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Melanogenic effect of purified mushroom tyrosinase on B16F10 Melanocytes: A Phase Contrast and Immunofluorescence Microscopic Study

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ABSTRACT

Cutaneous melanin pigment plays a critical role in camouflage, mimicry, social communication, and protection against harmful effects of solar radiation. It has been proven that tyrosinase plays a pivotal role in melanocytes dendrite formation; however, the molecular mechanism underlying this process has not been fully elucidated. The morphological changes were observed under a phase contrast microscope. These changes were evident, with globular cell bodies and increased numbers of tree branch-like dendrites. The present work aimed to study the morphoanatomic effects of purified tyrosinase to determine its skin-darkening potential using B16F10 melanocyte, which has not been done to date. Phase contrast and immunofluorescence microscopic analysis of B16F10 melanocytes has been done after treatment with various concentrations of purified tyrosinase along with standard tyrosinase (Sigma) in order to explore the mechanism of action of purified tyrosinase induced skin darkening. The phase contrast microscopic results showed that the number of melanocytes with melanin-loaded dendrites has increased significantly in purified tyrosinase treated cells in a dose dependent manner leading to skin darkening. In addition, immunofluorescence microscopic analysis revealed purified tyrosinase increase cellular tyrosinase expression in doze dependent manner due to tyrosinase absorption in B16F10 melanocyte. Present findings proved that purified tyrosinase possesses a skin darkening potential and could be used as a safe melanogenic agent for the treatment of hypopigmentation disorders or vitiligo.

1. Introduction

The unique distribution of pigments throughout the body results in different colors and patterns in all organisms. Pigmentation is highly heritable and is regulated by genetic, environmental, and endocrine factors that modulate the amount, type, and distribution of melanin [1,2]. Melanin is a unique pigmented biopolymer that is synthesized by specialized cells known as melanocytes, dendritic cells that comprise a relatively minor portion of the cells present in the dermal-epidermal border of the skin. In addition to its role in the determination of phenotypic appearance and protective coloration, melanin is involved in a number of important functions, such as balance and auditory processing, absorption of toxic drugs and chemicals, and neurologic development during embryogenesis [3]. Melanocytes, the specialized skin cells are mainly involved in regulating the skin

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color by producing the pigment melanin. The loss of melanin in the epidermis can increase a person's risk in acquiring skin cancers and result in hypopigmentation [4,5]. Any defect that affects melanocytes or their functions often results in pigmentary disorders leading to enhanced, reduced or complete loss of skin pigmentation. The most common pigment disorders are not disorders of melanin quality, but rather of the pigment-producing cell itself, which may be reduced in number, absent, or hyperactive and commonly, with regional localization. Hypomelanosis can either be acquired, e.g., vitiligo, or congenital via inheritance of mutations in pigment-related genes, e.g., albinisms and piebaldisms. Pigment excess (hypermelanosis) can be associated with inflammatory responses, as in keloid scars, or with local abnormal melanocytes functions, as in dysplastic nevi or malignant melanoma. Melanogenesis takes place in special organelles, melanosomes, present in the melanocytes. The biosynthetic pathway of melanogenesis has been elucidated, where two types of melanins are synthesized within melanosomes: eumelanin and pheomelanin. The first step of melanogenesis is initiated with tyrosine oxidation to dopaquinone catalyzed by the key enzyme, tyrosinase. Regulation of melanogenesis in mammals is controlled at different levels and is complex at each level [2,6].

In the present scenario various therapies are in use to cure hypopigmentry disorders by use of synthetic agents but they are still unsatisfied for treatment of skin hypopigmentation disease conditions, and there is a great demand to develop more safe and effective melanogenic agents. Recently, many efforts have been made to develop new therapeutic agents against hypopigmentation abnormalities, especially using novel biologically active compounds from natural mushroom flora. New melanogenic agents that cause or initiate melanogenesis can be developed from indigenous species of mushrooms. Natural plant extracts have powerful phytochemical properties which are now being exploited world over and there is a sudden surge in ayurvedic or traditional uses of plant wealth in treatment of diseases like cancers, arthritis, sterility, psoriasis and diabetes. For the treatment of hypopigmentation or vitiligo, plant extracts of *Psoralea corylifolia* and *Ammi majus* have been used since time immemorial. Recently, it has been reported that *Psoralea corylifolia* and its active ingredient psoralen causes melanin granule dispersion leading to skin darkening of *Channa punctatus* and *Bufo melanostictus*. Several reports have concluded that the melanin granule dispersion caused by *Psoralea corylifolia* and its active ingredient psoralen involve muscarinic cholinergic receptors [2,7–9].

Medicinally, mushrooms have an established history of use in traditional oriental medicine. Many traditionally used mushrooms from genera *Auricularia*, *Flammulina*, *Ganoderma*, *Grifola*, *Lentinus*, *Trametes* (*Coriolus*) and *Tremella* have been demonstrated to possess significant medicinal properties [10]. *Pleurotus ostreatus* have been used traditionally as well as medicinally in various ailments such as anti-tumor, immunomodulatory, hypocholesterolaemic, anti inflammatory, anti microbial and antiviral activities [11]. Reviewing the literature it becomes evident that there are no conclusive data available with regard to the effects of various mushroom tyrosinases on melanogenesis in different vertebrate species including human beings. Despite this, to the best of our knowledge, there are no studies indicating extracts of mushroom as melanogenic agents, except for the work of Zehtab et al. [12], who reported that mushroom tyrosinase prevented experimental autoimmune vitiligo. Suppression of clinical and histological disease was observed when the animals received mushroom tyrosinase but exact mechanism is still unknown, so in the present work, it is not clearly known that whether mushroom extracts induce increase arborization of melanocytes, dendrites or increase the number of melanin granules, or work through tyrosinase or any other signal transduction pathway in reactivating melanin polymerization within the pigment cells or B16F10 melanocytes.

2. Material and Methods

For the present study, the compound mushroom tyrosinase (lyophilized powder ≥1000 unit/mg solid), was purchased from Sigma-aldrich St. Louis, Missouri, United States. Goat anti-murine tyrosinase IgG antibody and IgG Alexa Flour ® 594 donkey anti-goat IgG (H + L) (2 mg/mL) was purchased from life technologies North America, United States. Dulbecco's Modified Eagle Medium (AT006A-5L) Fetal bovine serum (RM10432-100ML),Anitibiotic Antimycotic Solution 100X (A002-20ML),Trypsin-EDTA solution1X (TCL042-5 × 100ML), MTT {[3-(4, 5 Dimethylthiazol -2-y l)]-2, 5- diphenyltetrazolium bromide}(TC191-500MG), 4', 6-diamidino-2-phenylindole (DAPI) (TC229-5MG),Phosphate buffered saline (RM7385-1PK) and Trypan blue, Certified (RM263-5G) were purchased from HiMedia Laboratories Pvt.Ltd. Mumbai.

2.1. Preparation of mushroom tyrosinase

Tyrosinase from *P. ostreatus* was purified by ammonium sulphate precipitation, dialysis followed by gel filtration chromatography on Sephadex G-100, and DEAE-Cellulose ion exchange chromatography [13].

2.2. Preparation of melanocyte culture

Melanocyte cell line B16F10 used in the study was procured from the National Center for Cell Science, Pune and were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% heat inactivated fetal bovine serum (FBS), 1.5 g/L NaHCO3, 2 mM L-glutamine, 10,000 units penicillin, 10 μg/mL streptomycin, and 25 μg/mL amphotericin B and incubated at 37° C with 5% CO_2 in a humidified atmosphere. To inhibit the bacterial contamination 2% Benzalkonium chloride was kept in incubator. The cells were subcultured in a ratio of 1:3 on every third day. For cell expansion and experiments with isolated cells, the B16F10 cells were detached with 1X Trypsin-EDTA (0.25% Trypsin and 0.1% EDTA in Hank's Balanced Salt Solution). Generally, after 3-4 passages, the cells were discarded and

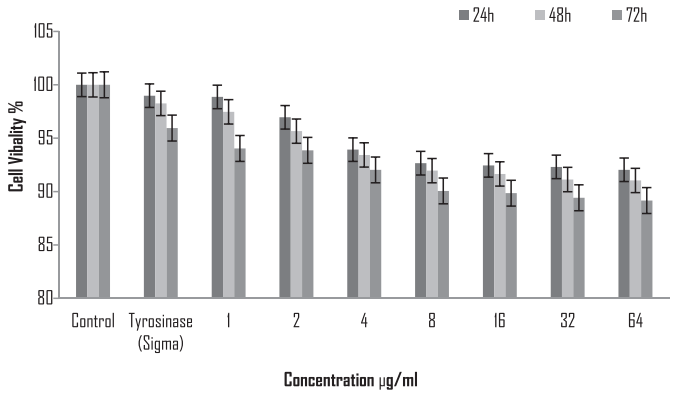


Fig. 1. Effect of purified tyrosinase of *P. ostreatus* (1-64 μg/mL) on cell viability of B16F10 melanocytes at 24, 48 and 72 h. The results are presented as the mean ± SEM from triplicate experiments. $p < 0.005$ indicated that data was significant.

when necessary the cells preserved in liquid nitrogen were used as fresh culture.

2.3. Cell viability assay of B16F10 melanocytes

MTT {[3-(4, 5 Dimethylthiazol -2-y l)]-2, 5- diphenyltetrazolium bromide} assay is colorimetric assays for measuring the mitochondrial activity of enzymes that reduce MTT to formazan dye, giving a purple color. When 70% confluency of B16F10 melanocytes were attained, trypsinization and seeding was done in 96 well microtitre plates at a density of 10^4 cells/well in DMEM media supplemented with 10% FBS and 10,000 units penicillin, 10 μg/mL streptomycin, and 25 μg/mL amphotericin B antibiotic solutions. After overnight incubation, media of each well was replaced and the cells were treated with desired stimulants to perform MTT assay Kim et al. [14], to examine any cytotoxic effect of extracted tyrosinase of *P. ostreatus* along with standard control tyrosinase (Sigma) in B16F10 cells over the concentration range of 1, 2, 4, 8, 16, 32, and 64 μg/mL at different incubation periods of 24, 48 and 72 hr respectively. At the completion of incubation stimulant-containing media were discarded and fresh DMEM media containing 1 mg/mL of MTT was added to each well and incubated at 37 °C for 4 h. The solution was replaced with 0.04 N HCl–isopropyl alcohol solution and further incubated at room temperature for 30 min. Harvested solution was centrifuged at 13,000 rpm for 5 min and absorbance of supernatant was measured at 570 nm using microplate ELISA reader (Thermo Multiskan Ascent SN#354.00429).

2.4. Phase contrast Microscopy

For morphologic observation under phase contrast microscope, the cell suspension of 1×10^5 B16F10 cells was prepared in DMEM supplemented with 10% FBS and (10 mL) antibiotics (10 μg/mL penicillin, 10,000 unit streptomycin and 25 μg/mL amphotericin B). On day 0, B16F10 cells in complete DMEM were dispensed into the 24 well plate (10^5 cells per well). After an overnight incubation at 37°C in 5% CO_2 and humidified atmosphere, purified tyrosinase of *Pleurotus ostreatus* to the final concentration of 1, 2, 4, 8, 16, 32, and 64 μg/mL were added in the coated wells. The plates were incubated at 37°C for 24, 48 and 72 hrs respectively. The cells were observed under microscope.

2.5. Immunofluorescence Microscopy

In immunofluorescence microscopy, immunofluorescence staining of B16F10 cells was carried out to determine the tyrosinase expression Cheung et al. [15]. Immunofluorescence is a technique used for light microscopy with a fluorescence microscope. This technique uses the specificity of antibodies to their antigen to target fluorescent dyes to specific biomolecule targets within a cell, and therefore allows visualisation of the distribution of the target molecule through the sample and may be used to analyse the distribution of proteins and small biological and non-biological molecules. The cells were grown on glass cover slips placed in each well of a 12 well plate (2×10^4 cells/well) for 24 h. After an overnight incubation, the purified tyrosinase of *Pleurotus ostreatus* and standard control mushroom tyrosinase (Sigma) were added in triplicate at different concentration 1, 2, 4, 8, 16, 32, and 64 μg/mL for 48 h. After the incubation the cells were rinsed briefly in 1X PBS, fixed at room temperature for 20 min with 3% paraformaldehyde and permeabilized with 0.1% Triton X-100 in PBS followed by blocking with 10% BSA in PBS for 30 min at room temperature. Cells were then incubated for 15 min with 100 μL of goat anti-murine tyrosinase IgG antibody. After washing thrice with PBS keeping the cells for 10 min at each wash the cells were incubated for 1 h at room temperature with secondary antibody, donkey anti-Goat IgG conjugated to AlexaFluor-594 in PBS buffer. Cells were then washed with PBS and treated with 1 μg/mL 4', 6-diamidino-2-phenylindole (DAPI) for 10 min at room temperature to stain their nuclei. The cells were viewed and photographed using inverted fluorescence microscope.

3. Results

3.1. Cell viability assay of B16F10 melanocytes

MTT assay was performed on B16F10 cells to observe the effect of treatment of purified mushroom tyrosinase of *P. ostreatus* on cell viability at concentration ranges

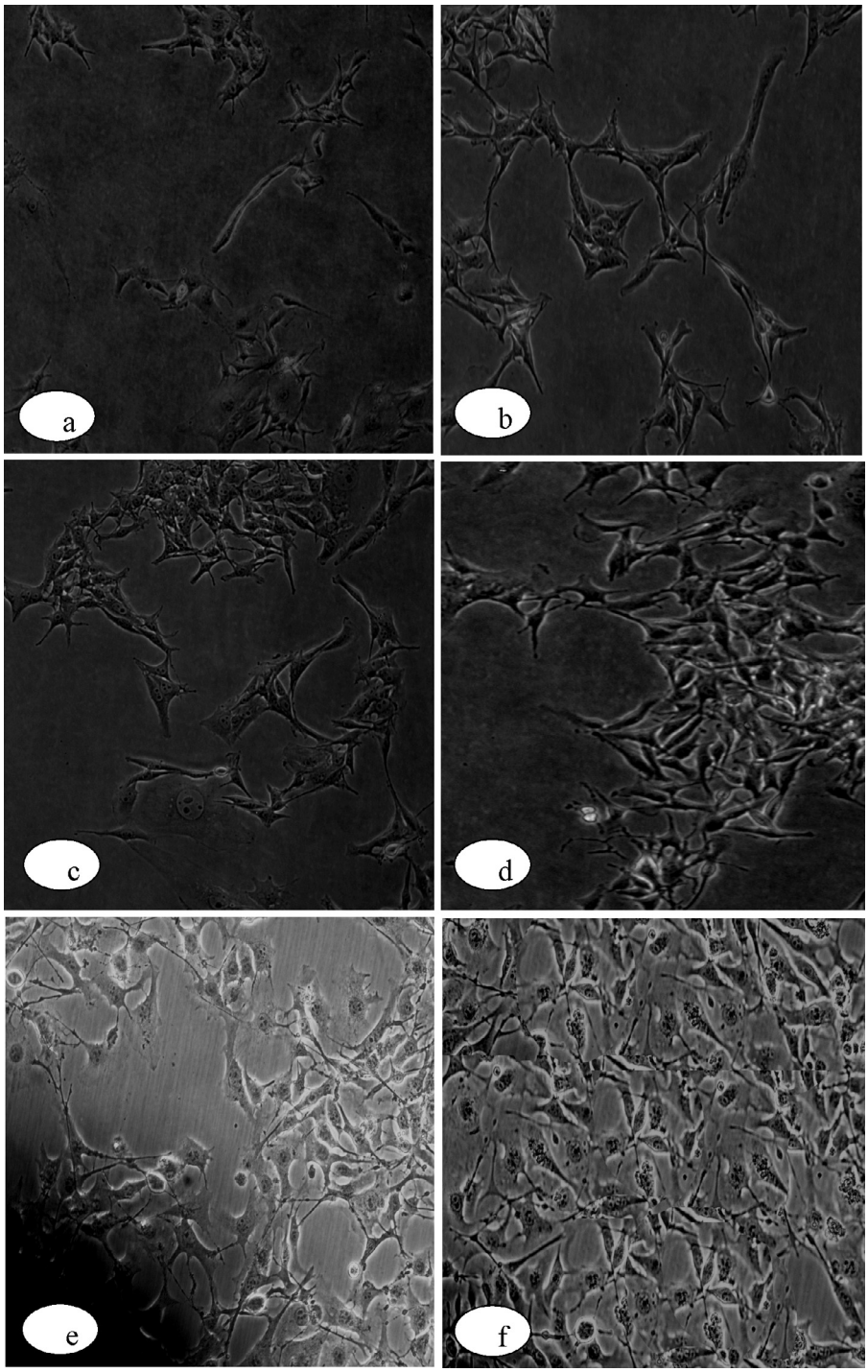


Fig. 2. Morphological appearance of B16F10 melanocytes. Melanocytes exhibited only 1-2 dendrites with clear cytoplasm (a), significant morphological changes which showed less dendritic extensions with clear cytoplasm (b,c), large dendrites and formed a confluent monolayer (d,e), multipolar highly branched dendritic network (F). All photographs are under phase contrast microscope equal magnification of 200 X.

of 1 to 64 μg/mL. Tyrosinase of *P. ostreatus* on B16F10 cells showed decrease in cell viability with increase in concentration of purified mushroom tyrosinase at 1 to 64 μg/mL showing 98% to 92% viability. Maximum viability was observed at a concentration of 2 μg/mL 98% (0.376 ± 0.5 mg/1×10^5cells) ($p > 0.005$). Standard mushroom tyrosinase (Sigma) was found to proliferate maximum 99.73% (0.381 ± 0.5 mg/1×10^5cells) ($p < 0.005$) with respect to control as 100% (0.382 ± 0.5 mg/1×10^5cells). Whereas, when the period of treatment was extended

from 48 to 72 h; along with an increase of the dose above 8 μg/mL, there was no further enhancement in cellular proliferation and a gradual decrease in growth was observed above this dose which had reached to 58.11% (0.222 ± 0.5 mg/1×10^5cells) ($p < 0.005$) as compared to control of 100% (0.382 ± 0.5 mg/1×10^5cells) (Fig. 1).

3.2. Phase contrast microscopic observation of B16F10 melanocytes

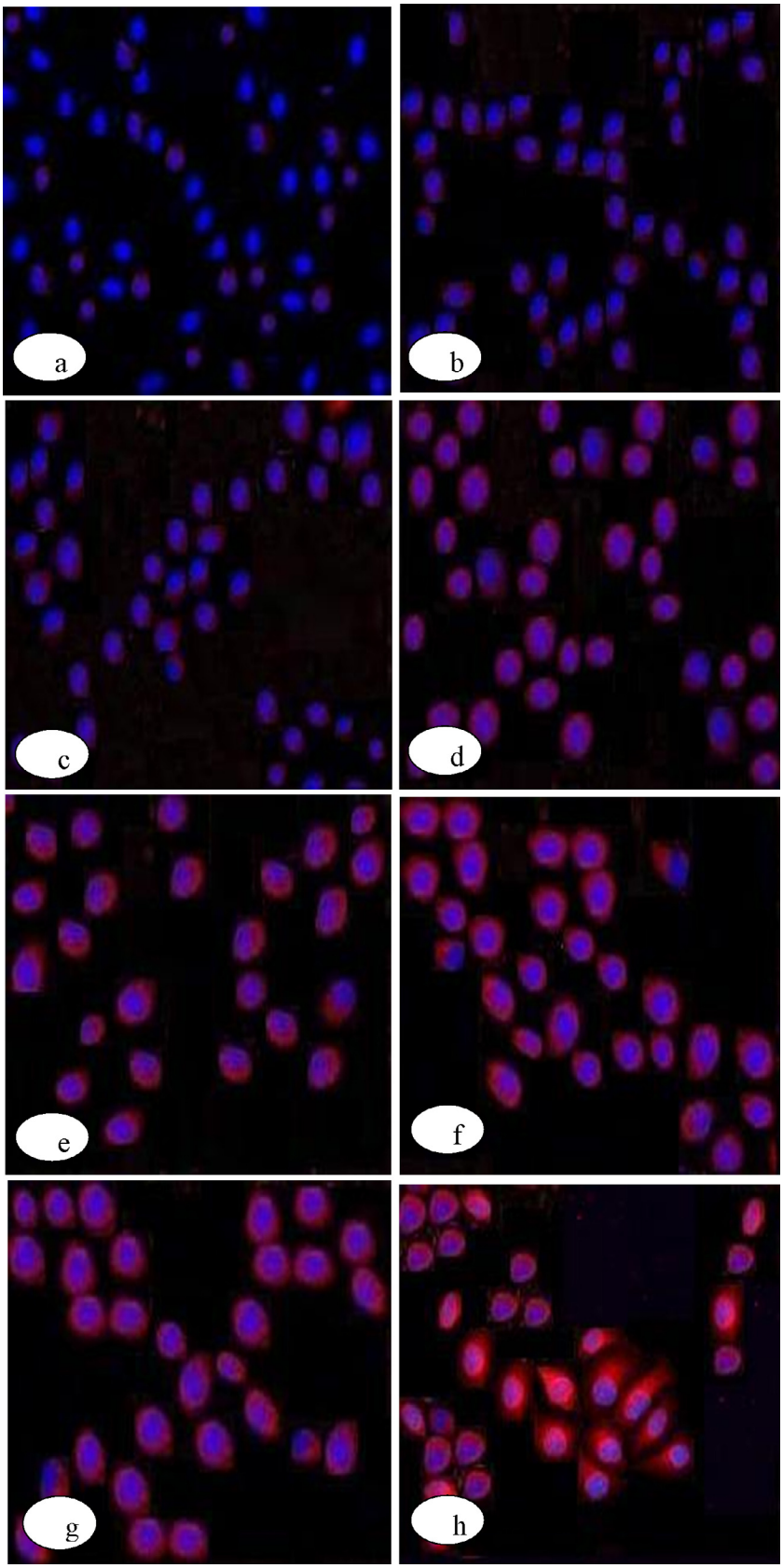
Dendrite formation and extension comprise a characteristic morphology of B16F10 melanocytes in the skin representing one of the functional activities of melanocytes which is the ability to transfer melanosomes into neighbouring keratinocytes. In phase contrast microscopic observation, assessment of the purified tyrosinase of *P. ostreatus* on the morphology and proliferation ability of B16F10 melanocyte showed that the number of cells proliferation and dendrite formation increased which were treated in dose dependent manner (1 to 64 μg/mL) for a period of 24 and 48 h. While most of the untreated cells had only two dendrites. Standard tyrosinase (Sigma) also increased the melanocytes centricity and proliferation at concentration of 8 μg/mL. It was found that the treated melanocytes developed dendrites which could be visually observed. The morphological changes in B16F10 melanocytes induced by purified tyrosinase of *P. ostreatus* were evident, with markedly globular cell bodies and significantly increased numbers of tree branch-like dendrites as compared to untreated cells, which exhibited only 1-2 dendrites with clear cytoplasm (Fig. 2a). The minimum concentration of 1-4 μg/mL purified tyrosinase of *P. ostreatus* caused significant morphological changes in the B16F10 melanocytes, which showed less dendritic extensions with clear cytoplasm when they were treated, (Fig. 2b,c). At increasing concentration of purified tyrosinase of *P. ostreatus* from 8 and 16 μg/mL, it was observed that B16F10 melanocytes produced large dendrites and formed a confluent monolayer within 24 to 48 h in culture (Fig. 2d,e). At highest concentration of 32 and 64 μg/mL purified tyrosinase of *P. ostreatus* under the same culture conditions, it was found that multipolar highly branched dendritic network was formed and dense pigmented granules appeared in the cytoplasm of the B16F10 treated cells (Fig. 2f).

3.3. Immunofluorescence microscopic observation of B16F10 melanocytes

Enhanced enzyme formation at the protein level is evident at the first instance to account the increase in tyrosinase activity. Thus, immunofluorescence study was performed to assess the effect of purified mushroom tyrosinase of *P. ostreatus* on tyrosinase protein absorption on B16F10 melanocytes. In immunofluorescence microscopic observation distinctly higher expression of the tyrosinase protein was evident in melanocytes by intense orange red fluorescence, when treated with purified tyrosinase of *P. ostreatus* at concentration (1 to 64 μg/mL) for 48 h. Whereas, tyrosinase expression in most of the untreated cells was negligible as compared to treated cells (Fig. 3a). Expression of the tyrosinase in B16F10 melanocytes was observed on treating with purified tyrosinase of *P. ostreatus* at concentration (1 to 64 μg/mL) for 48 h. B16F10 melanocytes showed higher tyrosinase levels, concentrated in the perinuclear region. The minimum concentration of 1-8 μg/mL purified tyrosinase of *P. ostreatus* increase tyrosinase expression in dose dependent manner and changes were observed in the B16F10 melanocytes (Fig. 3 b- d). The maximum tyrosinase expression of treated cells was observed at concentration varying from 16 to 64 μg/mL, where intense orange red fluorescence was very much equivalent within the concentrations (Fig. 3 e-g) and tyrosinase (Sigma) exhibited maximum tyrosinase expression at 8 μg/mL (Fig. 3h). B16F10 melanocytes showed slight decrease in intensity of florescence which is directly related to decrease in the cellular tyrosinase at 72 h of incubation with the purified mushroom tyrosinase at concentration (1 μg/mL to 64 μg/mL).

4. Discussion

For the past few decades, mushroom tyrosinases have been a great concern solely due to the key role in mammalian melanogenesis. Melanogenesis has been defined as the entire process leading to the formation of dark macromolecular pigments, i.e., melanin. Melanin is formed by a combination of enzymatically catalyzed and chemical reactions. The enzyme extracted from the champignon mushroom *Agaricus bisporus* is highly homologous with the mammalian ones, and this renders it well suited as a model for studies on melanogenesis. In fact, almost all studies on tyrosinase inhibition conducted so far have used mushroom tyrosinase because the enzyme is commercially available [16]. The present findings suggested that tyrosinase of *P. ostreatus* on B16F10 cells showed decrease in cell viability with increase in concentration of purified mushroom tyrosinase at 1 to 64 μg/mL showing 98% to 92% viability. Maximum viability was observed at a concentration of 2 μg/mL 98%. Standard mushroom tyrosinase (Sigma) was found to proliferate maximum 99.73% with respect to control as 100%. Whereas, when the period of treatment was extended from 48 to 72 h; along with an increase of the dose above 8 μg/mL, there was no further enhancement in cellular proliferation and a gradual decrease in growth was observed above this dose which had reached to 58.11% as compared to control of 100%. These findings are in agreement with those of Mallick et al. [17], who reported the similar effects of placental total lipid fractions (PTLF) on B16F10 melanocytes viability. It was observed that PTLF stimulated distinct proliferative response of B16F10 cells was evidenced from higher extent of [3H] thymidine incorporation. The mitogenic response was just initiated at a concentration as low as 1 mg/ml of PTLF ($101 \times 6\%$) and reached its maximum (152%) at 10 mg/ml as compared to the control (100%). At concentrations higher than 10 mg/ml, growth stimulation started to decline and nearly 95% of the control growth was observed at 200 mg/ml. The decline in growth was not due to cell death since there was no loss of cell viability. It had been also reported by Lee et al., 2005 [4] that the serial concentration of glycyrrhizin 0.2, 0.5 and 1.0 mM significantly increased the cell's viability in B16 melanoma cells but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significantly decreased when a concentration of 1.5 mM. The present findings are in full agreement with those of Yoon et al. [18] who reported that the effects of isopanduratin A and 4-hydroxypanduratin-A on cell viability, percentages of viable melan-a cells. Melan-a cells were treated with various concentrations (1-100m M) of the compounds for 72 h had no significant effect on the cell viability as well as morphology in B16F10 melanoma cells. The data of the present findings showed that purified tyrosinase of *P. ostreatus* increased cell viability significantly are in full corroboration with the detailed work of Jeon et al.[19] who have studied the effect of the main ingredients of lotus flower essential oils in human melanocytes. Supplement-reduced melanocytes were stimulated with 10 μg/ml of lotus flower oil for 5 days. The cell viability increased significantly at a concentration 12.5 μM and decreased at concentration of 25 and 50 μM. Simultaneously, the present findings are also in full agreement with those of Moreira et al. [20] who reported the effect of leaves and flower extracts of *P. venusta* on cell viability, it was found the vehicle used with leaves extract promoted a significant decrease in cell viability (12.4%), the extracts did not show any additional cytotoxic effect on cells when compared with the vehicle group. Besides, the cytotoxicity noted with the vehicle was not a concern since it was not able to interfere with melanin production. Cytotoxic or proliferative effect of xanthoxylin on B16F10 melanoma cells has been observed, it was found xanthoxylin at the lowest concentration significantly increased the cell's viability however; increased the concentration it was decreased described by Moleephan et al. [21]. The present findings have also proved that purified tyrosinase of *P. ostreatus* induce melanogenesis significantly without causing any cytotoxic effects, these data are in full validation with the recent work of Yamauchi et al. [22] who reported the synthesis of quercetin glycosides and their melanogenesis stimulatory activity in B16 melanoma cells.

Over the last few years, the molecular mechanisms responsible for melanocyte dendricity have attracted increasing attention in the field of melanocyte research. In the present study, the effect of purified tyrosinase of *P ostreatus* on morphoantomical changes in B16F10 melanocytes *in vitro* was observed. Marked morphoantomical changes were evident in B16F10 melanocytes treated with 1 to 64 μg/ml, with globular cell bodies and increased numbers of tree branch-like dendrites as compared to untreated cells, which merely exhibited 1–2 dendrites. The minimum concentration of 1-4 μg/mL purified tyrosinase of *P. ostreatus* caused the morphological change in the B16F10 melanocytes, where it showed less dendritic extensions with clear cytoplasm. At increasing concentration of purified tyrosinase of *P. ostreatus* from 8 and 16 μg/mL, it was observed that B16F10 melanocytes produced large dendrites and formed a confluent monolayer within 24 to 48 h in culture. At highest concentration of 32 and 64 μg/mL purified tyrosinase of *P. ostreatus* under the same culture conditions found that multipolar highly branched dendritic network was formed and dense pigmented granules appeared in the cytoplasm of the B16F10 treated cells. The present findings suggested that purified tyrosinase of *P. ostreatus* showed dendritic processes with the accumulation of the pigment granules which is similar to the findings of Lin et al. [23] who reported during a herbal screening programme that both *Piper nigrum* extract and piperine induced morphological alterations in melan-cells, with more and longer dendrites observed. Mallick et al. [17] have found that B16F10 melanoma cells grew with large dendrites and formed a confluent monolayer within two to three days after treatment with placental total lipid fraction (PTLF) in culture. Multipolar, highly branched dendritic network and also dense pigmented granules appeared in the cytoplasm of the treated cells. Whereas, Takeyama *et al.* [24], who demonstrated that quercetin (3,5,7,3',4'-pentahydroxyflavone) stimulates dendrites of melanocytes which extended towards the adjacent keratinocytes in human epidermis culture model. Similar data have been reported by Moleephan *et al.* [21] where they had reported xanthoxylin triggers significant increase in dendrites formation in B16F10 melanocytes in dose dependent manner, which is important in transporting melanosomes to keratinocytes during melanogenesis.

In this series of experiments, the qualitative measurement of tyrosinase expression was performed by immunofluorescence staining method. Enhanced enzyme formation at the cellular protein level is evident at the first instance to account the increase in tyrosinase activity. Thus, immunofluorescence study was performed to assess the effect of purified mushroom tyrosinase of *P. ostreatus* on tyrosinase protein absorption on B16F10 melanocytes. The findings of the present study distinctly exhibit higher expression of the tyrosinase protein as evident in melanocytes by intense orange red fluorescence, when treated with purified tyrosinase of *P. ostreatus* at concentration (1 to 64 μg/mL) for 48 h. Whereas, tyrosinase expression in most of the untreated cells was negligible as compared to treated B16F10 melanocytes which showed higher tyrosinase levels to be more concentrated in the perinuclear region when incubated for 48 h with purified mushroom tyrosinase. It was also observed that on longer incubation period (72 h) with the purified mushroom tyrosinase, B16F10 melanocytes showed slight decrease in intensity of florescence at all the concentrations (1 μg/mL to 64 μg/mL) employed, indicating decrease in the cellular tyrosinase. The present findings have proved distinctly higher expression of tyrosinase within the cytoplasm in the B16F10 melanocytes, treated with purified tyrosinase of *P. ostreatus*, this can be correlated with the findings of Mallick et al. [17], who have reported the effect of placental peptide on tyrosinase protein expression using immunofluorescence studies. It was found that B16F10

Fig. 3. Immunofluorescence labeling of B16F10 treated melanocytes. Tyrosinase expression negligible in untreated cells (a), minimum concentration of purified tyrosinase (1-8 μg/mL) increase tyrosinase expression in dose dependent manner (b-d), maximum tyrosinase expression observed at concentration of 16 to 64 μg/mL (e-g), tyrosinase (Sigma) exhibited maximum tyrosinase expression at 8 μg/mL (h). All photographs are under phase contrast microscope equal magnification of 200 X.

cells showed distinctly higher expression of the tyrosinase protein within the cytoplasm of the cells treated with 10 μg/ml PP and 50 μg/ml PPPF for 48 h compared to basal level expression of the protein in untreated control. Park et al. [25] have reported the effects of harmaline and harmalol on tyrosinase expression. It was observed that harmaline and harmalol stimulated melanin synthesis and tyrosinase activity, as well as expression of tyrosinase, TRP-1 and TRP-2. On the other hand, tyrosinase expression in the B16F10 melanocytes can be increased by exposure of the cells to agents that raise intracellular levels of cAMP, such as dibutyryl cAMP (dbcAMP), isobutylmethyl xanthine (IBMX), hydroalcoholic extracts of leaves and flowers of *P. venusta* and Kaliziri extract (KZ) [20,26,27]. Here we present a microscopic approach through which it becomes clear that tyrosinase of *P. ostreatus* has various biological advantages such as gentle effectiveness and without cytotoxicity. It is a potent mushroom tyrosinase for darkening and photoprotection. From the data of the present work, it is also concluded that tyrosinase is the key in regulation of melanin production; the study conducted on the effect of purified tyrosinase absorption and its effects has shown that mushroom tyrosinase as an effective agent for melanogenesis and can serve as a potent melanogenic agent to cure vitiligo.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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