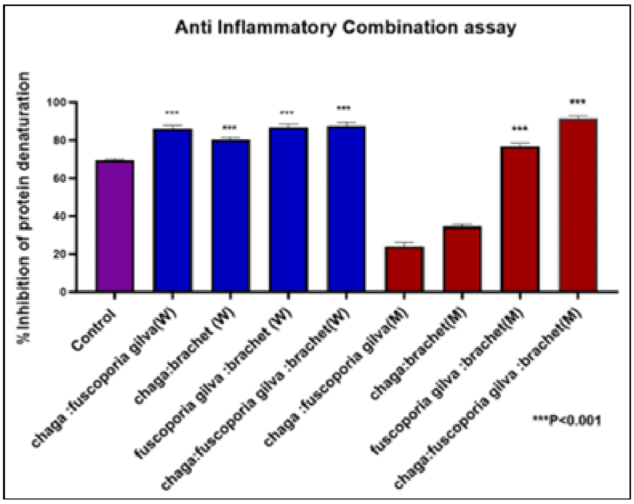

Figure 3.2 (b): Anti-inflammatory activity of Methanolic and Water extracts of mushrooms in various combinations.

With the exception of the combination of Chaga and *Fuscoporia gilva*, Methanol and Chaga, and *Bracket* methanol extracts, all the other combinations have significant anti-inflammatory activity.

3.3 Cytotoxicity Activity

The combination of Chaga, *Fuscoporia gilva*, and Bracket (1:1:1 ratio) extracts exhibited significant cytotoxicity, comparable to that of 5-Fluorouracil (5-FU), an antimetabolite drug widely used since 1957 to treat various cancers, including colorectal and breast cancer [37]. The mushroom combination's ability to exhibit cytotoxicity similar to that of 5FU makes it a potential candidate.

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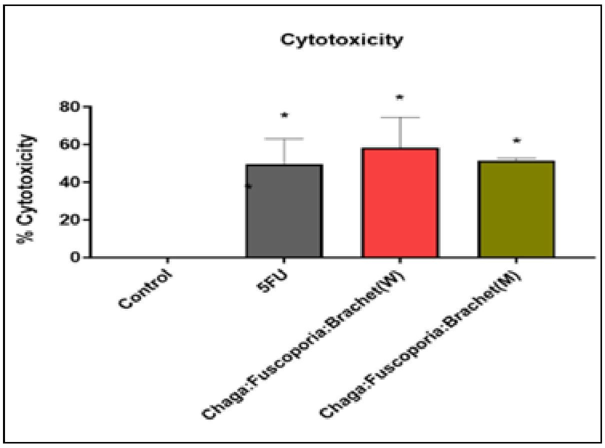
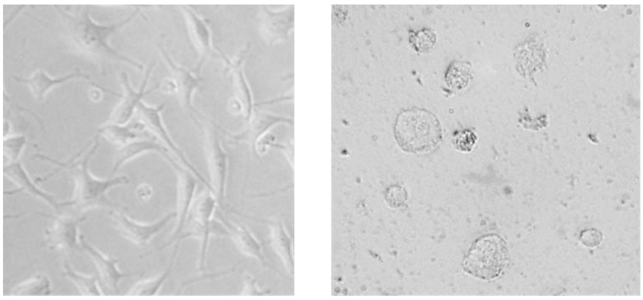


Figure 3.3: Cytotoxic effects of various combinations of mushroom extracts on L929 cells

3.4 Differentiation of L929 cells to mature adipocytes:

The L929 cells, known for their ability to spontaneously differentiate into adipocytes, were selected as an alternative to the conventional 3T3-L1 cell model. Unlike 3T3-L1 cells, which require a 10–14-day induction period, L929 cells differentiated into mature adipocytes within just 5 days, significantly reducing the processing time [41]. L929 cells cultured on gelatin-coated wells exhibited progressive differentiation during the incubation period. By day 5, the cells showed a marked increase in size, with a transition from spindle shape to a rounded morphology and the appearance of numerous small lipid droplets throughout the cytoplasm. After 1 week, the cells exhibited morphological features characteristic of mature adipocytes (MA-L929).



(a) **(b)**

Figure 3.4: (a) L929 Cells (b) Mature Adipocytes (MAL929)

3.5 Triglycerides levels

There is a significant increase in triglyceride levels in MA-L929 cells compared to L929 cells. The graph also shows that treatment with Chaga, *Fuscoporia*, and *Bracket* mushroom extracts, either individually or in combination, significantly reduced triglyceride levels in mature adipocytes. At the same time, this indicates that these mushroom extracts effectively lower intracellular lipid accumulation, supporting their anti-obesity potential as a nutraceutical approach.

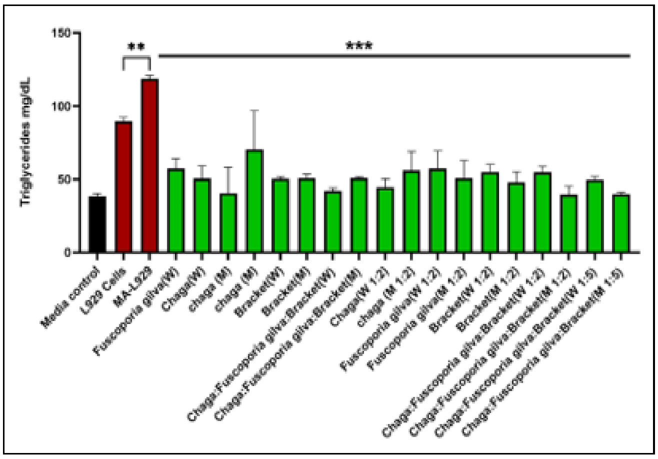


Figure 3.5: Measurement of Triglycerides in cells upon treatment with various Mushroom extracts

4. Discussion

The present study evaluated the anti-obesity potential of medicinal mushroom extracts, namely Chaga (*I. obliquus*), *Fuscoporia gilva*, and *Bracket* mushrooms, using adipocyte-based models, with particular emphasis on their potential application as nutraceutical agents [38]. Obesity is a multifactorial metabolic disorder characterized by adipocyte hypertrophy, excess lipid accumulation, and oxidative stress. In this context, nutraceuticals derived from medicinal mushrooms may offer a natural and potentially safer alternative to synthetic anti-obesity agents [34]. The three mushroom extracts demonstrated significant anti-adipogenic activity, as indicated by reduced triglyceride levels and suppressed lipid accumulation in differentiated adipocytes. The combined water extract of *Fuscoporia gilva*, Chaga, and *Bracket* mushrooms exhibited strong antioxidant activity, which may help attenuate oxidative stress and subsequently inhibit adipogenesis, consistent with earlier studies [39]. The *Bracket* extract also showed notable lipid-lowering effects, possibly through modulation of redox homeostasis and mitochondrial function. Among the individual extracts, *Fuscoporia gilva* and *Bracket mushroom* extracts produced the greatest reduction in triglyceride levels, suggesting potential lipolytic activity, possibly attributable to their bioactive polysaccharide and triterpenoid content. Overall, these findings suggest that the investigated mushroom extracts may regulate adipocyte metabolism and oxidative stress, supporting their potential use as nutraceuticals for obesity management [40].

5. Conclusion

The present in vitro findings demonstrate that medicinal mushroom extracts, particularly combination formulations of Chaga (*Inonotus obliquus*) and *Fuscoporia gilva*, may modulate oxidative stress, inflammatory responses, and lipid accumulation in adipocyte-like cells. The extracts reduced triglyceride accumulation while maintaining cell viability and exhibiting notable antioxidant and anti-inflammatory activities, suggesting potential anti-obesity-related nutraceutical value. Notably, combination treatments were more effective than individual extracts, suggesting possible synergistic effects on adipocyte metabolism. However, mechanistic validation, standardized adipogenesis models, dose-response characterization, and in vivo studies are necessary before definitive therapeutic conclusions can be established.

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