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Full Length Research Paper

Melanin inhibitory and melanin stimulatory effects of extracts of *Chlorophytum tuberosum* and *Chlorophytum borivilianum* on isolated fish scale melanophores

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The epidermal scale melanophores of fishes are disguised type of smooth muscle cells and behave differently when exposed to certain hormones and drugs; however, their responses to plant extracts have not been studied. *Chlorophytum* species holds an important position in Indian herbal medicine; hence, in the present investigation, effects of extracts of two species of *Chlorophytum,* that is, *Chlorophytum tuberosum* and *Chlorophytum borivilianum* have been studied on the isolated scale melanophores of the teleost fish, *Channa punctatus.* The lyophilized extract of tubers of *C. tuberosum* had a melanin aggregating effect causing paling of the skin; the action seems to be mediated through alpha adrenergic receptors present dominantly on fish melanophores. On the other hand, the extract of tubers of *C. borivilianum* had a melanin dispersing effect within the fish melanophores inducing darkening of the skin and the responses seem to be mediated probably through beta adrenergic receptors.

Key words: *Channa punctatus,* melanophore aggregation, *Chlorophytum tuberosum, Chlorophytum borivilianum.*

INTRODUCTION

India is bestowed with a wealth of medicinal plants, most of which have been used since ages in Ayurveda and Unani systems of medicines and by tribal healers. Safed musli (Chlorophytum species) holds an important position in Indian herbal medicine. The role of Chlorophytum species in medicinal world dates back to classical Ayurveda references of 10th and 11th century where its particular properties such as an aphrodisiac, digestive power, rejuvenator, and immunomodulator are well known. However, there are no studies on the effects of their extracts on pigment cells including human melanocytes. The epidermal melanophores of lower vertebrates which are disguised type of smooth muscle cells behave differently when exposed to certain hormones and drugs. Their prolonged stimulation can cause accumulation of large amount of cytocrine melanin in the granules, which control long lasting slow skin colour changes, as well as rapid physiological ones (Fujii,1969; Bagnara and Hadley, 1973), were found to be mediated through cholinergic drugs. Some other types of cellular receptors, mediating the intracellular melanin granule dislocation, have been reported in a large number of lower vertebrates such as fishes, amphibians and reptiles (Watanabe et al., 1962; Reed and Finnin, 1972; Katayama et al., 1990, 1999; Ali et al., 1995, 1998; Peter et al., 1996, 2011). As these melanophores have contractile melanosomes which contain black pigment melanin, they offer excellent opportunities for studying the effects of various compounds, drugs and hormones in order to understand the complex phenomenon of melanogenesis.

Except for a few reports, there are no scientific studies where the pigment cells, that is, melanophores and melanocytes have been investigated for the effects of

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various plant extracts, despite the fact that some preparations containing *Psoralea corylifolia* extracts have been used for centuries in tropical preparations in Chinese medicines for the treatment of vitiligo which is a melanocyte disorder. Similarly, another plant *Ammi majus* belonging to Apiaceae family has been used in traditional Unani system of medicine for treatment of vitiligo and leucoderma because of its high xanthotoxin contents (Ahmed and Garg, 1998). Thus, there are little or no studies conducted on the effects of extracts of traditionally known melanogenic or melanolytic plant extracts on the vertebrate melanophores / melanocytes including human beings.

In the present work, tuber extracts of two species of *Chlorophytum,* that is, *Chlorophytum tuberosum* and *Chlorophytum borivilianum* have been used to study their effects on isolated melanophores of *Channa punctatus*, in order to find whether any of the extracts have melanogenic or any other properties which can be used for exploring their candidature as a pigment receptor modulator.

MATERIALS AND METHODS

The tubers of *C. tuberosum* and C*. borivilianum* were sundried and powdered. Then the powdered material was dissolved in water overnight. The material was then centrifuged. The supernatant liquid was filtered through a 0.45 µ membrane filter paper. The filtrate was then lyophilized. The lyophilized material was used for further study. The freshly prepared aqueous solution of lyophilized powder was used for each experiment.

Experiments were performed on isolated epidermal melanophores of the teleostean fish, *C. punctatus*. The fishes were purchased from the local market and brought to the laboratory alive and were kept in a large aquaria at room temperature which ranged between 25 and 30°C. The fishes were fed with comme rcial fish food thrice a week. Care was taken to maintain the fishes in healthy conditions. Those fishes which had infections or which showed even slight sluggishness were immediately discarded. Experiments were conducted on either sex of the animals. Selection of fishes was done, in which, uniformity in size and body weight were kept in mind. All fishes used were of average length of 12 to 16 cm and body weight of 30 to 40 g. The scale melanophores were found to remain in an intermediate state of neither dispersion nor aggregation in 0.7% fish saline for about 30 min (Ali, 1983).

Experimental procedure

The scales were removed from the dorsolateral region of the live *C. punctatus* and immediately transferred in 0.7% fish saline in transparent glass Petri dishes of 8 cm size (Spaeth, 1913). The scales of *C. punctatus* from the dorsolateral region were found to have a uniform population of melanophores. Moreover, the size of the melanophores was found to be almost equal.

Measurement methods

Control recording of the actual size of the diameter of the melanophore was done by using Leitz Occular micrometer, calibrated previously with 10×10 magnification of the microscope (Bhattacharya et al., 1976). Actual diameter of 10 melanophores from each scale was recorded; the selection of such melanophores for measuring the size was done at random as almost all the melanophores in a scale were of equal size. The measurement involved the actual diameter of the melanophore (length × breadth of the melanophores with the process). The value was then multiplied by the unit of the micrometer which was 15 µ. Thereafter, the mean was calculated and this value was then divided by 100 to obtain the values in a digit with three decimal points. This was mean melanophore size index (MMSI).

Scales after being removed from the dorsolateral sides of *C. punctatus* were placed in 0.7% fish saline. Two of the scales were transferred in a Petri dish containing the same saline, and the MMSI of the group of ten melanophores was recorded from these control scales. For the treatment of melanophores with various compound concentrations, two scales were placed in each Petri dish, in 0.7% fish saline to which known concentration of the aqueous crude extract of lyophilized powder of *C. tuberosum* and *C. borivilianum* was added. As much as ten of such pairs of scales were used in different dishes, with each dish having a concentration of plant extracts. After a constant incubation period which ranged between 7 and 10 min, the MMSI of ten of such treated melanophores from each concentration was recorded. Thus a set of experiment comprised the measurement of responses of about hundred melanophores.

The standard error (±SE) of the mean values of MMSI was calculated using the standard methods of statistical analysis (Lewis, 1971). The drug solutions of *C. tuberosum* and *C. borivilianum* were prepared freshly before use in distilled water. In the present study, the concentrations of extracts are expressed in g/ml.

RESULTS

It was found that isolated scale melanophores of *C. punctatus* remained in the state where there was neither dispersion nor aggregation, in 0.7% fish saline. The mean melanophore size index (MMSI) of such control melanophores was found to be 4.296 ± 0.194 (Figure 1). The lyophilized extract of tubers of *C. tuberosum* was added in the incubating medium of the normal melanophores in the concentration range of 5×10^{-4} to 3×10^{-3} g/ml. The extract was in contact with the melanophores for about 5 to 7 min.

It was observed that the *C. tuberosum* tuber extract displayed a clear cut melanophore aggregation effect. Its extract in the aforementioned concentrations produced a very powerful aggregation effect on all the melanophores in a dose dependent manner. The maximal concentration of 3×10^{-3} g/ml of *C. tuberosum* extract decreased the MMSI level from a control value of 4.296 ± 0.194 to 0.89 ± 0.682 (Figure 1). The melanophores at this stage had aggregated to a great extent.

When these *C. tuberosum* extract treated scales with highly aggregated melanophores were again immersed in normal saline, and 2 to 3 saline changes were made; it was observed that the aggregation effect began to diminish and after 10 min this effect had completely disappeared. The melanophores had re-dispersed to normal state. The MMSI of these melanophores was

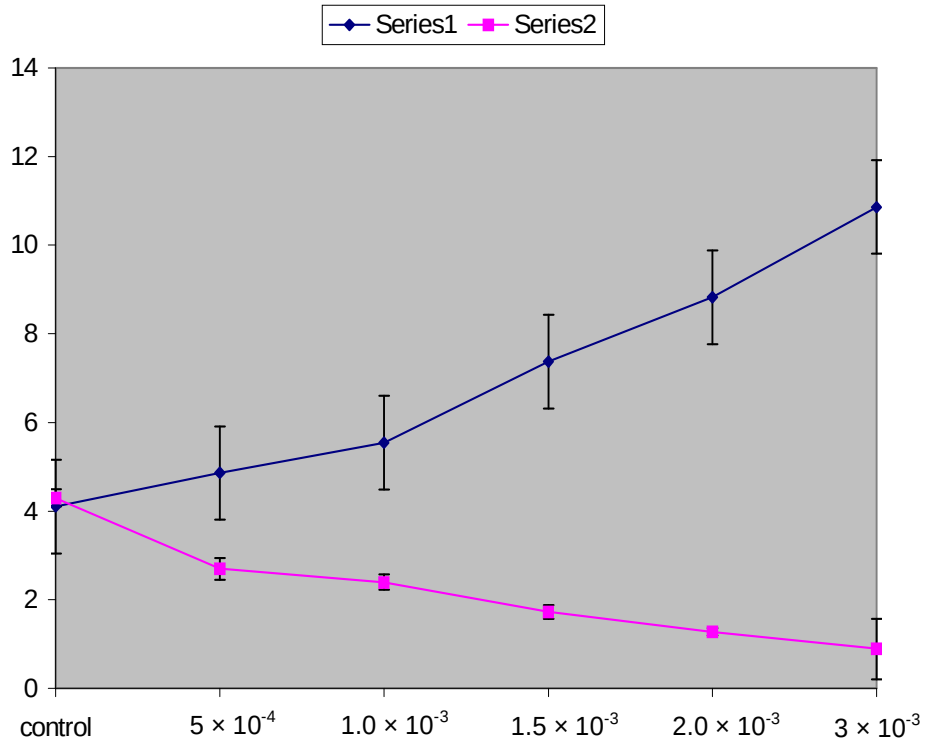

Figure 1. Series 1: MMSI of *C. borivilianum*. Series 2: MMSI of *C. tuberosum.*

found to be 4.00 ± 0.186. The dose response curve for the melanophore aggregation effect of *C. tuberosum* extract is shown in Figure 1.

On the other hand, when the lyophilized extracts of tubers of *C. borivilianum* were tested for their effects on the isolated melanophores of *C. punctatus*, it was found that the extract evolved an opposite effect that is, causing severe melanophore dispersion, making the scales to appear dark. The dose as low as 5×10^{-4} g/ml induced an almost immediate measurable dispersal response within 4 to 5 min. The MMSI increased from a control value of 4.104 ± 0.232 to 4.86 ± 0.264 by this concentration of the extract of *C. borivilianum*.

Further increase in concentrations of *C. borivilianum* extract, increased the magnitude of the dose dependent dispersal response of the melanophores (Figure 1). At the maximal concentration of 3×10^{-3} g/ml, *C. borivilianum* extract caused a full dispersion of all the melanophores. The intensity of dispersion of melanophores is shown in Figure 1. The MMSI had reached a level of 10.864 ± 0.338. The dispersal effect induced by *C. borivilianum* extract was found to be reversible as placing the *C. borivilianum* extract dispersed the melanophores in 0.7% fish saline reducing the MMSI from 10.864 ± 0.338 to 4.42 ± 0.85 within 8 to 10 min (Figure 1).

DISCUSSION

The results of present study have shown that the extract of tubers of *C. tuberosum* induced dose dependent melanophore aggregation leading to paling of fish scales which seems to be mediated by alpha adrenergic receptors present dominantly on the fish melanophores. It appears that extracts of *C. tuberosum* chemically contain adrenaline-like compounds which activate the dominantly present alpha adrenergic receptors, to induce melanin aggregation of the fish melanophores. These data are in corroboration with findings of many earlier workers who have reported that adrenaline causes aggregation of fish melanophores (Svensson et al., 1993; Aspengren et al., 2009; Reed and Finnin, 1972).

In the present study, the aggregation responses of *C. punctatus* melanophores caused by tuber extracts of *C. tuberosum* are similar in action to that of most of the aggregating agents like adrenaline, nonadrenaline, phenelephirine and reserpine which induce distinct aggregation of *C. punctatus* melanophores (Ali et al., 1985a, b; Ali, 1986; Aspengren et al., 2003). The aggregation response in pigment cells is mediated through alpha 1 and alpha 2 adrenergic receptors which are found to be dominantly present in these species (Ali and Ovais, 1983).

On the contrary, it was found that in the present study, the extracts of tubers of *C. borivilianum* caused a powerful dose dependent melanophore dispersion leading to darkening of the fish skin. The action seems to be mediated by beta adrenergic receptors present on teleost fish melanophores. These data are in corroboration with earlier findings of some workers who stated that adrenaline can also cause melanophore dispersion, in selected concentrations. The beta adrenergic receptors are stimulated by the chemical compounds present in the extract. This activation of the beta receptors induced melanophore dispersion via adenyl cyclase second messenger system (Breeder and Rasquin, 1955; Miyashita and Fujii, 1975; Ali et al., 1985).

These data are supported by the earlier observation (Ovais, 1994; Ali, 1983; Ali et al., 1985a, b) that beta receptor activation via cyclic AMP, as a second messenger, induces melanophore dispersal in the same fish *C. punctatus*. Earlier studies on frog melanophores also substantiate the fact that there exists a mosiac population of cellular receptors on melanophores which when activated cause dispersion or aggregating response (Longshore and Horowitz, 1981; Ali et al., 1995, 1998, 2011). These data are quite interesting as for the first time it is being documented that extracts of the *Chlorophytum* species can cause both melanin inhibitory as well as melanin stimulatory effects.

Burton (2008) has reported that *Pseudopleuronectus americanus* melanophores display pattern related transition ranges in noradrenaline concentrations between those including beta adrenoceptors mediated melanophore dispersion and those stimulating alpha adrenoceptor mediating aggregation. These findings have demonstrated that a physiological concentration of noradrenaline has the capacity to produce simultaneously, melanosome aggregation and dispersion in different localized areas of the colour patterns in fishes. It has also been found that catecholamine and related substances caused aggregation *in vivo* and *in vitro* confirming the presence of an aggregating mechanism in *Oreochromis mosambica*. The melanophores of this species have been demonstrated to possess predominant alpha 2 receptors causing aggregation (Acharya and Ovais, 2007). In adult scales, propranolol enhanced the melanosome aggregating response of epinephrine and isoproterenol, but not norepinephrine, indicating that beta adrenoceptor mediates melanosome dispersing response in adult Zebrafish. Similar response was not observed in embryos until 60 h post – fertilization (hpf). The melanophore adrenoceptor blocking effects of phentolamine and propranolol in embryos were much lower than that in adult Zebrafish, suggesting that these adrenoceptors in developing melanophores are less sensitive to the classical antagonists (Xu and Xie, 2011). Very recently, Galgut and Ali (2011) have reported that ethanolic extract of *Arachis hypogeae* and its active ingredient resveratrol induced melanophore aggregation of tadpole *Bufo melanostictus* via adrenergic receptors. Thus, these findings open new areas where plant extracts and their active ingredients can be used as novel depigmenting agents with low toxicity.

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