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Effect of elemental iodine and hair dye on pigmentation of *Duttaphrynus melanostictus species*

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Abstract
The preliminary investigation was conducted to investigate influence of iodine and hair dye on pigmentation during metamorphosis of *Duttaphrymus* species. The series of iodine and hair dye concentration as 0.5, 1.0, 1.5, and 2.0 ppm were maintained along with control. The rate of pigmentation during metamorphosis was very early on the 18th day at the concentrations of 1.0 and 1.5 ppm of iodine than control than control (on 24th day). In case of hair dye there was an initiation of the pigmentation was very late compare to the control one. Our result indicates the effect of hair dye on the pigmentation of the *Duttaphrymus* species.

Keywords: Pigmentation, *Duttaphrymus melanostiotus*, metamorphosis, iodine, hair dye

1. Introduction
Amphibians are the best examples for the progressive metamorphosis. It is most important developmental stage in tadpoles with gradual changes from aquatic to terrestrial life. The progressive metamorphic changes include progressive development of the limbs, which increase in size and differentiation. The amphibian metamorphosis is under neuro-endocrine control, involving neuro-secretory cells in the brain (the hypothalamus) and two endocrine glands, the pituitary (anterior pituitary) and the thyroid. During this stage there are number of morphological changes such as feeding habit, keratinization, the appearance of limbs and total regression of tail due to apoptosis. The process of apoptosis occurs in four stages. I. Decrease in protein synthesis in striated muscle cells of the tail region [1, 2, 3]. II. Increase in concentrations of digestive enzymes within the cells, such as lysosomal proteases, RNase, DNase, collagenase, phosphatase, and glycosidases all rise in the epidermis, notochord, and nerve cord cells [2]. III. Cell death is due to release of metalloproteinase inhibitor [3]. The gills of the tadpole slowly close over as the animal develops lungs and becomes a mouth breather, as such its mouth becomes wider [4]. The trigger to metamorphosis may be an environmental signal affecting the larval brain through the nervous system, this drastic change in amphibian metamorphosis is due to diffusion of the hormones such as thyroxine (T4) and tri-iodothyronine (T3). Among these, T3 is more active hormone. The release of T3 is under the control of the hypothalamus of brain. The other hormone Thyroxine (T4) is more organ specific than tissue specific in action. The importance of the thyroid gland and thyroxine on metamorphosis is proved [5], Krishnapriya *et al*., 2014 [1]. When a thyroid gland is removed from young tadpoles, they fail to metamorphose [6]. Normally, anurans tadpole is about 60-70mm in length. But thyroidectamised tadpole grows to a length of about 123 mm, when a thyroid-less tadpole is fed with dried thyroid gland, it proceeds to metamorphosis. Similarly a thyroid-less tadpole can be stimulated to undergo metamorphosis by rearing the tadpole in water containing powdered thyroid gland [7].

The Asian common toad *Duttaphrynus melanostictus* is a widely distributed species in South Asia. It inhabits India, Sri Lanka, Taiwan and south western including southern China, southward through Southeast Asia to Indonesia (Shieh 1993). Adult females of this species grows to almost 20 cm long [3]. This species is commonly distributed in villages, towns, and open areas, and only seen occasionally in primary forest. In winter, the species can hibernate in mud holes (Nguyen *et al*. 2005; Lin *et al*. 2011). Most investigations of anuran reproduction are those species inhabiting the temperate zone where reproductive patterns reflect the annual climatic cycle (Jørgensen *et al*. 1986; Wells 2007). *Duttaphrynus melanostictus sp*. was found in terrestrial habitat, restricted in vegetations surrounding ponds in and around Mysuru district, Karnataka. All clutches of eggs were placed directly above water, thus protected from the sun. Number of eggs in one clutch is variable. Breeding female will lay all of her eggs, clumped into a single clutch. Eggs are unpigmented, whitish in color, coated with sticky jelly substances. Fertilized eggs will hatch after 15-16 days and tadpoles will emerge in water. Tadpoles will stay for 48-50 days growing its body before moving to the next stage. After about two months tadpoles will develop into stage where hind limb develops. In the next 10 days forelimb will emerge. It takes about three months for an egg to become a fully metamorphosed frog [20]. Present study is taken to assess the developmental changes during have undergone feeding experiments for assessment of developmental changes during metamorphosis of *D. melanostictus* species with different concentrations of Iodine and hair dye. The hair dye naturals (Ammonia free) permanent hair colour cream is used as test same as early survey indicated good number of people use this as a hair dye. The wash off from the hair /scalp is entering into water bodies affecting the fauna. The hair dye contains more than 100 chemicals. All hair dye has common ingredient resorcinol (dihydroxy benzene). Benzene is one of the most basic petrochemical, known to increase the risk of causes. It also inhibits thyroid peroxidase enzymes disruptions thyroid harmone synthesis Para-phenylenediamin causes skin problem like swelling, utricaria leading to depigmenatation. The present study is undertaken to study and correlate the effect of hair dye on the neuroendocrine system leading to formation of pigmentation in metamorphosis larvae.

2. Materials and Methods

Experiments were conducted on tadpoles of *Dattaphrynus melanostictus* spices hatched from the same egg mass collected from a small temporary pond in Mysuru, Karnataka. Adult species from the pond was caught and brought to laboratory for identification by a Zoologist Dr. Rajanna. K.

Chemicals and Apparatus

Iodine anhydrous, Sigma (St. Louis, MO, USA). Black Garnier Brand NH_3 free (hair dye purchased from local market), Glass jars (5 liters), Pond water, measuring glass Jar, Glass trough, Hand Lens.

Methodology

70-80 Healthy tadpoles of 2-3 days old were collected from an artificial tank in Mysuru, Karnataka, which was mentioned above. The collected tadpole were identified as *Dattaphrynus melanostictus.* The various concentrations of 0.5ppm, 1ppm, 1.5ppm and 2ppm, of black hair dye in triplet concentrations along with control were prepared using pond water. Nearly 15 tadpoles were weighed, was noted and transferred into glass jars with a capacity of 5 liters. Jars were labeled according to their concentration. The environmental factors such as temperature, pH, aeration, light and water supply were kept uniform. Each culture consisted of ten larvae in 2L of water collected from their natural habitat. The water was changed periodically to prevent fouling. The above procedure was followed for iodine feeding assay according Swingle (1917) [18]. The tadpoles were maintained in different concentrations of iodine and hair dye of black and observed every day to see the rate of pigmentation in comparison to other groups. (Krishnapriya *et al.*, 2014) [1].

Statistical Analysis

All the test samples were performed out independently in triplicates, data is expressed as the numbers of pigments formed and their mean value is denoted by star symbol the results were processed using SPSS 10.

3. Results

Iodine embryonic development of *D. melanostictus* sp. was studied in the laboratory to assess the effect of Iodine and hair dye on pigmentation. The tadpoles measuring around 1.5 cm in length, which were utilizing yolk since their emergence from the egg jelly, were let into container having iodine and hair dye. They completely metamorphose into more or less adults till they retrieve their tail approximately after 45 ± 50 days. The effect of anhydrous iodine on pigmentation formation of *D. melanostictus* spices was given in the table 1. No change was observed until 8 days in the cultures. After 8 days careful microscopic examination with the helpful of dissection microscopic and magnifying lens revealed that 1.0ppm and 1.5ppm iodine group showed slow pigmentation in the tadpoles starting from 18 days and it was very clear till 36th day which was 24th day in control one. At the concentration of 1.0 ppm on 36th day there was pigmentation which was towards the shrinking tail; this was not observed in control or in other concentrations of iodine groups (Table1). Interestingly, in the higher concentrations at 2.0 ppm there was observation of death of tadpoles with no indication of pigmentation.

Table 2. Indicates the effect of pigmentation in *D. melanostictus* tadpoles exposed to upon hair dye. The tadpoles in hair dye variable in pigmentation process in tadpoles. The initiation of pigmentation was observed on 36th day for 0.5 ppm; and it was clearly notified till 45th day; in case of 1.5 ppm concentration there was no such clear pigmentation was observed on 36th day it was started on 40th day and it was on 45th day in higher dose (2.0 ppm). In case of control group pigmentation was observed from the day 28th, it was clearly notified till end of the 45th after it was difficult to indentify clearly. There was variation in the formation of pigmentation in compare to the day with different dose.

4. Discussion

The pigmentation rate in *D. melanostictus* varies as the metamorphosis proceeds. As the metamorphic rate increases the pigmentation rate also increased. (Table 1 & 2). According to some reports (*Krishnapriya et al., 2014*) [1] feeding of iodine and its compounds; iodoform and potassium iodide- greatly accelerate metamorphosis of tadpoles. With very high iodine percentage, metabolism is stimulated to such an extent that the animal emaciate rapidly and die early before there is time for cellular differentiation. In comparison lower to iodine percentage, the size of animals is roughly inversely proportional to the percentage of iodine administered. Overdose with iodine or thyroid extract leads to decrease in size and great emaciation of the animals [18]. The iodine in minimum doses stimulates growth, though not to any great degree, and in large doses it leads to cessation of growth and tissue disintegration or absorption. Iodine feeding has little effect on pigmentation. Our results also suggests that higher dose of the iodine has also lead to the death of the *D. melanostictus (*from the day 24th) tadpoles.

According to table 1, it is known that administration of certain concentration of iodine (1.0ppm and 1.5ppm) will initiate the early pigmentation than compare to control one as well as in least concentration. This shows there is influence of the iodine in certain concentration of the studies. But this trend was not followed in the case of Hair dye feeding method (table 2). Careful scrutiny of the table 2 also suggest that there is a late pigmentation in the *D. melanostictus* when exposed to the different concentration of the Hair dye when compare to the control one. It also implies its endocrinological influence to bring out a comparative analysis of the influence of iodine and hair dye concentrations on pigmentation during the metamorphosis of *D. melanostictus.* Thus we can conclude elemental iodine administrated to *D. melanostictus* tadpoles in sufficient amount brings about rapid metamorphosis than the control one. This is not observed in case of the hair dye where there is effect on the pigmentation of the tadpoles.

Table 1: Pigmentation during metamorphosis of *D. melanostictus* upon iodine feeding

Day of development	0.5 ppm	1.0 ppm	1.5 ppm	2.0 ppm	Control
2	-	-	-	-	-
8	-	-	-	-	-
18	-	*	*	-	-
24	*	**	*	0	*
28	**	***	**	0	**
32	***	****	***	0	***
36	****	*****	****	0	****

-No Pigmentation;
* Initiation of pigmentation;
**Pigmentation was found to spread on the whole body;
***Pigmentation towards the hind limbs;
****Pigmentation towards fore limbs and head;
*****Pigmentation was towards the shrinking tail,
0= death

Table 2: Pigmentation during metamorphosis of *D. melanostictus* upon Hair dye feeding

Day of development	0.5 ppm hair dye	0.1 ppm hair dye	1.5 ppm hair dye	2.0 ppm	Control
2	-	-	-	-	-
8	-	-	-	-	-
18	-	-	-	-	-
24	-	-	-	-	*
28	-	-	-	-	**
32	-	-	-	-	***
36	*	-	-	-	***
40	*	*	*	-	****
45	**	**	*	*	****

- No Pigmentation;
*Initiation of pigmentation;
**Pigmentation was found to spread on the whole body;
***Pigmentation towards the hind limbs;
****Pigmentation towards fore limbs and head;
*****Pigmentation was towards the shrinking tail

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