Effect of *Emblica officinalis* (Gaertn) on lens regeneration in the frog, *Rana cyanophlyctis* (Schneider)

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*Emblica officinalis* (Amla) accelerated cell proliferation and dedifferentiation of pigmented epithelial cells of dorsal iris and consequently induced lens regeneration in *R. cyanophlyctis*. Further it enhanced the percentage of lens regeneration not only in young tadpoles but also in adult frogs. Lens regeneration ability declined with the age of animals in both control as well as treated groups.

**Keywords:** *Emblica officinalis*, Frog, Lens regeneration, Tadpole

Lens regeneration provides a clear example of transdifferentiation of differentiated cellular type having a distinct pattern of metabolic activities to another cellular type, which is morphologically and biochemically distinct from original. *Emblica officinalis* (Hindi:Amla) is full of medicinal properties\(^1,2\). Ascorbic acid, a constituent of *E. officinalis* has positive effect on inflammation and wound healing. Ascorbic acid enhances proliferation and dedifferentiation of iris pigmented epithelial cells in culture medium, and also increases the formation of lentoids\(^3\). It is used to treat diabetic cataract, watering of eyes, cough, asthma, bronchitis, peptic ulcer and cardiac disorders\(^4\). Further research of contemporary and traditional medical literature indicates that *Emblica* either in combination with other herbs or alone has been useful in the amelioration of colds, warts, skin afflictions, influenza, elevated cholesterol and as an immune restorative in cancer conditions. These multifarious medicinal properties of *Emblica* prompted the study of its effect on lens regeneration in the frog *Rana cyanophlyctis*.

**Materials and Methods**

The experiments were carried out on 180 animals of different developmental stages of *Rana cyanophlyctis*. Tadpoles of different developmental stages were raised from the spawn of *R. cyanophlyctis* collected from local ponds during mansoon season.

The lentectomy of anaesthetized animals was done under the stereoscopic binocular microscope. A longitudinal slit was made in the cornea of the right eye extending across the middle of the pupillary space. The lens was extracted through incision (Fig 1a, b). The left eye was kept intact. Out of these operated animals, 90 were treated with *Emblica* and remaining 90 were kept untreated and served as control. The mode of treatment was given in two ways depending upon the stage of the animal:

1. Young and mature tadpoles were reared in *Emblica* solution (5 ml *Emblica* extract/500 ml water).
2. 0.12 ml *Emblica* was injected intra-peritoneally into adult frogs.

The doses were given on alternate days upto the day of termination of experiment (40th day after operation). The animals of different stages viz. young and mature tadpoles and adult frogs were categorized into 3 groups: Groups A, B and C

The operated animals of both treated and untreated groups were preserved at different time intervals in Bouin’s solution for histological evaluation. After 24 to 30 hr of preservation in Bouin’s solution, 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.

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Preparation of Emblica extract — Amla powder (50g) was mixed with 1.5 l tapwater and kept for 12 hr. The solution was boiled to make it 250 ml of the whole solution. This extract was treated as standard solution. To make the working solution 5 ml of this standard solution was mixed with 500 ml of tap water. Half of the operated tadpoles (considered as treated group) were reared in the Emblica solution; treated adults were injected 0.12 ml solution of Emblica on alternate days.

Results

The *E. officinalis* induced lens regeneration from dorsal iris (Table 1). The results show that lens regenerative power is found in the tadpoles of *R. cyanophlyctis* however, it declined with the age of the animal. It occurred in 23.3% cases of young three toe stage tadpoles (Group A, A1) in 16.7% in cases of mature five toe stage tadpoles (Group B, B1) and in 6.7% cases of adult *R. cyanophlyctis* (Group C, C1). In contrast, Emblica was found to induce and accelerate lens regeneration in different developmental stages of the frog. It occurred in 93.3% cases of young three toe stage tadpoles (Group A, A2), in 86.7% cases of mature five toe stage tadpoles (Group B, B2) and 80% in adult frogs (Group C, C2) (Table 1). Thus the declining trend of regeneration with age was seen in Emblica treated animals also. 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, i.e. 40th day. The regenerated lens was found normal in its function, which was tested by its normal response obtained when intact eye was closed by putting black tape on it.

| Table 1 — Percentage of cases of lens regeneration in untreated (control) and Emblica treated groups young, mature and adult *Rana cyanophlyctis*. |
|-----------------------------|-------------|----------------|----------------|----------------|----------------|-----|
| Stage of animal             | Sub Groups  | Day of preservation | No. of operated animals preserved | Normal Regenerates | No. of Lentoids | Regeneration (%) |
| Young Tadpoles (3 toe stage) | A1 (Untreated) | 3 5 | - | - | 5 | 23.3 |
|          | A2 (Treated)   | 5 5 | 1 | - | 4 | 93.3 |
|          |                | 7 5 | 1 | - | - | - |
|          |                | 15 5 | 2 | - | - | - |
|          |                | 40 10 | 3 | - | - | - |
| Mature Tadpole (5 toe stage)| B1 (Untreated) | 3 5 | - | - | 5 | 16.7 |
|          | B2 (Treated)   | 5 5 | 1 | - | 4 | 86.7 |
|          |                | 7 5 | 1 | - | - | - |
|          |                | 15 5 | 2 | - | - | - |
|          |                | 40 10 | 2 | - | - | - |
| Adult frog | C1 (Untreated) | 3 5 | - | - | - | 6.7 |
|          | C2 (Treated)   | 5 5 | 3 | 1 | 1 | 80 |
|          |                | 7 5 | 3 | 1 | 1 | - |
|          |                | 15 5 | 4 | - | 1 | - |
|          |                | 40 10 | 8 | 1 | 1 | - |
Fig. 1(a) — Photograph showing insertion of needle into the right eye of adult frog *Rana cyanophlyctis*. (b): Newly extracted (lentectomy) lens (EL) with lens capsule. Fig. 2 — Microphotograph of a section passing through dorsal iris (D1) of *Emblica* treated tadpole of *R. cyanophlyctis* showing depigementation of PECs, dorsal iris becomes swollen and knob like. (× 200). Fig. 3 — Microphotograph of a section passing through lentectomized eye of 3 days *Emblica* treated tadpole of *R. cyanophlyctis* showing cleft formation in between two laminae of dorsal iris. (× 400). Figs 4,5 — Microphotograph of a section passing through lentectomized eye of 5 days *Emblica* treated tadpole of *R. cyanophlyctis* showing formation of lens vesicle at the tip of dorsal iris. (× 200)
The histological changes occurred during lens regeneration in all the three age groups animals were found almost similar. In both, control and Emblica treated animals lens regeneration occurred from pigmented epithelium cells of dorsal iris. Histological observations revealed that during lens regeneration after lentectomy the two layers of pigmented epithelium cells of dorsal iris began to thicken and the nuclei of iris cells changed their shape (Figs 2, 3). The pupillary margin of the iris became knob like. The formation of this knob-like structure continued until the free margin became a swollen loop like structure. Scattered mitotic figures were also observed. These changes continued up to day 7 after operation in Emblica treated animals. Then the cells started to dedifferentiate as they threw out their melanosomes. These melanosomes were ingested by macrophages that entered the wounded site. Dorsal iris cells continued to divide forming a vesicle-like structure in the region of the removed lens (Figs 4-6). Now the vesicle differentiated into a new regenerated lens (Figs 7, 8). Once the new lens formed, the cells of dorsal iris ceased mitosis. The newly formed lens was surrounded by a lens epithelium cells of which cuboidal and slightly taller than before. In addition, lens fiber formation began in the inner surface of the vesicular lens. Cells began to elongate and entered the lumen of the vesicle. The lumen was filled by primary lens fiber nuclei before the secondary lens fibers began to form (Fig. 8). Later, the secondary lens fibers began to differentiate and grow around the central nucleus and the regenerated lens became a better-defined structure. In the next stage the regenerated lens got detached from the dorsal iris and returned to its normal position (Fig. 9). At last, the nuclei of the secondary lens fibers progressively disappeared. Figures 9, 10 show operated eyes of Emblica treated animals with regenerated lens. Figure 10 shows the placement of regenerated lens into normal position by day 40 after operation.

In untreated operated adult frogs, lens regeneration occurred in only two cases in which a nodulated lentoid structure was found to develop. Histological study revealed that the structure so developed was almost similar in its origin to normal lens, originated from dorsal iris in lentectomized iris but was of smaller size. Whereas, in treated adult frogs regenerated lenses were similar to normal intact lenses. Only in some cases regenerated lenses were of lentoid type.

Discussion

The results of the present study showed that E. officinalis induced lens regeneration in not only in young and mature tadpoles but also in adult R. cyanophlyctis, and that lens regeneration capacity declined with the age of animals; similar trend was also found both in untreated and Emblica treated groups. In both of the groups results clearly show that lens regeneration declined with the age of the animals. Similar trend was found both in untreated and in Emblica treated groups.

The mechanism of Emblica effect on lens regeneration is not clear. The literature on this Ayurveda drug is very scanty. However, it is reported that ascorbic acid (a constituent of Emblica) has accelerating effect on wound healing and enhances proliferation and dedifferentiation of iris pigmented epithelial cells in culture medium. Thus accelerating effect on lens regeneration in all the developmental stages of the animal R. cyanophlyctis is a peculiar feature observed in the present study.

Lens regeneration from non-ocular tissue (dorsal iris) has been well-documented in urodele amphibians. However, it has been observed that the ability of iris and retinal pigmented epithelial cell (PECs) to transdifferentiate into lens is not restricted to urodele amphibians but is widely conserved in vertebrates. Good lens regeneration from PECs in vivo has been seen in a few species of fishes and some other amphibians. Vitamin A is known to induce and accelerate lens regeneration in amphibian tadpoles, froglets, chick embryos and Swiss albino mice. In cell culture study, PECs of almost all vertebrates can switch differentiation to acquire the characteristics of
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lens. \(^{13-16}\) Pigmented epithelial cells dissociated from fully grown human eyes readily trans-differentiated into lens phenotypes in the manner observed in chick embryo PECs. \(^{13}\)

Lentectomy stimulates the iris epithelial cells of the newt’s eye to undergo DNA synthesis and proliferate. \(^{7-19}\) Concomitantly with these processes, melanosomes disappear from the pigmented epithelial cells of iris and thus undergo dedifferentiation. Similar events have also been reported in present experiment. After completion of the phase of dedifferentiation, some cells retreat from the cell cycle, elongate and proceed to synthesize lens specific proteins and transform into lens fibers. \(^{18,19}\)

It is found that *Emblica* can enhances dedifferentiation of iris pigmented epithelial cells of tadpoles and adults of *R. cyanophlyctis* resulting in lens regeneration. This suggests that *Emblica officinalis* may be usefully employed for investigations of the molecular mechanisms responsible for homeotic transformation. Lens regeneration appears to be a suitable system for such investigation. The discovery opens the possibility that researchers may one day enhance the endogenous regenerative capacity of mammals by inducing cellular dedifferentiation in vivo.

References