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Differentiation of skin and skin glands of *Bufo melanostictus* (Schneider) under the influence of vitamin A

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Abstract: The effect of vitamin A on differentiation of skin of various regions with particular reference to regional distribution of skin glands of *Bufo melanostictus* (Schneider) tadpoles of various developmental stages was evaluated. All experiments were carried out on young tadpoles of stage 25, 30, 34 and 36 of this toad species. Vitamin A has been found to affect differentiation of skin in the toad tadpoles and the effect is more severe on younger tadpoles as compared to the older ones. The tadpoles of stage 25 when treated with vitamin A, distinct effect was swelling of tadpoles particularly in the ventral region. Integument became transparent and the cellular organization of integument has disrupted severely and cells appear to be swollen mucoid type. Such condition is known as mucous metaplasia. Vitamin A treatment causes mucous mataplasia both in the dorsal and ventral region in all stages (stage 25, 30, 34 and 36) of tadpoles of *Bufo melanostictus*. Vitamin A induced mucous metaplasia is greater on the ventral region as compared to the dorsal region of the body. Vitamin A treatment has been found to inhibit differentiation of skin glands. The characteristic swelling as a result of vitamin A treatment is in not found in the tadpoles of older stages such as stage 36. Tadpoles of discontinuous treated group showed gradual recovery from the damaging effects produced by vitamin A in the skin in all stages, although tadpoles do not acquire complete normal morphology but they undergo metamorphosis. Glandular differentiation is resumed in tadpoles of discontinuous group of all stages.

Keywords: Anuran, Dermis, Epidermis, Melanophores, Mucous metaplasia, Tadpoles.

Vitamin A and its derivatives (retinoids) are known to affect differentiation, morphogenesis and growth of vertebrates. Besides influencing morphogenesis in a wide variety of cells and tissues, retinoids have been observed to produce specific effect on cell differentiation particularly that of epithelial cells and appendages[1].Vitamin A has been observed to cause complete suppression of epidermal keratinization and transformation of epidermal cell into ciliated and secretory type[2]. In mammalian system it was observed that in the presence of modified vitamin A levels trachiobranchial epithelium of *Rabbit* became squamous type which ultimately differentiated into keratinocytes in retinol free medium[3]. Similarly the transformation of avian foot scale into feather bearing scale by vitamin A[4]. It has also been found to affect metamorphosis of anuran tadpoles[5, 6, 7]. When frog and toad tadpoles were given exogenous administration of their rearing medium, besides, variety of teratological effects, their metamorphosis was delayed or inhibited[7]. The studies related to development of skin glands have been described with respect to particular region of skin. Treatment of tadpoles with exogenous thyroxine had revealed differences in the rate of development among the two types of glands i.e. mucous and granular glands[8, 9]. Normal development of the skin glands of the dermal plica has been observed in *Rana pipiens* [10,11] and *Ranas ylvatica* [12]. The development of the skin glands had been found to be directly dependent upon thyroid in the tadpoles[8, 13, 14]. The sensitivity of the epidermis to thyroid appears very early but the response to the hormone manifested by development of these glands occurs much later and at different times in different regions of the body[8, 13, 11, 15, 16]. Verma[14] reported that the skin glands of *Rana pipiens* develop sequentially at different rates in different regions of the body. Very less is known how vitamin A influences differentiation of skin and its glands in amphibians. In view of the above the present study was carried out to study differentiation of skin and skin glands in different regions of the body of tadpoles of *Bufo melanostictus* (Schneider) at different stages of development.

Materials and Methods

The present study was under taken on young and advanced tadpoles of the common Indian toad, *Bufo*

melanostictus (bufonidae, anura, amphibian). This toad is found in abundance in and around Jaipur and Ajmer. It hibernates during winter and in other seasons it remains hidden during the day and from March on wards it comes out at dusk and can be collected easily in the nights and like other many Anuran, it breeds during monsoon. The spawning takes place in shallow pools and ponds where the eggs are found in long double strings on the surface of water or entangled in between water plants. Generally these animals lay eggs in the early hours of the morning after a rain following a warm day. In laboratory conditions (29-32*C) hatching takes place in less than 24 hours after spawning and the larval period lasts for about four weeks from hatching to the end of metamorphosis. The spawn collected from the field hatched in the laboratory aquaria. The tadpoles were maximally fed with semi-boiled spinach every day. The tadpoles were distributed in several tanks and plastic troughs to avoid overcrowding. The water of aquaria and troughs was also changed every day to avoid contamination. The tadpoles grew well in such conditions and there was negligible mortality. All experiments were carried out on young tadpoles of stages 25, 30, 34, and 36 of this toad species. The stagination was done according to the normal table of development of *Bufo melanostictus* [17]. For observations living tadpoles were anaesthetized by immersing them in 1:4000 working solution of MS 222 (Ethyl-m-aminobenzoatemethanosulfonate Sandoz) within few minutes and they could remain (1)under anaesthesia for about 30 minutes without any fatal results. On being transferred to ordinary water they revived in a few minutes.

Experimental design

Tadpoles at each developmental stage were divided into three experimental groups: (1) Group A, tadpoles were reared in ordinary water throughout the period of experiment (control group), (2) Group B, tadploes were reared in vitamin A palmitate (1 1U/ml-sigma) and (3) Group C, tadpoles were treated with vitamin A palmitate 1 IU/ml (sigma) for three days and then transferred to tap water for the remaining twelve days.

Chemicals used:

(1) Nuclear fast red: 0.1 gm of nuclear fast red was dissolved it in 100 ml of 5% Alumimium sulphate in water. Heated and stirred the solution cautiously up to 2-3 hours and then cooled and filtered it. (2) Azan: 0.5 gms aniline blue, 2.0 gms Orange G and 2.0 gms Oxalic acid were dissolved in 100 ml distilled water, boiled for 10 minutes and allowed it to cool for some time and finally filtered it. (3) Phospomolybdic Acid: 1.0gmphosphomolybdic acid was dissolved in 100 ml of distilled water. (4) Vitamin A palmitate , the preparation used was water dispersible powder of vitamin A palmitate , type VII (sigma) . To study its effects, the tadpoles were immersed in aqueous suspensions of IU / ml of this vitamin for the required period of time according to the experimental design. One gram of this powder contains 250000 international units (IU/U.SP) of vitamin A palmitate. The aqueous suspensions containing 1 IU /ml of the vitamin A were prepared by dispersing 4 mg of the powder, respectively, in one liter of tap water. Normal development and differentiation of skin was observed in the tadpoles of *Bufo melanostictus* at stage 25, 30, 34, and 36 of development. Effect of vitamin A on skin gland differentiation was observed for these developmental stages. For vitamin A treatment tadpoles of different development stages were reared in 1 IU/ml solution of vitamin A palmitate (Sigma) for varying periods.

Schedule of Fixation:

Tadpoles of different experimental groups were fixed at 1 day, 2 day, 3 day, 4 day, 5 day 6 day, and 15 day following treatment.

Parameters of study:

Temporal and spatial pattern of differentiation of skin and glands. Tadpoles fixed at different close intervals were sectioned serially and stained for visualization of various components of skin, particularly the basement membrane and skin glands. The serical sections stained with modified azan[18] were also used for histo-chemical localization of muein, collagen and fibers. For morphological studies, the tadpoles were examined under steresoscopic binocular microscope. They were sketched with the help of camera Lucida and representative cases were photographed. For histological examination, the

tadpoles were processed through the steps of dehydration and clearing and then embedded in paraffin wax. The tadpole was sectioned transversely and serially at 6u thickness and then stained with aniline blue and orange G according to the modified azan staining technique[18]. The steps in sequence for this technique are given below:

1.	Xylene	15 minutes
2.	Xylene	15 minutes
3.	Absolute Alcohol	10 minutes
4.	90% Alcohol	10 minutes
5.	70% Alcohol	10 minutes
6.	50% Alcohol	10 minutes
7.	Distilled water	10 minutes
8.	Nuclear fast red	30 minutes
9.	Distilled water	Wash for 3-4 minutes
10.	Phosphomolybdic Acid	1 minutes
11.	Distilled water	Wash for 2 minutes
12.	Azan	5 minutes
13.	Distilled Water	Wash for ½ minutes
14.	Differentiate in 90% alcohol	Few d
15.	Absolute alcohol	15 minutes
16.	Xylene	15 minutes
17.	Xylene	15 minutes
18.	Mount in D.P.X using No.0 or No. 1 cover glass.	

Results

Stage 25 Untreated (control) Group A

In the stage 25 (Fig.1) differentiation of skin is not much advanced and is represented as a single layer of epidermis without skin glands throughout the body. In the subsequent three days skin is still represented by single layer of epidermis with a thin basement membrane but skin glands were absent. Pigments were present in the integument which is clearly indicated even in the morphological appearance of the tadpoles. The pigment melanophores were present even in skin of very young tadpoles. These melanin containing chromatophores were present mainly in the dermis. Melanophores were generally absent on the ventral side. Tadpoles had reached to stage 36 on the 15th day. These tadpoles were having well differentiated skin with pigmentation. Skin was still made up of 1 to 2 cell layer thick epidermis. On the dorsal side mucous glands were present. In morphological appearance iridophores, the reflecting pigments also indicated.

Stage 25 Vitamin A treatment (continuous) Group B

The tadpoles of stage 25 treated with vitamin A palmitate for fifteen days indicated that vitamin A treatment affects differentiation and development of skin of *Bufo* tadpoles (Fig. 2). There was no great effect of vitamin A treatment up to 2nd day. On 3rd day the distinct effect was observed as swelling particularly on the ventral region of tadpole. Integument became transparent. The cellular organization of integument had disrupted severely affected and cells appeared to be swollen and mucoid type. Such type of condition is known as mucous metaplasia. The skin of dorsal region did not show mucous metaplasia as compared to the ventral region. Melanophores were present in the dermis but basement membrane became irregular and wavy. Subsequent treatment of vitamin A caused disruption of basement membrane and dermis resulting in disorganization of epidermis. Most of the tadpoles died on 15th day post treatment and those who survived exhibited highly retarded morphogenesis. In these, skin became thin and transparent particularly in the ventral region. Due to disruption of basement membrane the epidermal organization became wavy and irregular. The tadpoles became swollen because of excessive mucous secretion by the epidermal cells. Mucous glands and serous glands are generally absent in these treated tadpoles.

Stage 25 Vitamin A treatment (discontinuous) Group C

The tadpoles which were removed from vitamin A solution after three days of treatment gradually showed recovery from the vitamin A effects. One day after removal of vitamin A the basement membrane appeared below the epidermis (Fig. 3). In these tadpoles melanophores were present in the dermal region and basement membrane is present below the single cell layered epidermis. The epidermis of ventral region still remained wavy as observed on 2nd day after removal from vitamin A and their outermost layer is covered by a layer of mucous. Third day after

removal the swelling caused by vitamin A treatment is reduced and tadpoles showed normal appearance. Tadpoles of discontinuously treated group showed almost normal skin architecture in the dorsal region including pigments and occasional skin glands. In the ventral region also epidermis became normal and cells did not show muco metaphasis.

Stage 30 Untreated (control) Group A

In the tadpoles of stage 30 also organization of skin was very simple similar to that of control of stage 25(Fig.1).

Stage 30 Vitamin A treatment (continuous) Group B

Tadpoles of stage 30 treated with vitamin A for one day showed mucoid skin on dorsal as well as ventral and lateral regions (Figs. 4). Mucous glands were present in the epidermis and dermis became very thin. Melanophores were present both in the epidermis as well as in the dermis. The epidermis became very wavy; this feature was very prominent as vitamin A caused thinning of skin of ventral region and due to excessive secretion of mucous there is swelling. However, such swelling and thinning of epidermis was not observed in the dorsal region. Mucous metaplasia has further advanced in the tadpoles treated with vitamin A for 4th and 5th day. In these cases vitamin A caused degradation of dermal organization and pigmentation, mucous glands had significantly increased in the dorsal and ventral region. Skin of the ventral region becomes very thin and wavy. Dermis was nearly absent in treated tadpoles in the ventral and lateral region. Subsequent treatment of vitamin A from 6 to 15 days severely affected organization of skin of both dorsal and ventral region. Most of the tadpoles died between day after vitamin A treament Histogenesis of those which survived showed cells lost their compact arrangement, basement membrane and dermis gets diffused or absent. Morphology of tadpoles of stage 30 treated with vitamin A for fifteen days showed in ventral and dorsal region. In histology cellular organization was totally disrupted in the skin and distinct epidermal cells and dermis were absent and skin appeared to be dead.

Stage 30 Vitamin A treatment (discontinuous) Group C

The tadpoles treated with vitamin A for first three days and then transferred to ordinary water for the remaining period of experiment showed recovery from the effects of vitamin A in a gradual manner (Figs. 5). One day after rearing in water following three days of vitamin A treatment showed epidermis, a basement membrane and dermis in the dorsal region of tadpoles. Similarly such recovery features were observed in the tadpoles which were reared in water for 2 to 3 days following first three days of vitamin A treatment. Morphology of these tadpoles showed normal pigment epidermis of the dorsal region. There was swelling due to excessive secretion of mucous in the ventral part of the body and relatively thin skin still persisted even three days of discontinuous treatment. On 15th day the skin of the dorsal region showed significant recovery from the effect which was produced as a result of excessive vitamin A treatment. These tadpoles showed even skin glands in the dorsal region while the skin of ventral region was still like muciod type. In the morphology the body is still showing oedema due to mucoid nature of ventral skin but dorsal region showed quite a normal skin.

Stage 34 Untreated (control) Group A

Integument of stage 34 tadpoles of group A observed on first day after rearing in water show thin epidermis and dermis. Pigments were present both in epidermis as well as in dermis (Fig. 6). Skin of the ventral region of the body was also very thin and melanophores were either rare or absent. After three days of rearing in water skin showed initiation of differentiation of glands in the dorsal region. However, integument of the lateral region still remains free from glandular differentiation whereas on the ventral side epidermis showed development of mucous glands. Distribution of melanophores was uniform throughout the dorsal part of the body of the tadpoles. Tadpoles of stage 34 after four days showed quite advanced features of differentiation of integument while in the dorsal region nest of cells which forms mucous were observed. Another type of skin glands found in the dorsal region was known as granular glands or serous glands. Both types of glands are epidermal in origin but after differentiation, they yet embedded in the

dermis which is now compact and thick in the hind limbs. Glandular differentiation could also be observed in the dorsal region of skin of hind limbs. Both mucous and serous glands are found in the dorsal skin of the tail region. Tadpoles of group A of 5th day also showed well differentiated skin having 4 to 5 cell layer thick epidermis. Melanophores are present both in the epidermis and ventral region. The lumen of serous gland is filled with granular substance. However the skin of ventral region still showed 1 to 3 cell layer thick epidermis without a prominent and thick dermis. On the lateral side of the body there were more serous glands than the mucous glands. The tadpoles of stage 34 reached to metamorphic climax or get metamorphosed on rearing in water for fifteen days. In these skin was fully differentiated and epidermis was showing advanced stratifications. Distribution of skin glands in different parts of body on the dorsal side of the head region and there were large number of mucous glands present which were having nest of cells surrounding a lumen and embedded in the dermis. The serous glands were having a comparatively larger lumen and their number was less in the dorsal region of head. Distribution of skin glands in the hind limb showed both serous and mucous glands in the dorsal part of the skin but these glands were either few or absent on the ventral side of the skin.

Stage 34 Vitamin A treatment (continuous) Group B

Vitamin A treatment to the tadpoles of stage 34 produces variety of effects on differentiation of integument (Fig.7). The vitamin A treatment of two days resulted in thin basement membrane or absent, epidermal cells became mucoid type. Three days vitamin A treatment caused changes in the morphological features of tadpoles. The skin of ventral region became thin and transparent, little swelling was observed due to excessive mucous production. In the dorsal region epidermis lost its compactness and stratification. Skin glands were generally absent in such cases. On the ventral side skin became very mucoid type of epidermis without skin glands and basement membrane was absent in these cases. Four days of vitamin A treatment caused mucous metaplasia both in the dorsal and ventral region. Dermis and basement membrane were either indistinct or absent. Subsequent treatment of vitamin A (5 to 15 days) resulted in disruption of cellular organization of skin throughout the body. In most of such cases epidermis became mucoid type, basement membrane either got disappeared and cells of ventral region became wavy type. On 6th day also skin appears to have got severely affected by vitamin A. The basement membrane and dermis were becoming discontinuous and diffused. Fifteen days vitamin A treatment to the tadpoles of stages 34 prevented them to undergo metamorphosis. These tadpoles failed to metamorphose even after fifteen days when their corresponding controls were all metamorphosed. In these, the architecture of skin became severely disorganized leaving no skin glands, basement membrane, dermis etc.

Stage 34 Vitamin A treatment (discontinuous) Group C

Those tadpoles of stage 34 which were transferred to water after initial three days of Vitamin A treatment showed persistence of vitamin A effect even three days after rearing in water (Figs. 8). In these, skin still remains 1 or 2 cell layer thick with a thin basement membrane. Glandular differentiation was absent in the dorsal and ventral region. The skin of ventral region is thin and transparent and is still oedematic. Tadpoles of group C when reared in water for twelve days after initial three days of Vitamin A treatment showed excellent recovery from the vitamin A effect. Although the tadpoles do not acquire normal morphology similar to the controls but they underwent metamorphosis. Skin of dorsal region of such tadpoles had multilayered epidermis, basement membrane, thick dermis and metanophores both in epidermis and in the dermis. Both mucous and serous glands were found embedded in the dermis. These features were indicative of recovery effects in the tadpoles which were transferred to water after three days of vitamin A treatment.

Stage 36

The development of skin and skin glands in tadpoles of stage 36 was almost similar to that of stage 34 (Fig. 7). The vitamin A treatment adversely affected

the development of skin of tadpoles, however, the characteristic swelling and mucous metaplsia observed in younger tadpoles were not observed in this stage.

Discussion

In the tadpoles of *Bufo melanostictus* both mucous and granular glands had been noticed in the present study. The skin glands observed in present study were similar to the general features of skin glands described by Dawson[19]. The granular glands of many amphibians particularly the toads protects them from being eaten up by many enemies because their secretion is repelling and irritating and hence are referred as poison glands. Abel and Match[20] described the poisonous secretion of some bufonids as Bufogin (C_{18} H24 $O_{4)}$. In the present study serial cross sectional examination of skin in the dorsal, ventral and lateral regions in very young tadpoles of stage 25 and in some key metamorphic stages (30, 34 and 36) has revealed certain interesting features regarding development and distribution pattern of skin glands. Although basic pattern of differentiation of skin is similar in this anuran species[21,22] compared with other amphibians yet there are certain variations which are noticed only in this particular bufonid. Tadpoles of stage 25 showed one or two cell thick epidermis and very thin dermis. In these, glandular differentiation has been observed in the skin. The gland rudiments first develop at stage 30(limb bud stage) and show glandular differentiation up to stage 34(1st toe indentation stage).In many rainds (*Rana pipens* and *Rana sylvatica)* also skin gland differentiation starts after stratum spongiosum of dermis below the basement membrane is formed which accommodates the developing mucous and serous glands[10,11,12]. Verma[14] observed first gland rudiments in the mid larval period i.e. stage 13 in *Rana pipiens* (equivalent to stage 34 of *Bufo melanostictus*). The enlargement and subsequent migration below the epidermis of these glands takes place gradually during the period of metamorphosis.

Verma[14] studied the skin of *Rana pipiens* around the jaw and posterior lip region and no sequential development was traced for the skin glands in other regions to traced priority of differentiation of mucous or serous glands. In the present study observation were made in *Bufo melanostictus* mainly confined to the skin of dorsal, ventral and lateral regions immediately posterior to the head region. In this bufo species during larval development the number of glands are more in the dorsal region as compared to the ventral and lateral regions. Among these two types of glands, mucous glands are more abundant in the dorsal skin then in the ventral and lateral skin. Similar observations were made in ranid amphibians. According to Le QuangTrong[23, 24, 25] the ventral skin of young tadpoles generally lacks serous glands. The distribution of glands in different amphibians may be related to differences in their habitat[25]. In *Bufo melanostictus* it has been observed that development of the two type of gland is independent without intermediate or transitional phase. During their development some glands contain both mucous and granular material at the same time (intermediate phase). This is in conformity with Bovbjerg[11] who observed in *Rana pipiens* that the two types of glands develop independtly without intermediate or transitional phase. On the contrary Mc Manus[26] found that the granular cells in the amphibian *Desmo gnathusfuscus* pass through a mucoid stage during histogenesis. The skin comprises of very thin epidermis and simple dermis throughout the mid larval period in this bufo species. The basement membrane becomes distinct and epidermis and dermis becomes comparatively thicker immediately before onset of metamorphic climax.

Kertainization of outermost epidermal cells is not observed up to stage 34. These features of bufonid skin are quite similar to the skin characteristics described in other bufonids[27]. Comparatively thinner epidermis and dermis of ventral region can be attributed to the various physiological functions related to aquatic mode of life of these amphibians. Like many amphibian species[22] in the tadpoles of *Bufo melanostictus* also characteristic skin colour is produced by definite pattern of distribution of chromatophores located either in the epidermis and dermis. Epidermal melanophores are forced in the skin of dorsal region. The light reflecting chromatophores i.e. iridophores are also found in the skin of *Bufo melanostictus* predominantly in the

dermis of dorsal region. The melanophores present in the dermis concentrated at regular intervals and gives black-brown colour to the dorsal skin. Dermal melanophores are generally absent in the ventral and lateral skin.

Retinoids (Vitamin A and derivates) have been observed to regulate vision, growth and differentiation, reproduction and healthy status of epithelium. It is well known that vitamin A is directly involved in the process of vision because it serves as important component of rhodopsin. There are reports that hypervitaminosis causes mucous metaplasia and other histological deviations on developing mammalian skin[28]. Vitamin A has been found to affect differentiation of skin and its glands and the effect is stage dependent. Tadpoles of stage 25 when treated with 1 IU/ml vitamin A continuously for fifteen days showed complete inhibition of differentiation of skin. However, in the discontinuously treated group, when they were transferred to water on 3rd day they showed beginning of skin gland differentiation on 15th day. is clearly indicates that Vitamin A does not change the fundamental properties of skin when it is administered even at very early phase of differentiation. Once the treatment is withdrawn cells come out of inhibitory influence of this drug. But when treatment is prolonged beyond three days the effects are irreversible. Similar effects of Vitamin A was also recorded in the tadpoles of stage 30 who demonstrated beginning of differentiation of skin glands on 6th day (equivalent to stage 34) after rearing in water. However, this experimental design does not answer the question whether effect of vitamin A is direct on epidermal and dermal differentiation or is mediated through systemic influences such as hormones.

Tadpoles of stage 34 show first differentiation of mucous glands and serous glands which were visible after three days of rearing in water (Stage 36). Three day of vitamin A treatment to these tadpoles does not inhibit development of mucous glands but adversely affected development of serous glands. Continuous treatment for six days causes disappearance of both mucous and serous glands. However, those tadpoles which were transferred from vitamin A to water `after initial three days of treatment showed redifferentiation of both types of glands within three days, although mucous glands appeared first followed by serous gland. When effect of vitamin A was studied on well developed skin glands in the tadpoles of stage 36, mucous glands were observed in the skin after five days of continuous treatment but the number of serous gland were greatly reduced. In the discontinuously treated group normal gland pattern is re-established within two days after transfer to water. While studying the effect of vitamin A on skin gland differentiation it was also noticed that vitamin A treatment causes mucous metaplasia of epidermis, decreases in number of pigments, swelling of abdomen due to accumulation of mucous and degeneration of horny teeth and jaw. Similar general effects of vitamin A have been reported by other investigators in different anuran species[6,7,30,31,32]. How vitamin A affects various components of skin of other anuran tadpoles at different developmental stages is not yet known. Studies concerning the effects of vitamin A during amphibian limb regeneration have been carried out in many amphibian species and it has been reported that prolonged exposure of blastema to vitamin A causes inhibition of regeneration[33, 34, 35]. Discontinued treatment results in whole limb regeneration[6,36,33,30,37,38,39,40,41,42]. The effect of vitamin A is stage dependent[36, 43, 44]. Vitamin A maintains healthy status of regenerating epithelium[45].

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Legends of figures:

Fig. 1- Morphology and Histology of skin of untreated (control) tadpoles of stage 25. 1(A) general morphology of dorsal region (0.7x), 1(B) ventral region (0.7x) and 1(C) skin with pigmentation and mucous glands on the dorsal side (10x). M- mucous glands, P- pigments.

Fig. 2 - Morphology and histology of skin of vitamin A treatment (continuous) tadpoles of stage 25. 2(A) skin becomes thin and transparent (0.7x) in the dorsal region, 2(B) epidermal organization becomes wavy and irregular (40x) in the ventral region and 2(C) swelling of skin and it becomes thin and transparent and show mucous metaplasia (0.7x) in the ventral region.

Fig. 3 - Morphology and histology of skin of vitamin A treatment (discontinuous) tadpoles of stage 25. 3(A) absence of melanophores (0.7x) ventral region, 3(B) showing pigments (0.7x) in the dorsal region and 3(C) skin show pigments and skin glands (40x) in the dorsal region.

Fig. 4- Morphology and histology of skin of vitamin A treatment (continuous) tadploes of stage 30. 4(A) swelling of tadpole in the ventral region (0.7x), 4(B) no swelling in the dorsal region (0.7x) and 4(C) skin shows mucous metaplasia (40x) dorsal region.

Fig. 5- Morphology and histology of skin of vitamin A treatment (discontinuous) tadploes of stage 30. 5(A) skin shows significant recovery and serous glands are present (10x) in the dorsal region, 5(B) skin shows pigmentation (0.7x) in the dorsal region and 5(C) oedema (0.7x) in the ventral region. S- serous glands.

Fig. 6 - Morphology and histology of skin of untreated (control) tadpoles of stage 34. 6 (A) distribution of melanophores in the dorsal region (0.7x), 6(B) mucous and serous glands are present (10x) in the dorsal region and 6(C) mucous and serous glands are present (40x) in the dorsal region. M- mucous glands, S- serous glands.

Fig.7 - Morphology and histology of skin of vitamin A treatment (continuous) tadpoles of stage 34.7(A) skin shows disruption of cellular organization (40x) dorsal region, 7(B) tadpoles failed to metamorphose (0.7x) and 7(C) tadpoles failed to metamorphose and skin becomes thin and transparent (0.7x) in the ventral region.

Fig. 8- Morphology and histology of skin of vitamin A treatment (discontinuous) tadpoles of stage 34. 3(A) recovery from the vitamin A effects (0.7x) in the dorsal region, 8(B) recovery from the vitamin A effects (0.7x) in the ventral region and 8(C) multilayered epidermis, thick dermis with pigmentation, mucous and serous glands are present in the dermis (10x) in the dorsal region. E- Epidermis, M- mucous glands, S- serous glands.

Figure -1

Figure -2

Figure -3

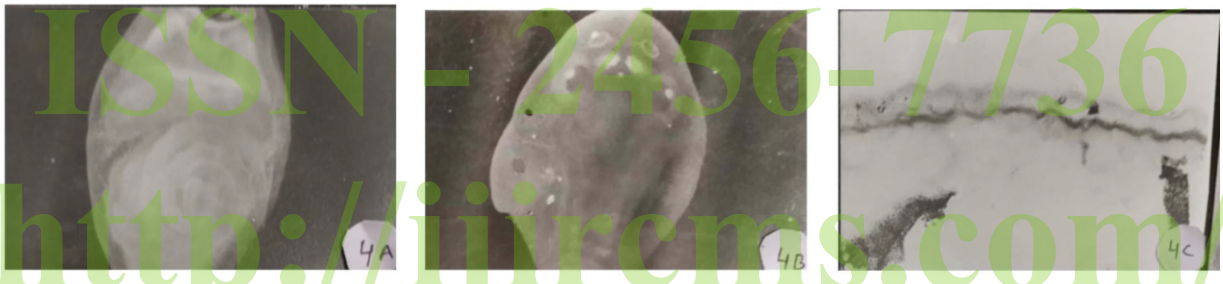

Figure -4

Figure -5

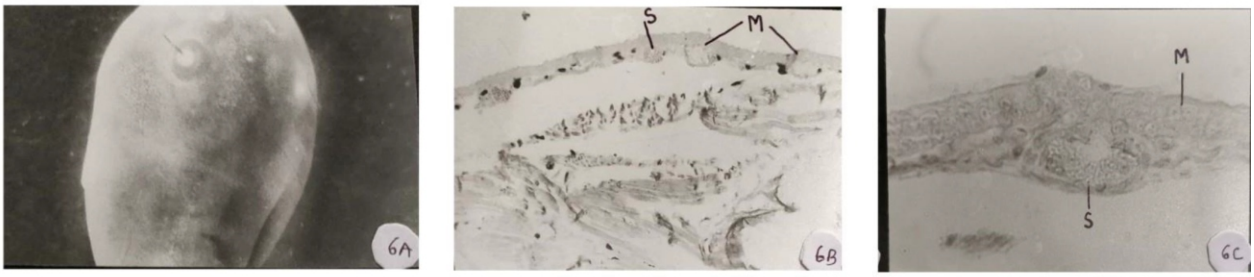

Figure -6

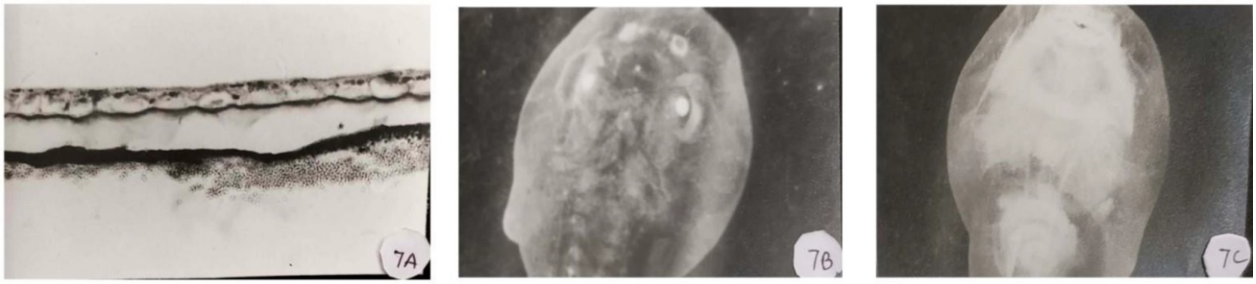

Figure -7

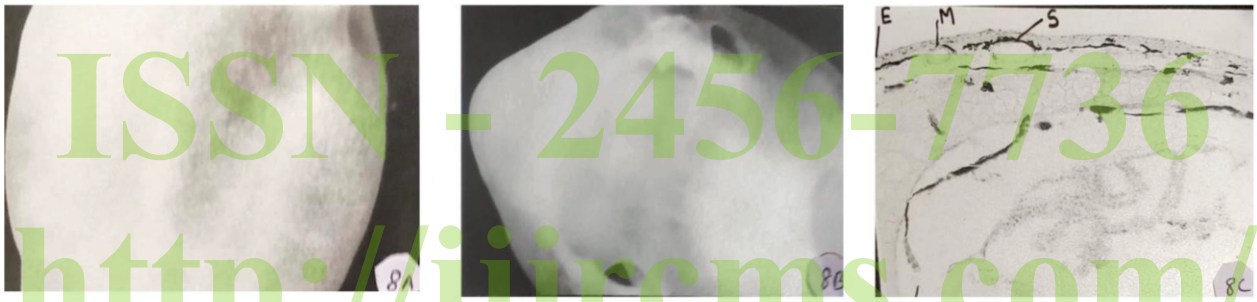

Figure -8