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EFFECT OF *ARNICA* ON LENS REGENERATION IN ADULT FROG *RANA CYANOPHLYCTIS*

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The present research aims at showing the role played by *Arnica mont* Q. in inducing lens regeneration even in the adult frog *Rana cyanophlyctis* which otherwise have lost this capacity. It has been observed that certain chemicals which increase the mitogenic activity and dedifferentiation may cause lens regeneration in amphibians. *Arnica mont* Q. was found to accelerate cell proliferation and dedifferentiation of pigmented epithelial cells (PECs) of dorsal iris and consequently induced the lens regeneration in adult frog. Arnica was found to induce lens regeneration in 80% adult frogs of treated group whereas lens regeneration was not reported even in a single animal of control group. Most of the regenerated lenses were small in size and nodulated in shape. However, 40% regenerated lenses were found normal in size, shape transparency and histological features. The most common feature of *Arnica mont* Q. treated regenerates, was precocious differentiation of lenses, cytoplasmic vacuolization and herniated lens fibers. It is speculated that this precocious differentiation caused by arnica, might be one of the reasons for smaller size of regenerated lenses.

INTRODUCTION

Lens regeneration provides a clear example of transdifferentiation of differentiated cellular type having a distinctive pattern of metabolic activities to another cellular type, which is morphologically and biochemically distinct from original. *Arnica mont* Q. have positive effect on wound healing and inflammation. Arnica was once use to treat colds, fevers, sore throat of infection, upper respiratory tract and in flok tradition it was ready for heart problems, uterine bleeding, fatigue boils, insect bite and inflamed veins (Blumenthal *et al.*, 2000; Reider *et al.*, 2001). This motivated us to experimentally use the drug on lens regeneration in adults of *Rana cyanophlyctis.*

It has been found that some chemicals which increase the mitotic activity and differentiation may cause lens regeneration in amphibians. *Arnica mont* Q. might also accelerate lens regeneration through increased proliferation and dedifferentiation of dorsal iris cells. Therefore, this chemical was selected for testing the effect on lens regeneration in adult frog *R. cyanophlyctis.*

MATERIALS AND METHODS

The experiments were carried out on 50 adult frog *R. cyanophlyctis.* Lentectomy was done under local anesthesia (2% xylocane). A fine longitudinal slit was made in the cornea of right eye under a stereoscopic binocular microscope. The complete intact lens was extracted through the incision (Fig. 1). Out of these operated animals, 25 were treated with *Arnica mont* Q. and remaining 25 were kept untreated that served as control. Following the operation 0.1 ml of *Arnica* solution was given as orally on alternate days up to the day of termination of experiment (40^{th} day afte operation). Same treatment was given to the animals of untreated group. For treatment, mother tincture of *Arnica mont* Q. was used.

The operated animals of both Arnica treated and untreated control groups were preserved at different time intervals in Bouin's solution for histological evaluation. After 24 to 30 hrs of

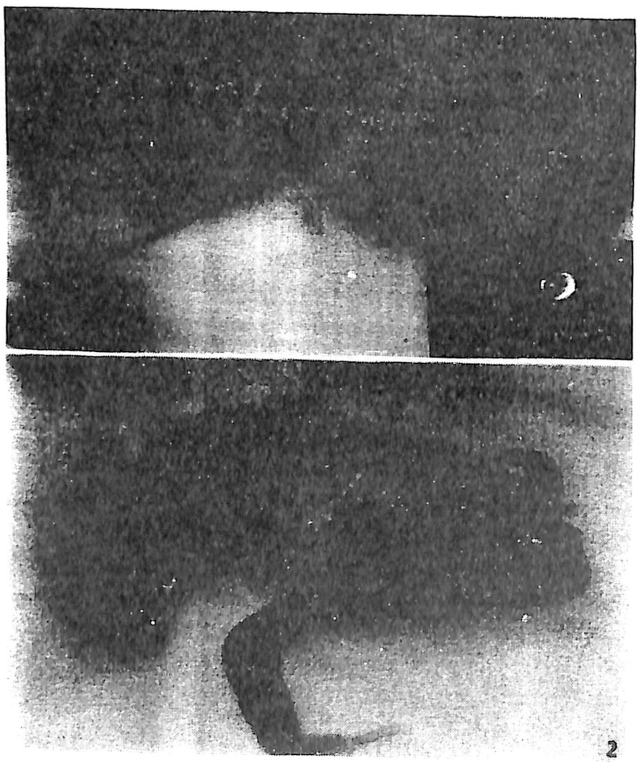
preservation, animals were transferred to 70% alcohol for histological slide preparation, eyes were removed from the preserved animals and dehydrated in alcohol series, cleaned in xylene and embedded in paraffin wax. These were sectioned and stained in haematoxyline and counter stained with eosin.

RESULTS

In the present experiment the *Arnica mont* Q. induced the lens regeneration from dorsal iris. Results obtained are presented in Table I.

Table I : Lens regeneration in adult frog under the influence of *Arinca mont* Q.

Group	No. of animals	No. of regen-erates	No. of non reg-enerates	% of regen-eration	Mode of regeneration		% of regenerated	
					Lens	Lentoid	Lens	Lentoid
Arnica Mont Q. Treated	25	20	05	80	08	12	40	60
Control	25	Nil	25	00	-	-	-	-



Figs. 1 & 2 : 1. Technique of lens extraction from eye, insertion of needle in to the right eye and pushing the intact lens out; 2. Normal looking right operated eye with regenerated lens.

Histological study of lens regeneration showed that lens regeneration had occurred in 20 out of 25 operated animals. Out of 20 regenerates only 8 lenses were found normal and rest 12 were of lentoid shape. Cytoplasmic vacuolization and herniated lens fibers were common features in regenerated lens/lentoids. The percentage of lentoid formation was found to be high (60%). In the animals of control group the lens regeneration was not observed even in a single case.

The morphological features of regenerated lenses like shape, size and transparency were found similar to that of normal intact lenses. Externally, it was difficult to distinguish the right operated eye (with regenerated lens) from the normal intact eye on the day of termination of experiment (Fig. 2). The operated eye with regenerated lens was found normal in its function. It was tested by its normal response given when intact left eye was closed by putting black tape of it.

The results show that *Arnica mont* Q. can successfully induce lens regeneration in adult frog *R. cyanophlyctis*. Lens regeneration was not reported even in a single case of untreated control group animals; while in the Arnica treated animals of the same age lens regeneration occurred in 80% individuals.

Histological study revealed that during lens regeneration the two layers of pigmented epithelium of dorsal iris began to thicken and iris cells changed their shape. Then the pupillary margin of the iris becomes knob-like (Figs. 3 & 4). The formation of this knob-like structure continued until the free margin became a swollen loop-like structure; scattered mitotic figures were also observed. All these changes continue up to day 7 after operation in Arnica treated animals. Then the cells started to dedifferentiate, they threw out their melanosomes (Fig. 5). These melanosomes were ingested by macrophages that entered from the wounded site. Dorsal iris cells continued to divide forming a vesicle like structure in the region of the removed lens (Fig. 6). Now the vesicle differentiated into a regenerated lens. Once the new lens formed, the cells of the dorsal iris ceased mitosis. The newly formed lens was surrounded by a lens epithelium whose cells were cuboidal and slightly taller than before. In addition lens fiber formation was initiated in the inner surface of the vesicular lens. Cells began to elongate and entered the lumen of the vesicle. Gradually, the lumen was filled by primary lens fiber nuclei before the secondary lens fibers began to form (Fig. 7). Later, the secondary lens fiber began to differentiate and grow around the central nucleus and the regenerated lens became a better defined structure. In the next stage, the lens detached from the dorsal iris and returned to its normal status (Fig. 8). At last the nuclei of the secondary lens fibers progressively disappeared. The distinguishing feature of *Arnica mont* Q. treated regenerated lenses were the arrangement of differentiated secondary lens fibers. All the fibers were arranged in a transverse fashion.

The most common feature of *Arnica mont* Q. treated animals in lens regeneration was precocious differentiation of lens, cytoplasmic vacuolization and herniated lens fibers. The percentage of lentoid formation was also found higher (60%). The development of lens and the lentoid was almost similar except precocious differentiation in the later. The lentoids were of different shapes, mostly eliptical, nodulated and spherical. Most of them were having vacuolated lens fibers (Fig. 9).

DISCUSSION

In the present study it has been found that *Arnica mont* Q. induces lens regeneration in adult frog *R. cyanophlyctis*. However, the regenerated lenses were found smaller in size and lens fibers of the most of the regenerated lenses were herniated and irregularly arranged. In 8 cases, the regenerated lenses were found normal in shape, size and transparency. The remaining regenerated lenses were of lentoid type.

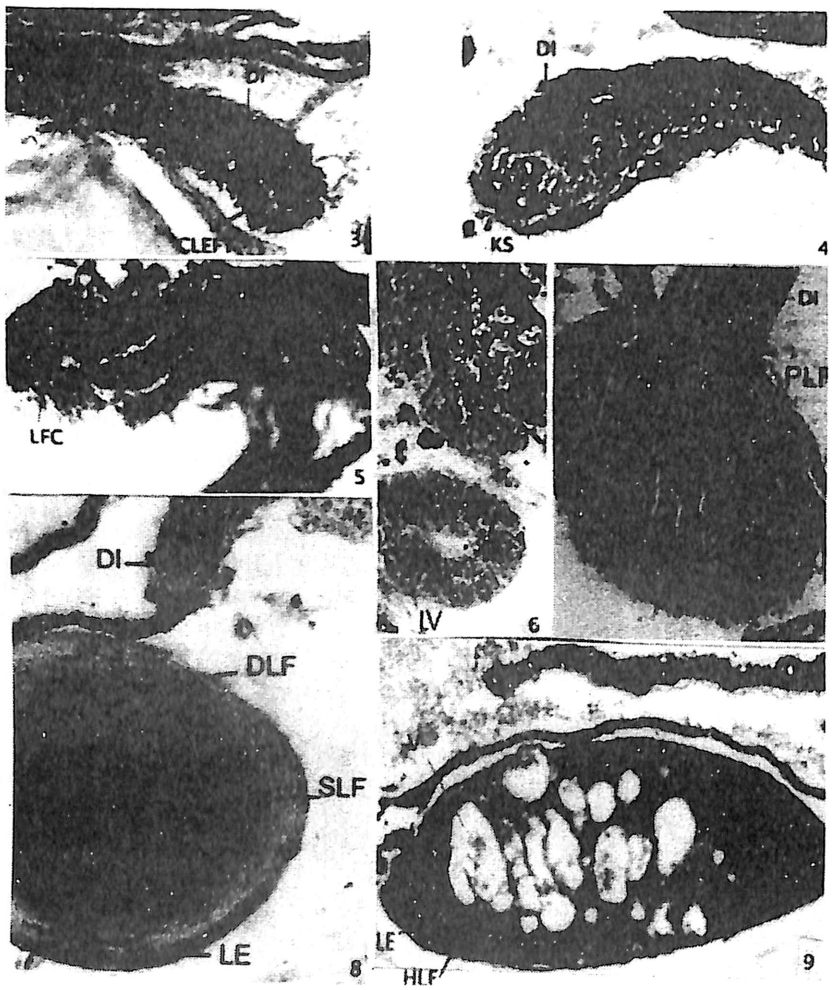


Fig. 3-9 : 3. Bilaminar layers of dorsal iris with a visible cleft in between two layers (100x); 4. Swollen knob like structure at the tip of dorsal iris (100x); 5. Lens forming cells in the cleft of dorsal iris (100x); 6. Cellular lens vesicle at the tip of dorsal iris in Arnica treated frog (100x); 7. Early differentiation of regenerated lens of Arnica treated frog (100x); 8. Well differentiated regenerated lens of Arica treated frog (50x); 9. Vacuolated regenerating lens/lentoid (50x).

(IL=Intact lens; OERL=Operated eye with regenerated lens; DI=Dorsal iris; KS=Knob-like structure; LFC=Lens forming cells; LV=Lens vesicle; PLF=Primary lens fibers; DLF=Differenting lens fibers; SLF=Secondary lens fibers; LE=Lens epithelium; V= Vacuole; HLF=Herniated lens fibers)

The exact mechanism of *Arnica mont* Q. on lens regeneration is not yet clear and the literature on this homeopath drug (with this respect) is very scanty. However, Acharaya (2003) reported inductive and accelerating effect of *Arnica mont* Q. on lens regeneration in Swiss albino mice. It is also reported by several homeopath doctors that Arnica promotes the wound healing and tissue regeneration (Blumenthal *et al.*, 2000; Reider *et al.*, 2001). It also improves the circulation and mitotic activity in the injured tissues. Thus precocious differentiation of regenerating lens is a peculiar feature observed in the present study.

The precocious differentiation activity might be one of the reasons of small regenerated lenses and high percentage of lentoids in the present observation. This type of effect of Arnica gives similar impact to that of thyroxine effect on limb regeneration in amphibian tadpoles. Jangir (1980) suggested that thyroxine hormone induces precocious differentiation of blastemal cells during limb regeneration in the tadpoles of *Bufo melanostictus* and also enhances cell division in the blastemal cells. Cell proliferation by itself is not so important for normal and proper regeneration. What is important is whether this proliferation occurs in dedifferentiating cells or in redifferentiating cells matters much. The former leads to good regeneration while the later would be associated with defective regeneration. For normal regeneration to take place it is necessary that the usual temporal pattern of wound healing, dedifferentiation, proliferation and establishment of a proper blastema prior to the beginning of redifferentiation must not be disturbed. It seems that thyroxine interferes with this normal pattern by inducing an early and precocious morphogenesis, histogenesis and growth in the regenerates. Consequently, these redifferentiation processes begin before cells liberated from the stump tissues have dedifferentiated sufficiently to acquire full morphogenetic potencies to give rise to a fully normal limb. This appears to be reason why regenerates of thyroxine treated tadpoles ultimately turn out to be more oligodactylous, malformed or deficient in other ways.

In the present study too, regenerating lenses of *Arnica mont* Q. treated animals were found to be abnormal most of them were smaller in size, having herniated lens fibers and were highly vacuolized.

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REFERENCES

ACHARYA, P. 2003. Histological and Biochemical studies on lens regeneration in adult mice under the influence of different chemicals. *Ph.D. Thesis, University of Ajmer, India.*

BLUMENTHAL, M., GOLDBERG, A. & BRINCKMANN, J. 2000. *Herbal medicine : Expended commission.* Monograph Copyringt American Botanical Council. Publ. by Integrative Medicine Communications, 1029 Chestnut Street, Newton, MA 02464. pp. 7-9.

JANGIR, O.P. 1980. Experimental studies on the ontogenesis and generation of limb in the anura *Bufo melanostictus. Ph.D. Thesis, University of Rajasthan, India.*

REIDER, N., KOMERICKI, P., HAUSEN, B.M., FRITSCH, P. & ABERER, W. 2001. The samy sid of natural medicines : Contact sensitization to arnica (*Arnica montana* L.) and marigold (*Calendula officinalis* L.). *Contact Dermatitis.* **45**(5) : 269-272.