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Original Article

Commercially Available Vitamin A Palmitate is Toxic and Teratogenic to the Tadpoles of *Microhyla nilphamariensis* (Anura: Microhylidae)

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ABSTRACT

Aim/Background: The present study, examined the impact of excess Vitamin A on feeding and pre-metamorphic tadpoles of *Microhyla nilphamariensis* to analyse its dose-dependent and stage-specific lethal and teratogenic effects. **Materials and Methods:** Feeding and pre-metamorphic stage tadpoles were subjected to varying concentrations of commercially available Vitamin A palmitate for different time intervals. The control and treated tadpoles were fixed 5, 10- and 15-Days Post Treatment (dpt) in neutral buffered formalin for morphological analyses, morphometry and histology. **Results:** In both feeding and pre-metamorphic stages, mortality was high when exposed to higher concentrations and longer duration (48–96 hr). When exposed for 24 hr, the treated tadpoles showed significant differences in morphometry, and abnormalities in the eye and intestine of the feeding stages and eye and kidneys of pre-metamorphic stages. Significantly, most of the treated tadpoles died before attaining metamorphosis and those that survived had a delayed metamorphosis with abnormalities. **Conclusion:** Vitamin A is lethal to the tadpoles of *Microhyla nilphamariensis* at high concentrations and at lower concentrations it affects the thyroid hormone function and causes abnormalities of various kinds.

Keywords: Tadpoles, Feeding stage, Pre-metamorphic stage, Histology, Morphometry.

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INTRODUCTION

Vitamins are essential micronutrients required in very small amounts to maintain the fundamental functions of the body. Vitamin A is a generic term for a large number of related compounds like retinal, retinoic acid, Vitamin A palmitate etc., having similar molecular structure and function. During human fetal development, Vitamin A acts as an essential morphogen causing cell growth, differentiation and organogenesis.[1] In regions, where Vitamin A Deficiency (VAD) is a public health issue, pregnant mothers are even recommended to take stipulated doses of Vitamin A supplementation to prevent night blindness.[2] However, excessive intake of Vitamin A during pregnancy has been considered teratogenic leading to malformations in various organs of human fetus.[3] Thus, Vitamin A elicits dose dependent effects during human fetal period.

Vitamin A is not only significant for human development but also affects development and physiology of other organisms. Vitamin A is considered as an important nutrient for health of both captive and wild population of amphibians.[4] Vitamin A is an important component in the diet and also used to treat hypovitaminosis in amphibians-maintained *ex situ* in various conservation breeding programs.[5] Though Vitamin A plays crucial role in maintaining good health, it has been associated with organ malformation in many organisms when used in excess amount. Vitamin A derivatives, retinoids, have always been regarded as one of the causative factors of limb malformations in amphibians.[6,7] Recently, Khan and Mahapatra,[8] reported anopthalmia in common Asian toad from Eastern Ghats of India, which they suspect to be caused by retinoids. Improper disposal of drugs and pharmaceuticals leading to pharmaceutical pollution of aquatic bodies is a big concern nowadays[9,10] and Vitamin A palmitate is a commonly prescribed drug for treating eye diseases[11] and skin care.[12] Retinoic acid is especially teratogenic to the larval stages of anuran amphibians where it causes hind limb malformations in *Xenopus laevis, Rana clamitans* and *R. septentrinolis.*[6] Vitamin A-induced limb abnormalities in tadpoles has also been reported in few sub-tropical anurans.[13,14] Mahapatra *et al.*[15] gave a comparative account of the morphological abnormalities of the

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tadpoles post treatment with Vitamin A in four different species of sub-tropical anurans. Still, it is important to understand the effects of Vitamin A at various life history stages of different anuran taxa to gauge the magnitude of alterations it can elicit. *Microhyla nilphamariensis* is a commonly available microhylid in eastern India[16] and relatively under-studied with regards to effects of Vitamin A. The present study aims to describe the effects of commercially available Vitamin A palmitate on feeding and pre-metamorphic stage tadpoles of Ornamented pygmy frog, *Microhyla nilphamariensis.*

MATERIALS AND METHODS

The model species of this study is *Microhyla nilphamariensis* Howlader, Nair, Gopalan and Merilä, which is commonly distributed in Baripada, the study area. The earlier record of *M. ornata* (Duméril and Bibron) from Odisha was now referred as *M. nilphamariensis* based on the work of Garg *et al.*[16] The *Microhyla* specimens collected from Baripada were diagnosed based on morphological characters such as lateral band starting from tip of the snout and approaching the groin on either side of the body. The bands were more prominently blackish brown and continuous. Ventral side was mottled with various sized blackish-brown spots, more prominently on throat, chest, and margins of the belly (Figure 1).

Collection and rearing of tadpoles

Eggs of *Microhyla nilphamariensis* were collected from undisturbed habitats in Baripada, Odisha, India (21°57′27.28′′N, 86°44′22.87′′E) during monsoon. The eggs were housed in plastic tubs (45 cm diameter) filled with conditioned water in the laboratory at room temperature (28±1°C). After hatching, the tadpoles were transferred to dechlorinated water (stored and aerated) that was changed every one-day interval. The tadpoles were staged according to Gosner.[17] When they reached feeding stage, i.e., Gosner stage 25, they were fed with boiled egg yolks and *Amaranthus* greens. All experiments were performed as per Institutional Animal Ethical Committee (IAECC) guidelines Maharaja Sriram Chandra Bhanja Deo University, Baripada, Odisha, India.

Treatment with Vitamin A Palmitate and mortality assessment

Water miscible formulations of commercially available Vitamin A palmitate (Abbott Pharmaceuticals, Mumbai, India) were prepared. Feeding stage (Gosner 25) and pre metamorphic (Gosner 28) stages were considered for Vitamin A palmitate treatment. Feeding stages is a developmental stage where the tadpoles start feeding from external sources. Pre-metamorphic stage tadpoles are advanced in development than feeding stages and have limbs in the form of limb buds. Different concentrations of Vitamin A palmitate treatment were given for 24, 48, 72 and 96 hr. Briefly, eight concentrations of Vitamin A palmitate viz., 1,2,5,10,20,30,40 and 50 IU (International Units) were prepared from a saturated stock solution. Feeding was stopped a day prior to the treatment. 20 tadpoles of feeding stages were treated with 1 litre of 1,2,5,10, 20 and 30 IU in glass bowls (20cm x 10 cm). Three replicates were done for each concentration. Similarly, for pre-metamorphic stages, 12 tadpoles each were treated with 1 litre of 1,2,5,10,20,30,40 and 50 IU Vitamin A palmitate. Control sets with 20 and 12 tadpoles of feeding and pre-metamorphic stages, respectively were taken without Vitamin A. The Vitamin A solution was changed every day for treatment beyond 24 hr. After treatment, the tadpoles were moved to normal conditioned water and observed up to their death or metamorphosis. Mortality was recorded as percentage death for each individual concentration at 24, 48, 72 and 96 hr for both stages.

Morphometry and Histology

Treated sets that showed less than 45% mortality at 24 hr were taken for morphological analyses, morphometry and histology. The control and the treated tadpoles were fixed 5, 10- and 15-Days Post Treatment (dpt) in neutral buffered formalin. For morphometry, Body Length (BL), Tail Length (TAL), Total Length (TL), Internarial Distance (IND), Interorbital Distance (IOD), Maximum Tail Height (MTH) and Tail Muscle Height (TMH) were considered (Figure 2).[18] All the measurements were taken with an ocular micrometer and photographs were taken with stereo zoom microscope (Hund, Wetzlar). Significant difference of the morphometrical parameters between the control and treated groups was calculated by Kruskal-Wallis test at 5% level of significance.

For histology, the tadpoles of control and treated sets were fixed 10 and 15 dpt in 10% neutral buffered formalin solution overnight. They were embedded in paraffin and the entire tadpole was sectioned at 7µ thickness using a rotary microtome machine. Sections were stained using haematoxylin and eosin stain. The sections were examined using a binocular microscope (Olympus CX21i) and photographs were taken with a Magnus Pro camera (Magcam DC5). Area of lens, width of various layers of eye and height of cells was measured using Image J and significant difference between control and treated were statistically analysed using *t*-test at 5% level of significance. Labelling and insertion of scale bars were done with Adobe Photoshop 7.0.

RESULTS

Mortality and Metamorphosis

Table 1 shows the percentage mortality of feeding stages treated with graded concentrations of Vitamin A palmitate at different time intervals. Mortality increased with increase in dose and duration of treatment. For the feeding stages, mortality was 100% for tadpoles treated with 10 IU and above and for more than 72 hr. The pre-metamorphic tadpoles treated with higher concentrations like 30, 40 and 50 IU also recorded 100%

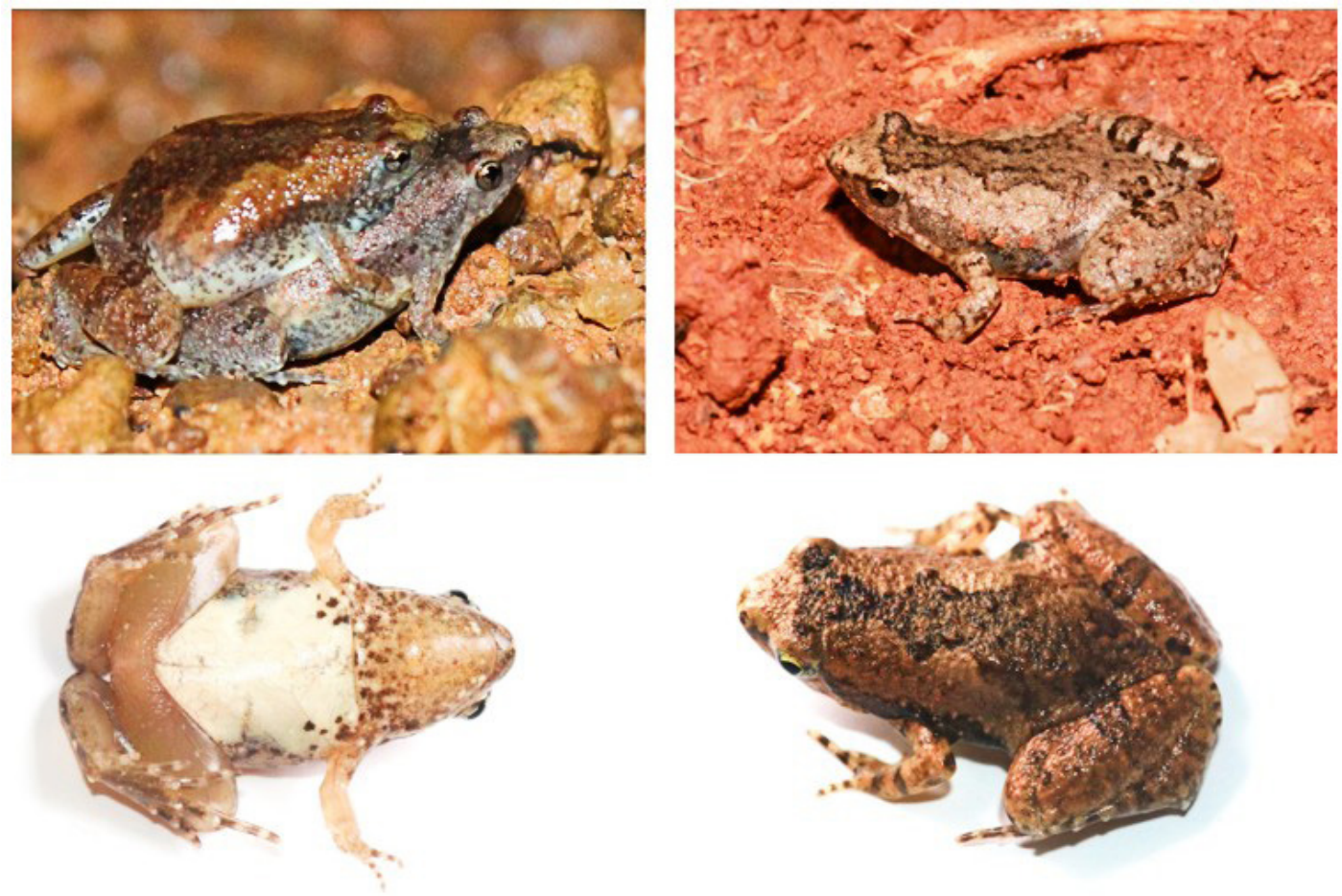

Figure 1: Images of *Microhyla nilphamariensis* showing amplecting pair, dorsal and ventral aspects of live individuals.

mortality when treated beyond 72 hr (Table 2). Survivability was very low even after removal of the test solution after the stipulated time period. Tadpoles in treatment groups having less than 45% mortality (except 1 IU 24 hr pre-metamorphic stage that had 0% mortality), survived up to 20dpt maximum but in treatment groups that recorded more than 45% mortality, all tadpoles (100%) died within 5dpt, irrespective of stage, concentration and duration of treatment.

The control tadpoles of feeding and pre-metamorphic stages metamorphosed within a range of 97–108 and 61–67 days respectively. However, in all treatment groups of feeding stages, the tadpoles died before attaining metamorphosis. Interestingly, in feeding stages that were exposed to 10IU and 20IU for 72 hr, only one tadpole survived in each treatment group up to 108dpt. However, both of them died before metamorphosis (Figure 3). All treated pre-metamorphic tadpoles, except 1 IU 24 hr, also died before metamorphosis. Only 10% tadpoles of 1IU 24 hr metamorphosed in 65-70 days.A: Gosner stage 41 tadpole; B: 10IU 72 hr treatment; C:20IU 72 hr treatment. Arrows show abnormalities. {Bars =0.4mm}.

Morphology and morphometry of Vitamin A treated tadpoles

For morphological and morphometrical analyses, we considered the treatment sets experiencing ≤45% mortality, since they survived for a longer period as mentioned above. Hence, we considered only 24 hr treatment sets to maintain uniformity in morphometrical comparisons. No substantial deformities were observed in the overall morphology and morphometry up to 10dpt. At 15 dpt, morphometrical comparisons showed significant differences due to Vitamin A treatment in both feeding and pre-metamorphic stages. Treated tadpoles of feeding stages, showed a significant increase in all morphometrical parameters indicating increase in size of the tadpoles (Table 3). The feeding stage tadpoles that survived up to 108 dpt in 10 and 20 IU 72 hr treatment groups showed facial anomaly like a triangular snout and wider body as compared to the control (Figure 3A). Additionally, the one exposed to 10 IU showed abnormalities in the tail (Figure 3B) while the one exposed to 20IU showed retardation in the length of femur and tibio-fibula (Figure 3C).

In pre-metamorphic tadpoles, Body Length (BL), Tail Length (TAL) and Total Length (TL) was significantly smaller at 1 IU and 10 IU but significantly greater at 20 IU. The Maximum Tail

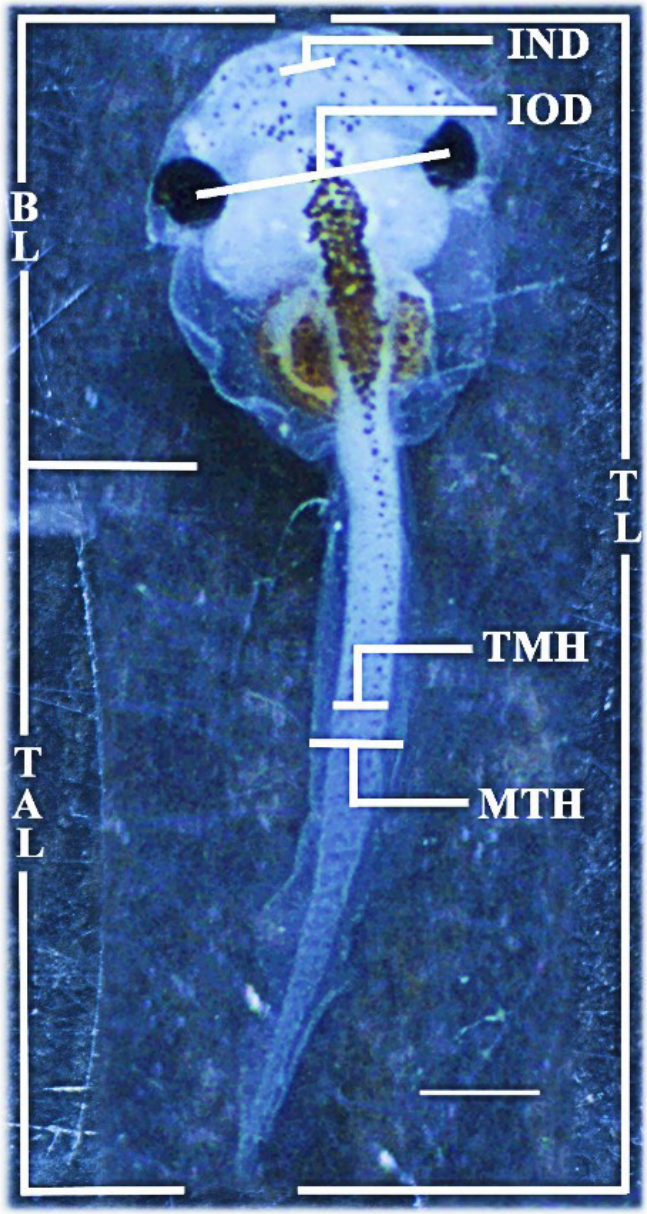

Figure 2: Dorsal view of tadpole of *Microhyla nilphamariensis*.BL: Body Length, TAL: Tail Length, TL: Total Length, IND: Inter Narial Distance, IOD: Inter Orbital Distance, TMH: Tail Muscle Height, MTH: Maximum Tail Height {Bar=0.4mm}.

Height (MTH) was significantly smaller in all treated groups but Tail Muscle Height (TMH) was significantly greater only in 20 IU (Table 4).

Histological analyses

Transverse sections through control tadpoles were compared with their corresponding sections in the treated ones for both feeding and pre-metamorphic stages. Histological comparisons did not reveal any important changes at 10dpt. However, at 15dpt, histology revealed Vitamin A palmitate mediated abnormalities in the eye and intestine of feeding stages while eye and kidney of pre-metamorphic stages. The effect of Vitamin A on other organs of both stages was not apparent histologically. Transverse section passing through eye of feeding stages showed an outer covering of the eye, Cornea (C), Circular Lens (L), and Choroid (CH). Retina consists of the inner Nuclear Layer (NL) towards choroid and Ganglion Cell Layer (GL) towards lens with a Plexiform Layer (PL) in between (Figure 4A). Tadpoles treated with 10IU vitamin A palmitate for 48 hr, showed a significantly thinner nuclear layer of Retina (NL) (0.039±0.022mm) than control (0.059±0.078mm). Besides, the lens fibres were also sparse (Figure 4B). The area of lens in treated tadpoles (0.035±0.26 mm^2) and the maximum lens to choroid distance (0.36±0.044 mm) was substantially greater than control (Area of lens: 0.031±0.06 mm^2; Lens to choroid: 0.34±0.013 mm). Transverse section passing through intestine in control tadpoles showed a monolayer of Intestinal Epithelium (E) Enclosing Lumen (L) filled with food particles. The intestinal epithelium consisted of cuboidal cells. The intestine was lined on the inner side by a connective tissue layer, the Serosa (S) (Figure 4C). However, in 10IU 24 hr treatment the intestinal epithelial cells were 1.69 times longer (0.026±0.017 mm) than control (0.016±0.037 mm). They were also interspersed with Apoptotic Cells (AC) having condensed cytoplasm (Figure 4D).

Transverse section passing through eye of pre-metamorphic stages showed similar anatomy like feeding stages with an outer covering, the Cornea (C), Circular Lens (L), and Pigmented Epithelium (PE) (Figure 5A). Pre-metamorphic stages treated with 10IU 24 hr Vitamin A palmitate showed two lenses, one attached to the Plexiform Layer (PL) and another towards the Cornea (CO). The Pigmented Epithelium (PE) surrounded the entire structure (Figure 5B). Out of the two lenses, the area of the bigger lens (0.032±0.004mm^2) of the treated tadpole was 2 folds smaller than control (0.065±0.005mm^2). However, the maximum length of the eye (lens to choroid) of the control was smaller (0.412±0.019mm) than the treated (0.450±0.015mm). Transverse section passing through kidney showed pronephric tubules lined with glandular wall (Figure 5C) but the pronephric tubules in 20IU 24 hr treated tadpoles did not maintain their tubular shape and appeared diffused and disintegrated (Figure 5D).

DISCUSSION

The present study used commercially available Vitamin A palmitate to evaluate its teratogenicity on anuran larval stages. Teratogenic effects were validated by morphological observations, morphometrical and histological comparisons. Morphometrical parameters normally used for taxonomic studies were used to describe teratogenic effects. Feeding stages were more sensitive as identical concentration of vitamin A palmitate caused higher mortality in feeding stages than pre-metamorphic stages. Additionally, the feeding stages experienced high mortality when exposed beyond 24 hr, even at lower doses. Thus, vitamin A showed a stage specific toxicity towards the tadpoles which was also dose dependent.

Vitamin A regulates thyroid hormone metabolism and inhibits thyroid hormone secretion.[19,20] Anuran larval development is divided into three stages based on the action of the thyroid hormones, such as pre-metamorphosis, pro-metamorphosis and climax.[21] During pre-metamorphosis, thyroid hormones are released in smaller quantities which peaks during metamorphic climax and brings about metamorphosis. Feeding stages precede pre-metamorphosis where tadpoles have just started to feed after hatching out of the egg jelly. Thyroid gland becomes functional in anuran tadpoles during pre-metamorphosis although thyroid hormone receptors are present in different tissues much before pre-metamorphosis.[22] In the present study, metamorphosis wasn't achieved in 100% of treated feeding stages indicating that Vitamin A probably alters the functioning of the thyroid hormone receptors due to which thyroid hormone signalling related phenomena like development of limbs, regression of tail and metamorphosis are impaired and tadpoles die before attaining metamorphosis. However, few tadpoles of pre-metamorphic stages treated with lower concentration for shorter duration (i.e., 1 IU 24 hr) metamorphosed although a little delayed. Thus, lower doses of vitamin A were unable to inhibit metamorphosis in older tadpoles suggesting a stage specific threshold for vitamin A palmitate that interferes with thyroid hormone signaling.

Effects of Vitamin A on the morphology and morphometry was also stage specific. Most of the treated tadpoles died very early, i.e., 5 dpt, without showing any distinct morphological deformities. Those that survived longer i.e., up to 20 dpt also did not show any remarkable morphological alteration. Only, single representatives of a few treatment groups of feeding stages that survived longer, showed hindlimb and tail deformities along with craniofacial anomalies. Retinoic acid, a vitamin A derivative produces phocomelia and micromelia in mice embryos where all long bones

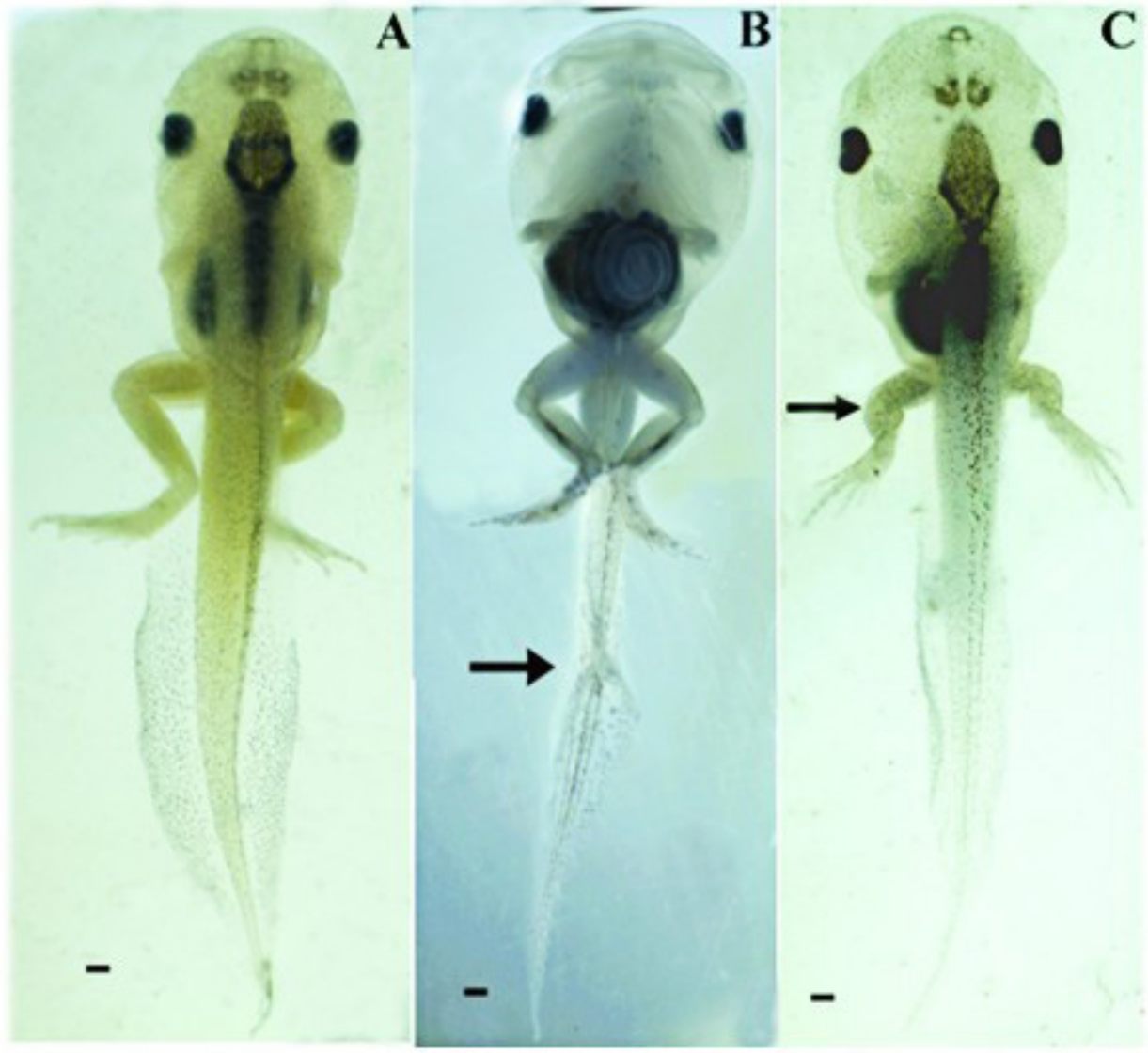

Figure 3: Vitamin A treated tadpoles of *Microhyla nilphamariensis* 108 dpt.
A: Gosner stage 41 tadpole; B: 10 IU 72 hr treatment; C:20 IU 72 hr treatment. Arrows show abnormalities. {Bars =0.4mm}.

Table 1: Mortality rate of feeding stages of *Microhyla nilphamariensis*.

Concentration of Vitamin A (IU)	Percentage (%) of death at different time interval (in hours)			
	24 hr	48 hr	72 hr	96 hr
1	10	23	38	18
2	23	49	73	63
5	33	74	88	97
10	40	81	100	100
20	65	90	100	100
30	83	95	100	100

Table 2: Mortality rate of pre-metamorphic stages of *Microhyla nilphamariensis*.

Concentration of vitamin A (IU)	Percentage (%) of death at different time interval (in hours)			
	24 hr	48 hr	72 hr	96 hr
1	0	14	16	20
10	18	43	65	67
20	45	60	75	100
30	60	75	100	100
40	60	100	100	100
50	47	62	100	100

Table 3: Morphometry of control and Vitamin A exposed feeding stages of *Microhyla nilphamariensis*.

Concentration (IU)	BL*	TAL*	TL*	IND*	IOD*	MTH*	TMH*
0	3.10± 0.11#	4.93± 0.31	8.03± 0.41	0.47± 0.00	2.13±0.10	0.97±0.10	0.47±0.09
1	4.42± 0.47	6.42± 0.72	10.9± 0.80	0.66±0.10	2.76±0.40	1.3±0.22	0.6±0.19
2	4.27± 0.05	5.93± 0.80	10.2± 0.85	0.8±0.14	2.8±0.14	1.57±0.05	0.73±0.09
5	4.13± 0.38	5.85± 0.22	9.98± 0.60	0.63±0.06	2.7±0.25	1.89±0.19	0.77±0.06
10	4.10± 0.17	5.23± 0.27	9.1± 0.35	0.7±0.00	2.1±0.07	1.2±0.12	0.7±0.05

All measurements are expressed as Mean ± standard deviation (in mm).*-Difference significant among control and treated at 5% level of significance.[BL = Body length, TAL = Tail length, TL = Total length, IND = Internarial distance, IOD = Interorbital distance, MTH = Maximum tail height, TMH = Tail muscle height].

Table 4: Morphometry of control and Vitamin A exposed pre-metamorphic stages of *Microhyla nilphamariensis*.

Concentration (IU)	BL*	TAL*	TL*	IND	IOD	MTH*	TMH*
0	6.93±0.15#	11.50±0.70	18.53±0.85	0.65±0.05	3.30±0.30	3.50±0.20	1.25±0.05
1	5.93±0.35	9.96±0.51	15.90±0.85	0.67±0.13	3.19±0.35	2.48±0.13	1.01±0.12
10	6.53±0.17	11.27±1.27	17.9±1.48	0.67±0.58	3.70±0.63	2.60±0.27	1.23±0.32
20	7.50±0.30	12.40±0.80	19.90±1.10	0.70±0.00	3.85±0.35	3.05±0.25	1.30±0.00

All measurements are expressed as Mean ± standard deviation (in mm).*-Difference significant among control and treated at 5% level of significance.[BL = Body length, TAL = Tail length, TL = Total length, IND = Internarial distance, IOD = Interorbital distance, MTH = Maximum tail height, TMH = Tail muscle height].

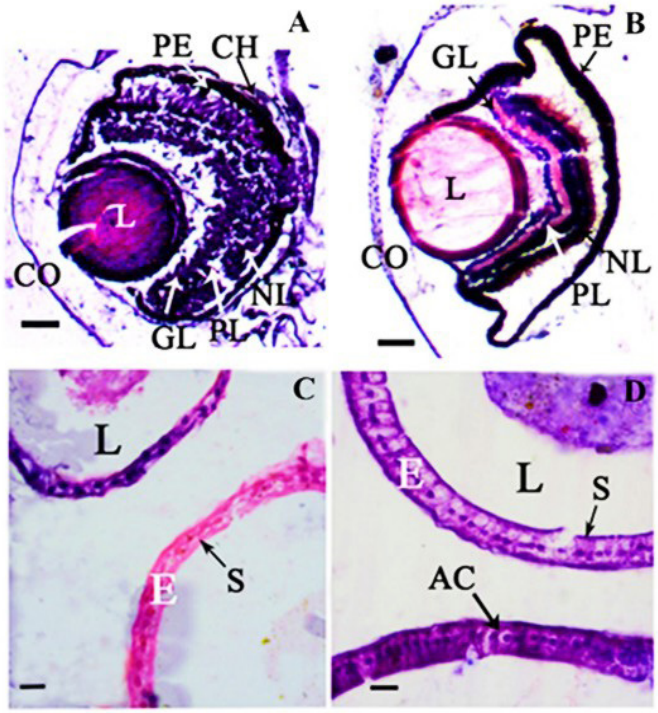

Figure 4: T.S. through control and Vitamin A treated tadpoles of *Microhyla nilphamariensis* 15 dpt.
A- Eye of control; B- Eye of 10IU 48 hr; C- Intestine of control; D- Intestine of 10IU 24 hr. {CO: Cornea, CH: Choroid, L: Lens, PE: Pigmented epithelium, NL: Nuclear layer, PL: Plexiform layer, GL- Ganglion cell layer, E: Intestinal Epithelium, L:Lumen, S: Serosa, AC: Apoptotic cell; Bars=50µm (A-B) and 20µm (C-D)}.

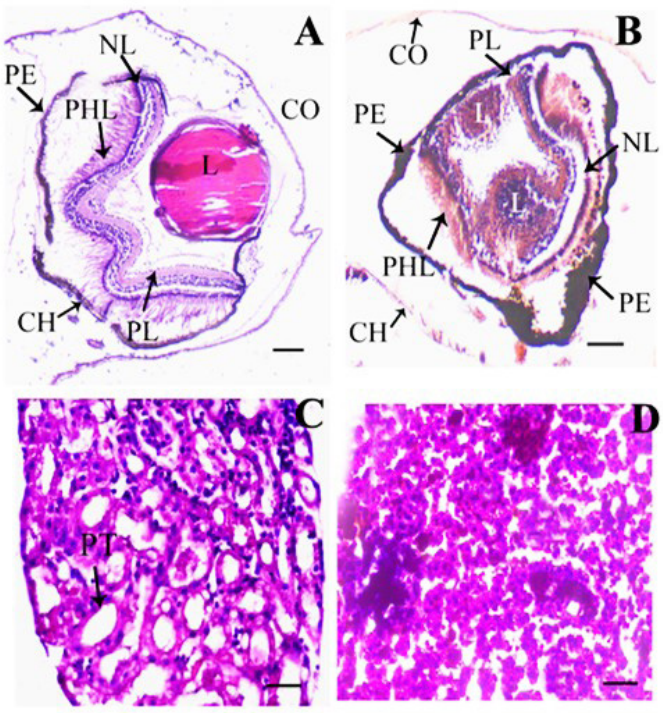

Figure 5: Transverse sections through control and vitamin A exposed pre-metamorphic tadpoles of *Microhyla nilphamariensis* 15 dpt.
A- Eye of control; B-Eye of 10IU 24 hr; C-Kidney of control; D-Kidney of 20IU 24 hr. {CO- Cornea, CH- Choroid, L-Lens, PE-Pigmented epithelium, PHL- Photoreceptor layer, NL- Nuclear layer, PL- Plexiform layer, PT- Pronephric tubule, Bars- 50µm}.

like femur and tibio-fibula are affected.[23] Administration of excess Vitamin A has been also known to cause anomalies of cranial and facial skeleton in rat embryos.[24] However, pre-metamorphic tadpoles did not show any visible morphological anomaly.

Morphometry revealed significant variations in both feeding and pre-metamorphic stages 15 days post treatment. Especially, the feeding stages showed overall increase in the size of the tadpoles. Dietary Vitamin A have shown significant growth in fishes Orange-Spotted Grouper-*Epinephelus coioides*,[25] Goldfish-*Carassius auratus*,[26] and Nile Tilapia *Oreochromis niloticus*.[27] But the pre-metamorphic stages showed varied responses at different concentrations. Low doses caused diminutive tadpoles while higher doses caused growth. Thus, Vitamin A treatment to feeding stages up to 10 IU, caused growth of the tadpole but impaired thyroid hormone function. Contrarily, in the pre-metamorphic stages lower doses of vitamin A caused degrowth in tadpoles and at the same time it was unable to inhibit metamorphosis indicating higher doses of vitamin A being associated with growth as well as impaired thyroid function. Decrease in the Maximum Tail Height (MTH) is an indication of thinning or degradation of tail muscles in Vitamin A treated pre-metamorphic stages. Hypervitaminosis A has been reported to cause accelerated myofibril degradation in rats.[28] However, Vitamin A caused expansion of tail muscles in feeding stages contrary to its usual effects.

Histological comparisons of control and treated feeding stage tadpoles revealed anomalies in the intestine where Vitamin A caused apoptosis in the intestinal epithelial layer along with changes in cell shape. Both feeding and pre-metamorphic stages treated with Vitamin A also showed abnormalities in the eyes. Thickening of pigmented epithelium, thinner nuclear layer of retina and damage to lens fibres in the feeding stages and duplication of lens in the pre-metamorphic stages were the remarkable anomalies detected that may lead to blindness in the tadpoles. Excess Retinoic Acid (RA) has been known to cause deleterious effects on eyes, resulting in dry eye symptoms[29] and thickening of choroid and reduced thickening of lens in chicks.[30] Recent report on anopthalmia in toads[8] could very well be a developmental anomaly caused by Vitamin A or its derivatives. Loss of renal tubules in pre-metamorphic stages at higher doses of Vitamin A corroborates to a study on humans where Vitamin A excess caused acute kidney injury.[31]

Although there are set of guidelines for safe disposal of expired drugs, in rural and semi-urban India expired drugs are generally dumped in dump yards or unused pits. During rainy seasons tadpoles and other aquatic fauna are exposed to such runoff

mixed with various drugs and active compounds. It is a well perceived fact that medical waste is a source of generation of hazardous biomedical waste.[32,33] Although the chemical or pharmaceutical waste constitute 3% of the total medical waste,[33] its impact on the ecosystem is not yet well studied in India. Most importantly the pharmaceutical waste such as expired medicines and the cytotoxic waste such as waste containing cytostatic drugs, often used in cancer therapy and genotoxic chemicals, are hazardous. A study from Brazil by Freitas and Radis-Baptista[34] highlights pharmaceutical pollution as an emerging public health concern worldwide, associated with the increased production and consumption of pharmaceutical and healthcare products. The study also emphasizes that inappropriate disposal of active pharmaceutical ingredients from medicinal and personal care products can be detrimental to the environments even at low concentrations. India has already experienced drastic decline of vulture population (*Gyps* spp.) due to extensive use of diclofenac, a Non-steroidal Anti-inflammatory Drug (NSAID), in veterinary medicine.[35] In 2018, Ministry of Health and Family Welfare, Government of India has released a guideline for disposal of unused medicines as Good Manufacturing Practices (GMP) and requirements of premises, plant and equipment for pharmaceutical products are laid down in Schedule M of the Drugs and Cosmetics Rules, 1945 (https://pib.gov.in/newsite/PrintRelease.aspx?relid=178039). The guideline prescribes the requirements for disposal of waste including the rejected drugs by medicine take-back programs, waste immobilization though encapsulation, flushing of certain medicines and incineration of hazardous pharmaceutical waste. However, proper implementation of existing policies and guideline, especially in rural and sub-urban India, requires technical directions, and awareness campaigns at various levels. Therefore, it is pertinent to mention that pharmaceutical waste in the environment not only can affect the aquatic species like frogs, but also may affect the species in terrestrial environment including humans. Previous studies have investigated the teratogenic aspects of vitamin A and its possible implications in the development and growth of anurans[6-7,13-15] but the present study not only affirms the teratogenic effects of vitamin A but also highlights the importance of proper guidelines for pharmaceutical waste management for conservation of aquatic life.

CONCLUSION

The present study provides additional information on the teratogenic effects of Vitamin A on a relatively unstudied anura *M. nilphamariensis*. Since Vitamin A biology of mammals has many similarities with the amphibians,[4] anuran tadpoles naturally become models to study Vitamin A mediated teratogenesis. In the present study, although, Vitamin A caused very high mortality it was found to elicit several effects in the tadpoles that were similar to mammals like thyroid hormone malfunction, cranio-facial anomalies, hindlimb malformations, muscle fibre degradation and damage to vital organs like kidney and intestine making it an effective model to study teratogenesis. Further studies can be designed to investigate the molecular aspects of Vitamin A mediated teratogenesis in this species. Besides, additional studies on other important life stages like pro-metamorphic and climactic stages of the larva along with adults can help deduce the threshold of Vitamin A that can affect various life stages of this anura. Most importantly, this study highlights the toxicity of Vitamin A palmitate, an extensively used drug, that can adversely affect the aquatic life especially anuran tadpoles if somehow finds its way to aquatic bodies due to improper disposal. This may impact the anuran species which specifically select temporary pools for breeding activities. Future studies in this regard are underway.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

IU: International Units; **dpt:** Days post treatment; **hr:** Hours; **BL:** Body length; **TAL:** Tail length; **TL:** Total length; **IND:** Internarial distance; **IOD:** Interorbital distance; **MTH:** Maximum tail height; **TMH:** Tail muscle height; **CO:** Cornea; **CH:** Choroid; **L-Lens, PE:** Pigmented epithelium; **NL:** Nuclear layer; **PL:** Plexiform layer; **GL:** Ganglion cell layer; **E:** Intestinal Epithelium; **L:** Lumen; **S:** Serosa; **AC:** Apoptotic cell; **PHL:** Photoreceptor layer; **PT:** Pronephric tubule.

SUMMARY

Continuous exposure to excess Vitamin A and its derivatives, the retinoids, is teratogenic to animals. Malformation in amphibians due to environmental perturbations have been many times linked to retinoids. The present study examined the impact of commercially available Vitamin A palmitate on feeding and pre-metamorphic tadpoles of *Microhyla nilphamariensis*. The treated tadpoles showed abnormalities in the eye and intestine of the feeding stages and eye and kidneys of pre-metamorphic stages. Significantly, most of the treated tadpoles died before attaining metamorphosis showing that Vitamin A palmitate wat lethal to the tadpoles. Few that survived the treatment had a delayed metamorphosis with various morphological abnormalities indicating the critical effects of Vitamin A on thyroid hormone

function. The study also discusses the potential implications of improper disposal of drug on the growth and development of anurans.

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