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RESEARCH ARTICLE

TNF-R2, TGFβ2 and HSP70 in Uranium-Exposed Larvae of Grass Carp, Ctenopharyngodonidella.

Fakhr El-Din M. Lashein

Department of Zoology, Faculty of Science, Sohag University, Egypt

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Corresponding Author

Fakhr El-Din M. Lashein

This work was conducted to study the effect of uranium on TNFR2, TGFβ2 and (HSP70) as an active cell cytokines and induced protein for their important protective role. So, newly hatched larvae (one day old) of grass carp, *Ctenopharyngodonidella*, were exposed to ascending concentrations of uranium equivalent to 1, 2, 4, 8mg/l for a week of exposure. Deformations and percentages of mortalities and hence LC50 was determined. Consequently, newly-hatched larvae were exposed to the lowest concentration to achieve the chronic treatment for 30 days in addition to the control. During the experimental period at 5^{th}, 15^{th} and 30 days, selected specimens of control larvae were fixed to follow up the expression of TNFR2, TGFβ2 and (HSP70) normally. Meanwhile, uranium- exposed larvae were fixed at the end of the experimental period to evaluate the effect of uranium on both TNFR2, TGFβ2 and (HSP70) compared to control. Sections were prepared for immuno-staining using specific antibodies.

The results revealed a concentration-dependent mortality and deformations. Increased expression of TNFR2, TGFβ2 and (HSP70) in different organs of control larvae was age-dependent except for the TGFβ2 in liver which tend to decrease from 15 – 30 days-old larvae and it is restricted to the brush border of the intestinal cells compared to that of either TNFR2 or HSP70. In uranium-exposed larvae inhibition of cytokines in contrast to the up-regulation of HSP70 were noted in the studied organs (brain, retina, liver, muscles and skin) compared to control.

In conclusion, the expression of both the cytokines and the heat shock protein in the larvae of the grass carp and its affects with chronic uranium exposure contribute to improve our understanding in the role of these components in protection and/or treatment against uranium-induced carcinogenicity in long term exposure.

Introduction

Uranium is a naturally occurring radioactive metal that has both chemical and radiological toxicity (Cooley and Claverkamp, 2000). Uranium's environmental prominence is currently increasing because of new mining and milling activities to support the resurging commercial nuclear power industry in response to energy production needs with low carbon output. Such anthropogenic activities can increase environmental concentrations of uranium from typical levels of 0.02mg/L in natural waters to approximately 2 mg/L (Colle*et al.*, 2001; Bonin and Blanc, 2001). Enhanced concentrations cause the metal to accumulate in biota. Uranium's chemotoxicity is known to

accumulate in and damage the liver in lake whitefish, *Coregonusclupeaformis* and in zebrafish, *Daniorerio* (Cooley and Claverkamp, 2000; Lereboursr*et al.*, 2009). Concentrations of 1 to 20mg/g fresh weight in the bivalve, *Corbiculafluminea* (Simon and Garnier-Laplace, 2005) were reported. In adults and larval stages of *D. rerio*, increased reactive oxygen species production and altered innate immune defense system at concentrations ranges from 20 - 1000µg/L were reported (Gagnaire*et al*., 2013). In plants uranium concentrations in shoots of *Brassica juncea* and *Brassica chinensis* grown in a uranium-contaminated soil was recorded (Huang et al., 1998). Increased uranium accumulation of crop plants including *Brassica juncea* and *Helianthus annuus*, by soil acidification was also noted (Huhle et al., 2008). Uranium translocation among tissues of the plant is in the relation of roots>shoots ≅ leaves being 3500 mg kg^{-1} dry weight in roots and 2000 mg kg^{-1} dry weight in shoots and leaves, respectively. Natural uranium is classified as both a radiological and toxicological agent and is the only radionuclide for which chemical toxicity is the limiting factor. The most important factor influencing the potential health impact of natural uranium is its solubility (Sheppard *et al.* 2005).

Recently, The Egyptian Nuclear Materials Authority announced the discovery of huge amounts of uranium ore, giving a glimpse of hope for improving the country's collapsing economy. In Egypt, uranium was discovered in high concentrations in five main areas, included Abu Zenima in Sinai, Abu Rashid on the Red Sea, Sila in Upper Egypt and the Gtar. Because uranium is considered an important strategic raw material, the plan is to use it mainly as fuel for energy production. Also, uranium concentrations in some Egyptian environmental samples like Toshki soil, Aswan iron-ore, and phosphate samples from El-Sibayia in the Nile Valley and El-Quseir in the Red Sea coast were determined (El-Taher, 2010). The author concluded that the phosphate rocks are rich natural sources of uranium among the other minerals forming the earth crust.

From the other hand, cell cytokine TNFR2 was found to induce apoptosis indirectly by up-regulation of transmembrane TNF which then secondarily stimulated TNFR1 (Vercammen*et al*., 1995; Grell*etal*., 1999). There is a further evidence from rat-mouse cytotoxic T-cell hybridoma PC60 that TNFR2 can also induce cell death independently through TNFR1 activation (Vandenabeele*et al.*, 1995). TNFR2 is necessary for angiogenesis (Pan *et al*., 2002), migration of intestinal epithelial cells and Langerhans cells (Corredor*et al.*, 2003), proliferation of myofibroblasts (Theisset al., 2005) and differentiation and survival of T-cells (Kim and Teh, 2001, Kim *et al.*, 2006). There is also evidence for its neuroprotective role (Arnett *et al.*, 2001; Marchetti , 2004). Also, a complementary cytokine TGFβ2 was found to participate in cell cycle regulation and apoptosis (Hanahan and Weinberg, 2000) and it has a role in hemopoiesis and angiogenesis during development (Lashein and Seleem, 2012). In addition, heat shock proteins Hsp70 is an important part of the cell's machinery and help to protect cells from stress by improving overall protein integrity and it is directly inhibits apoptosis (Beere*et al.*, 2000; Gupta *et al.*, 2010). So, the present work was carried out to investigate the normal expression of TNFR2 and TGFβ2 cytokines and the heat shock protein in the early life stages of C. idella as a model of vertebrates for their importance in biological activities. From the other hand (owing to the lack of nockout animal model) to clarify the effect of chronic exposure to uranium as a chemical pollutant on these cytokines and the heat shock protein for their importance in disease resistance as well as their possible therapeutic uses.

MATERIAL AND METHOD

Uranium stock solution was prepared from uranyle acetate equivalent to 5 g/L in acidified distilled water. On the day before test application, test media were prepared using the stock solution after dilution with dechlorinated tap water. Multiple concentrations were prepared, 1, 2, 4 and 8 mg/l for the test experiments. Newly hatched larvae (one day old) of grass carp, *C. idella*, were exposed to one of four concentrations of uranium plus a control. Each treatment consists of 4 replicates with 50 larvae/ replicate in a volume of ½ L test solution. During 7 days of exposure mortality was recorded daily just prior to stock and test solutions renewal. LC50 was calculated by Probit. Analysis (Finney, 1976) and morphological abnormalities were recorded and photographed using Canon 7 mega pixel digital camera and processed for scaling using ZEN Widefield – UI Automation CarlZeiss, 2011.

For chronic treatment, newly hatched larvae were exposed to the lowest concentration tested (1 mg/L), in which a slight morphological abnormalities were recorded, up to 30 days of experimental period in addition to the control group. Daily renewal of test media and fish food supply was carried out with permanent aeration. At 5^{th}, 15^{th}, and 30^{th} –day of the experimental period, larvae of control were fixed in Carnoy's fixative (Drurry and Wallington, 1976). While uranium-exposed larvae were fixed at the end of experimental period. Fixed larvae were processed for histological and immunohistochemical evaluation at 7µ thickness. In histological study, serial transverse and frontal sections of the selected days above of control were stained with hematoxylin and eosin stains and examined microscopically. Selected sections of brain, retina, liver of control of the selected days above were mounted in Superfrost/Plus glass slides for immunostaining to follow up the normal expression of TNFR2, TGFβ2 and HSP70

during larval development. In uranium-exposed larvae, selected sections representing the brain, retina, liver, intestine, muscles and skin at the end of experimental period (30 days) were processed for immune-staining for evaluation compared to control.

In immunohistochemical study, deparaffiniezedSuperfrost/Plus glass slides mounted sections were retrieved for antigen reantigenicity using 10 mM citrate buffer at pH6 at 100 ˚C for an hour (Buchlowalow and Bocker, 2010). After cooling at room temperature, sections were treated for 10 minutes with hydrogen peroxide block (0.3% hydrogen peroxide solution with less than 0.1% sodium azide) and then with protein block (phosphate buffer solution, pH 7.6, with 0.5% BSA, 0.5% casein and less than 0.1% sodium azide) for 10 minutes to block nonspecific background staining. Primary antibodies, rabbit anti-human polyclonal tumor necrosis factor receptor two (TNFR2)(1:50 dilution) in phosphate buffer, rabbit anti-human polyclonal transforming growth factor beta two (1:100 dilution) in phosphate buffer (TGFβ2) and rabbit anti-human polyclonal heat schock protein 70 (HSP70) ready to use were applied to tissue sections and incubated at room temperature according to the corresponding manufacture protocols (www.sprinbio.com) for each antibody. After washing in phosphate buffer, sections were coated with secondary antibody (Biotinylated Goat Anti-Rabbit), conjugated with Streptavidin Horseradish Peroxidase Enzyme and visualized with 3,3′ diaminobenzidine (DAB) chromogene in DAB substrate. In all cases, negative control sections in which the primary antibody not applied to tissue sections were carried out. Sections were dehydrated in ascending grades of ethanol, cleared in xylen and mounted with DPX mounting media. Sections were examined microscopically to evaluate the expression of TNF-R2, TGFβ2 and heat shock protein in control larvae at the selected days above (5, 15, 30 days) and in uranium-exposed larvae at the end point of the experiment compared to control.

RESULTS

Toxicological observation during 7 days of exposure period revealed a concentration-dependent mortality and larval deformation. The percentages of mortalities were 12, 32, 77 and 87% in the exposed larvae to 1, 2, 4 and 8mg/l uranium, respectively. LC50 value was about 3.5mg/l (Fig. 1). The encountered larval deformations including, edematous heart and body curvature (skyphosis) (Fig. 2B) in 1mg/l uranium-exposed larvae as compared to control (Fig. 2A). Heart edema and swelling of belly region were more frequent (Fig. 2 C) in either 2, 4 or 8mg/l uranium-exposed larvae.

In immuno-stained histological sections, both of TNF R2 and TGF β2 revealed differential expression in the developing brain (Fig. 3A, B) and the differentiated retina (Fig. 3D, E) of 5 days-old larvae. In the developing brain the expression is more intense in the gray matter than in the white matter. In retinal ganglion cells, inner nuclear layer, outer nuclear layer and lens epithelia, TGF β2 is more expressed than TNF R2 (Fig. 3D, E). In contrast, the expression of heat shock protein is faint for the developing brain (Fig. 3C) and is also faintly and restricted to the plexiforms of the differentiated retina and the lens epithelia (Fig. 3F).

In 15 days-old larvae, increased expression of both cytokines was observed in the developing ganglion and the nuclear layers of the retina (Fig. 3G, H) for TNF R2 and TGF β2, respectively. Concerning the heat shock protein, a sharp expression was observed in both the outer and the inner plexiform layers of the retina (Fig. 3I) compared to the faint expression observed in the retina of 5 days-old larvae. The increased expression of both cytokines (TNF R2 and TGF β2) and the heat shock protein was parallel to the expression in the outer corneal layer (Fig. 3J – L) and the increased expression in developing brain (Fig. 3M – O) of 15 days-old larvae.

In the developing liver of 5 and 15 days-old larvae, the expression of both cytokines (TNF R2 and TGF β2 and the heat shock protein) is consistent with that observed in the brain and the retinal tissues. TGF β2 is more expressed (Fig. 3Q) than either the TNF R2 (Fig. 3P) or heat shock protein (Fig. 3R).

In 30 days-old larvae, the structural organization of the retina is characteristic of vertebrate. In hematoxylin and eosin-stained sections, prominent ganglion cells are arranged in two rows with differentiated axons, thick inner plexiform, multilayered bipolar lamina (the inner nuclear layer), thin outer plexiform, outer nuclear layer and the pigmented epithelia (Fig. 4A the insert). In TNF R2 immuno-stained sections the nuclear layers and the ganglion cells with their axons showing intense expression (Fig. 4A) compared to the plexiforms in control larvae. TGF β2 immuno-staining revealed the same staining in addition to the differential expression among the inner nuclear layer being lighter toward the outer plexiform and darker toward the inner plexiform (Fig. 4C). Heat shock protein expression is restricted to the plexiforms being darker in the outer than the inner (Fig. 4E). In uranium-exposed larvae (1mg/l) the expression of both the TNF R2 and TGF β2 is down-regulated in both the ganglion and the nuclear layers (Fig. 4B. D) as compared to control. In contrast, the expression of heat shock protein is upregulated in the plexiforms being darker in outer than the inner (Fig. 4F) as compared to control. In cerebral region of the brain also, the expression of TNF R2 and TGF β2 is down-regulated in the granular and molecular layers in uranium-

exposed larvae (Fig. 5B, D) as compared to control (Fig. 5A, C). Heat shock protein exprssion is up-regulated throughout the cerebral layers in uranium-exposed larvae (Fig. 5F) as compared to control (Fig. 5E).

In 30 days-old larvae, the liver consists of hepatic parynchyma (Fig. 6 insert) showing increased expression of TNFR2 (Fig. 6A) as compared to that observed in 15 days-old larvae. In contrast, down-regulaton of both TGF β2 and the heat shock protein was noted (Fig. 6 C, E) as compared to that noted in 15 days-old larvae. In uranium-exposed larvae, downregulation of TNFR2 and TGF β2 expression (Fig. 6B, D) and up-regulation of heat shock protein expression (Fig. 6 F) were noted as compared to control in a manner similar to that observed in both the retina and cerebral region. In the intestine, TNFR2 is prominently expressed in the nuclei (Fig. 7A), TGF β2 at the epithelial brush border and the nulei of the muscular layer (Fig. 7C) while the expression of heat shock protein is distributed throughout the tissue and faintly expressed (Fig. 7 E). In uranium-exposed larvae, down-regulation of the expression of both the TNFR2 and TGF β2 (Fig. 7B, D) while increased expression of heat shock protein was noted at the luminal surface of the lining intestinal epithelia (the brush border) and the nuclei (Fig. 7F) as compared to control. Down-regulaton of TNFR2 and TGF β2 (Fig. 8B, D) and up-regulation of heat shock protein (Fig. 8F) in muscles and skin of uranium-exposed larvae were noted in a consistent manner similar to those noted in other tissues, as compared to control (Fig. 8A, C, E), respectively. Alternative light and dark bands are best observed in TNF R2 immuno-stained sections than in both TGF β2 and heat shock protein immune-staining. Obscure of muscle striation was noted in TNF R2 immuno-stained sections of uranium-exposed larvae.

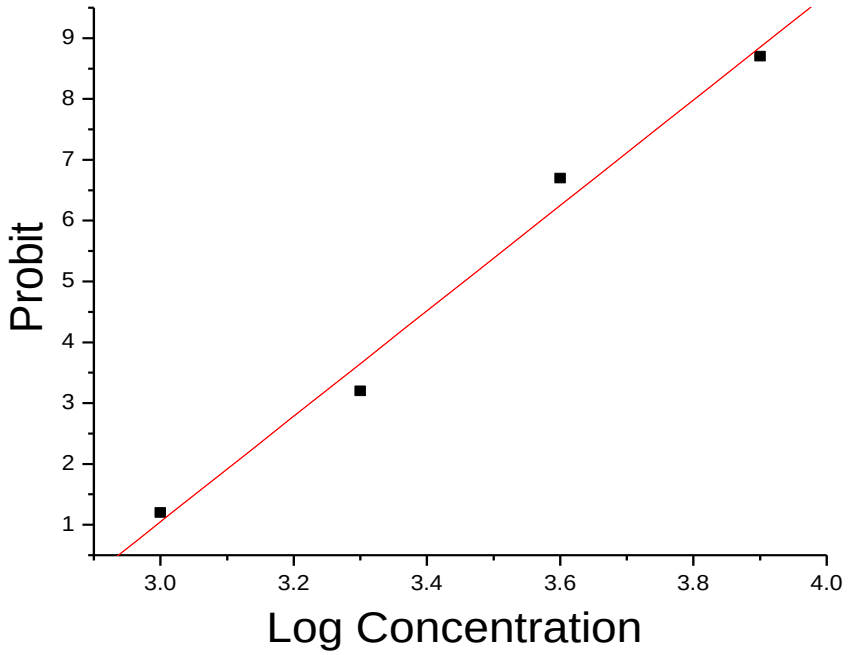

Fig. 1: LC50 curve of *C. idella* larvae exposed to uranium at 7-days of exposure period.

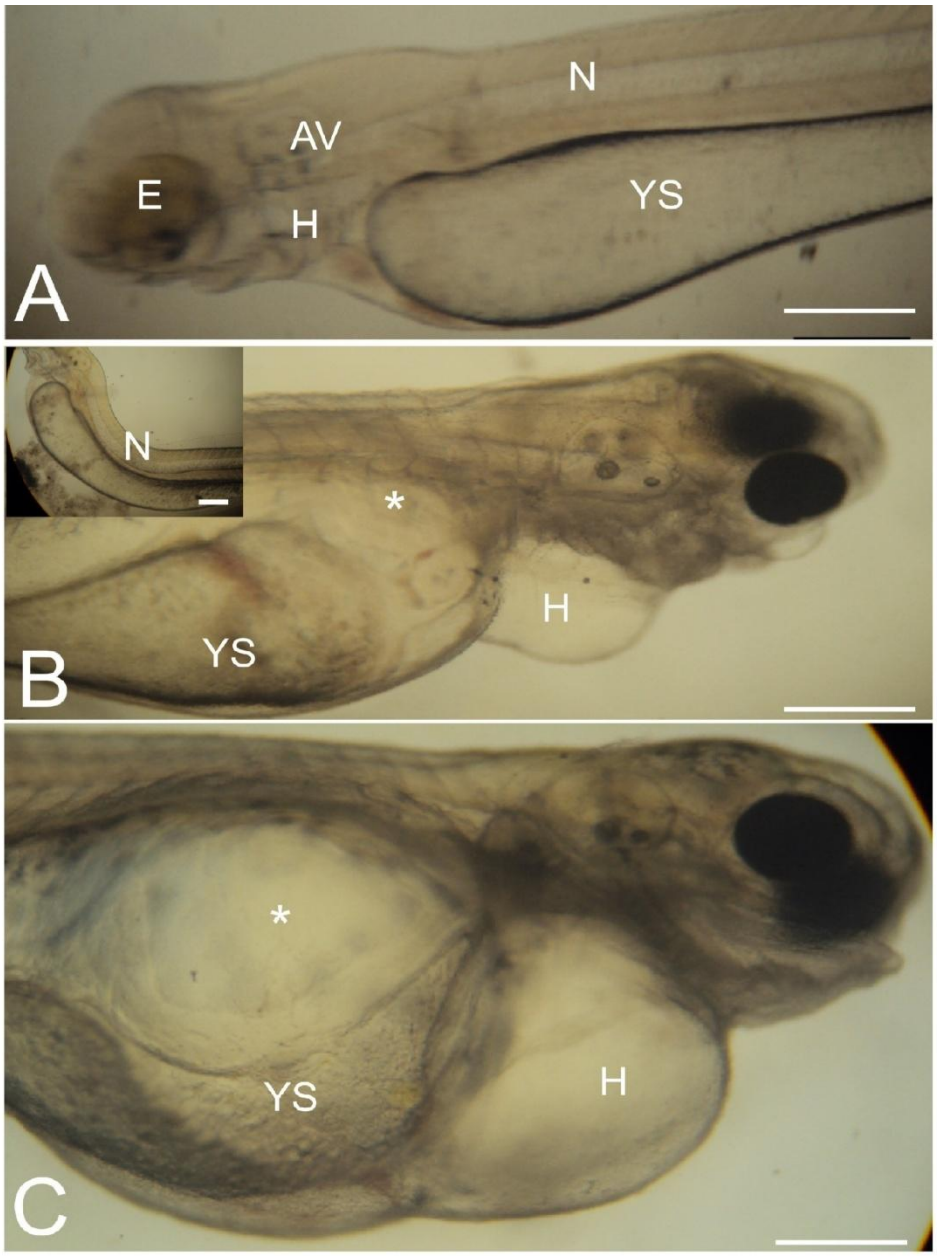

Fig. 2:

Fig. 2: Photomicrographs of 7-days-old larvae to show edematous heart (H), slight inflation of belly (*) dorsolateral to the remnant of yolk sac (YS), curvature of notochord (N) (the insert of **B**) in 1mg/l uranium-exposed larvae and in either 2, 4 or 8mg/l uranium-exposed larvae (**C**) compared to control (**A**) of normal heart (H), notochord (N), eyes (E),auditory vesicles (AV) and remnant of yolk sac. Scale bar 500µm.

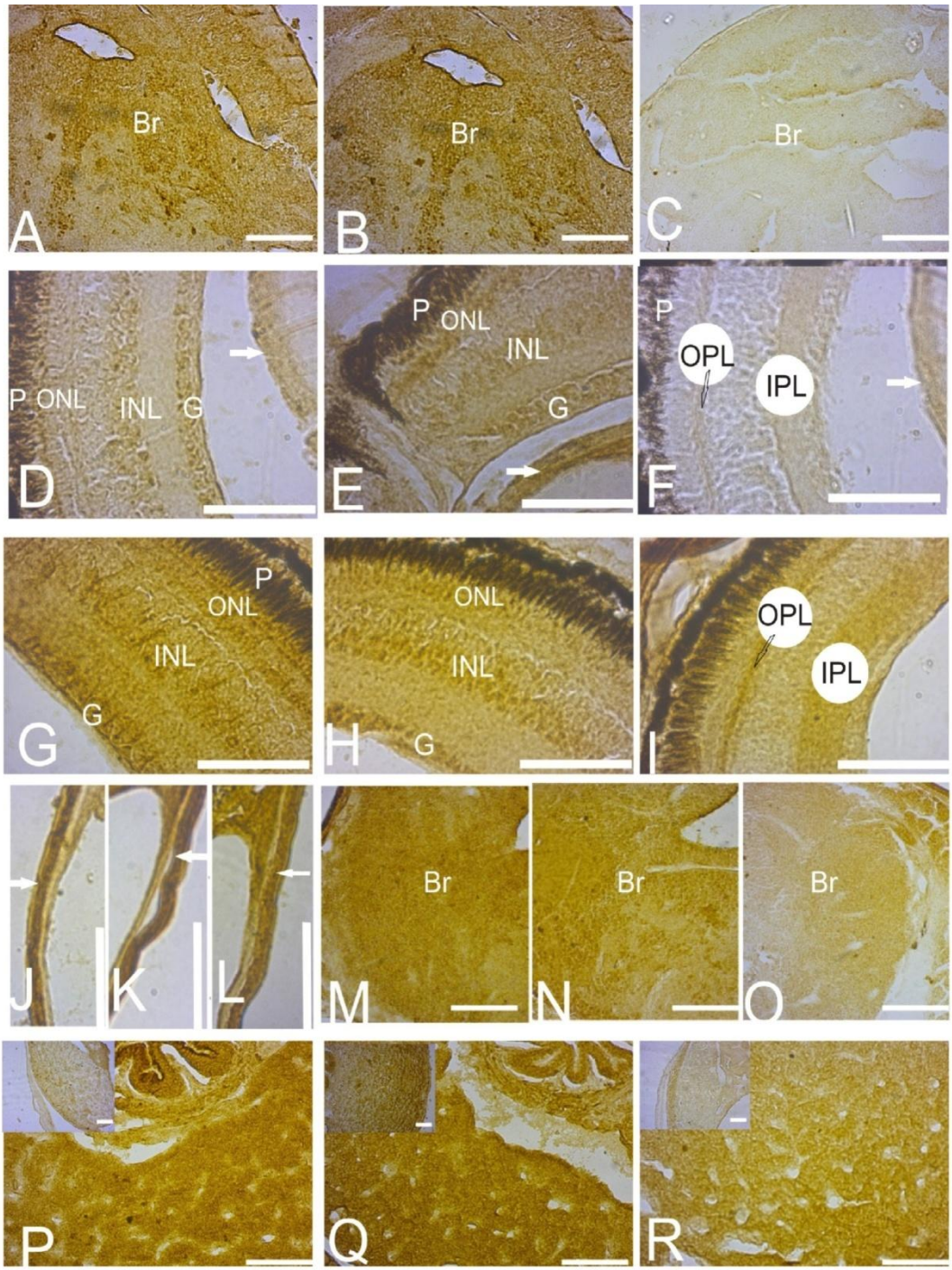

Fig. 3: Immuno-stained sections of the developing brain (**A – C**) and retina (**D – F**) of 5-days-old larvae showing the faint expression of HSP70 (**C, F**) against the well expressed TNFR2 (**A, D**) and TGFβ2 (**B, E**) in normal development. Increased expression in retina (**G – I**); cornea (**J –L**, arrows) and brain (**M – O**) of 15-days-old larvae. Coincide expression was detected in liver from 5 (the inserts) to 15-days-old larvae for TNFR2 (**P**) TGFβ2 (**Q**) and HSP70 (**R**), respectively. Brain (Br), pigmented retina (P), ganglion cells (G), inner nuclear layer (INL), outer nuclear layers (ONL), outer plexiform (OPL), inner plexiform (IPL), lens epithelia (arrows). Scale bar 50μm.

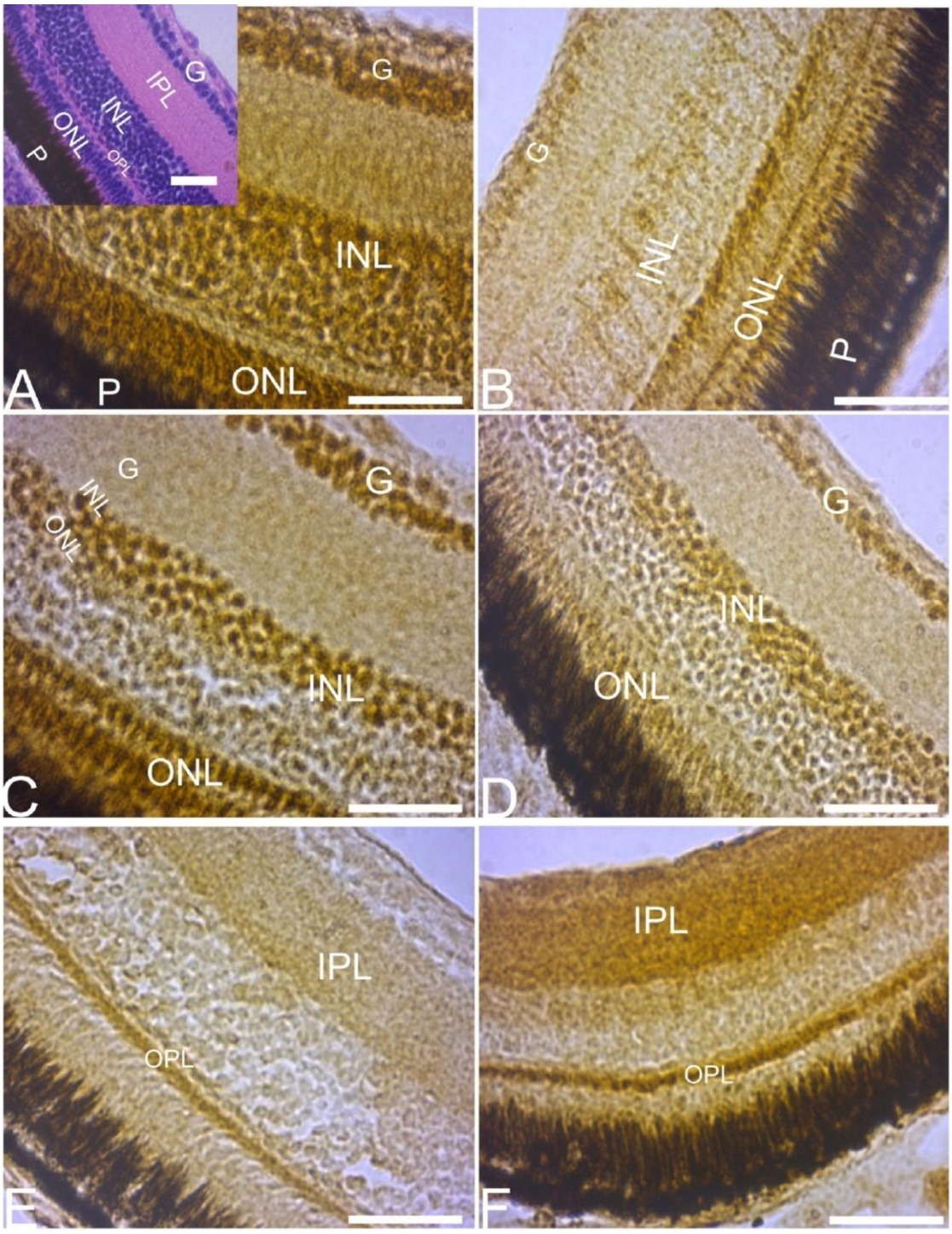

Fig. 4: Immuno-stained sections of 30-days-old control larvae show a well organized retina (insert of A, H$E stain, Scale bar 20µm.) and the increased expression of TNFR2 (A) and TGFβ2 (C) in ganglion (G), inner nuclear (INL) and outer nuclear layer (ONL) and the increased expression of HSP70 (E) in both the inner (IPL) and the outer (OPL) plexiforms of the retina compared to those in 15-days-old larvae. Downregulated expression of TNFR2 (B), TGFβ2 (D) and the increased expression of HSP70 (F) in the retinal layers corresponding to those of control is shown. Scale bar 50µm.

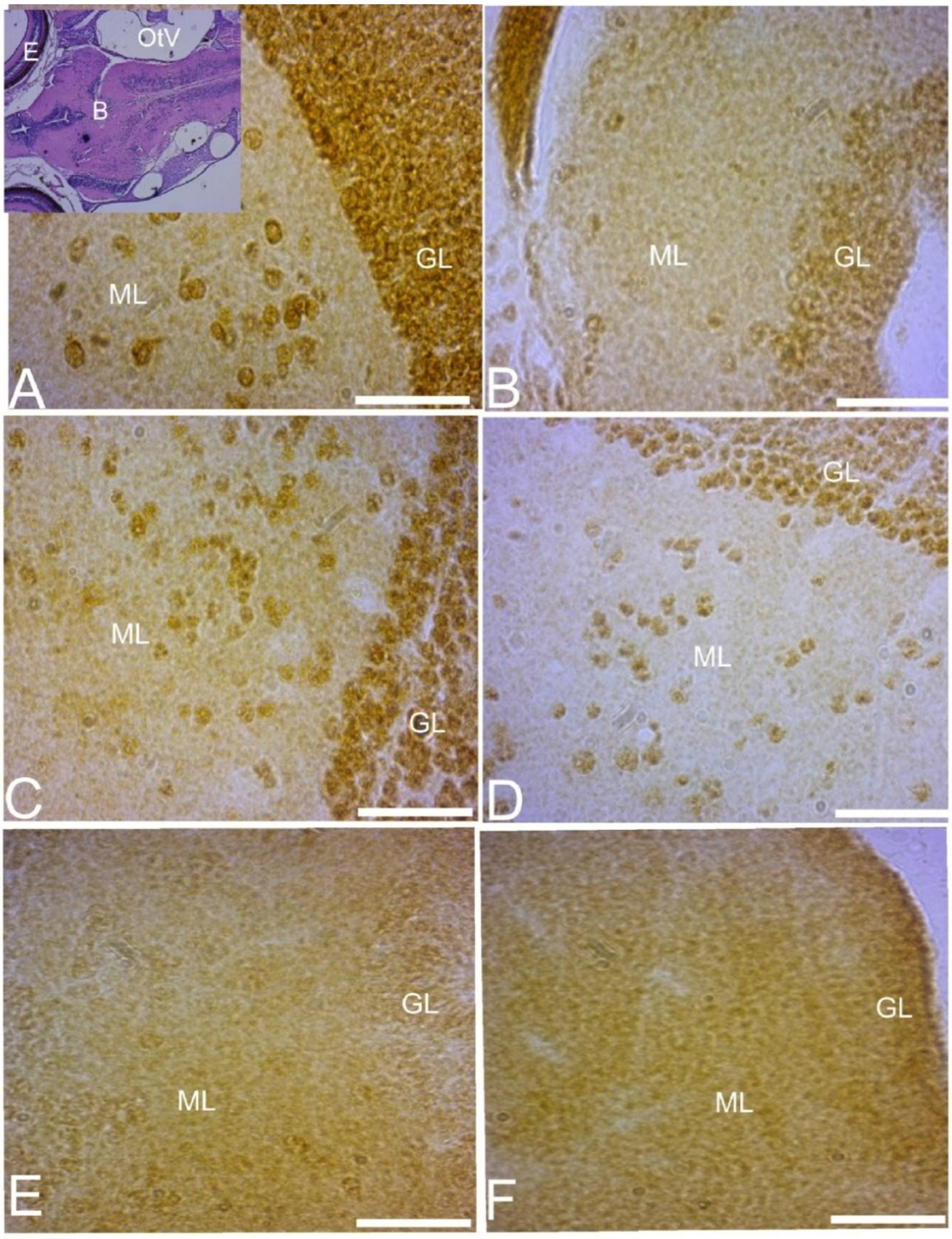

Fig. 5: Immuno-stained section of 30 days-old control larvae to show the expression of TNFR2 (**A**), TGFβ2 (**C**) in both the granular (GL) and molecular (ML) layers. HSP70 expression (**E**) is homogenous throughout the granular (GL) and the molecular (ML) layers. Inhibition of TNFR2 (**B**), TGFβ2 (**D**) and up-regulation of HSP70 (**F**) in uranium-exposed larvae corresponding to control was noted. The insert of (A,) represent the developed brain (B) and the sense organs, eyes (E) and otic vesicles (OtV). Scale bar 50µm.

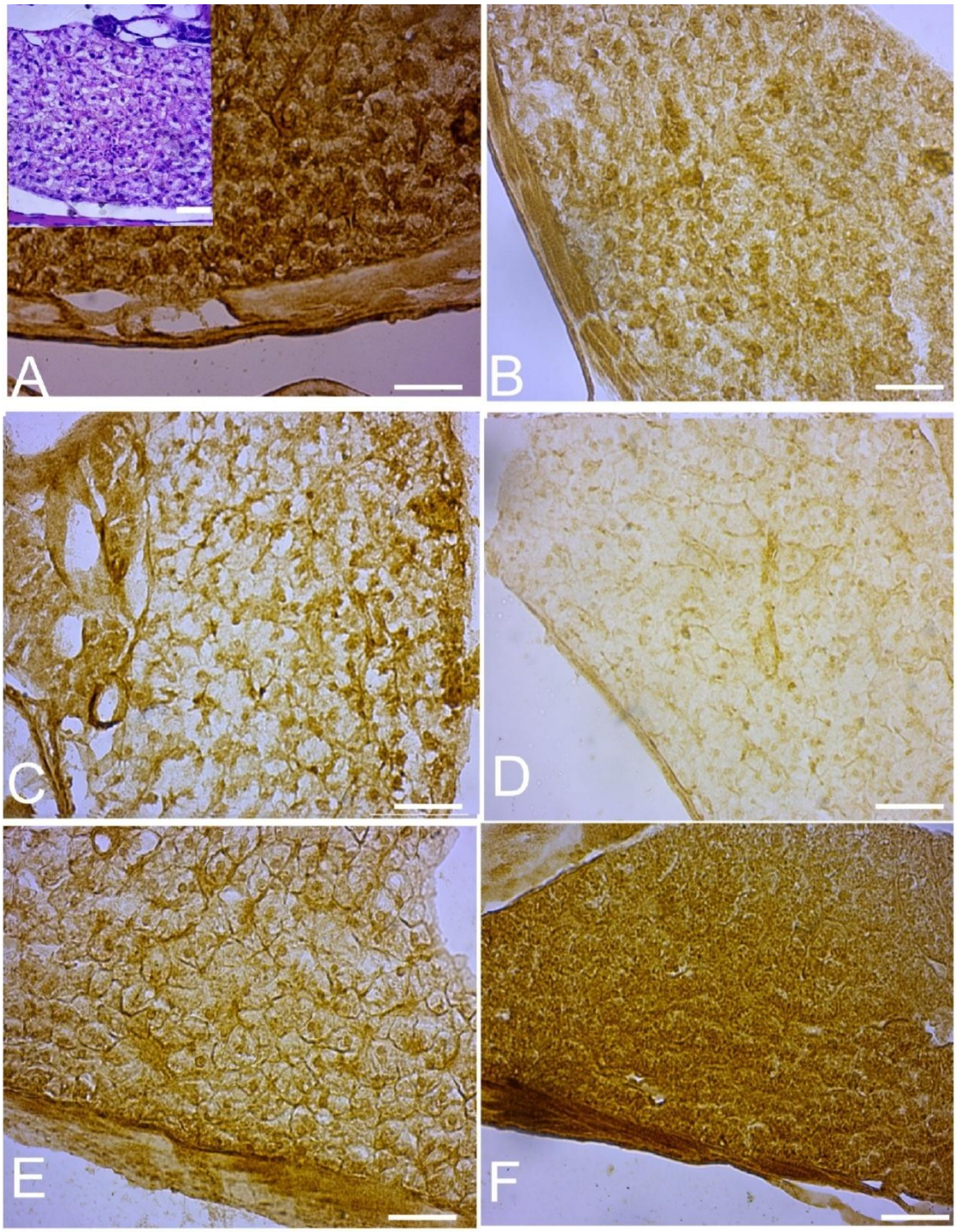

Fig. 6: Photomicrographs of immuno-stained section of 30 days-old control larvae through the liver showing the increased expression of TNFR2 (**A**) against the decreased expression of both TGFβ2 (**C**) and HSP70 (**E**) compared to the expression in 15 days-old larvae. Inhibition of both TNFR2 (**B**) and TGFβ2 (**D**) against upregulated expression of HSP70 (**F**) in uranium-exposed larvae is shown. The insert of (**A**) showing hepatic parynchyma in H$E stain. Scale bar 20μm

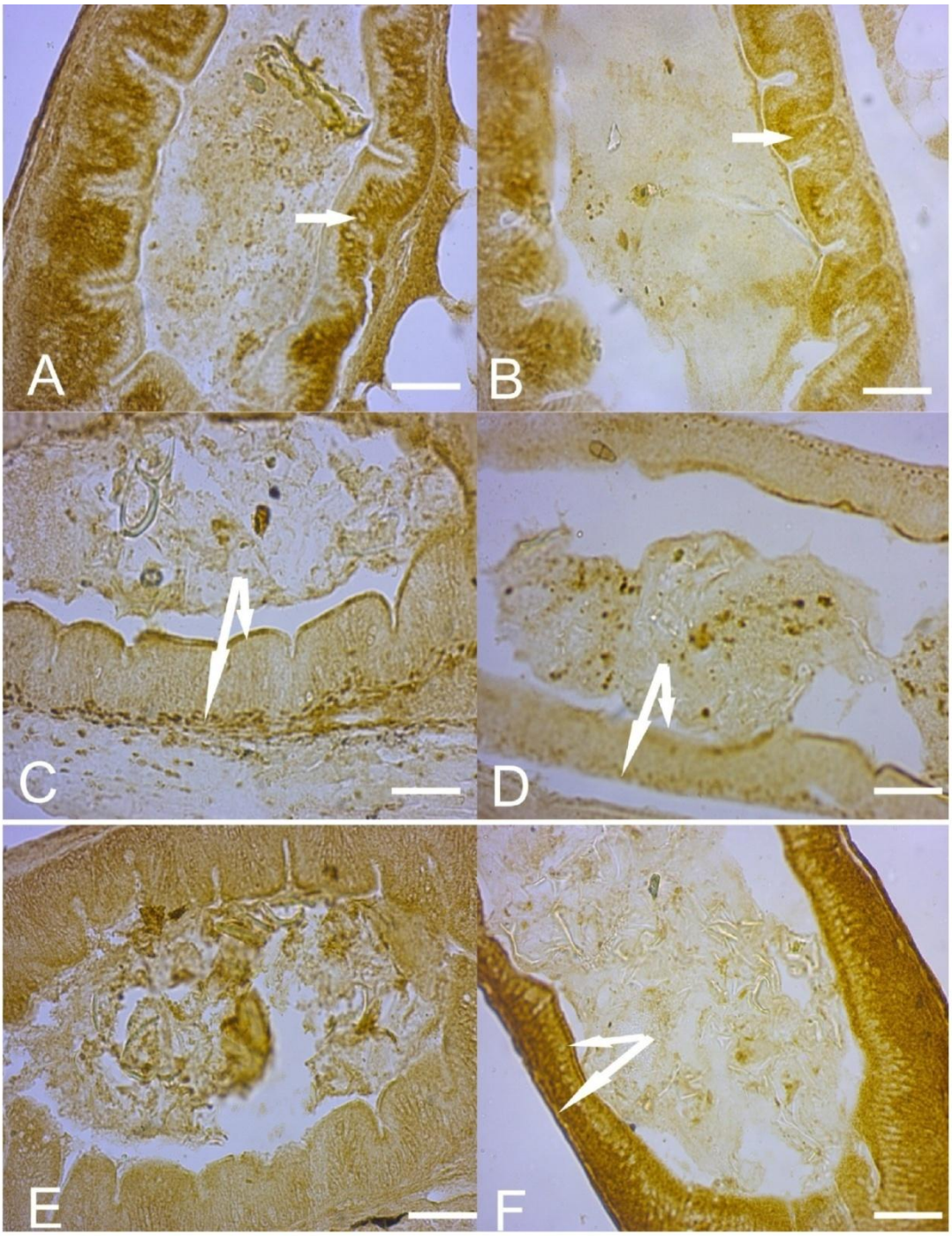

Fig. 7: Photomicrographs of immuno-stained section of 30 days-old control larvae through the intestine showing stained nuclei (arrow) for TNFR2 (**A**), staining of TGF β2 (**C**) in the brush border of epithelia and the nuclei of muscularis (arrows) and homogenously expressed HSP70 (**E**) throughout the intestinal tissue. Inhibition of both the TNFR2 (**B**) and TGFβ2 (**D**) aginst the upregulated expression of HSP70 (**F**) in uranium-exposed larvae comparable to control is shown. Scale bar 20µm.

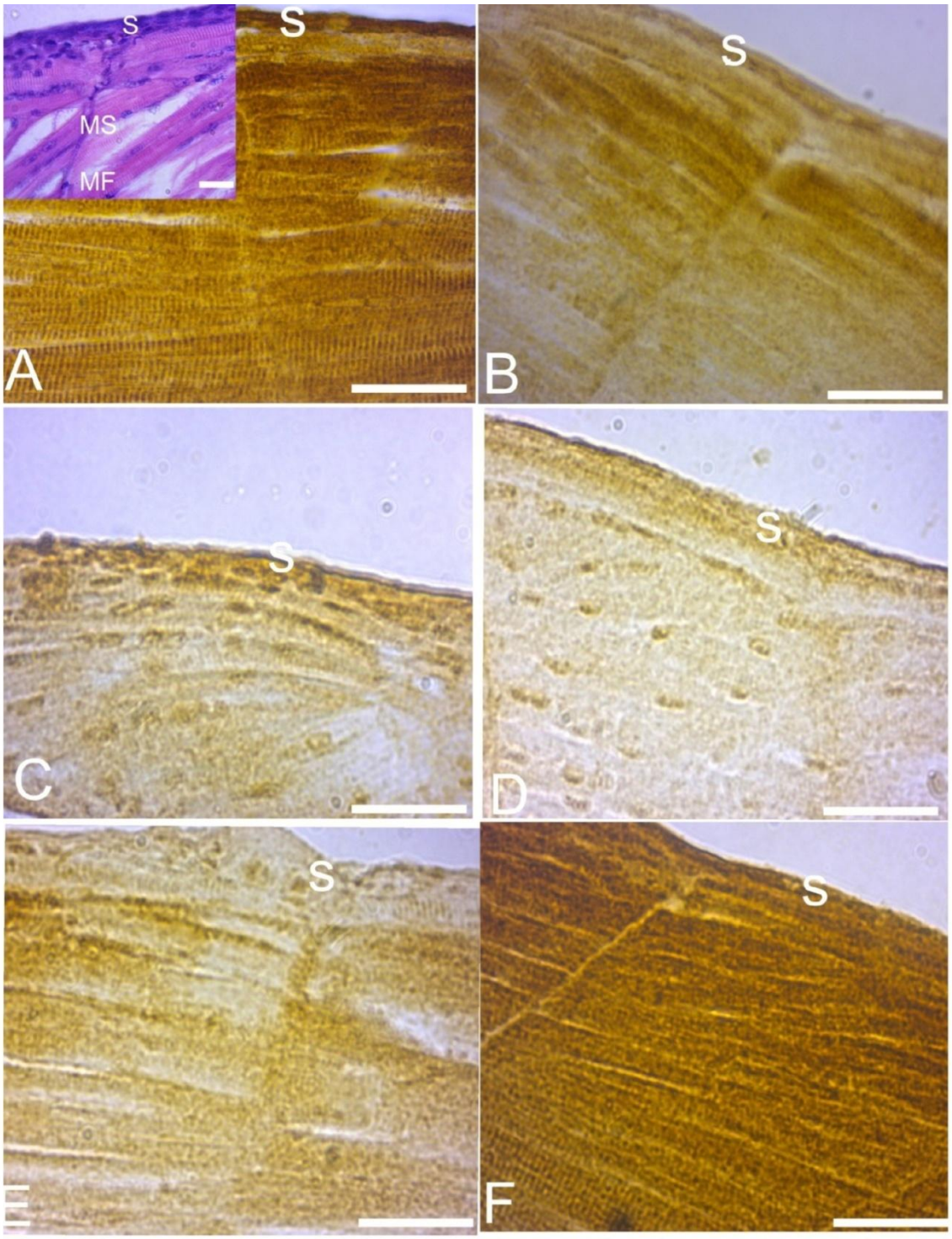

Fig. 8: Photomicrographs of immuo-stained section through the muscles and skin (S) of uranium-exposed larvae showing down-regulation of cytokines TNFR2 (**B**) and TGFβ2 (**D**) and up-regulation of HSP70 (**F**) compared to control (A), (C) and (E), respectively. The insert of (A) represent the the developing muscls. Muscle fibers (MF), myoseptum (MS), skin (S). Scale bar 20μm

DISCUSSION

The present study revealed a concentration-dependent mortality and deformation concerning edematous heart, body curvature and belly inflation. The LC50 for 7 days of exposure was 3.5 mg/l. By reviewing literature, it is obvious that species differences influence the toxicity of uranium to fish species. Hence, Parkhurst*et al*. (1984)reported a no observable effect concentration of greater than 9.0 mg/l for uranium in brook trout embryos and larvae. Species differences LC50s of uranium toxicity in fish of 7-d-old were 2.66, 1.22, 0.73 mg/l for black-banded rainbowfish (***Melanotaenianigrans***), chequeredrainbowfish (***Melanotaeniasplendidainornata)*** **and**Mariana's hardy-head (***Craterocephalusmarianae***) (Bywater*et al*., 1991). An a no observable effect concentration of less than 404 µg/L was determined for gudgeon larvae above which body length and weight were affected (Holdway 1992). No-Observed-Effect Concentrations (NOEC) ranging from 18–810 µg/L, for 5 species of fish were reported (van Dam *et al.,* 2002). The results of the toxicity tests completed in this study for 7 days of exposure revealed a concentration-dependend lethality and deformation with LC50 about 3.5 mg/l indicating species differences among the heavy metals in general.

Concerning the cytokines expression, it was found that an increased expression of TNF R2 in the various organs (brain, retina, liver) of larvae from 5 – 30 days-old. TNFR2 is also expressed in both the developing intestine and muscle striation in 30 days-old larvae. Chronic uranium exposure led to decreased expression of the receptor in all studied organs. Tumor necrosis factor-α (TNFα) is a powerful pro-inflammatory cytokine produced and released mainly by mononuclear phagocytes that regulates endothelial cell functions (Miura *et al*., 2006). TNFα exerts its functions through interaction with two specific cell surface receptors: the 55 kDa tumor necrosis factor receptor superfamily member TNF R1 (P 55) and the 75 kDa TNF R2 (P 75). TNFR1 is expressed in most cell types whereas TNFR2 function seems to be restricted to immune and endothelial cells (Aggarwal, 2003). Recent studies with deficient mice have shown that TNFR2 promotes remyelination of nerve fibers, neuroprotective signaling pathways, tissue repair, regeneration and T cell survival and activation with high suppressive capacity (Arnett *et al*., 2001; Aggarwal, 2003; Marchetti*et al*., 2004; Kim *et al.*, 2006; Rauer*et al*., 2010). The present investigation revealed, beside TNFR2 gene conservation in the developing fish larvae, it express in normal development in various tissues, not restricted to the immune or endothelia, including brain, eyes, liver, intestine and striated muscles of the developing larvae under investigation. In addition to the inhibition of TNFR2 receptor in chronic uranium exposurethat is consequently downstream signaling of TNF, thereby affecting TNF-dependent processes in the studied organs including differentiation, regeneration, repair, neuroprotection ….etc.

In addition, co-expression of the transforming growth factor β2 with TNFR2 was noted in the studied organs (brain, ganglion and nuclear layers of the retina and cornea) of the developing larvae from 5-30 days. In liver, decline of TGFβ2 expression was noted from 15 – 30-days old larvae compared to that of TNFR2 with concomitant expression at the apical cell part of the intestine and throughout the developing muscles and skin. The transforming growth factor-beta (TGF-beta) family constitutes of dimeric proteins that regulate the growth, differentiation and metabolism of many cell types. To our knowledge, research on TGF-β in fish has been limited to its involvement in the immune system (Harms*et al*., 2000; Haddad*et al*., 2008; Castillo*et al*., 2009) and in reproduction (Kohli*et al*., 2003; Kohli*et al*., 2005; Tan *et al*., 2009). Similarities of fish TGFβ2 to human with varying percentages were recorded, 93% in common carp (Sumathy*et al.*, 1997) and 72% for zebrafish (Wyatt *et al*., 2007) were recorded. Recently, Funkenstein*et al*., (2010) identify a novel transforming growth factor-beta (TGF-beta6) gene in fish that participate in muscle growth. These findings confirm our finding concerning the expression of TGFβ2 in different organs of fish larvae that is participating in different tissue functions. Moreover, the results suggest a role of TGFβ2 in the intestinal mucosa by the virtue of its differential expression as compared to that of TNFR2. From the other hand, uranium exposure led to inhibition of TGFβ2 expression which consequently alters the functional activities of this cytokine in the tissues under investigation.

Moreover, the results confirmed that Hsp70 could be used as a tool for evaluation, a kind of potential biomarker, and could be a disease resistance factor against agents induced depletion of bioactive cytokines in addition to its involvement in normal development. In contrast to the down-regulation of both TNFR2 and TGFβ2 cytokines in uranium-exposed larvae, up-regulation of HSP70 in plexiforms of retina, brain, liver, intestinal mucosa, muscles and skin was noted. HSP70 induction is of importance for tissue protection against uranium induced alteration in cytokines expression under investigation and could mitigate the adverse effects of uranium chemo-toxicity at long term exposure. In this context, HSP70 is widely distributed group of HSPs found in numerous organisms from bacteria to mammals, and its expression is markedly induced in response to environmental stresses, such as heat shock, UV and γ-irradiation, and chemical exposure (Georgopoulos and

Welch 1993; Wu 1995). Two distinct isoforms of HSP70 cDNA have been identified from both rainbow trout and zebrafish (Yamashita *et al*., 2004; Ojima et al. 2005). Many studies have examined gain- or loss-of functions to elucidate the biological roles of HSP70 as a molecular chaperone in animal cells *in vitro* and *in vivo*. The chaperone functions of HSP70 appear to be closely related to stress tolerance in animal cells, and over-expression of HSP70 enhances anti-apoptotic activity against cellular stress (Mosser*et al*. 2000; Xanthoudakis and Nicholson, 2001). HSP70 translocated into the lysosomes were found to accelerate protein degradation and catabolism under both stressed and normal conditions (Yamashita *et al*., 2010). Results of the present investigation may help in improving our knowledge concerning the role of both cytokines and the heat shock protein in cancer induction by environmental pollution. From the other hand, these cytokines and the heat shock protein may contribute to the disease treatment.

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