



EFFECT OF THYROXINE ON THE MORPHOMETRIC STUDIES OF TOAD, *BUFO STOMATICUS*

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ABSTRACT

The present study deals with the effect of thyroxine on the young toads of *Bufo stomaticus*. The Toads were exposed to two different doses of thyroxine i.e. 10^{-2} thyroxine and 10^{-4} thyroxine. Experiment was conducted for 15 days. The young toads were examine for various morphometric parameters viz:- Snout to Vent Length (SVL), Head Length (HL), Head Width (HW), Diameter of Eye (DOE), Inter Orbital Space (IOS), Hand Length (HAL), Foot Length (FL), Tibea Length (TL), Leg length (LL), Arm Length (AL), First Finger Length (FFL) and Second Finger length (SFL).The results were statistically analyzed and significant changes were observed on the selected parameters.

Key words: Toad, Metamorphosis, Thyroxine, Morphometry.

INTRODUCTION

There are continued concerns about endocrine disrupting chemical effects, and appropriate vertebrate models for assessment of risk are a high priority. Toad tadpoles are very sensitive to environmental substances because of their habitat and the complex processes of metamorphosis, regulated by the endocrine system, mainly thyroid hormones. During metamorphosis, marked alteration in hormonal factors occurs, as well as dramatic structural and functional changes in larval tissues. There are a variety of mechanism determining thyroid hormone balance or disruption directly or indirectly. Directly acting agents can cause change in thyroxine synthesis or secretion in thyroid, thyroidal iodide uptake, deiodinase and proteolysis. Indirect action may result from biochemical processes such as sulfaction and deiodination. Thyroxine treatment definitely affected growth of the toad.

The relationship between the thyroid glands, adrenal and gond has been described by a number of workers. Metamorphosis and growth process are effectively influenced by thyroxine treatment [1]. Thyroxine is arguably the most important hormone in anuran development and affects development through exogenous and endogenous means [2]. *Bufo stomaticus* lives in low water surface, moist soil and mud. They appear only in monsoon season. Information regarding their morphometry is mearge. Present studies mainly deal with the statistical relationship between morphometric parameters. The morphometric information available on Indian anuran is mainly based on size analysis from systematic description [3-7].Effect of Thyroxine on some digestive enzymes of the adult male toad, *Bufo melanostriectus* already done [8]. Effect of exogenous thyroxine on developmental polyphenism in *spadefoot* toad tadpoles [2]. Thyroid hormone induced gene experssion programma that orchestrate amphibian metamorphosis. In contrast to

anurans, many salamanders do not undergo metamorphosis in nature. However they can be induced to undergo metamorphosis via exposure to thyroxine. In Mexican axolotl (*Ambystoma mexicanum*) induced metamorphosis by using thyroxine [9].

Reproducibility of experiment is a key issue in science [10] and thus a key issue in research in the field of complementary medicine [11,12]. An experiment with thyroxine and amphibians has been published. The effect of thyroidal hormone (Thyroxine) on the absorption process of the isolated rat intestine was done [13]. Present piece of work has been designed to study the statistical relationship of between morphometric parameters of *Bufo stomaticus*.

MATERIALS AND METHODS

Collection of samples, the young toads of *Bufo stomaticus* were collected from fields and temporary pools of water situated near Ram Niwas Garden and Sanganer area of Jaipur. They were brought to the laboratory in polythene bags.

Experimental set up:

The toads were kept in large and deep glass trough covered with net to prevent them from jumping and brought weight which means was used. The animals were fed with chironomous larvae, snails, small insects and algae. Collected specimens were divided into three groups of 25 animals. Group I (control), Group II (10⁻² Thyroxine) and group III (10⁻⁴ Thyroxine) for 15 days. The experiments were carried out at room temperature (28°C -32°C). The rearing medium was changed on every alternate day. To study the changes, the animals were anaesthetized with diethylether and fixed in Bouin’s fixative, preserved in 70% alcohol and examined under stereoscopic binocular microscope for morphometry. The morphometric parameters used were - snout to vent length (SVL), head length (HL), head width (HW), diameter of eye (DOE), inter orbital space (IOS), hand length (HAL), foot length (FL), first finger length (FFL), second finger length (SFL). All the parameters were measured with the help of calipers and ocular and stage micrometer scale. Morphometric variables were statistically calculated and compared with each other with the help of correlation coefficient and t-value significance.

Table 1: Mean, standard deviation and standard error of morphometric measurement of young toad (*Bufo stomaticus*)

Sl.No.	Morphometric Parameters	Control			10 ⁻² thyroxine			10 ⁻⁴ thyroxine		
		Mean length (mm)	S.E.±	S.D. (mm)	Mean length (mm)	S.E.±	S.D. (mm)	Mean length (mm)	S.E.±	S.D. (mm)
1.	SVL	12.15	±0.325	1.028	11.15	±0.247	0.783	11.75	±0.318	1.006
2.	HL	5.45	±0.138	0.437	4.58	±0.202	0.639	4.73	±0.240	0.764
3.	HW	4.75	±0.214	0.677	4.42	±0.218	0.689	4.60	±0.194	0.614
4.	DoE	2.29	±0.090	0.284	2.04	±0.026	0.084	2.02	±0.019	0.063
5.	IoS	2.03	±0.055	0.176	2.04	±0.026	0.084	2.02	±0.019	0.063
6.	HAL	6.72	±0.213	0.675	6.15	±0.269	0.851	6.25	±0.226	0.716
7.	FL	5.72	±0.238	0.754	6.25	±0.170	0.540	6.40	±0.145	0.459
8.	TL	3.73	±0.108	0.343	3.57	±0.079	0.249	3.62	±0.089	0.282
9.	LL	11.13	±0.380	1.203	11.40	±0.323	1.021	12.00	±0.333	0.054
10.	AL	2.66	±0.083	0.263	2.48	±0.096	0.304	2.20	±0.104	0.329
11.	I st FL	1.05	±0.050	0.158	1.19	±0.072	0.228	1.03	±0.029	0.094
12.	II nd FL	2.05	±0.050	0.158	2.20	±0.081	0.258	2.17	±0.105	0.333

SVL= Snout vent Length
 HL= Head length
 HW= Head width
 IoS= Inter orbital space
 DoE= Diameter of eye
 HAL= Hand length
 FL= Foot length
 LL= Leg length Ist
 FL= First finger length
 TL= Tibia length
 AL= Arm length
 IInd FL= Second finger length

Table 1: Statistical relationship between different morphometric parameters in young toads (*Bufo stomaticus*)

Sl.No.	Morphometric Parameters	Control		10 ⁻² thyroxine		10 ⁻⁴ thyroxine	
		r-value	t-value significance	r-value	t-value significance	r-value	t-value significance
1	SVL with TL	-0.486	3.922*** P< 0.001	+0.271	3.922*** P< 0.001	+0.089	3.222*** P< 0.001
2	TL with FL	+0.072	3.922*** P<0.001	+0.259	3.922*** P<0.001	+0.208	3.922*** P<0.001
3	HL with FL	-0.396	2.878** P>0.01	+0.171	2.878** P>0.01	-0.074	2.878** P>0.01
4	SVL with HL	+0.764	3.922*** P<0.001	+0.191	3.922*** P<0.001	+0.095	3.922*** P<0.001

** P> 0.01 Significant

*** P<0.001 Highly Significant

RESULTS AND DISCUSSION

It was observed that in Group I (control) percentage of survivability was 100% and no mortality was noticed, the animals were normal in all respects. Table-1 represents mean length of the body which showed an increase upto 12.15mm in snout to vent length. Snout pointed, nostrils nearer to tip of the snout than the eye . Inter orbital space broader than width of upper eyelids, first finger shorter than the second finger. The upper surface was dark with patches on the body.

In case of Group II (10⁻² Thyroxine) mean length of the body was less than the control, it was 11.15 mm. Inter orbital space (IOS) and foot length (FL) were more than the control. First Finger Length (FFL) and Second Finger Length (SSL) were less than the control (Table-1). In case of Group III (10⁻⁴ Thyroxine) mean length of the body was more than the Group II but less than the control. Inter orbital space (IOS) was less than the Group II. Second Finger Length (SFL) was more than the Control (Table-1).

Normal thyroid gland activity is concerned mainly with energy metabolism in nearly all tissues of the body. Development of the hyperthyroid state in vertebrates elevates basal metabolic rate due to increment in the rate of oxygen consumption in target tissues [14].

Direct effect of thyroxine on mature rats can be considered as a part of its overall catabolic action. The different response of immature rats to thyroxine compared to older animals could be attributed to the difference in thyroxine metabolism and the development pattern [15]. The amount of thyroxine acts on metamorphic tissues during development. Difference in development treated with difference in receptor or metamorphic gene activity in addition to possible difference in amount of circulating thyroid hormone [2]. Present findings are supported by above authors.

Thyroxine treatment initially inhibited growth of the body in young toadlets in Group- II, it was decreased (Table-1). Morphometric relationship between SVL with TL, TL with FL, HW with FL and SVL with HL were analysed statistically 10⁻² thyroxine concentration was more effective there was positive correlation between different morphometric parameters in toads (Table1 & 2) Head length was highly significant in 10⁻² thyroxine and 10⁻⁴ thyroxine concentration (Table-2).

In axolotl metamorphosis can be precisely induced and studied in juveniles or adults. Functional genomics approaches are beginning to reshape the way transcription is conceptualized during amphibian metamorphosis [16, 17]. Thus T₄ concentration is known to affect gene expression and mortality in anurans

[18, 19]. A low concentration of T₄ delays the initiation timing of morphological period [9]. When the result of previous experiments it was found that Thyroid hormone treated animals being slower development than the control animals [20-22].

CONCLUSION

In conclusion, our data indicate that T₄ influences the antioxidant defense system on over all the body during metamorphosis. In this study we showed the effect of thyroxine on the morphometric parameters in young toads, it depends upon the different doses of thyroxine.

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REFERENCES

1. McGarry M P and Vanable J W, The role of Thyroxine in the formation of Gland Rudiments in the skin of *Xenopus laevis*, 1969, Dev. Bio. 20, 426-434.
2. Storz and Brain L, "The Role of Thyroxine in spadefoot toad Development". 2003, Electronic Thesis, Treatises and Dissertations. Paper 1553.
3. Mohanty H and Dutta S K, Life history of the common Indian tree frog, *Polypedates maculatus* (Anura : Rhacophoridae). J. Bombay Nat. Hist. Soc. 1981, 85,512-517.
4. Shaffer HB, Size and scaling in the Indian frogs, *Nyctibatrachus* and *Nanno batrachus* (*Ranidae*). Fieldian Zool.1988, 46,1-10.
5. Sekar A G, a role of the morphometry of *Rhacophorus malabaricus*, the malabar gliding frog. J. Bombay Nat. Hist. Soc.1988, 85,527-628.
6. Mohanty A K, Biology of Indian Paddy Field frog, *Rana limnocharis*. 1994, Ph. D. thesis, Utkal University, Orissa.
7. Saxena S and Sharma R, Effect of testosterone on the morphometric studies of toad, *Bufo fergusonii*. J. Exp. Zool. India, 2003, 3, 231-235.
8. Bhattacharayya SK, Chaki KK and Misra KK, Effect of thyroxine on some digestive enzymes of the adult male toad, *Bufo melanostictus*. Folia Biol (Krakow).2002, 50(1-2) : 83-90.
9. Page R B, Voss SR, Samules AK, Smith JJ, Putta S and Beachy CK, Effect of thyroid hormone concentration on the transcriptional response underlying induced metamorphosis in the Mexican axolotl (*Ambystoma*). J. of Bio. Med. Genomics. 2008, 9:78.
10. Richter SH, Garner JP, Auer C, Kunert J, Wuerbel H, Systematic variation improves reproducibility of animal experiments. Nat. Methods, 2010, 7(3):167-168.
11. Bellavite P, Orolani R, Conforti A, Immunology and homeopathy. 3. Experimental studies on animal models. J. of Evid based Complement Alternat Med.2006, 3 (2): 171-186.
12. Stoc-Schroerer B, Albrecht H, Betti L, Reporting experiments in homeopathic basic research – description of the checklist development, J. of Evid Based Complement Alternate Med. 2011, (10), 1093,170.
13. Matty A J and Seshadri B, Effect of thyroxine on the isolated rat intestine. Int. J. of Gastroenterology and Hepatology, 2013. Gut, 6 (2), 200-202.
14. Videla L A, Energy metabolism, Thyroid calorigenesis and oxidative stress: functional and cytotoxic consequences.2000, Redox Rep 5:265-275.
15. Saicic Z S, Mijalkovic D N, Nikolic A L, Blagojevic DP and Spasic M B, Effect of thyroxine on antioxidant defense system in the liver of Rats of Different age.2006, Physiol. Res. 55:561-568.
16. Das B, Cai L, Carter MG, Piao YL, Sharov AA, Ko MSH, Brown DD, Gene expression changes at metamorphosis induced by thyroid hormone in *Xenopus laevis* tadpoles. Dev. Biol.2006, 291:342-355.
17. Buchhaloz DR, Heimerier RA, Das B, Washington T, Shi YB, Pairing morphology

- with gene expression in thyroid hormone-induced intestinal remodeling and identification of a core set of TH-induced genes across tadpole tissues. *J. of Dev. Biol.* 2007, 303:576-590.
18. Zhang F, Degitz SJ, Holcombe GW, Kosian PA, Tietge J, Veldhoen N, Helbing CC, Evaluation of gene expression end points in the context of a *Xenopus laevis* metamorphosis based bioassay to detect thyroid hormone disruptors. *Aquat Toxicol*,2006, 76:24-36.
 19. Helbing CC, Ji L, Bailey CM, Veldhoen N, Zhang F, Hol Combe GW, Kosian PA, Tietge J, Korte JJ, Degitz SJ, Identification of gene expression indicators for thyroid axis disruption in a *Xenopus laevis* metamorphosis screening assay Part 2. effects on the tail and hindlimb. *Aquat Toxicol*,2007, 82: 215-226.
 20. Lingg G, Endler PC, Highland amphibians – recalculation of raw data from 1990 to 2010 on the effect of highly diluted thyroxine. *Int. J. High Dilution Res*, 2011, 10(37):311-324.
 21. Carrasco S, Ferreira CM, Bonamin LV, Ultra high dilution of triiodothyronine modifies cellular apoptosis in *Rana catesbeiana* tadpole tail in vitro. *J. of Homeopathy* ;2011, 100:220-227.
 22. Harrer B, Replication of an experiment on extremely diluted thyroxine and highland amphibians. *J. of Homeopathy*. 2013, 102,25-30.

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