

Histopathological lesions in the selected tissues of *Cirrhinus mrigala* (Ham.) fingerlings exposed to a sublethal concentration of mercury

Ashok Kumar Gupta and Ashwani Kumar

Department of Aquaculture, College of Fisheries
Maharana Pratap University of Agriculture & Technology, Udaipur-313 001, India

(Received: 1 March, 2004; Accepted: 29 September, 2004)

Abstract: In the present investigation histopathological lesions in the selected tissues such as gills, kidney and eye of the fingerlings of *Cirrhinus mrigala* exposed to a sublethal concentration of mercury, i.e. 240 µg/l from static bioassay have been studied. The primary gill lamellae showed degeneration in the middle and distal region. In distal region, spherical structure showing complete degeneration and fusion of pillar, epithelial, mucus producing gland and blood cells in the secondary gill lamellae. Degeneration and necrosis in glomerulus, interstitium tissue and epithelium lining of renal tubules in kidney have also been seen in mercury treated fingerlings. Whereas periorbital region of eye showed exudation in choroids layer and degeneration in oedematous tissue or adjacent sclera. The results have been discussed in relation to respiratory and excretory physiology and probable cause of fish death due to mercury poisoning.

Key words: Histopathology, *Cirrhinus mrigala*, Mercury, Gill, Kidney, Eye.

Introduction

Large scale of mortality of spawn, fry and fingerlings in most of the water bodies has been reported due to discharge of metallic ions including mercury in the form of industrial effluents. Limited studies on the effect of mercury at cellular level particularly in gills, liver, kidney and other vital organs in the fish have been available (Kendall, 1977; Mustafa, 1995; Gupta and Rajbanshi, 1995; Prasad *et al.*, 1995; Banerjee and Bhattacharya, 1997). In the present study histopathological changes have been investigated in gill, kidney and eye of *Cirrhinus mrigala* fingerlings exposed to a sublethal concentration of mercury from 96 hr static bioassay.

Materials and Methods

Short-term (96 hr) static bioassay was conducted to evaluate the toxicity of mercury (as HgSO₄) for *C. mrigala* fingerlings (average length 5.8 cm and weight 2.352 gm.) following standard methods (APHA, 1989). The 96 hr LC₅₀, presumable safe and dischargeable concentration of mercury at a temperature of 35°C for *C. mrigala* fingerlings have been computed 342.164, 73.510 and 1.118 µg/l respectively as per methods of Finney (1971) and Hart *et al.* (1945). During the bioassay test, pH, DO, alkalinity and hardness of the water were 8.5, 5.0, 700 and 270 mg/l respectively.

After 96 hr of bioassay, treated fingerlings were carefully removed from a sub-lethal concentration of mercury, i.e. 240 µg/l and sacrificed immediately. The samples of gills, kidney and eye were excised carefully, washed in running water and fixed in 4% neutral formaline. The serial sections of 5-8 µ thickness were cut and stained using eosin and Mayre's haematoxylin. The histopathological changes if any in the tissues of treated fish were observed carefully.

Results and Discussion

Sublethal concentration of 240 µg/l of mercury brought about marked changes in the gill structure of the fingerlings of *C. mrigala* (Figs. 1 and 2). The gill filaments showed severe pathological changes in the distal region as compared to that of middle region. The secondary lamellae as a whole, including the flanges of the pillar cells have been fused together forming large spherical structure in the distal region of the gill filaments. The fusion was of the highest order and no distinction could be made among pillar cells, epithelial cells, blood cells and mucus cells in such region (Fig. 2). The blood cells were also found accumulate in this spherical structure.

Kidney of the fingerlings of *C. mrigala* exposed to a sublethal concentration of 240 µg/l of mercury revealed various histopathological changes (Fig.3). The most remarkable histopathological change was the expansion of the renal tubules to such an extent that it covers the area of the lumen. Further, the cells lining the lumen also ruptured causing tubular necrosis. In lumen of the renal tubules, cellular debris was also noticed. At places the glomeruli found engorged with blood. The haemopoietic tissue of the renal interstitium was also considerably reduced and degenerated.

The choroids tissue of eye showed considerable exudation by inflammatory cellular infiltration of the periorbital tissue (Fig.4). Whereas remarkable degeneration was seen in oedematous tissue or adjacent sclera of the periorbital tissue.

In the present investigation mercury ions induced severe histopathological changes in gill such as degeneration in the middle and distal region in the primary lamellae. In the distal region spherical structure was formed due to degeneration and fusion in pillar, epithelial and mucus producing gland cells along with large number of blood cells of the secondary gill lamellae.

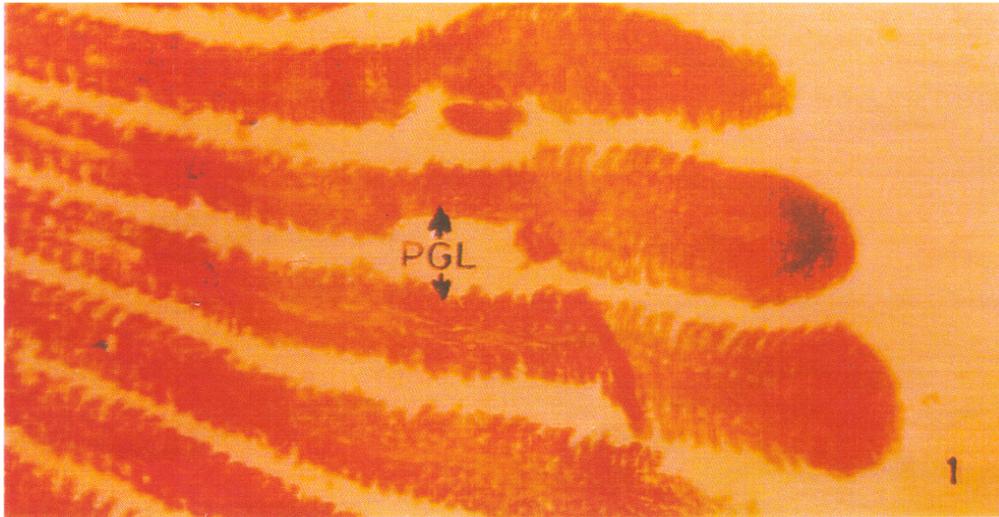


Fig. 1: Photomicrograph of the section of gill filament of *Cirrhinus mrigala* fingerlings treated with a sublethal concentration of 240 µg/l of mercury for 96 hr (X-70). PGL-Primary gill lamellae showing degeneration in the middle and distal region.

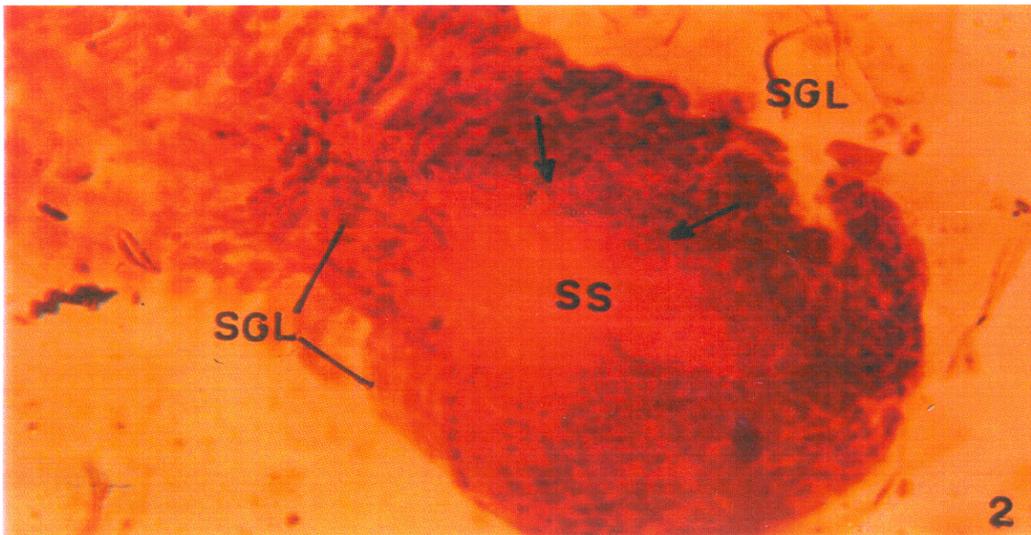


Fig. 2: Photomicrograph of the section of distal region of the gill filament of *Cirrhinus mrigala* fingerlings treated with a sublethal concentration of 240 µg/l of mercury for 96 hr (X-700). SS - Spherical structure showing complete degeneration and fusion of pillar cells, epithelial cells, mucus producing gland cells and the blood cells in the secondary gill lamellae (SGL), arrows indicate blood cells accumulated inside the spherical structure.

Khargarot and Somani (1980) have also exposed fish *Puntius sophore* to high levels of dissolved inorganic Hg or methyl mercury (MeHg) and suggested that mercury ions severely damaged the gill. Gupta and Rajbanshi (1995) have also studied the surface architecture of the gill of *Rasbora daniconius* treated with a sublethal concentration of mercury (0.05 mg/l). These authors (op. cit.) also observed significant changes such as damage, fusion and clumping in the middle and distal parts of the gill lamellae resulting into considerable decrease in the overall surface area. Whereas, Ribeiro *et al.* (1996) reported an increased proliferation in the interlamellar region lead to a thickening of the secondary lamellae of the gills

of *Trichomycterus brasiliensis* exposed to mercury ions. It is suggested that severe damage in gill of fish exposing to high levels of dissolved inorganic mercury or methyl mercury (MeHg), interfered with physiological processes involving the gills, including gas exchange and ions regulation (Renfro *et al.*, 1974; Borquegneau, 1977; Lock *et al.*, 1981; Stinson and Mallat, 1989; Gupta and Rajbanshi, 1995). Skidmore (1970), Eisler (1971), Burton *et al.* (1972) and Bilinski and Jonas (1973) have also described the deleterious effect of heavy metals and suggested that the death of fish in acute poisoning was due to disruption of respiratory process caused by the damage of gill epithelium. Further, Skidmore (1970) suggested that the

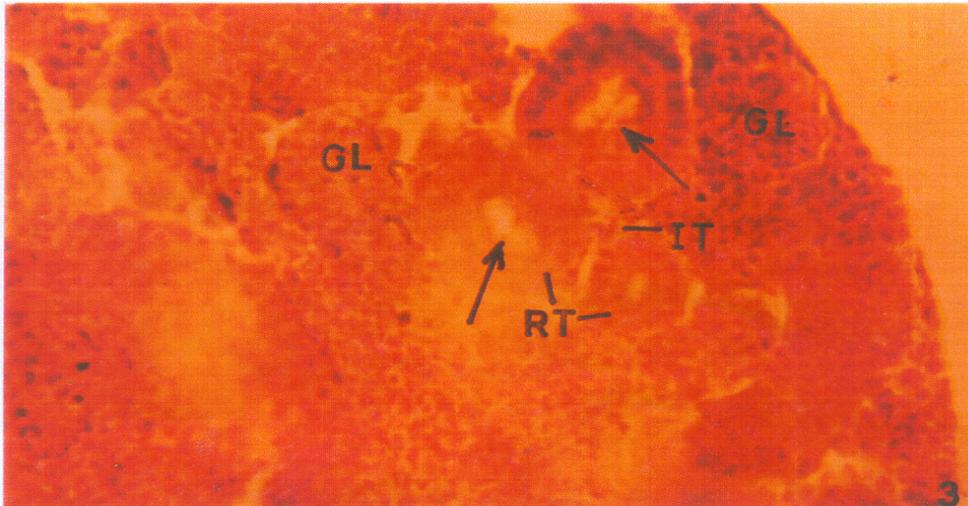


Fig. 3: Photomicrograph of the section of kidney of *Cirrhinus mrigala* fingerlings treated with a sublethal concentration of 240 $\mu\text{g/l}$ of mercury for 96 hr (X-700) RT- Renal tubule expanded, Arrows indicate necrosis in epithelial lining the lumen. GL- Degeneration in the glomerulus, IT- Degeneration in interstitium tissue.

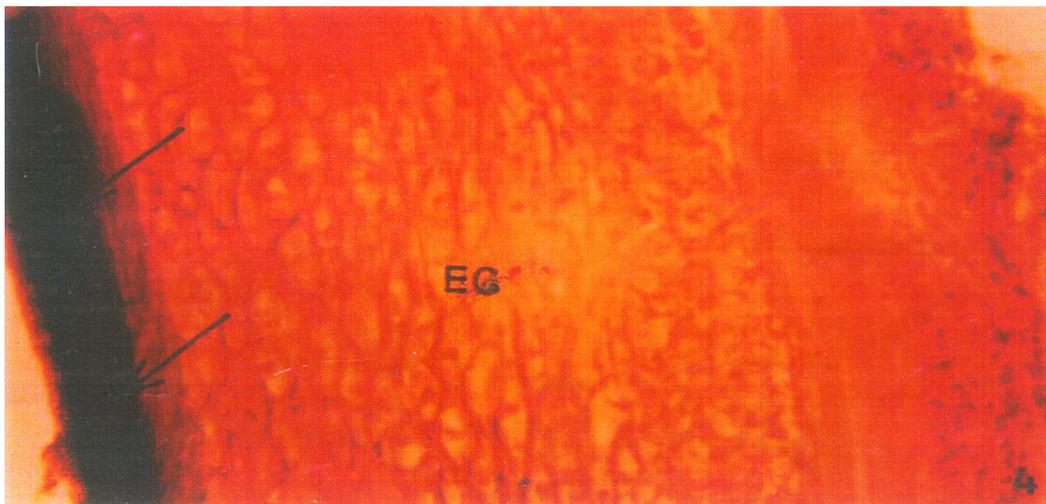


Fig. 4: Photomicrograph of the section of periorbital region of the eye of *Cirrhinus mrigala* fingerlings treated with a sublethal concentration of 240 $\mu\text{g/l}$ of mercury for 96 hr (X-700). EC- Exudated choroid region, Arrows-degenerated oedematous tissue of adjacent sclera.

respiratory handicap imposed by lifting of the epithelium (as swellings) must outweigh any protective effect against pollutant uptake in the later stage of acute poisoning. Burton *et al.* (1972) demonstrated that tissue hypoxia precedes death when maximum gill ventilation is no longer efficient to supply the oxygen needs of trout because of gill deterioration due to which the gas exchange system is altered. Gardner and Yevich (1970), Skidmore and Tovell (1972), Bilinski and Jonas (1973), Gardner and LaRoche (1973) and Gupta and Rajbanshi (1981, 1982, 1988 a,b and 1995) have the same opinion that the depression in respiratory activity is a common feature in acute metallic poisoning caused due to alterations in the cellular component of the gill of the fishes.

The pathological changes noticed in the kidney of mrigal fingerlings by a sublethal concentration of mercury include degeneration in the glomerulus and interstitium tissue, necrosis in epithelium lining the lumen and expansion in renal tubules which possibly caused the impairment of kidney function. Sastry and Agrawal (1977) have also noticed desquamation of the tubular cells, enlargement and pycnosis of nuclei, shrinkage of glomerular network, rupture of the glomerular wall and haemorrhages in the interstitial haematopoietic tissue in the kidney of *C. mrigala* treated with mercuric chloride. Whereas Ribeiro *et al.* (1996) have reported that the kidney of *Trichomycterus brasiliensis* treated with mercury were disorganised and tubule cells decrease in number

and change in size. Gupta and Rajbanshi (1979, 1986) have observed pathological symptoms in kidney of *Heteropneustes fossilis* and *Channa punctatus* exposed to different concentrations of copper include modifications in renal tubules by expansion and necrosis in the outer and inner epithelial lining and accumulation of cellular debris. Presumably, the observed pathological alterations in the kidney of mrigal fingerlings may be due to the internal exhaustion that brought about a change in the metabolic activity as a result of interaction between mercury ions and renal tissue through blood, resulting in disorder of the divalent ions as also suggested by Rasquin and Rosenbloom (1954). Trump and Bulger (1967), Kendell (1977) and Trump *et al.* (1975) in their superb studies have described both light microscopical and ultrastructural changes in the tubules associated with mercury poisoning in the southern flounder. Such studies have shown the main effects of levels of mercury as binding S-H groups to the protein of cell membranes, thus inhibiting enzyme system.

The *C. mrigala* fingerlings exposed to a sublethal concentration of 240 µg/l of mercury ions also revealed histopathological lesions in the periorbital region of eye include exudation by inflammatory cellular infiltration in choroids tissue and remarkable degeneration in oedematous tissue or adjacent sclera. Weis and Weis (1977b) studied the development of killifish in the methyl mercury-exposed groups revealed a spectrum of optic and cephalic abnormalities, the appearance of which correlated with dose level and length of time exposure. Weis and Weis (1977a) further also reported that even in more severe cases the entire axis is reduced in size and the embryo can consist of only a cyclopic head, anophthalmic head or in some cases no axis at all. Ribeiro *et al.* (1996) have recorded that optic nerve of *Trichomycterus brasiliensis* in mercury concentrations show disorganised disposition of axons and mainly disruption and dissociation of myelin sheath, leading to decrease in motility and coordination. The pathological lesion in the periorbital region of the eye may in turn produce abnormalities in the optic nerve and cephalic region of the brain leading to decrease in motility and coordination in the fingerlings of *C. mrigala*.

References

- APHA (American Public Health Association): American water works association and water pollution control federation. Standard methods for the examination of water and wastewater. 18th Edn. APHA. Washington D.C. (1989).
- Banerjee, S. and S. Bhattacharya: Histopathological changes induced by chronic non-lethal levels of elsan, mercury and ammonia in the liver of *Channa punctatus* (Bloch). *J. Environ. Biol.*, **18**(2), 141-148 (1997).
- Bilinski, E. and R.E.E. Jonas: Effects of cadmium and copper on the oxidation of lactate by rainbow trout (*Salmo gairdneri*) gills. *J. Fish. Res. Bd. Can.*, **30**, 1553-1558 (1973).
- Borqueneau, J.M.: ATPase activity in mercury intoxicated eel. *Experientia*, **33**, 941-942 (1977).
- Burton, D.T., A.H. Jones and J. Cairns Jr: Acute zinc toxicity to rainbow trout (*Salmo gairdneri*): Confirmation of the hypothesis that death is related to tissue hypoxia. *J. Fish. Res. Bd. Can.*, **29**, 1463-1466 (1972).
- Eisler, R.: Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. *J. Fish. Res. Bd. Can.*, **28**, 1225-1234 (1971).
- Finney, D.J.: Probit analysis. University Press, Cambridge (1971).
- Gardner, G.R. and G. La Roche: Copper induced lesions in estuarine teleosts. *J. Fish. Res. Bd. Can.*, **30**, 1363-1368 (1973).
- Gardner, G.R. and P.P. Yevich: Histological and hematological responses of an estuarine teleost to cadmium. *J. Fish. Res. Bd. Can.*, **27**, 2185-2196 (1970).
- Gupta, A.K. and V.K. Rajbanshi: Pathological changes resulting from bioassay of copper to *Heteropneustes fossilis* (Bloch). Proceeding Environmental Biology, published by AEB. pp. 167-172 (1979).
- Gupta, A.K. and V.K. Rajbanshi: (Measurement of acute toxicity of copper to the freshwater teleost, *Mystus bleekeri* (day) using bioassay, statistical and histopathological methods. *Arch. Hydrobiol.*, **19**, 427-434 (1981).
- Gupta, A.K. and V.K. Rajbanshi: Cytopathological studies resulting in cadmium bioassay with *Heteropneustes fossilis* (Bloch). *Acta Hydrochim. Et Hydrobiol.*, **10** (4), 345-352 (1982).
- Gupta, A.K. and V.K. Rajbanshi: Cytotoxicity of copper ions to certain tissues of a freshwater murrel, *Channa punctatus* (Bloch). *Poll. Res.*, **5**(3 & 4), 97-101 (1986).
- Gupta, A.K. and V.K. Rajbanshi: Alterations in the architecture of the gill surface produced by water-borne copper in *Heteropneustes fossilis* (Bloch). *Acta Hydrochim. Et. Hydrobiol.*, **16**(3), 325-331 (1988a).
- Gupta, A.K. and V.K. Rajbanshi: Acute toxicity of cadmium to *Channa punctatus* (Bloch). *Acta. Hydrochim. Et. Hydrobiol.*, **16**(5), 525-535 (1988b).
- Gupta, A.K. and V.K. Rajbanshi: Mercury poisoning: Architectural changes in the gill of *Rasbora daniconius* (Ham.). *J. Environ. Biol.*, **16**(1), 33-36 (1995).
- Hart, W.B., P. Doudoroff and J. Greenbank: The evaluation of the toxicity of industrial wastes, chemical and other substances to freshwater fishes. *Attant. Refinding Co. (Phill)*, pp. 317 (1945).
- Kendall, M.W.: Acute effects of methyl mercury toxicity in Channel catfish (*Ictalurus punctatus*) liver. *Bull. Environ. Contam. Toxicol.*, **18**, 143-151 (1977).
- Khargarot, B.S. and R.C. Somani: Toxic effects of mercury on the gills of freshwater teleost, *Puntius sophore* (Ham.). *Curr. Sci.*, **49**, 832-834 (1980).
- Lock, R.A.C, P.J.M. Craijsen and A.P. VanOverbeeke: Effects of mercuric chloride and methyl mercuric chloride on the osmoregulatory function of the gill in rainbow trout, *Salmo gairdneri* (Rich.) *Comp. Biochem. Physiol.*, **68C**, 151-159 (1981).
- Mustafa, C.: Effect of mercury, chromium and nickel on some blood parameters in the carp, *Cyprinus carpio*. *Turk. J. Zool.*, **19** (4), 305-311 (1995).
- Prasad, M.S., P. Prasad, S.D. Peters and M. Shil: Light and scanning electron microscopic studies on the effects of mercuric chloride in the catfish, *Heteropneustes fossilis*: Histopathology of the air breathing organs. *Proc. Ind. Ntl. Sci. Acad. (part B). Biol. Sci.*, **61** (5), 363-370 (1995).
- Rasquin, P. and L. Rosenbloom: Endocrine in balance and tissue hyperplasia in teleosts maintained in darkness. *Bull. Amer. Mus. Nat. Hist.*, **104**, 359-426 (1954).
- Renfro, J.L., B.Schmidt-Nielsen, D.Miller, D.Benos and J. Allen: Methyl mercury and inorganic mercury: Uptake, distribution and effect on osmoregulatory function in fishes. *In: Pollution and physiology of*

- marine organisms, (Eds: F. J. Vernberg and W.B. Vernberg) Academic Press, New York, pp. 101-122 (1974).
- Ribeiro, C.A., F.E. Oliveira., N.M. Turcatt, R.J Cardoso and C.S. Carvalho: Lethal effects of inorganic mercury on cells and tissues of *Trichomycterus brasiliensis* (Pisces: Siluroidei). *Ecotoxicol. Environ. Saf.*, **33**, 160-166 (1996).
- Sastry, K.V. and M.K. Agrawal: Histochemical localization of alkaline and acid phosphatases in the kidney of *Ophiocephalus punctatus* treated with mercuric chloride. *Folia. Histochem. ET. Cytochem.*, **15**, 243-247 (1977).
- Skidmore, J.F.: Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. *J. Exp. Biol.*, **52**, 481-494 (1970).
- Skidmore, J.F. and P.W.A Tovell. : Toxic effects of zinc sulphate on the gills of rainbow trout. *Water Res.*, **6**, 217-230. (1972).
- Stinson, C.M. and J. Mallat: Branchial ion fluxes and toxicant extraction efficiency in lamprey (*Petromyzon marinus*) exposed to methylmercury. *Aquatic Toxicol.*, **15**, 237-252. (1989).
- Trump, B.F. and R.E. Bulger: Studies of cellular injury in isolated flounder tubules. I. Correlation between morphology and function of central tubules and observations of phagocytosis and mechanical cell damage. *Lab. Invest.*, **16**, 453-482 (1967).
- Trump, B.F., R.T. Jones and S. Sahapong: Cellular effects of mercury on fish kidney tubules. In: The pathology of fishes (Eds: W.E. Ribelin and M. Migaki). Wis. Univ., Wisconsin Press, 585-612 (1975).
- Weis, J. S. and P. Weis: Effects of heavy metals on development of the killifish, *Fundulus heteroclitus*. *J. Fish. Biol.*, **11**, 49-54 (1977a).
- Weis, P. and J.S. Weis : Methyl mercury teratogenesis in the killifish, *Fundulus heteroclitus*. *Teratol.*, **16** (3), 317-326 (1977b).

Correspondence to:

Dr. A.K. Gupta

T-9, Tilaknagar, Sector 3

Udaipur-313001 (Raj.), India

E-mail: ashok gupta4U25@yahoo.co.in