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Histopathological Alterations in some Body Organs of Adult *Clarias gariepinus* (Burchell, 1822) Exposed to 4-Nonylphenol

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1. Introduction

Endocrine-disrupting chemicals (EDCs) include synthetic and naturally occurring chemicals that affect the balance of normal functions in animals (Razia et al. 2006). It has been found that exposure to natural and synthetic estrogenic chemicals may adversely affect wildlife and human health (Colborn et al. 1993). In vitro exposures (Soto et al. 1992; Soto et al. 1994; Toomey et al. 1999) have confirmed the effects of EDCs on tissue structure and cellular processes. Nonylphenol ethoxylates (NPEs) are EDCs which are used globally in the production of plastics, pesticides, and cleaning products and are present in sewage effluents around the world (Talmage, 1994). It has been reported that NP is the most important degradation product of NPEs because of its enhanced resistance towards biodegradation, toxicity, ability to bioaccumulate in aquatic organisms, and estrogenicity (Ahel et al. 1994). NP is found in surface waters, aquatic sediments, and ground water (Bennie, 1999; Talmage, 1994) and it is estrogenic in various aquatic animals (Nimrod and Benson, 1996; Talmage, 1994; Servos, 1999).

The application of environmental toxicological studies on non-mammalian vertebrates is rapidly expanding; and for aquatic system, fish have become valuable indicator for the evaluation of the effects of noxious compounds (Khidr and Mekkawy, 2008). Histology and histopathology can be used as biomonitoring tools for health in toxicity studies (Meyers and Hendricks, 1985). Histoplathological alterations are biomarkers of effect exposure to environmental stressors, revealing alterations in physiological and biochemical function (Hinton et al. 1992). Histopathology, the study of lesions or abnormalities on cellular and tissue levels is useful tool for assessing the degree of pollution, particularly for sublethal and chronic effects (Bernet et al. 1999). More than one tissue may be studied for assessment of the biological effects of a toxicant on localized portions of certain organs and also for assessment of subsequent derangements (degradations) in tissues or cells in other locations and this allows for diagnoses of the observed changes (Adeyemo, 2008). NP has been shown

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to cause histopathological changes in the germ and Sertoli cells of the male eelpot (Christiansen et al. 1998). The skin of fish is continuously exposed to and in direct contact with the environment pollutants such as NP. Histological changes in skin of rainbow trout with mucosomes in goblet cells were recorded after exposure to 10 µg/l of 4-nonylphenol. Several studies demonstrated the high susceptibility of skin to environmental pollutant impacts (Burkhardt-Holm et al. 1997; Iger et al. 1995; Shephard, 1994). Burkhardt-Holm et al. (1997) hypothesized that in trout, xenobiotic estrogens might affect the skin, like natural estrogens, via the steroid receptor.

In trout species, nonylphenol was found to accumulate in the liver, gill, skin, gut, fat, and kidney tissue (Ahel et al. 1993; Coldham et al. 1998; Lewis and Lech, 1996). So that, 4-nonylphenol may affects those organs in corresponding with its impacts on reproductive ones. Most of NP studies revealed sever effects on the liver and gonads of fish tissues (Christiansen et al. 1998; Jobling et al. 1996; Lech et al. 1996) and the corresponding metabolism. The liver is important in many aspects of nutrition, including lipid and carbohydrates storage and alterations in liver structure may be useful as biomarker that indicate prior exposure to environmental stressors (Hinton and laurén, 1990). Stressors-associated alterations of hepatocytes may be found in the nucleus or cytoplasm or both (Marchand et al. 2008). Malik and Hodgson, (2002) reported that the liver plays a major role in complex enzymatic processes of thyroid hormones conversion. So, liver dysfunction and disease affects thyroid hormone metabolism. Although gills are not only the prime organs for gaseous exchange, they perform several other physiological functions including osmoregulation and excretion. Parashar and Banerjee, (2002) reported that changes in environmental parameters often damage this delicate vital organ which has direct contact with aquatic environment. Many studies demonstrated that increased concentrations of different pollutants including several heavy metals seriously damage the gills of teleostean fish (Dutta et al. 1996; Wendelaar Bonga, 1997)).

African catfish (*Clarias gariepinus*), an omnivore freshwater fish, is a popular delicacy relished throughout tropical Africa (Nguyen and Janssen, 2002) due to fast growth rate, high stocking-density capacities, high consumer acceptability and high resistance to poor water quality and oxygen depletion (Adewolu et al. 2008; Akinwole and Faturoti, 2007; Karami et al. 2010). Because it is a prominent culture species (Adeyemo, 2008), the African catfish has been used in many fundamental experimental researches (Mahmoud et al., 2009).

The present work is an extension of previous studies of the present authors (Mekkawy et al., 2011; Mahmoud et al., 2011; Sayed et al., 2011) to determine to what extent the histopathological variations in some organs of the adult catfish, *Clarias gareipinus* (Burchell, 1822) are simultaneously correlated with biochemical and physiological NP-induced changes especially in respect with endocrine disruption.

2. Materials and methods

2.1 Specimen collection

Specimens of adult catfish *C. gariepinus* were collected from the River Nile at Assiut and then were transported to Fish Biology Laboratory of Zoology Department, Faculty of Science, Assiut University. The fish (500–1200 g) were fed on a commercial pellet diet (3% of body weight per day) and kept together in 100 l rectangular tanks containing tap water

(conductivity 2000 ls/cm; pH 7.5; oxygen 88–95% saturation; temperature 27-28 ^{o}C; photoperiod 12:12 light: dark). After 2 week acclimatization, fishes were used for the experimental setup.

2.2 4-nonylphenol

4-Nonylphenol was obtained from Sigma- Aldrich (Schnelldrof, Germany)

2.3 Experimental setup

The adapted adult fish classified into four groups (10 fish per each): control, 4-nonylphenol-treated group (for15 day/ for 0.05mg/l day), 4-nonylphenol-treated group (for 15 day/for 0.08mg/l day), and 4-nonylphenol-treated group (for 15 day/for 0.1 h/ day). In the present study, the range of NP exposures was 0.05-0.1 mg/l and the exposure concentrations are environmentally relevant. The conditions of the experiment were as that of acclimatization with changing all the tap water and concentrations of 4-nonylphenol every day.

2.4 Hematoxylin-Eosin (HE) and Masson's Trichrome (TRI) histopathological preparations

For microscopic preparations, after 15 days, 3 surviving fish of each group were removed and dissected. Small pieces of the liver, kidneys, gills, and skin were taken and immediately fixed in 10% neutral buffered formalin. Fixed tissues were processed routinely for paraffin embedding technique. Embedded tissues were sectioned at 5-7µ in thickness and then stained with Harris' hematoxylin and eosin stain (H & E) and Masson's Trichrome (TRI) stain according to Bancroft and Steven, (1982). Sections were visualized and studied using OLYMPUS microscope model BX50F4 (Olympus optical Co., LTP. Japan).

2.5 Transmission electron microscope (TEM)

Small pieces of liver of newly scarified fish were fixed in 2 % glutaraldehyde, washed in cacodylate buffer and post-fixed in 1% osmium tetroxide. Dehydration was carried out in ascending grads of alcohol and then embedded in epon-araldite mixture. Semithin sections of liver were cut at 1µm and stained with toluidine blue for examination under a light microscope. Ultrathin sections were stained with uranyl acetate followed by lead nitrate (Johannessen, 1978). Electron micrographs were obtained using a Jeol JEM 1200 EX Transmission Electron Microscope at Electron microscope center of Assiut University.

2.6 Ethical statement

All experiments were carried out in accordance with the Egyptian laws and University guidelines for the care of experimental animals. All procedures of the current experiment have been approved by the Committee of the Faculty of Science, Assiut University, Egypt.

3. Results

Throughout the duration of the experiment, the number of fish that died was 1.2, 3.75, 5.5, and 8 % for control, 0.05, 0.08 and 0.1 mg/l 4-nonylphenol respectively. Lesions were observed in the gills, skin, kidneys, and liver of sampled fish for all 4-nonylphenol at all

exposure concentrations and durations. The occurrence and degree of alterations were positively related with the concentrations of 4-nonylphenol while samples taken from the control group remained normal for all the organs throughout the duration of the experiment.

3.1 Histopathological changes in the gills

Histologically, the gills of the adult catfish *Clarias gariepinus* are composed of primary lamellae (pl), secondary lamellae (sl), epithelial cell (epc), mucous cell (mc), and chloride cell (chc) (Fig. 1a). The initial lesions in the gills were manifested in groups exposed to 0.05, 0.08, and 0.1 mg/l of 4-nonylphenol for 15 days (Fig. 1b, c, d, e). The anomalies include epithelial lifting, edema, deformed secondary lamellae in fishes exposed to 0.05 mg/l 4-nonylphenol (Fig. 1b) while in fishes exposed to 0.08 mg/l 4-nonylphenol, desquamation and necrosis were recorded (Fig. 1c). As Fig. (1d, e) shows gills with degeneration of cartilaginous bar malformed secondary lamellae, increase in chloride cell size and number, epithelial hyperplasia, diffusion of secondary lamellae and increase number of mucous cells in fishes exposed to 0.1 mg/l 4-nonylphenol for 15 days.

3.2 Histopathological changes in the skin

Normal structure of skin of adult catfish, *Clarias gariepinus* was shown in fig. (2a), where it consists of alarm cell (ac), mucous cell (mc) and epithelium (ep) with pigment cell (p). The fishes exposed to 0.05 mg/l of 4-nonylphenol showed enlarged alarm cell (eac), with vacuoles (va) in their skin structure (Fig. 2f). As shown in fig. (2c) other changes such as ruptured epithelial cells (repc) and enlarged mucous cells (emc) in the skin of fishes exposed to 0.08 mg/l of 4-nonylphenol for 15 days. Severe damage was recorded in fishes exposed to 0.1 mg/l of 4-nonylphenol as in Fig. (2b, d, e) in which ruptured epithelial cells, necrotic cell (nc), granuled cells (gc), vacuoles (va) and fat cells (fc) were recorded.

3.3 Histopathological changes in the kidney

The functional units of the kidney of the control fish, *Clarias gareipinus* are nephrons which are composed of renal corpuscles (rc) and renal tubules (rt); these structures are surrounded by haemopoietic tissue (ht). The shape of the renal corpuscle is roughly spherical consisting of a double membraned capsule (Bowman's capsule) enclosing a tuft of blood capillaries (glomerulus) (g). Bowman's space; a space between the glomerulus and the capsule (Fig. 3a). Examination of kidney sections of fish exposed to 0.05 mg/l of 4-nonylphenol for 15 days revealed edema in the epithelium lining of some renal tubules (e) and some showed degeneration (d) and rupture of Bowman's capsule (r). Hypertrophy of the glomerulus (hyt) was observed with shrinkage (sh). Moreover, necrosis (n) and pyknosis (p) were observed in some renal tubules (fig. 3b). After 15 days of exposure to the 0.08 mg/l of 4-nonylphenol, similar histological changes were observed, however, proliferation in renal tubules and haemopoieatic tissue (pr) with dissociation in some tubules (di) were recorded. Dilated blood vessels (dbv) and mealnomacrophages (m) were also observed (fig. 3c). The sections of kidney in fishes exposed to 0.1 mg/l of 4-nonylphenol showed severe damage or complete degeneration with obliterated Bowman's space (obs). Masson's Trichrome stain indicated the degeneration of connective tissue and degeneration of renal tubule and glomerulus (fig. 3d).

3.4 Histopathological changes in the liver

The liver of the control fish *Clarias gariepinus* appears as a continuous mass of hepatic cells; hepatocytes (h) which cord-like pattern interrupted by blood vessels and sinusoids (bs). The cords of hepatocytes are arranged around the central vein (cv). The hepatocytes are large in size, polygonal in shape with centrally located nuclei. The hepatocytes have homogenous eosinophilic cytoplasm. The sinusoids are seen as communicating channels occupied by blood cells with Küffer cells (kc) (fig. 4a). Examination of liver sections after exposure to 0.08 mg/l of 4-nonylphenol for 15 days showed degeneration (d) in the form of disintegration in most cytoplasmic contents. Lymphatic aggregations (la), necrosis (n), pyknosis (p), were observed (Fig. 4b). Also, melanomacrophages, pyknosis and rupture of hepatocytes (r) were recorded (fig. 4c). Less damage occurred in liver sections after exposure to 0.05 mg/l of 4-nonylphenol for 15 days (Fig. 4d). As Fig. (5a, b, c, d, e) shows marked severe damage occurred in fishes exposed to 0.1 mg/l of 4-nonylphenol for 15 days. Pyknosis indicated by arrows, fat cell (fc), lymphatic infiltration indicated by arrows, pigments diffusion, aggregation of fibers around central vein and rupture of hepatocytes were recorded. Masson's Trichrome stain indicated this severe damage in liver tissue (Fig. 5f).

3.5 Electron microscope examination of hepatocytes

The fine structure of the hepatocytes shows parallel cisternae of rough endoplasmic reticulum (rer), polygonal centrally located vesicular nuclei (n) with nucleolus (nu), numerous mitochondria (m) with different shapes and sizes and Golgi complex (g) near the nucleus (Fig. 6a).

Hepatocytes of animals exposed to 0.05 mg/l of 4-nonylphenol appeared swollen or hypertrophied with dense bodies (db), karyolysis in nucleus (fig. 6b) and damaged mitochondria (dm), rarified cytoplasm (rc) and vacuoles (v) (fig. 7b). Also, degenerative changes, shrunken and indented nuclei with cytoplasmic fat droplets (fd) were observed (Fig. 8b). In fishes exposed to 0.08 mg/l of 4-nonylphenol the cytoplasm shows tiney vacuoles (cv), the nuclei appeared irregular in shape with nuclear indentation. Some hepatocytes showed signs of karylyosis (Fig. 7a, 9a). Moreover, damaged mitochondria, degenerative rough endoplasmic reticulum (drer), increase in number of lysosomes (ly) were recorded. Some nuclei showed condensation and migration of chromatin at the nuclear periphery with prominent nucleolus with some apoptotic changes in the form of nuclear envelope (Fig. 9a). Some hepatocytes appeared swollen with large rarified areas in the cytoplasm resulting in disorganization and dissociation of cellular organelles (Fig. 7a). Hepatocytes of fishes exposed to 0.1 mg/l of 4-nonylphenol showed similar changes as those exposed to 0.05 and 0.08 mg/l of 4-nonylphenol, however, degenerative changes, hypertrophied, karylyosis, blood sinusoids collapsation, apoptosis and vacuolated hepatocytes (Fig. 10, 11). Mitochondria were swollen with destructive cristae; electron dense materials appeared at the periphery of these mitochondria (em) (Fig. 9b, 10a, 11,a, b). Concentric whorly organization of rough endoplasmic reticulum (cw) with detached ribosomes were also seen (Fig. 11a). Other regions of the reticulum appeared as parallel cisternae with electron lucent cytoplasm between its cisterna also, circular arrays of RER

were appeared (Fig. 10a, 11a). An increase in the number of lysosomes and fat drops were seen (Fig. 10b, 11b).

4. Discussion

It has been reported that NP like E2 is estrogenic and affects the histology of developing immune and endocrine organs and those in direct contact with aquatic environment (Yokota *et al.,* 2001; Kang *et al.,* 2003; Seki *et al.,* 2003; Razia *et al.,* 2006). Skin and gills are highly sensitive to pollutants due to their direct contact to aquatic environment. It has been reported that the skin is sensitive to steroid hormone activity (Pottinger and Pickering, 1985). The present results showed severe damage in the skin epithelial cells and necrosis reflecting such sensitivity to NP. NP exposure of rainbow trout resulted in a specific granulation pattern of epidermal mucous cells visible as irregularly shaped and large mucosomes (Burkhardt-Holm *et al.,* 2000). The unique granulation pattern in skin of rainbow trout represents a suitable bioindicator for nonylphenol exposure (Burkhardt-Holm *et al.,* 2000). These latter authors stated that the structural alterations in the skin of the estradiol-injected trout is pointed to a physiological response such as, detached pavement cells, vacuolation of the cytoplasm and severely deformed cell nuclei at a dose of $10\mu gl^{-1}$ which is lower than those in the present study. The damage occurred in the rainbow trout skin is similar to the quantitative changes of the mucous composition induced by hormones or environmental acidifications (Balm *et al.,* 1995). Schwaiger *et al.,* (1999) reported vitellogenin induction in the liver after exposure to 10 μgl^{-1} nonylphenol.

The present results exhibited severe damage in liver tissue of *C. gariepinus* including necrosis and decrease in the cell number along with vacuolation. Similar results were recorded by Uguz *et al.* (2003) who reported a significant increase in the Küpffer cells after one week of 4-nonylphenol exposure. Hughes *et al.* (2000) have shown NP-induced cell death. Galembeck *et al.* (1998), Hughes *et al.* (2000), and Uguz *et al.* (2003) reported that the disappearance of the cell membranes could be due to the lytic activity of alkylphenols.

In the present study, the liver cell borders disappeared and nuclei became larger after two weeks of exposure to $0.1mgl^{-1}$ of 4-nonylphenol, this is similar to the findings of Uguz *et al.* (2003) who reported that this may be due to the increase in the DNA/ RNA ratio which was been observed in carcinogenic cells induced by NP (Chiriboga *et al.,* 2000a, b). The increase in the connective tissue with regenerating hepatocytes instead of normal liver tissues recorded in the present work was similar to those of Uguz *et al.* (2003). Such changes can be interpreted as an indication of carcinogenic development in the liver (Chiriboga *et al.,* 2000a, b; Calmak, 2001). Generally, the lesions detected in cells, tissues or organs are represent an integration of cumulative effects of physiological and biochemical stressors and therefore, can be linked to the exposure and subsequent metabolism of chemical contaminants (Adeyemo, 2008).

The gills are the primary initial target of toxicity, and the cytological changes in gill morphology in fish usually occur as a result of contaminant exposure. Gills have an extensive surface area and minimal diffusion distance between dissolved O_2 and blood capillary for efficient gaseous exchange. The fusion occurred in gills of fishes exposed to

4-nonylphenbol in this investigation may cause a drastic reduction in the respiratory surface area. However, very little is known about the toxic impact of 4-nonylphenol on the functional morphology of the gills. The present results indicated such toxic impacts. Increase in the number of mucous cells in gills of fishes exposed to 0.1 mgl^{-1} of 4-nonylphenol was recorded. It has been reported that the immediate morpho-pathological response of the gills to ambient xenobiotics is often manifested by a significant increase in the density of its mucous cells (Dutta, 1997, Hemalatha and Banerjee, 1997). The large quantity of mucous secretion acts as a defense mechanism against several toxic substances (Handy and Eddy, 1991; Mazon *et al.*, 1999). Similar to the findings of Dutta *et al.* (1996), the present results included many alterations such as increase in mucous and chloride cell number and size, necrosis, rupture of epithelium, desquamation, deformed secondary lamellae and oedema.

According to Peuranen *et al.* (1994) any discontinuity of epithelial lining of the gill lead to a negative ion balance and to changes in the haematocrite and mean cellular haemoglobin values of the blood. The number of chloride cells increased in the present study and this is similar to the results of Parashar and Banerjee, (2002). They stated that the number of chloride cells in the epithelial linings of both primary lamellae and secondary lamellae of *Heteropneustes fossilis* increased significantly following exposure to lead nitrate solution. Dutta *et al.* (1996) summarized the increased number of chloride cells in the gills of fishes following exposure to a variety of toxicants.

Increased ion permeability and sodium efflux of gill epithelial cells due to ethoxylate nonylphenol were reported in rainbow trout (Pärt *et al.*, 1985). Similar results in the present work were recorded in the histology of gills under NP-stress and confirmed by the increased NP-induced anion gap.

The kidney of fishes receives the largest proportion of the post-branchial blood and therefore renal lesions might be expected to be good indicators of environmental pollution (Cengiz, 2006). Many studies used histological characteristics of kidney as an indicators of pollution especially nonylphenol (Srivastava *et al.*, 1990; Banerjee and Bhattacharya, 1994; Ortiz *et al.*, 2003; Cengiz, 2006). In the present work, histological changes in the kidney after exposure to 4- nonylphenol were necrosis, hypertrophy of glomerulus, degeneration and dissociation of renal tubules and Bowman's capsule, proliferation in the renal tubule and haemopoieatic tissue, shrinkage of glomerulus, pyknosis, dilated blood vessel, rupture of Bowman's capsule, and obliterated Bowman's space. Similar results were reported in fishes after exposure to other pollutants (Cengiz, 2006; Khidr and Mekkawy, 2008; Abdel-Tawab and Al-Salahy, 2009).

From the results of the current study, it could be suggested that the exposure of adult catfish, Clarias gariepinus to sublethel doses of 4-nonylphenol caused moderate and severe damage to some organs such as gills, skin, kidney, and liver. These adverse effects of NP in gills, skin, kidney and liver were simultaneously correlated with sever biochemical, physiological changes in addition to endocrine disruption (Mekkawy et al., 2011; Mahmoud et al., 2011; Sayed et al., 2011) So, it is concluded that NP works as estrogenic and non-estrogenic factor leadings to general and specific metabolism disruption in different pathway.

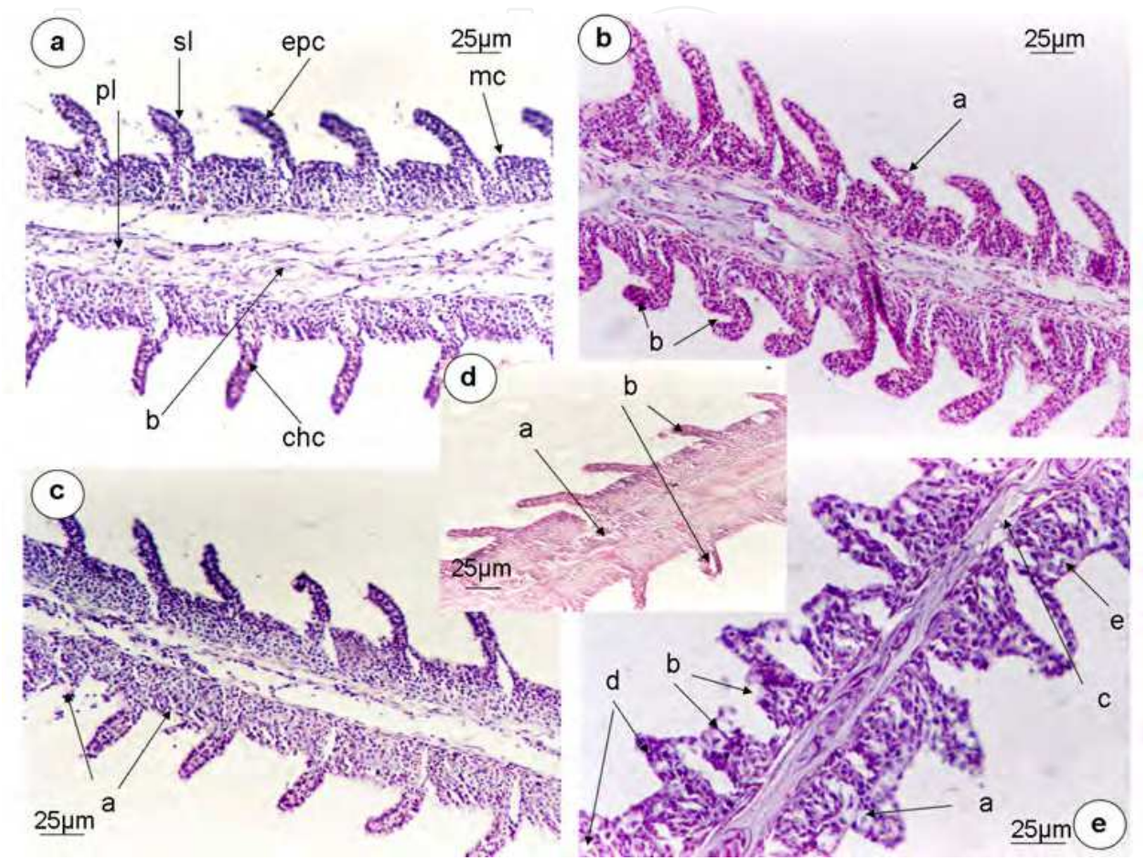

Fig. 1. (a) Gill structure of control adult fish *Clarias gariepinus*. (pl), primary lamellae; (sl) secondary lamellae; (epc) epithelial cell; (mc) mucous cell; (chc) chloride cell. (b) Gill tissue exposed to 0.05 mg/l 4-nonylphenol for 15 days showing a, epithelial lifting and oedema and b, deformed secondary lamellae. (c) Gill tissue exposed to 0.08 mg/l of 4-nonylphenol for 15 days showing a, desquamation and necrosis. (d)) Gill tissue exposed to 0.1 mg/l of 4-nonylphenol for 15 days showing a, degeneration of cartilaginous bar and b, malformed secondary lamellae. (e) Gill tissue exposed to 0.1 mg/l of 4-nonylphenol for 15 days showing a, increase in chloride cell size and number; b, epithelial hyperplasia and diffusion of secondary lamellae; c, degeneration and vacuolation of cartilaginous bar; d, desquamation and necrosis and e, increase number of mucous cells. Stain H& E. Magnification a, b, c, e (400X) and d (100X).

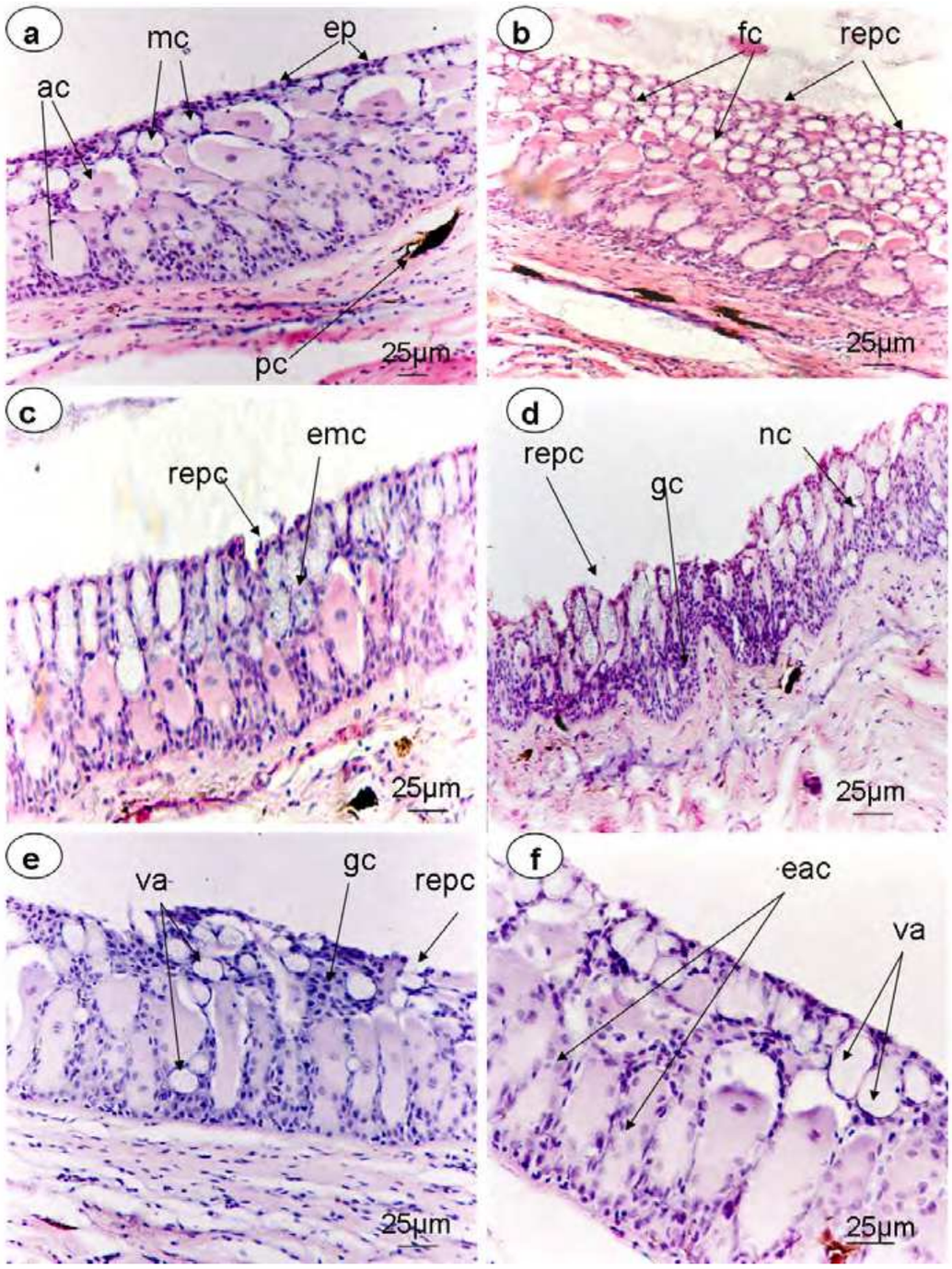

Fig. 2. Vertical sections of the skin of adult catfish *Clarias gariepinus* stained with H&E. (a) skin of control fish showing ac, alarm cell; mc, mucous cell; ep, epithelium; p, pigment cell. (b, d, e) skin of fish exposed to 0.1 mg/l of 4-nonylphenol for 15 days showing fc, fat cell; repc, ruptured epithelial cells; nc, necrotic cells; gc, granuled cells and va, vacuoles.(c) skin of exposed fish to 0.08 mg/l 4-nonylphenol for 15 days showing repc, ruptured epithelial cells and emc, enlarged mucous cells. (f) skin of exposed fish to 0.05 mg/l 4-nonylphenol for 15 days showing eac, enlarged alarm cells and va, vacuoles. Magnification a, b, c, d, e (100X) and f (400X).

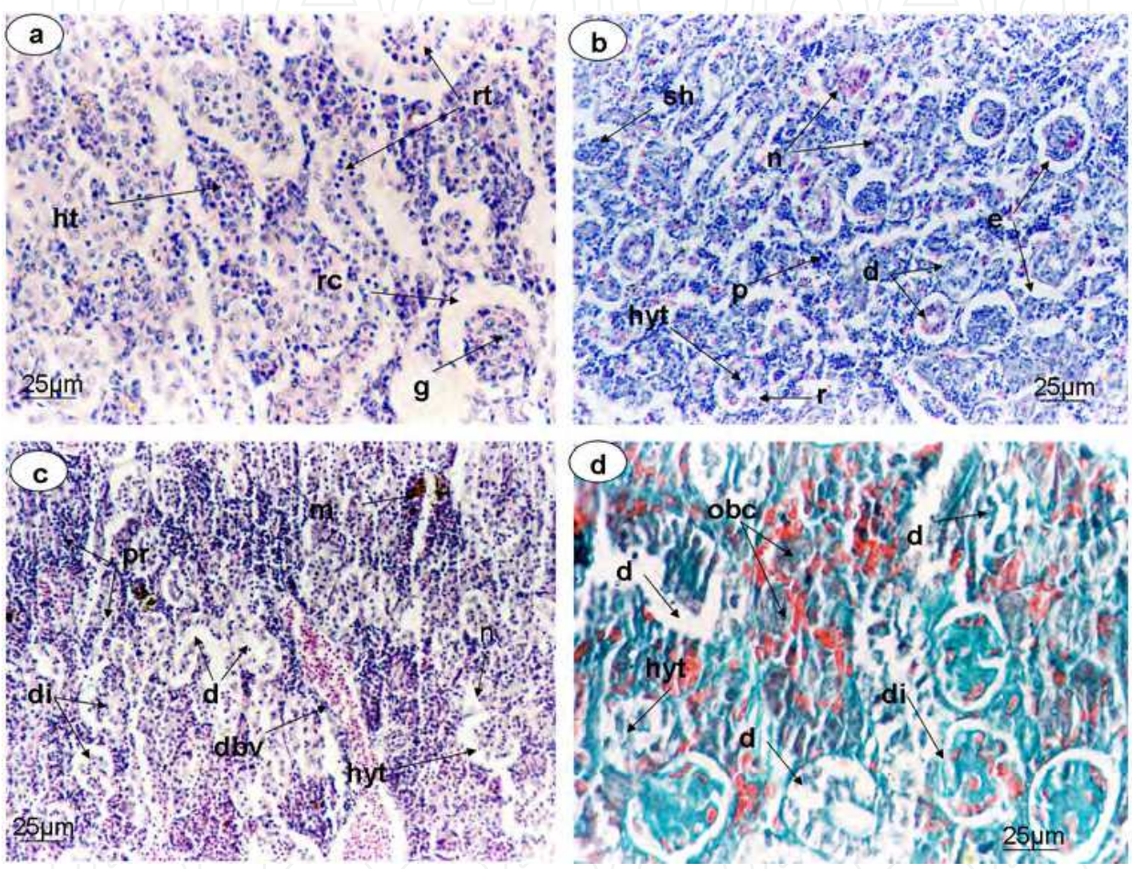

Fig. 3. Transverse sections of kidney of the *C. gariepinus.* (a) Control, (b) fish exposed to 0.08 mg/l of 4-nonylphenol for 15 days, (c) fish exposed to 0.05 mg/l 4-nonylphenol for 15 days, (d) fish exposed to 0.1 mg/l 4-nonylphenol for 15 days. ht, haemopoietic tissue; g, glomerulus; rt, renal tubules; rc, renal corpuscles; n, necrosis; hyt, hypertrophy of glomerulus; d, degeneration; di, dissociation; e, edema of renal tubules and Bowman's capsule; m, melanomacrophages; pr, proliferation in the renal tubules and haemopoieatic tissue; sh, shrinkage of glomerulus; p, pyknosis; dbv, dilated blood vessel; r, rupture of Bowman's capsule; obs, obliterated Bowman's space a, b and c Staind with H&E and d stained with masson's trichrome. Magnification a and d (400X), b and c (200X).

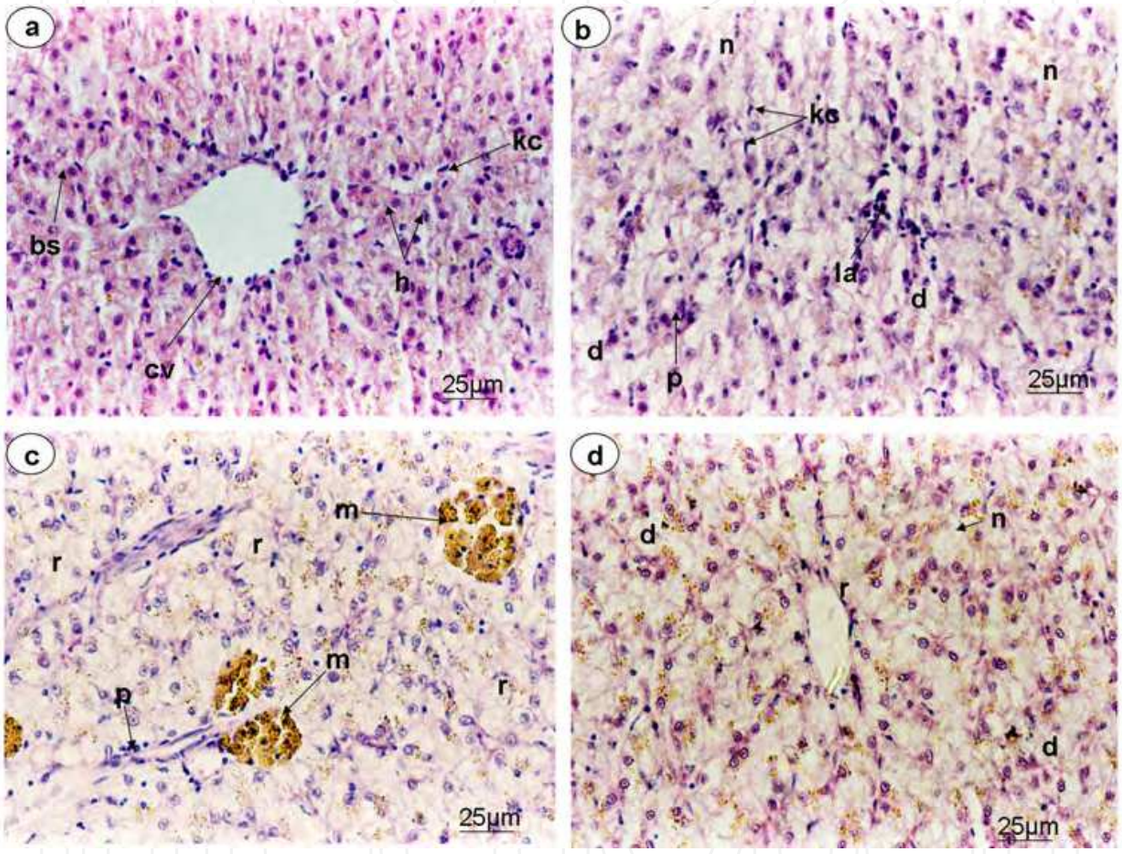

Fig. 4. Sections of liver of the *C. gariepinus.* (a) Control showing cv, central vein; bs, blood sinusoids and h, hepatocyte (b) fish exposed to 0.08 mg/l of 4-nonylphenol for 15 days showing n, necrosis; kc, küpffer cell; la, lymphatic aggregation; d, degeneration and p, pyknosis (c) fish exposed to 0.08 mg/l of 4-nonylphenol for 15 days showing m, melanomacrophages; p, pyknosis; r, rupture of the hepatocytes (d) fish exposed to 0.05 mg/l of 4-nonylphenol for 15 days showing d; d, degeneration; r, rupture of the cell membrane of central vein; n, necrosis. a, b, c and d stained with H&E, Magnification (400X).

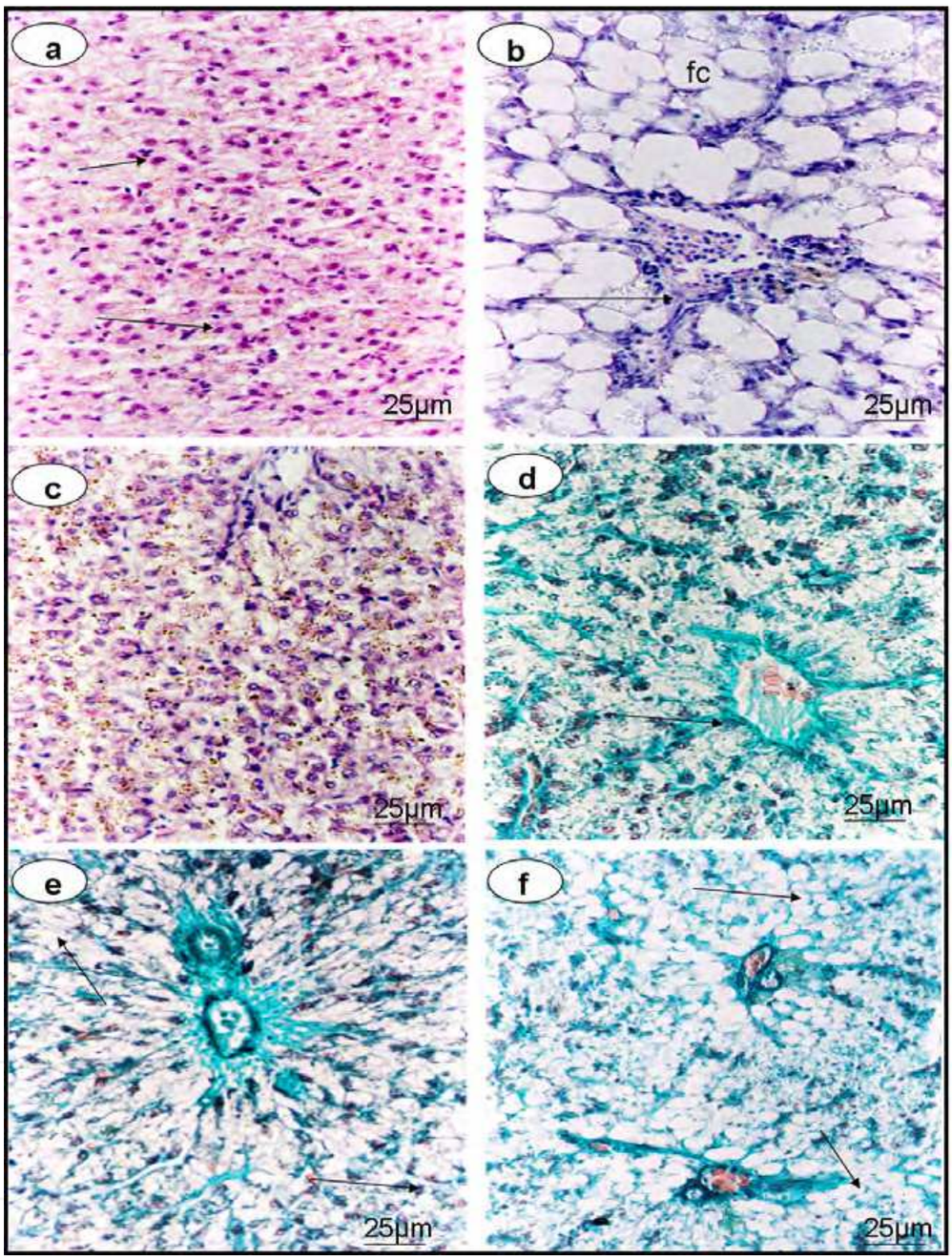

Fig. 5. Sections of liver of the *C. gariepinus.* (a) Fish exposed to 0.1 mg/l of 4-nonylphenol for 15 days showing pyknosis (arrows) (b) fish exposed to 0.1 mg/l of 4-nonylphenol for 15 days showing fc, fat cell and lymphatic infiltration (arrows) (c) fish exposed to 0.1 mg/l of 4-nonylphenol for 15 days showing pigments diffusion (d) fish exposed to 0.1 mg/l of 4-nonylphenol for 15 days showing aggregation of fibres around central vein (arrows) (e) fish exposed to 0.1 mg/l of 4-nonylphenol for 15 days showing rupture of hepatocytes and aggregation of fibres around the central vein (arrows) (f) fish exposed to 0.1 mg/l of 4-nonylphenol for 15 days showing accumulation of fats as fat cells. a, b and c staind with H&E and d, e and f stained with mssson's trichrome. Magnification (400X).

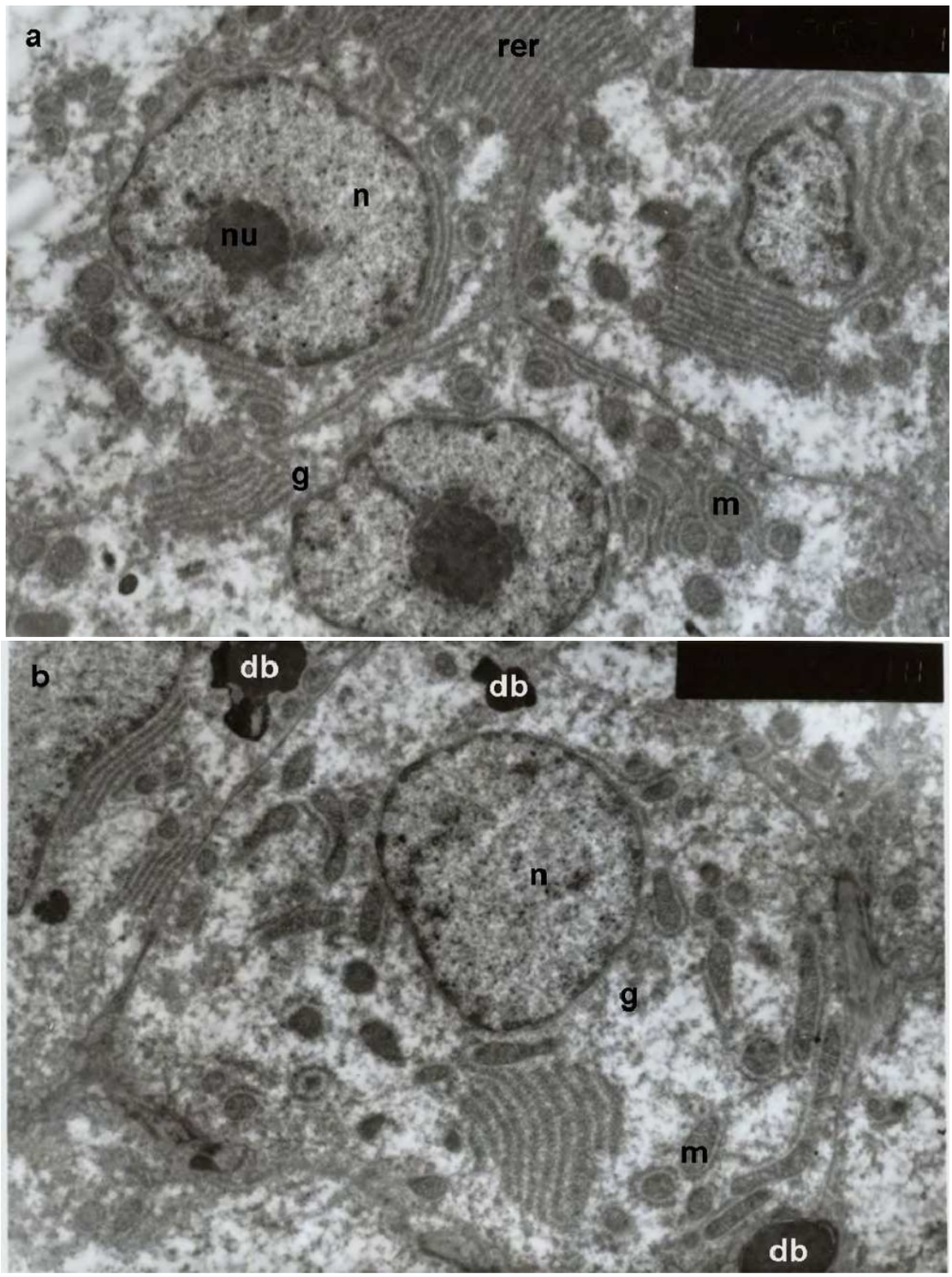

Fig. 6. Transmission electron micrograph of hepatocytes of catfish *Clarias gariepinus*. (a) control (X5000), (b) fish treated with 0.05 mg/l of 4-nonylphenol for 15 days (X5000). (n) nucleus, (nu) nuculeolus, (m) mitochondria, (rer) rough endoplasmic reticulum,(g) Golgi apparatus and (db) dense body.

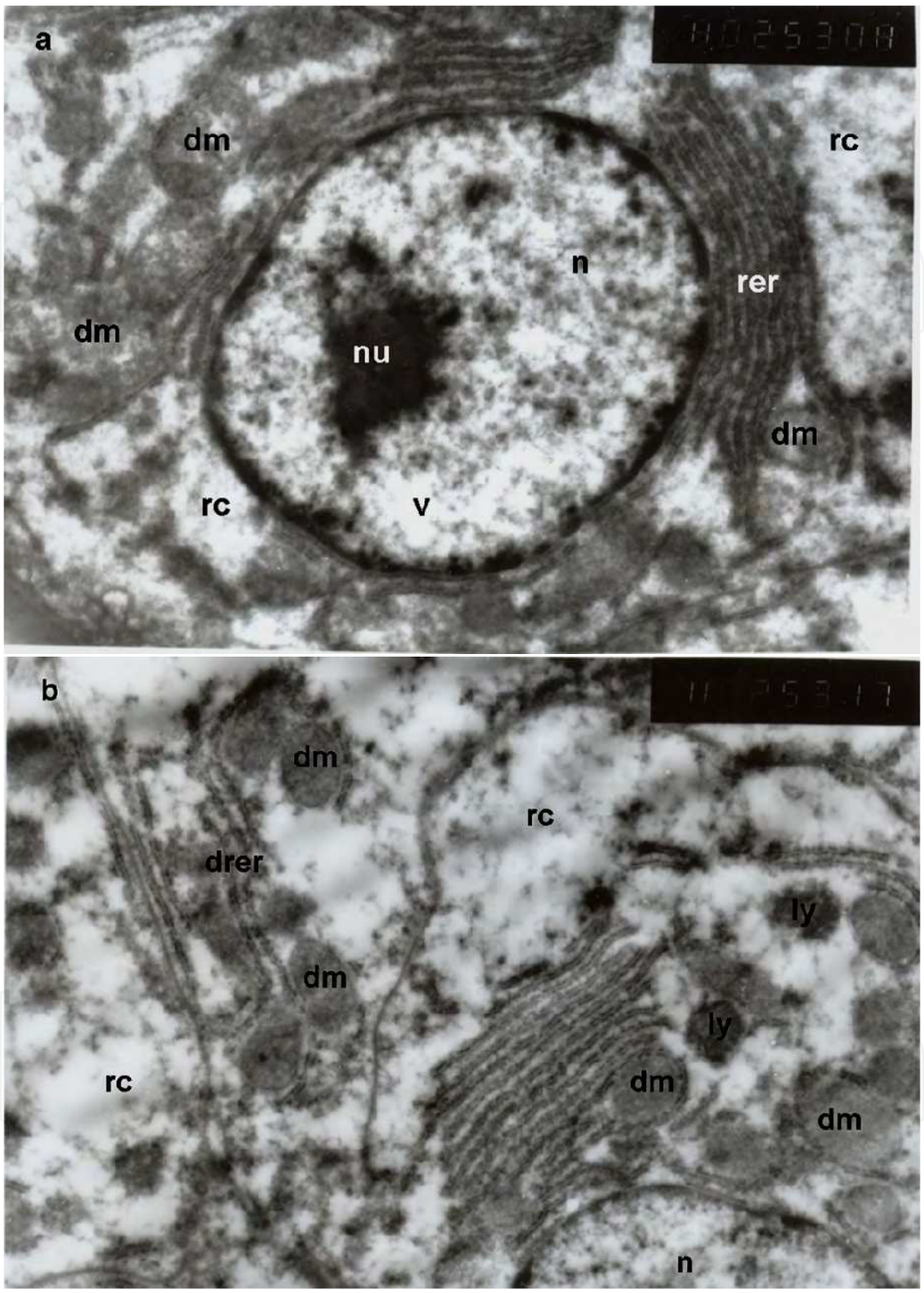

Fig. 7. Transmission electron micrograph of hepatocytes of catfish *Clarias gariepinus* showing marked degeneration of hepatocytes. (a) fish treated with 0.08 mg/l of 4-nonylphenol for 15 days (X8000), (b) fish treated with 0.05 mg/l of 4-nonylphenol for 15 days (X8000). (n) nucleus, (nu) nuculeolus, (dm) damaged mitochondria, (drer) degenerated rough endoplasmic reticulum,(rc) rarfied cytoplasm (v) vacuoles and (ly) lysosomes.

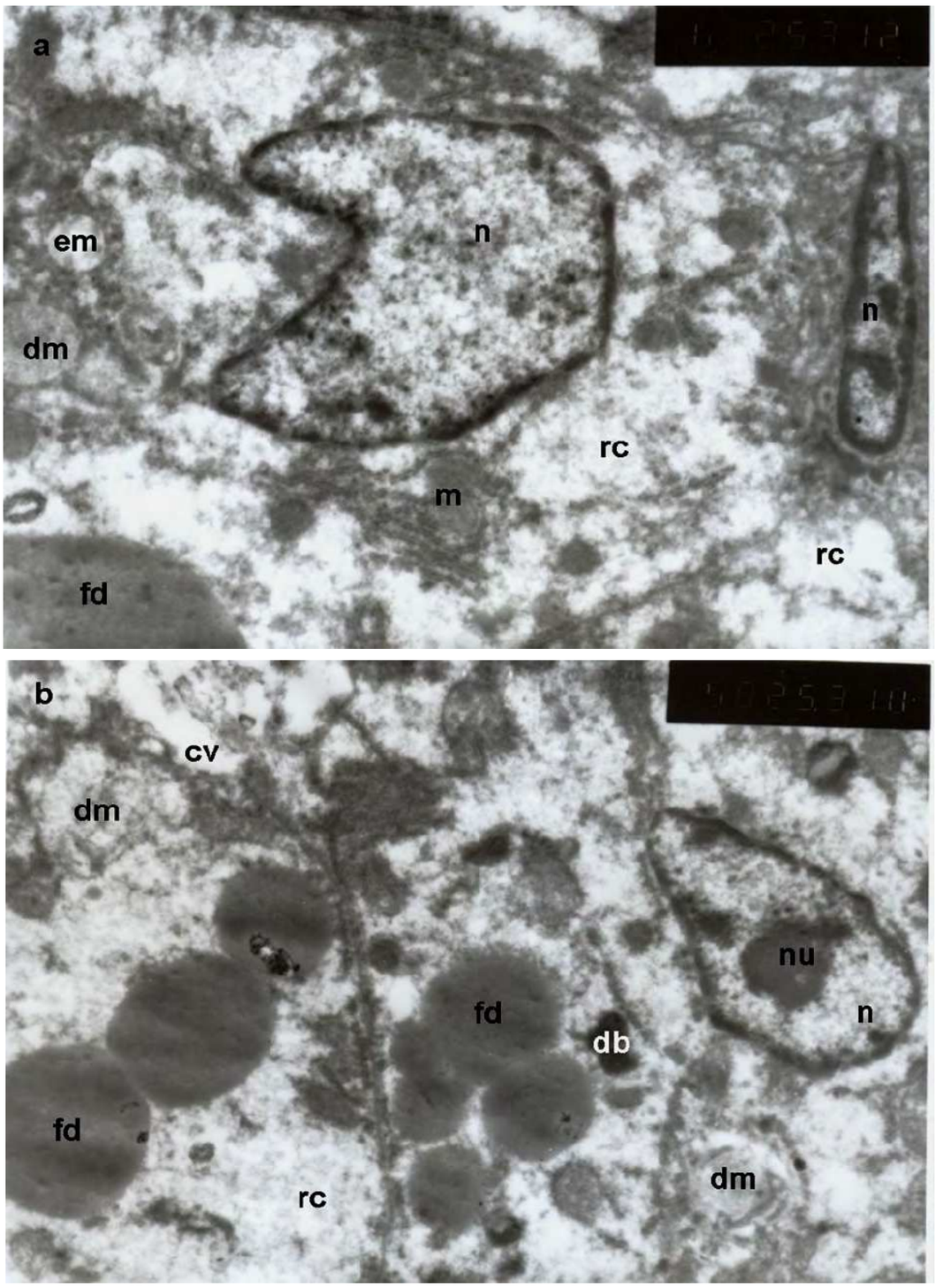

Fig. 8. Transmission electron micrograph of hepatocytes of catfish *Clarias gariepinus* showing marked degeneration of hepatocytes. (a) fish treated with 0.1 mg/l of 4-nonylphenol for 15 days (X5000), (b) fish treated with 0.05 mg/l of 4-nonylphenol for 15 days (X5000). (n) nucleus, (nu) nucleolus , (dm) damaged mitochondria, (em) empty mitochodria, (m) mitochondria, (rc) rarified cytoplasm (fd) fat drops and (db) dense body.

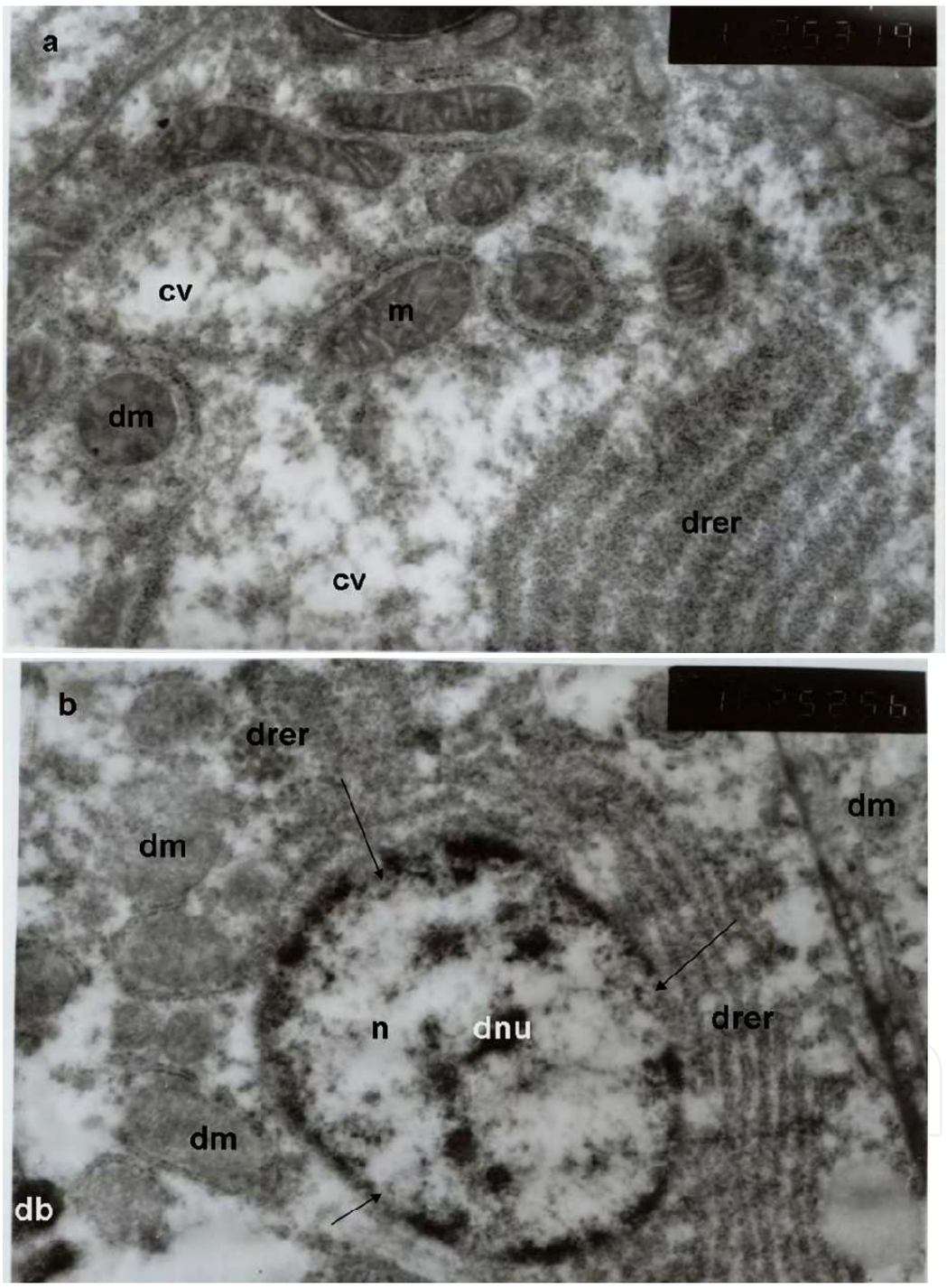

Fig. 9. Transmission electron micrograph of hepatocytes of catfish *Clarias gariepinus* showing marked degeneration of nuclear envelope (arrows) of hepatocytes. (a) fish treated with 0.08 mg/l of 4-nonylphenol for 15 days (X8000), (b) fish treated with 0.1 mg/l of 4-nonylphenol for 15 days (X8000). (n) nucleus, (nu) nuculeols, (dnu) degenerated nucleolus, (dm) damaged mitochondria, (em) empty mitochodria, (cv) cytoplasm vacuoles, (drer) degenerated rough endoplasmic reticulum and (db) dense body.

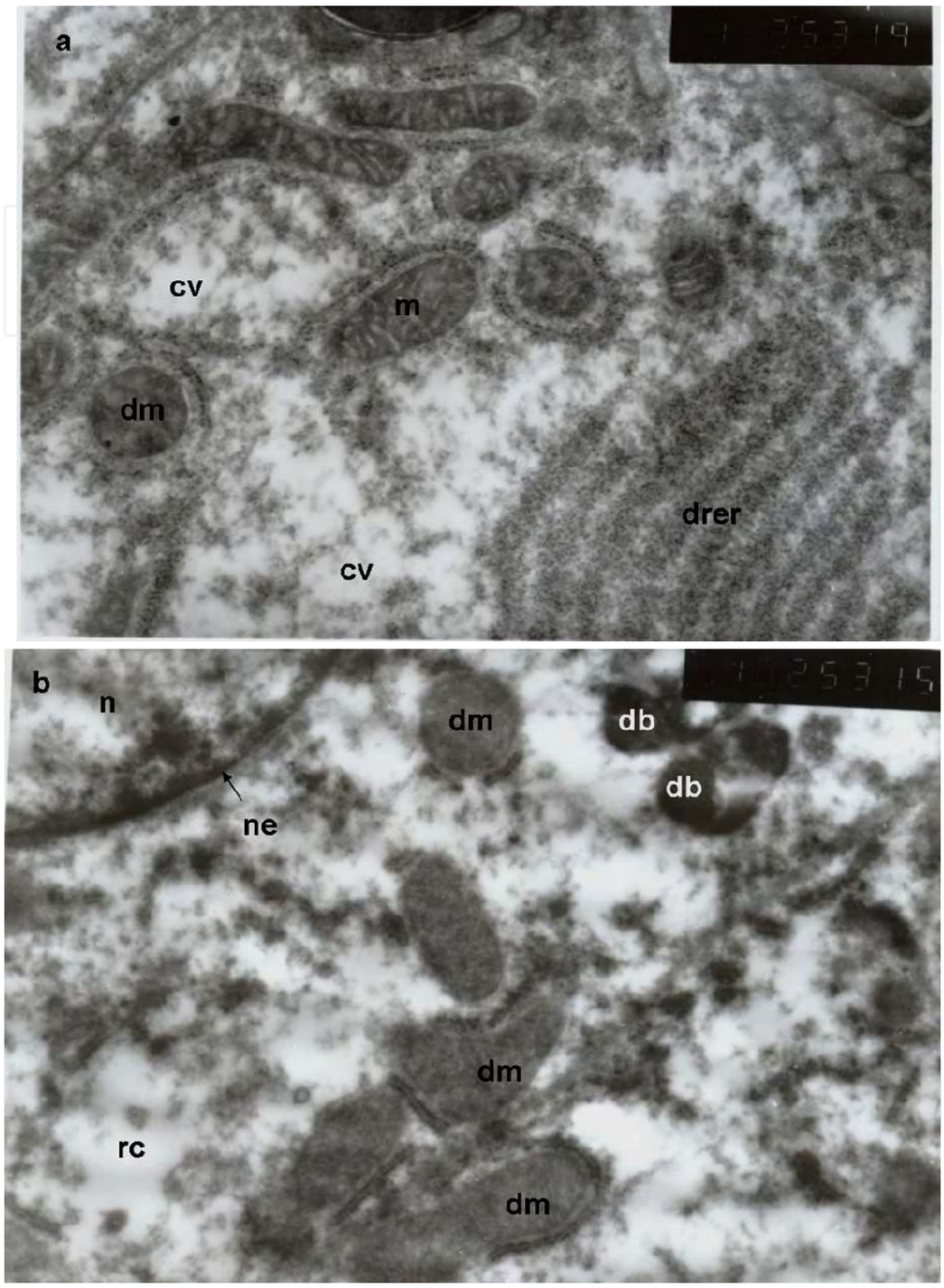

Fig. 10. Transmission electron micrograph of hepatocytes of catfish *Clarias gariepinus* showing marked degeneration of hepatocytes. (a) fish treated with 0.1 mg/l of 4-nonylphenol for 15 days (X14000), (b) fish treated with 0.1 mg/l of 4-nonylphenol for 15 days (X5000). (n) nucleus, (nu) nucleolus, (drer) degenerated rough endoplasmic reticulum, (dm) damaged mitochondria, (m) mitochodria, (rc) rarified cytoplasm, (cv) cytoplasm vacuoles, (fd) fat droplets and (db) dense body.

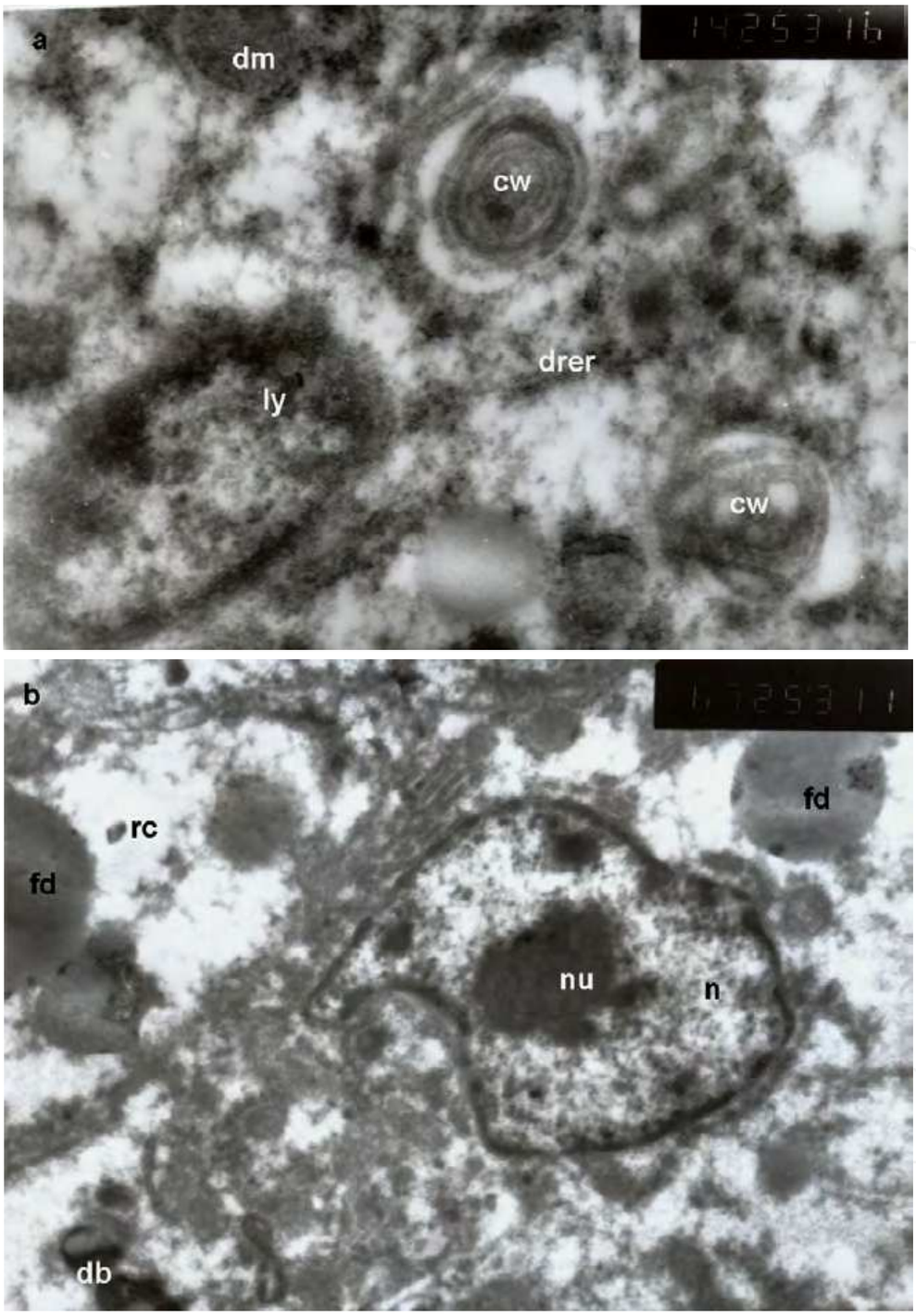

Fig. 11. Transmission electron micrograph of hepatocytes of catfish *Clarias gariepinus* showing marked degeneration of hepatocytes. (a) fish treated with 0.1 mg/l of 4-nonylphenol for 15 days (X14000), (b) fish treated with 0.1 mg/l of 4-nonylphenol for 15 days (X10000). (n) nucleus, (ne) nucleolus envelope, (drer) degenerated rough endoplasmic reticulum, (dm) damaged mitochondria, (cw) concentric whorls appearance of RER, (rc) rarified cytoplasm, (ly) lysosomes and (db) dense body.

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