Induced eye lens cataract in *Cyprinus carpio communis* upon exposure to monocrotophos, an organophosphate

The present studies are based on the feedback provided by Punjab fish farmers practising composite fish culture of Indian major and exotic carps. According to them, during the southwest monsoon, in ponds in the vicinity of toxicant (monocrotophos)-treated paddy fields, the eye lens of most of the culturable fishes became opaque. As a result, there was drastic decrease in the acceptability of supplementary feed, resulting in poor overall growth both in weight and length. The incidence of eye lens opacification has been reported in wild salmon, which has been linked with the use of organophosphate pesticide-treated fishery nets[1].

The opaqueness of the eye lens inhibits or reduces the entry of light on the retina, ultimately regulating the reproductive cycle of fishes, resulting in decreased fecundity and poor fish yield. In the wild this situation can lead to decline in fish diversity and fishery stocks.

In mammals and human beings, drugs and toxicants are known to cause cataractous-like effects[2–6]. The biochemical changes that occur as result of cataractous lens are the increase in oxidative activity evidenced by a fall in glutathione concentration[7], and the consequent structural modification of proteins, resulting in an increase of insoluble proteins[8–12]. In addition, the cholinesterase level is an important neurological–toxicological test to determine the toxicity of the pesticide (organophosphate) in a particular tissue. This enzyme mainly affects the nervous tissue by inhibiting cholinesterase[13–16].

Based on earlier information[1–16], an experiment was set up to pinpoint the reasons for the appearance of opaqueness in the eye lens in the commonly cultured fish, *Cyprinus carpio communis*. It was exposed to increasing concentrations of monocrotophos, i.e. 0.038 ppm (1/10th LC_{50}), 0.062 ppm (1/6th LC_{50}) and 0.126 ppm (1/3rd LC_{50}) for 30 days and subjected to biochemical analyses of insoluble protein, glutathione and acetylcholinesterase along with scanning electron microscopic studies, to ascertain whether the development of cataract was due to toxicant exposure or not. Immediately after sacrificing the experimental fish, the eyeball of the fish was taken out in chilled phosphate buffer and the eye lens was separated. One part of the tissue was semi-dried with filter paper, weighed and homogenized in phosphate buffer using motor-driven Teflon pestle homogenizer at 4°C to obtain 10% (w/v) homogenate. Acetylcholinesterase, glutathione (reduced) content and protein were estimated in this 10% homogenate by standard biochemical methods[17–20] and the other part of the tissue was fixed in 2.5% gluteraldehyde and dehydrated for viewing under scanning electron microscopy.

The acetylcholinesterase activity in the fish lens showed slight variation in its contents at the lowest concentration (0.038 ppm) of pesticide toxicity apparently due to allergic reaction of the pesticide, whereas in the higher dose (0.062 ppm) exposure, an increase up to the level of 15% was observed (Figure 1), where the fish tries to cope with the allergic reaction caused by monocrotophos toxicity. It has been found that at the highest dose (0.126 ppm) it was drastically reduced by 36%, thus causing severe poisoning. It can be inferred that sublethal concentration of monocrotophos is one of the causative reasons for imbalance in the biochemical activities of eye lens (Figure 1). The interpretation was made on the basis of decrease in cholinesterase level which is as follows: if the decreases is 15–25%, the toxicant is slightly poisonous, 25–35% moderately poisonous and 35–50% decline causes severe poisoning[14].

Fish eye lens formation begins during embryogenesis and must retain functionality through its lifespan. The inability to maintain protein stability over time leads to the formation of cataract. Cumulative damage to the proteins causes loss of enzymatic activity and increases the likelihood of protein aggregation, a component of cataract formation. Supporting this view point, in the present studies the insoluble protein has been found to increase by 30% in comparison to the normal. Upon increasing assault with the toxicant, an increase of more than 50% insoluble protein was observed (Figure 2). It is suggested that the trend of increase in insoluble protein corresponds to the cataractous lens.

A healthy eye lens contains glutathione in high concentrations, whereas its low levels adversely affect the eye-lens transparency. Glutathione is synthesized in the lens and is essential for its normal metabolism. It benefits lens functioning by preserving the physico-chemical integrity of proteins in the lens, maintaining water balance, action of the sodium–potassium transport pump, molecular integrity of lens fibres (protein), maintaining molecular integrity of lens fibre membranes, acting as a free-radical scavenger to protect membranes and enzymes from

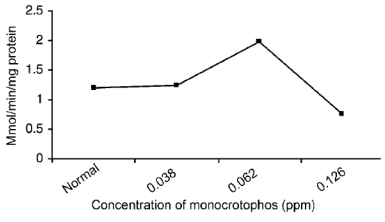

Figure 1. Variation in acetylcholinesterase in the eye lens of *Cyprinus carpio communis* L. upon exposure to different concentrations of monocrotophos.

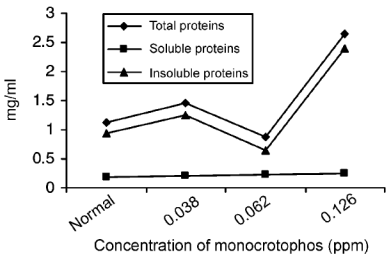

Figure 2. Variation in the soluble, insoluble and total proteins in the eye lens of *C. carpio communis* L. upon exposure to different concentrations of monocrotophos.

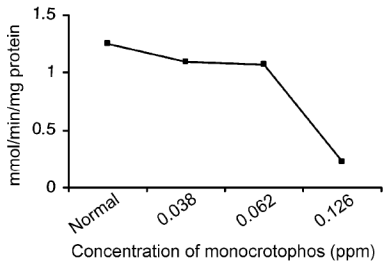

Figure 3. Variation in the reduced glutathione of eye lens of *C. carpio communis* L. upon exposure to different concentrations of monocrotophos.

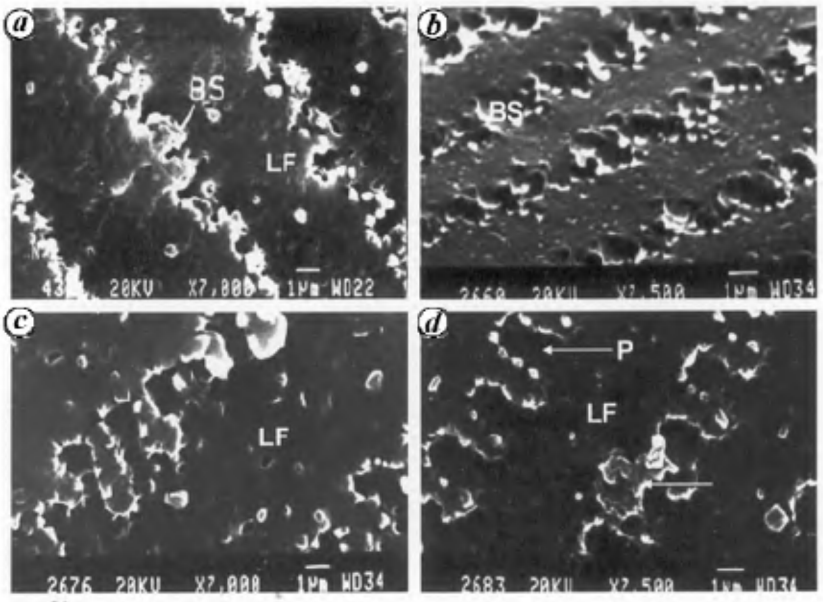

Figure 4. Scanning electron photomicrographs of eye lens of *C. carpio communis* L. ***a***, Normal lens fibres (LF) showing ball and socket (BS) joints. ***b***, Treated eye lens at 0.038 ppm concentration showing widening of intracellular spaces (arrow mark) at interdigitations of BS joints of LF. ***c***, Treated eye lens at 0.062 ppm concentration showing upliftment and loosening of LF. ***d***, Treated eye lens at 0.126 ppm concentration showing degeneration of BS joints (arrow mark) and increase in porosity (P).

oxidation, preventing free-radical-induced photochemical generation of harmful by-products and reactivating oxidized vitamin C which improves antioxidant capability in the lens[21–23]. Reduced glutathione was significantly decreased at the highest dose (0.126 ppm) of the toxicity group, but in the case of lower concentration there was decrease in reduced glutathione which was non-significant at 0.038 and 0.062 ppm concentrations, but at the highest dose of 0.126 ppm there was drastic decrease (Figure 3). The reduction of glutathione indicates increase in oxidized glutathione, which again is one of the important parameters for cataract development. At the ultrastructural level, it has been observed that widening of intracellular spaces at interdigitations of ball and socket joints of lens fibres resulted in their upliftment and loosening of compact structure of lens fibres with increase in concentration of monocrotophos. The degeneration of balls and sockets as well as increase in porosity of lens fibres increased on exposure (Figure 4). This in turn resulted in the loss of compactness between two layers of the lens fibres, affecting the transparency of lens and resulting in cataract. Similar changes were reported in lens fibres of human cataractous lens[5,6].

There are earlier reports regarding the biochemical changes of protein and glutathione related to the development of cataract produced by a variety of substances fed or injected in experimental animals to cause cataract[24–27]. The biochemical assay conducted on the lens, specifically on insoluble protein and reduced glutathione, showed a similar trend in the present study as reported in the development of the cataractous phenomenon[9–12]. The concentration of insoluble protein increased on the whole. At low concentration it appeared to be non-significant due to neurological allergen phase, but at higher concentration the change was significant, which is comparable to the changes in case of cataract development. The reduced glutathione decreased significantly and this further added to the development of cataract. Electron photomicrographs also proved the increase and widening of the distance between the lens fibres thus affecting lens transparency, which is one of the reasons for the development of cataract. It is thus suggested on the basis of biochemical studies at the ultrastructural level that the eye lens of *C. carpio communis* develops cataract when exposed to organophosphate pesticide, even at the sublethal doses.

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Thorium-rich zircons in granitoids of the Ladakh Batholith, Indus–Tsangpo Suture Zone, Ladakh, India

In northern India, the Ladakh batholith is an important geologic entity, located between two major tectonic suture zones – the Shyok to the north and the Indus–Tsangpo to the south. These sutures mark the closing of different branches of the Tethys Ocean and finally the collision of India with Asia at 60–50 Ma. The former represents a collisional boundary between the Karakoram block and the Kohistan–Ladakh–Gangdese island arc, and the latter represents the main boundary zone between the Indian and Asian plates. The Ladakh batholith is part of the Andean-type Trans Himalayan Plutonic Belt that extends for 2500 km from Afghanistan in the west, to east of Lhasa in Tibet[1]. It is composed of multiple calc-alkaline intrusions that vary in composition from olivine–norite and gabbro to granite[1]. The batholith trends roughly WNW–ESE and is approximately 600 km long and 30–50 km wide. The crystallization ages of the batholith[2,3] range from 102 to 65–49 Ma, suggesting that magmatism was contemporaneous with the Gandese batholith (southern Tibet) and that magmatic activity ceased with the collision of India and Asia[1,2]. The geological structure of the Indus and Shyok suture zones has been discussed elsewhere[4,5].

Recently, Ladakh granitoid samples were collected from several localities in the Ladakh area, including Chang La, Daah-Hanu and Hunder, and were processed to obtain U–Pb geochoronological data on zircons separated from them. Preliminary studies reveal that the granitoids at Chang La, Daah-Hanu and Hunder contain abun-

Table 1. U and Th contents (in ppm) of zircons in granitoids of the Ladakh batholith. Samples were analysed by TIMS at Isotope Laboratory of the University of Tuebingen, Germany under the aegis of the Alexander von Humboldt Fellowship

Sample no.	Location	Sample weight (separated zircon in mg)	Uranium (ppm)	Thorium (ppm)
1(i)	Daah-Hanu granitoid	0.024	993	6115
(ii)	Daah-Hanu granitoid	0.0339	1182	4329
(iii)	Daah-Hanu granitoid	0.0354	889.2	4146
(iv)	Daah-Hanu granitoid	0.0159	1346.2	9231
2(i)	Daah-Hanu granitoid	0.241	64	6090
(ii)	Daah-Hanu granitoid	0.0158	262	9289
(iii)	Daah-Hanu granitoid	0.0269	139	5456
(iv)	Daah-Hanu granitoid	0.244	152	6015
3(i)	Hunder granitoid	0.0293	738	5009
(ii)	Hunder granitoid	0.0122	706	12,030
(iii)	Hunder granitoid	0.0079	393	18,900
(iv)	Hunder granitoid	0.016	544	9173
4(i)	Chang La granitoid	0.017	313	8634
(ii)	Chang La granitoid	0.012	380	12,230
(iii)	Chang La granitoid	0.0254	309	5778
(iv)	Chang La granitoid	0.0282	368	5205