Vol. 12. No. 4. 2023

DOI: 10.13140/RG.2.2.16595.76322

Contents available at:

http://www.crdeepjournal.org

International Journal of Basic and Applied Sciences (ISSN: 2277-1921) (CIF:3.658 ; SJIF: 6.823)
(A Peer Reviewed Quarterly Journal)

Full Length Research Paper

A Histological Study of Lens Regeneration in Swiss Albino Mice under the Influence of *Emblica officinalis*

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ARTICLE INFORMATION

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Article history:
Received: 25-11-2023
Revised: 30-11-2023
Accepted: 08-12-2023
Published: 12-12-2023

Key words:
Swiss albino mice, lens, regeneration, *Emblica officinalis*, pigmented epithelial cells.

ABSTRACT

Regeneration is a very fascinating process by which an organism restores and replaces its damaged, lost or amputed body part. In the present study, it has been shown that how an aphakic mice regenerates its own lens from dorsal iris. Lens regeneration is a clear case of metaplasia or trans differentiation where other than lens tissue, non- lens ocular tissue do the job. The study was carried out on 120 animals belonging to two different developmental stages .Half of the animals were taken as 7 days old and another half were 60 days old swiss albino mice .Both the developmental stages were treated with Emblica officinalis. Doses were given in the form of intraperitonial injections. Injections were given on alternate days. Control groups were given sham injections in similar way. Experiment was terminated on 45th day after operations. Histological study revealed that newly regenerated lenses were very much similar to natural lens. It has also proved that process of lens regeneration is age dependent as lens regeneration capacity declines when animal reached to advanced age. Emblica officinalis promotes lens regeneration not only in young mice, but also in sexually mature mice proving that it has capacity of accelerating cell proliferation and inducing differentiation in pigmented epithelial cells of dorsal iris.

Introduction

Regeneration in biology, the process by which some organisms replace or restore lost or amputated body parts or the processes of renewal, restoration and tissue growth that makes genomes cells or organisms and ecosystems resilient to natural fluctuations or events that cause disturbance or damage. It reflects the reawakening of the development processes of morphogenesis and differentiation in adult when such process cease in post embryonic life of Organism. This ability of regeneration is present throughout the animal kingdom but in different extent Termbley first discovered this process in Hydra (coelenterate) in 1740. In invertebrates, the phylum coelenterates have the greatest ability of regeneration. Fair regeneration ability can be seen in other organisms like Planaria, Nemerteane and annelids but mollusks nematodes, arthropods and echinoderms show poor power of regeneration. Among vertebrates it is remarkably present in reptilians and amphibians. Birds and mammals show regeneration is lesser extent. Regeneration can be seen in many organs like tails, feathers, Limbs. Lens shows a very good example of regeneration in frogs, mice and pigs.

Fig 1 : Image showing the newly born pups of Swiss albino mice.

Lens is a transparent structure located in eyes and surrounded by a thin transparent membrane called lens capsule which protects, shields and maintains the shape of lens. Lens is composed of transparent proteins known as crystalline and capsules consist of collagen fibres. Due to the lamellar arrangement of fibres, it is highly elastic. The eye with natural lens is called Phakic eye and after the removal of lens eyes become aphakic. Function of lens is to focus the light rays passes through it in order to create clear Image of objects positioned at various distance Lens is highly flexible structure and suspended behind the Iris. Lens is a very good example of trans-differentiation (reviewed in Broke and Tosh 2005). In mice a new lens is formed by transformation of cells from one differentiated tissue type to another. Lens regeneration is a very complicated process consists of many events. Non-lens ocular tissue trans-differentiate in to lens observed in vertebrates like fishes and avian (Eguchi and Itoh 1981, Okada 2000). Lens regeneration in newts after lentectomy was observed by Eguchi and Kodama in 1995. An abundant literature exist on Lens regeneration in amphibians (Reyer, 1971; Reyer 1990; Stone 1959; Yamada 1967; Jangir et al 1995) Non Lens ocular tissues like iris and retinal pigmented epithelial cells (PECs) have the capability of trans-differentiation and differentiate in to lens. This is observed almost all vertebrates including mammals. In cell culture, PECs of almost all vertebrates trans-differentiate into Lens (Eguchi 1997, Okada 2000). *Emblica* exhibits strong anti-oxidant activity. It is one of the most important plants in traditional Ayurvedic medical system as well as in other traditional health systems for immunomodulatory, anti-inflammatory, antiulcer, hepatoprotective and anti cancer actions. The plant extract (aqueous) was tested for its radio protective properties against sublethat gamma radiation in Swiss albino mice. Experiment conducted on *Emblica officinalis* (amla) suggested acceleration of cells proliferation and differentiation of PECs of dorsal Iris and consequently induced lens regeneration in R. Cyanophlyctis. Further it increases the rate of regeneration not only in young tadpoles but also in adult frogs. Lens regeneration ability declines with age of animals in both control as well as treated groups. (The regenerative property of *Emblica* motivates to explore their influence on lens regeneration in Swiss Albino mice).

Experimental conducted by Banot J, OP Jangir, Mansi Sharma (2008) indicate *Emblica officinalis* induce and accelerate lens regeneration in different development stages of frog. Sumitra et al (2009) reported that *Emblica* increase cellular proliferation and cross linking of collagen at the wounded site and thus leading to the rapid healing of wound. G.K. Gupta, Digvijay Singh Shekhawat OP Jangir et al (2013) reported E. Officinalis enhance differentiation of myocardial tissue cells of tadpole of R.Cyanophlyctis.

Material and Method

Experimental was conducted on newly born (7 days old) Swiss albino mice and sexually mature (60 days young) Swiss albino mice for study of trans-differentiation ability of non-lens Ocular tissue. For this purpose 6 male and 18 females were procured from the Animals house of Choudhary Charan Singh University, Hisar. Mice colonies were prepared by placing one male mice with three females in each cage. Lentectomy was done on 120 mice in which 60 mice were newly born and 80 mice were sexually mature. It was carried out under local anesthesia (2% xylocaine). A longitudental slit was made in the cornea of the right eye under a stereoscopic binocular microscope.

Fig 2 : Image showing the removal of lens through lentectomy

Fine needles, curved forceps, crossway scissors are used to extract lens along lens capsule from right eyes. *Emblica* extract of 100µl/100ml was prepared for experimental purpose from mother tincher of *Emblica* officinalis. Operated animals of developmental stages were divided into two groups. One group was taken as control and another as treated group. Newly born mice were given a dose of 0.25 ml of 100µl/100ml *Emblica* extract intraperitonially. Mature mice were treated with a dose of 0.5ml of 100µl/100ml *Emblica* extract intraperitonially *Emblica* extract. Treated group were given *Emblica* extract on alternate days. Control groups were not given any drug but treated with Sham injections on alternate days. Animals were preserved on 3,5,9,25,45 days after operation. Bouin's solution was used for preservation. Animals were sacrificed with cervical dislocation and whole Animal was preserved in Bouin's solutions for 24 hrs and Then eye balls were removed for histological study.

The eye tissues were transferred to 70% alcohol. After then tissue were transferred to 90% & 100% alcohol twice for 15 minutes. After then tissue were transferred to xylene for 15 minutes twice. Tissues were embedded in to paraffin wax and blocks were prepared for histological study. Thin slices of tissues (ribbons) were prepared by cutting blocks with microtome. Dehydration of tissues was done with alcohol series of 30%, 50%, 70%, 90% and 100%. Xylene was used as clearing agent. Eosin and haematoxylin dyes were used for staining.

Results

Complete intact lens was regenerated from Pigmented epithelial cells (PECS) of Iris on 45 days after operation. Newly regenerated Lens was morphologically similar to natural intact lens. Size, shape and transparency of regenerated lens was exactly similar to natural lens. Distinguation between natural lens and regenerated lens seemed very difficult. When experimentally tested, regenerated lens were found normal in function. Lens regeneration was seen in both control and *Emblica* treated group. Young animals have high percentages of lens regeneration than the adult mice which confirmed that regeneration. Capacities decrease with age. In control group Adult animal had very less ability to regenerate the lens from PECs. Lentoid formation can be seen in Adult animals, but *Emblica* treated both group shows high percentages of lens regeneration. In treated group of adult animals lens regeneration were seen in high percentage in comparison to control group adult animal.

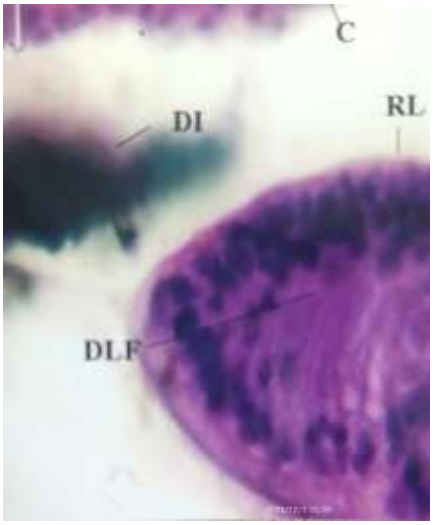

Fig 3 : Microphotograph of regenerated lens in Swiss albino mice showing differentiating lens fibre after 15 day of operation.

Histological studies revealed that after lentectomy, two layers of Pigmented epithelial cells of dorsal iris began to thicken and nuclei of Iris cell began to change their shape. The pupillary margin of Iris became knob like. Knob like structure perpetually formed until free margin take the shape of a swollen loop like structure. Intercellular communication between cells of pigmented epithelial cells reduced rapidly as a result of inflammatory reactions.

Table 1. Influence of *Emblica* on different developmental stages of Swiss albino mice

Group	Developmental Stage	Sub Group	No. of animal employed	Day of preservation	No. of animal preserved	Lens regeneration	Non regeneration	Percentage
A	7 days old Swiss albino mice	A_1 (Control)	30	3	5	0	5	
				5	5	1	4	
				9	5	1	4	26.66%
				25	5	2	3	
				45	10	4	6	
		A_2 (*Emblica* treated)	30	3	5	3	2	
				5	5	4	1	
				9	5	4	1	73.33%
				25	5	3	2	
				45	10	8	2	
B	60 days old Sexually mature Swiss albino mice	B_1 (Control)	30	3	5	-	5	
				5	5	-	5	
				9	5	-	5	10%
				25	5	1	3	
				45	10	2	8	
		B_2 (*Emblica* treated)	30	3	5	2	3	
				5	5	3	2	
				9	5	3	2	53.33%
				25	5	2	2	
				45	10	6	3	

During initial phase the condensed chromatin of PECs nuclei became progressively dispersed, nuclear volume increased and melanosomes moved towards the peripheral region of each PEC at the dorsal margin of Iris. PEC then discharged melansomes by active participation of macrophages, which appeared around the dorsal margin of Iris. PECs cells then started to de differentiate

after discharging of melanosomes. Dorsal Iris Pigmented epithelial cells continued to divide and then formed a vesicle like structure. This vesicle is then differentiated into a new regenerated lens. After that dorsal Iris cells ceased division. Lens epithelium cells surrounding the newly formed lens were cubodial in shape and taller than before Lens fibre formation were seen in inner surface of vesicular lens. Cells began to elongate and entered the Lumen of vesicle. Lumen was completely filled with primary lens fibre nuclei. Later on secondary Lens fibre began to form and differentiate then grow around central nucleus. Regenerated lens get converted into well-defined structure. At last regenerated lens got detached from dorsal Iris and returned to its natural position. And secondary lens fibre disappeared progressively. In control group of adult Lentectomized mice, Lens regeneration was seen in very less amount most of adult mice of control group developed only nodulated lentoid structure.

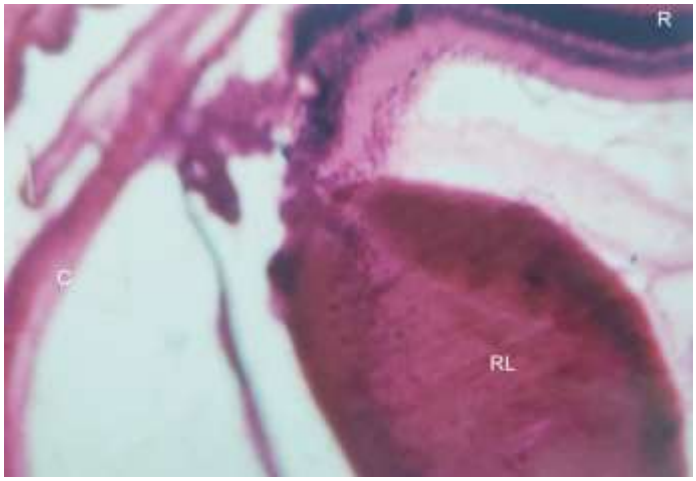

Fig 4 : Microphotograph through eye section of Swiss albino mice after 45 day of lentectomy showing regenerated lens cornea and retina.
C : Cornea, RL : Regenerated Lens, R : Retina.

Discussion

Experiment conducted on different age group of Swiss albino mice proves that *Emblica officinalis* has accelerating and proliferative ability. Differentiation of dorsal iris cells enhanced in presence of *Emblica officinalis.* Wound healing fastened with application of *Emblica*. It has also been noticed that the regeneration process is age dependent process. With the advancement of age the regeneration ability goes declines. After the 45th day of operation and termination of experiment. A complete intact lens is regenerated in place of old lens. Morphologically and physiologically regenerated lens is similar to native lens but slightly irregular in shape. Many signaling mechanism involved during lens regeneration like wnt, hedgehog, fibroblast growth factor and retinoic acid. In the experimental study it has been found that the regeneration ability of control group is 26.66% in 7 days young mice and which declines with advance age of 60 days old Swiss albino mice. Percentage of lens regeneration in these sexually mature mice were found negligible around 10% *Emblica* treated 7 days young mice showed 73.33% of lens regeneration and *Emblica* treated 60 days old sexually mature mice shows 53.33% lens regeneration which proves that *Emblica officinalis* enhance lens regeneration ability even in sexually mature Swiss albino mice. In the previous studies conducted by authors Banot J, OP, Mansi Sharma (2008) on frogs on explain that *Emblica officinalis* induces and speed up lens regeneration in different developmental stage of frogs. Sumitra et al (2009) studied effective role of *Emblica* on cellular proliferation and also reported wound healing properties of *Emblica* officinalis. From experimental studies, it has been found that *Emblica* increases cross-linking of collagen fibres on wounded site. Lens regeneration from non-occular tissue has been studies in amphibians (Reyes, 1954, 1977, Eguchi and Itoh 1982, Eguchi 1988) Lens regeneration ability is not confined to amphibian only but also shown by vertebrates like rabbit, pigs and mice.

The main finding of this study is that at early stages of development mice has the capacity to regenerate a complete eye lens. This ability of regeneration can be enhanced by the adequate dose of *Emblica offcinalis*. It works not only on young Swiss albino mice but also on sexually mature Swiss albino mice. A high percentage of lens regeneration was seen even in *Emblica* treated adult Swiss albino mice. In control group of similar developmental stage showed a negligible amount of Lens regeneration. After the removal of lens in newts, the iris pigmented epithelial cells got stimulated and undergo DNA synthesis and proliferation (Eisenberg and Yamada 1966. Yamada and Roese 1969, Reyer 1971). As reported by Yamada (1967) after completing the phase of de-differentiation, some cells reenter the cell cycle, elongate and starting synthesizing lens specific protein and get converted in to lens fibres. Tsonis et al (1998) studied (distinct) different type of proteins from pigment epithelial cells of dorsal Iris. Yet the *Emblica officinalis* is an Ayurvedic drug having many health benefits but the clear mechanism of lens regeneration through *Emblica* is until known. But ascorbic acid constituent of *Emblica* is well known for its accelerating property. It is found that it enhances the capacity of wound healing. It is also found that *Emblica* accelerates proliferation and de-differentiation of iris pigmented epithelial cells in vitro. Lens regeneration from dorsal Iris is not restricted to amphibians only but also seen in vertebrates. It was found experimentally that Pigmented epithelial cells of all the vertebrates can undergo trans-differentiation in vitro in culture medium.

Conclusion

From the present study, it can be deduced that *Emblica* can induce and accelerate lens regeneration not only in young mice but also in adults. A notable discovery from this study is the intriguing observation that pigmented epithelial cells (PECs) has the potential to trans-differentiate into structure resembling the lens .The dosages of *Emblica* utilized in the study were determined to be effective and can be regarded as optimal ,because no mortality was seen after the administration of dosages. In future ,this lens regeneration technology will provide new treatment ideas and hopes for variety of difficult to treat disease as well as age related cataracts, as in older ages the regenerative capacity declines

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