



Journal of Medical Sciences

ISSN 1682-4474

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Tarek A. El-Ghamrawy
Departments of Histology and Anatomy, Faculty of Medicine, Cairo University, Egypt

Chloroquine-Induced Retinopathy in the Rat. Immunohistochemical and Ultrastructural Study

Dina H. Abdel Kader,
Safinaz Salah El Din Sayed and Tarek A. El-Ghamrawy

The aim of this study is to detect the changes induced by chloroquine administration on the retina of rat using immunohistochemical and ultrastructural methods. Thirty-two male adult albino rats were used, divided into four groups (n = 8 each): 1, 2, 3 and 4, further subdivided into control (n = 2) and experimental (n = 6) subgroups. The experimental subgroups received chloroquine for one, two, three and four months, respectively. The eye balls were processed for light microscopic, immunohistochemical and ultrastructural evaluation, followed by morphometric assessment and statistical analysis. Examination of haematoxylin and eosin stained sections showed significant progressive thinning of Outer Reticular Layer (ORL) and Inner Nuclear Layer (INL) associated with decreased Ganglion Cell (GC) count. Positive Amyloid Precursor Protein (APP) immunostaining was first noticed in the GCs. Later, it was observed in the INL as well. Ultrastructural examination revealed cytoplasmic electron-dense membranous inclusion bodies in the ganglion, horizontal, amacrine and bipolar cells. The photoreceptors were involved on prolonged duration of administration.

Key words: Retinopathy, ganglion, bipolar cells, ultrastructural study, immunohistochemistry, amyloid precursor protein (APP), chloroquine

INTRODUCTION

Chloroquine, a lysosomotropic agent, had been used for decades in the treatment of chronic inflammatory diseases such as malaria, rheumatoid arthritis and systemic lupus erythematosus (Maenpaa *et al.*, 2004).

Ocular side effects of this antimalarial agent include keratopathy, ciliary body involvement, lens opacities and retinopathy. The latter adverse effect is considered as the major manifestation of ocular toxicity, as other ocular side effects although more common, yet they are benign (Yam and Kwok, 2006). Patients usually complain of difficulty in reading, blurred vision, light flashes, field defects and colour vision disturbances (Ingster-Moati *et al.*, 2006). Despite dose limitations and ophthalmologic monitoring, irreversible retinal damage can occur (Tripp and Maibach, 2006).

Although these serious side effects can warrant discontinuation of therapy, their pathogenesis is unclear (Tzekov, 2005). Furthermore, elucidation of the precise mechanism of retinal damage is required for the development of better pharmacokinetic models (Tripp and Maibach, 2006).

Thus, the aim of the present study was to elicit the retinal alterations associated with chloroquine administration and to determine the duration-effect relationship of the drug, using histological, immunohistochemical and ultrastructural techniques.

MATERIALS AND METHODS

Thirty-two male albino rats, weighing 150-200 g were used in this study. They were maintained in 12 h room light and 12 h of darkness. They were on normal rat chow and water ad libitum. The rats were divided into four groups (n = 8): 1, 2, 3 and 4; each of which was further subdivided into two subgroups: Control (n = 2) and experimental (n = 6). The control subgroups were injected daily with saline intraperitoneally. The experimental subgroups (1, 2, 3 and 4) received a single daily intraperitoneal injection of 50 mg kg⁻¹ of chloroquine (Alexandria Co. for Pharmaceuticals, Alexandria, Egypt) for one, two, three and four months respectively (Yoshida *et al.*, 1997). The eye balls were dissected under chloroform inhalation anesthesia. The right eyes were used for light microscopic examination. The left ones from experimental subgroups 1 and 4 were processed for electron microscopic examination.

Light microscopic examination: The right eye balls were fixed in bouin's solution, processed, embedded in paraffin, sectioned and stained with Haematoxylin and

eosin and immunohistochemical stain for APP using a primary antibody, which is an epitope specific rabbit antibody (catalog number E2651), purchased from Spring Bioscience Co. Immunostaining required boiling tissue sections in citrate buffer (pH 6.0) in microwave for 3 min, followed by cooling at room temperature before application of the primary antibody for 30 min at room temperature. Ultravision detection system, purchased from Lab Vision Corporation (catalog number TP-015-HD) was used to detect the immunoreaction using the streptavidin-biotin-peroxidase technique, followed by counterstaining with Mayer's haematoxylin (Bancroft and Gamble, 2002).

Morphometric study: The H and E stained sections were submitted for image analysis using image analyzer computer system Leica Qwin 500 C (Cambridge, UK). The thickness of both the Outer Reticular Layer (ORL) and the Inner Nuclear Layer (INL) in addition to the ganglion cell number were measured in all groups at X400 magnification in 10 non-overlapping fields/section. The data obtained were statistically analyzed by comparing mean values of different groups by one-way ANOVA test using SPSS 9 software. Results were considered statistically significant when p-value was <0.05 (Petrie and Sabin, 2005).

Electron microscopic examination: Left eye balls were fixed in 3% glutaraldehyde, post fixed in 1% osmium tetroxide, dehydrated and embedded in resin. Semithin sections were cut and stained with toluidine blue. Ultrathin sections were stained by uranyl acetate and lead citrate (Dawes, 1980). Examination was done on the TEM Zeiss 10S (Carl Zeiss, Obekochen, Germany), in the Histology Department, Faculty of Medicine, Cairo University.

RESULTS

Light microscopic results

Haematoxylin and Eosin and Morphometric results: The control retinae showed, from outside inward, the photoreceptors layer, the nuclei of rods and cones in the ONL, the ORL, the INL, the IRL and the GCL (Fig. 1). The retinae of animals treated by chloroquine for one month (group 1) revealed no evident changes when compared with those of the control rats (Fig. 2). However, by the lapse of two months of treatment (group 2), areas of focal thinning could be perceived within the ORL (Fig. 3). This became more evident in those treated for three months (group 3) as it reached up to complete merging between the ONL and the INL in some areas. This was associated with detectable ganglion cell loss (Fig. 4). Thinning of the

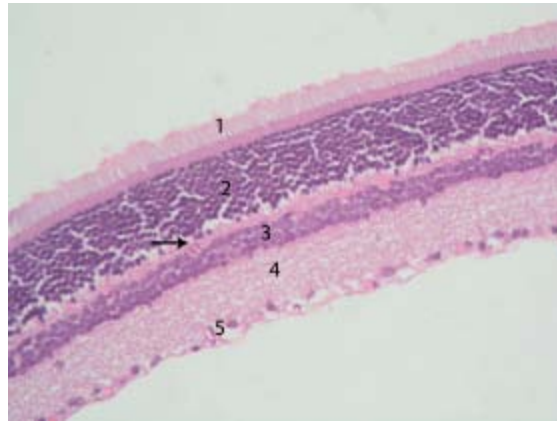


Fig. 1: A photomicrograph of a section in control retina showing photoreceptors layer (1), outer nuclear layer (2), outer reticular layer (arrow), inner nuclear layer (3), inner reticular layer (4) and ganglion cell layer (5). H and E x400

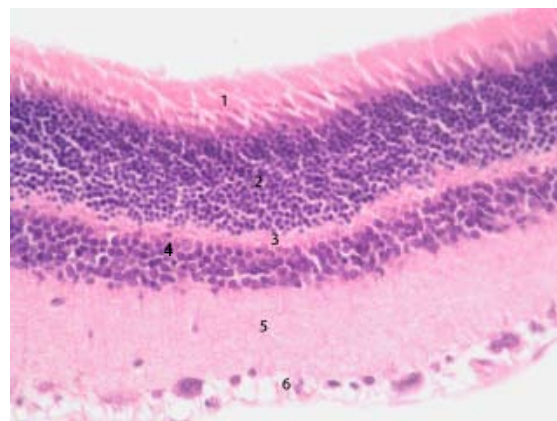


Fig. 2: A photomicrograph of a section in the retina of a rat receiving chloroquine for 1 month showing apparently normal photoreceptors layer (1), outer nuclear layer (2), outer reticular layer (3), inner nuclear layer (4), inner reticular layer (5) and ganglion cell layer (6). H and E x400

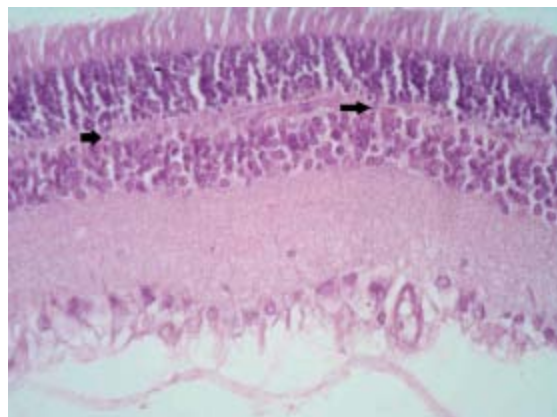


Fig. 3: A photomicrograph of a section of retina of a rat receiving chloroquine for 2 months showing focal thinning of the outer reticular layer (arrows). H and E x400

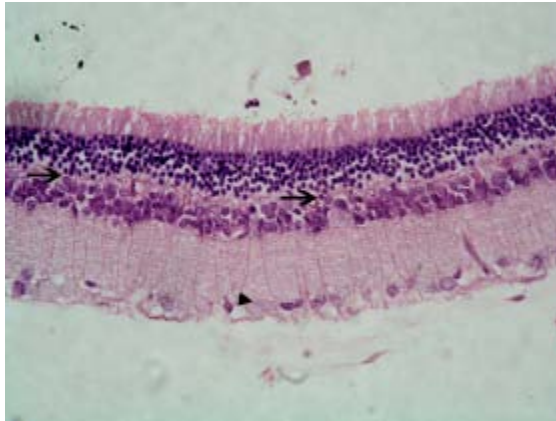


Fig. 4: A photomicrograph of a section of retina of a rat receiving chloroquine for 3 months showing evident thinning of the ORL with areas of merging of INL and ONL (arrow). Note the decreased number of ganglion cells (arrowhead). H and E x400

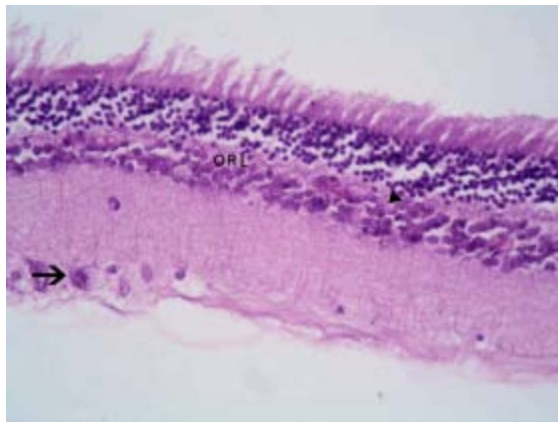


Fig. 5: A Photomicrograph of a section of a retina of a rat receiving chloroquine for 4 months showing areas of fusion between INL and ONL (arrowhead) within the markedly thinned outer reticular layer (ORL). Note the presence of few of ganglion cells some of which are swollen (arrow). H and E x400

Table 1: Mean outer reticular layer (ORL), inner nuclear layer (INL) thickness and ganglion cell (GC) number of the different groups

Groups	ORL		INL		GC	
	Mean±SD (um)	p	Mean±SD (um)	p	Mean±SD (Cell count)	p
Control	14.633±3.598		39.509±4.477		10.6±1.35	
Group 1 (1 month)	11.306±3.001	0.054	27.902±2.882	0.001*	9.5±1.27	0.281
Group 2 (2 month)	9.580±2.91	0.001*	19.525±2.345	0.001*	9.0±1.33	0.042*
Group 3 (3 month)	6.614±1.822	0.001*	13.102±2.183	0.001*	8.9±1.29	0.027*
Group 4 (4 month)	6.073±1.147	0.001*	11.637±1.415	0.001*	8.3±0.82	0.001*

SD = Standard deviation; * = Statistically significant as compared to control (p<0.05)

ORL was more marked after four months of treatment (group 4) and was also associated with thinning of the INL. The marked thinning resulted in more areas of fusion between the ONL and the INL. Few ganglion cells, where some of which were swollen, were detected as well (Fig. 5).

Estimation of the thickness of the ORL revealed a gradual thinning of this layer in the treated groups which was statistically significant in groups: 2, 3 and 4 as compared to control. The thinning of the INL was also gradual and statistically significant for all the experimental subgroups when compared to control.

The ganglion cell count decreased gradually in the treated groups and was statistically significant for groups: 2, 3 and 4 when compared to control (Table 1).

Immunohistochemical results: In the control retinae, APP immunoreactivity was almost absent from all retinal cells, apart from faint reaction in few ganglion cells (Fig. 6). The retinae of group 1 revealed weak positive APP immunoreactivity within some cells of INL (Fig. 7), while, others showed stronger positivity (Fig. 8). In group 2, moderate APP positive immunoreactivity was notable within some swollen cells of the INL and others were displaced into the IRL. Weak positive immunoreactivity was detected in some swollen ganglion cells (Fig. 9). In group 3, moderate to strong APP immunoreactivity was detected in cells within INL and some of which were

displaced into the ORL (Fig. 10). It was also detectable in the horizontal, bipolar, amacrine and Muller cells (Fig. 11) as well as in some ganglion cells (Fig. 12). In group 4, positive APP immunoreaction was detectable in some cells which were displaced into the ORL (Fig. 13).

Toluidine blue results: Semithin sections of the control retinae showed the photoreceptor layer, the ONL, the ORL, the INL, the IRL and the ganglion cells (Fig. 14 a, b). Retinae of group 1 rats showed dark granules in the cells of the INL extending through the IRL as well as in the ORL (Fig. 15). The dark cytoplasmic granules were also evident in the ganglion cells (Fig. 16). In group 4, the dark cytoplasmic granules were seen within the cells of the INL as well as in the ganglion cells. The nuclei of rods could also be seen displaced into the thinned ORL (Fig. 17).

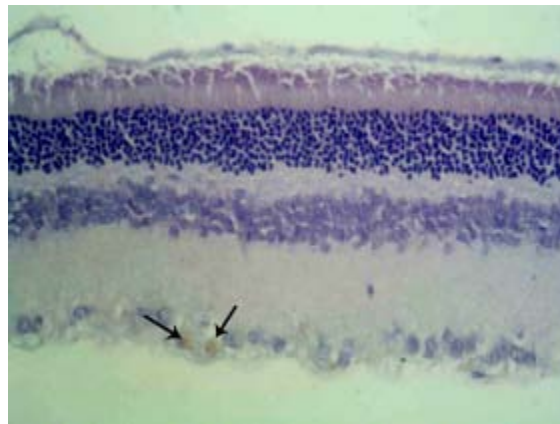


Fig. 6: A photomicrograph of a section of retina of a control rat showing faint APP positive immunoreactivity in few ganglion cells (arrow). APP immunoreaction x400

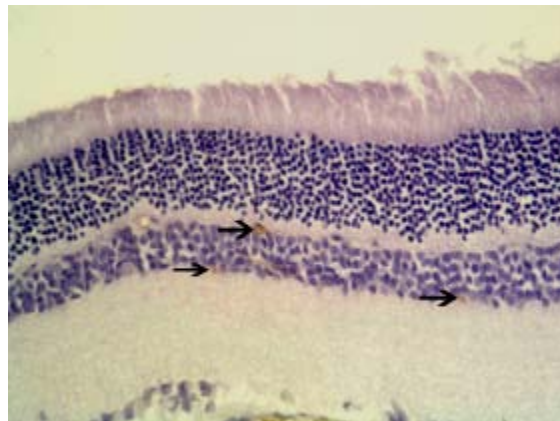


Fig. 7: A photomicrograph of a section of retina of a rat receiving chloroquine for 1 month showing mild APP positive immunoreactivity involving cells (arrows) of the inner nuclear layer. APP immunoreaction x400

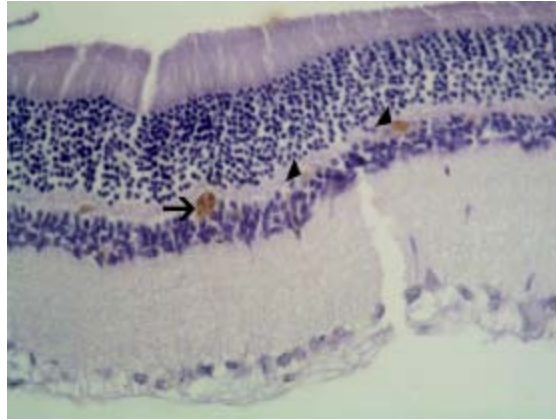


Fig. 8: A photomicrograph of a section of retina of a rat receiving chloroquine for 1 month showing strong APP positive immunoreaction in a large swollen cell (arrow) of the inner nuclear layer, other cells (arrowheads) show mild reaction. APP immunoreaction x400

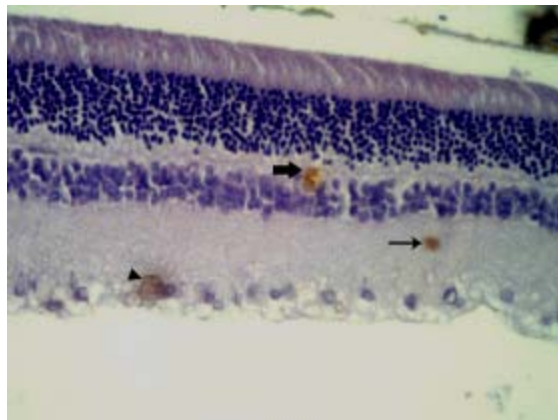


Fig. 9: A photomicrograph of a section of retina of a rat receiving chloroquine for 2 months showing moderate APP positive immunoreaction in some swollen cells (thick arrow) of the inner nuclear layer and ganglion cell layer (arrowhead). Note the positive immunoreactivity (thin arrow) extending into the inner reticular layer. APP immunoreaction x400

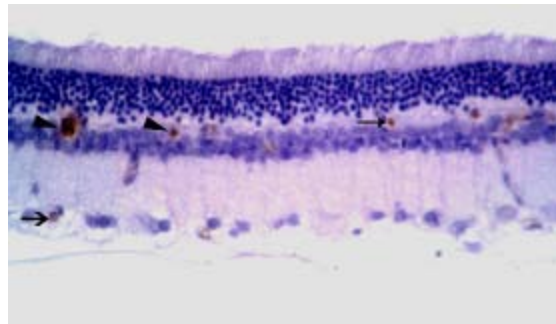


Fig. 10: A photomicrograph of a section of retina of a rat receiving chloroquine for 3 months showing strong APP positive immunoreaction in some cells (arrowhead) of the inner nuclear layer. Some are displaced (thin arrow) into the outer reticular layer. Note the positive immunoreactivity of ganglion cells (thick arrow). APP immunoreaction x400

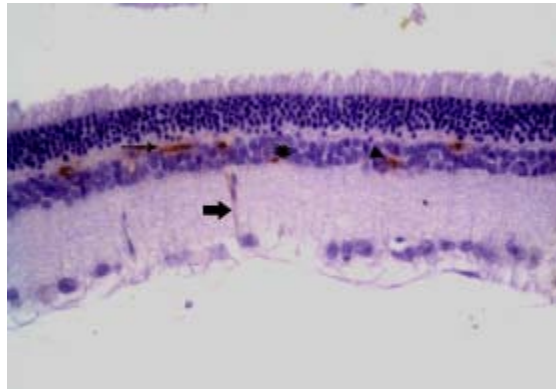


Fig. 11: A photomicrograph of a section of retina of a rat receiving chloroquine for 3 months showing APP positive immunoreaction in a horizontal (thin arrow), bipolar (arrowhead), amacrine (*) and Muller cell (thick arrow). APP immunoreaction x400

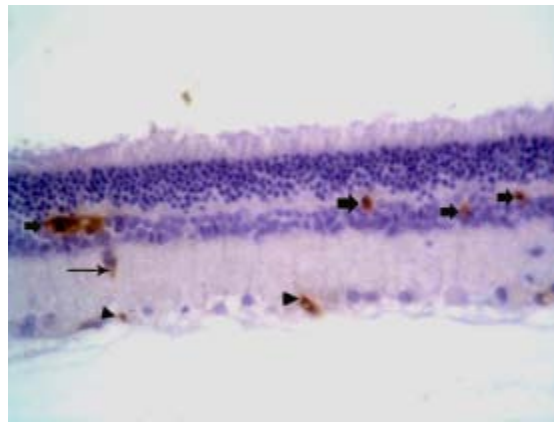


Fig. 12: A photomicrograph of a section of retina of a rat receiving chloroquine for 3 months showing strong APP positive immunoreaction in some cells (thick arrow) of the inner nuclear layer, process of a Muller cell (thin arrow) and ganglion cell layer (arrowhead). APP immunoreaction x400

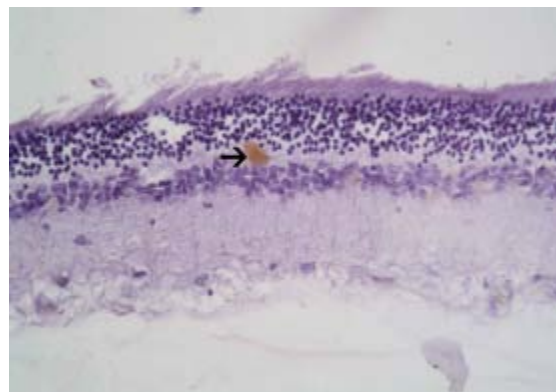


Fig. 13: A photomicrograph of a section of retina of a rat receiving chloroquine for 4 months showing strong APP positive immunoreaction in a cell (arrow) that is displaced into the outer reticular layer. APP immunoreaction x400

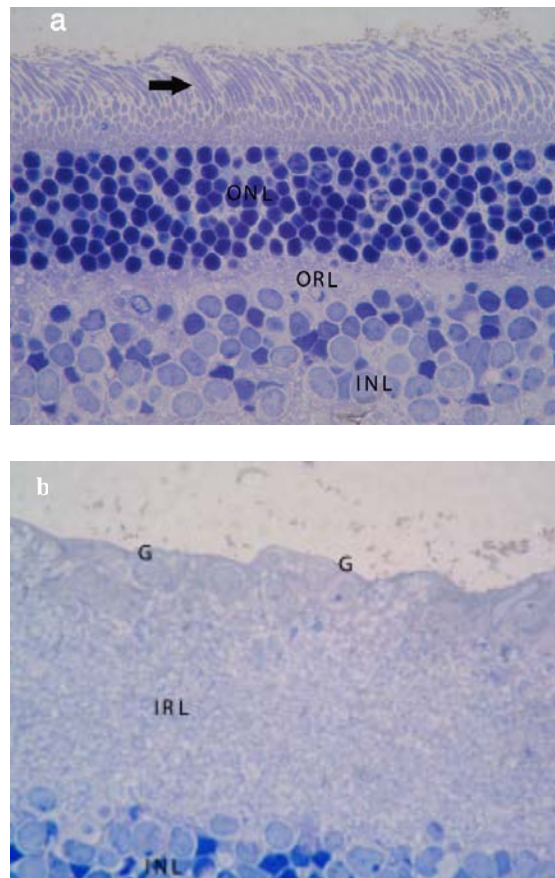


Fig. 14: Photomicrographs of semithin sections of retinae of control rats showing in: (a) Photoreceptor layer (arrow), outer nuclear layer (ONL), outer reticular layer (ORL) and inner nuclear layer (INL); (b) Inner nuclear layer (INL), inner reticular layer (IRL) and ganglion cells (G). Toluidine blue X1000

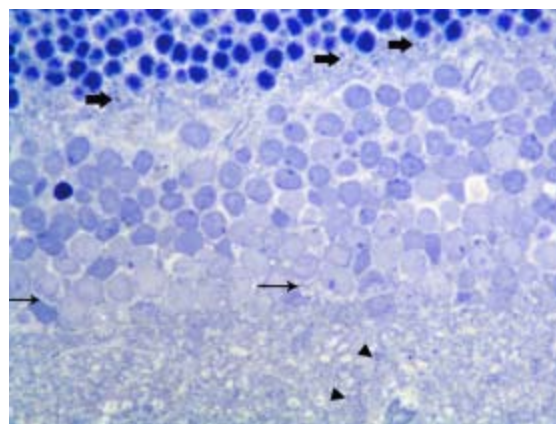


Fig. 15: A photomicrograph of a semithin section of the retina of a rat receiving chloroquine for 1 month showing dark granules in some cells (thin arrow) of the inner nuclear layer, extending through the inner reticular layer (arrow head) as well as the outer reticular layer (thick arrow)

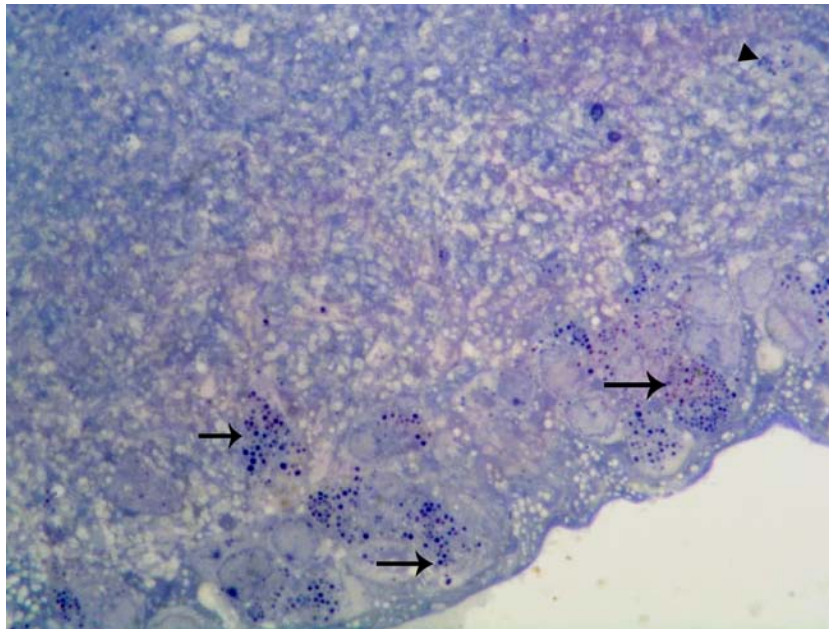


Fig. 16: A photomicrograph of a semithin section of retina of a rat receiving chloroquine for 1 month showing dark granules (arrow) in the cytoplasm of ganglion cells and inner reticular layer (arrowhead). Toluidine blue X 1000

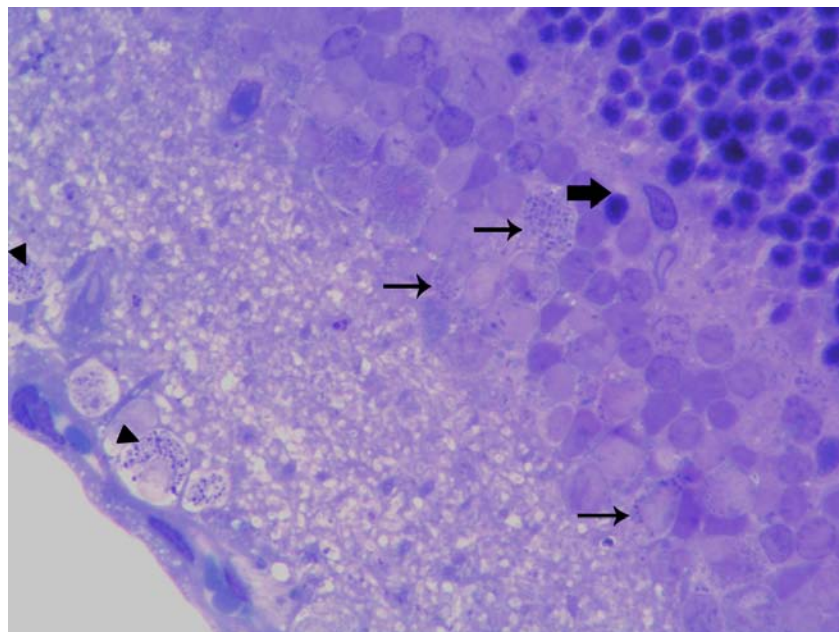


Fig. 17: A photomicrograph of a semithin section of retina of a rat receiving chloroquine for 4 months showing large amount of granules in the cytoplasm of cells of the inner nuclear layer (thin arrow) and that of ganglion cells (arrowhead). Note the nuclei of rods (thick arrow) which are in close contact to the cells of the inner nuclear layer. Toluidine blue X 1000

Electron microscopic results: Ultrastructural examination of the control retinae showed the nuclei of rods and cones in the ONL (Fig. 18a). In the INL, horizontal, bipolar, amacrine and Muller cell were observed (Fig. 18b). Ganglion cells with large euchromatic nuclei and moderate amount of organelles were also detected (Fig. 18c). While, examination of group I the retinae revealed early changes, in the form of membrane-bound electron-dense cytoplasmic inclusion bodies within the cone cells (Fig. 19a), the bipolar cells (Fig. 19b) and the ganglion cells (Fig. 19c). The cone cells are identified by their

presence just inner to the outer limiting membrane and their euchromatic nuclei. They are distinguished from the rods, that are more numerous and present at different levels within the ONL. Rods possess heterochromatic nuclei with characteristic aggregation of their chromatin in the centre. The cytoplasmic inclusion bodies sometimes appeared multilamellar (Fig. 19d). As regard group IV, the inclusion bodies were observed within the inner segment of cone cells (Fig. 20a) as well as in the bipolar cells (Fig. 20b). It also involved the cell body and cytoplasmic processes of some horizontal cells (Fig. 20c).

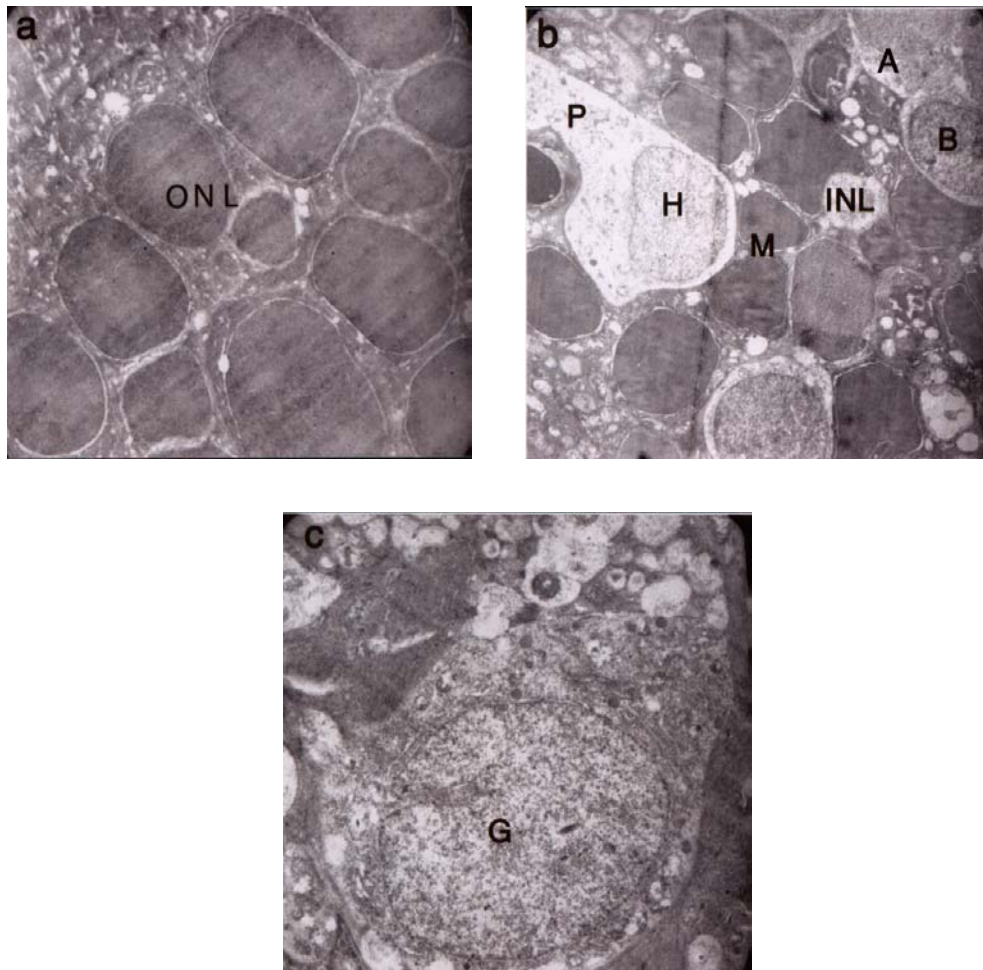


Fig. 18: Photomicrographs of ultrathin sections of retinae of control rats showing in: (a) The inner segment of photoreceptors, nuclei of rods and cones in the outer nuclear layer (ONL). X4000; (b) Horizontal cell (H) with its horizontal process (p), bipolar cell (B), amacrine cell (A) with its large rounded euchromatic nucleus and Muller cell (M) with irregular outline and electron dense nucleus, within the inner nuclear layer (INL). X2000; (c) A ganglion cell (G) with a large euchromatic nucleus. X4000

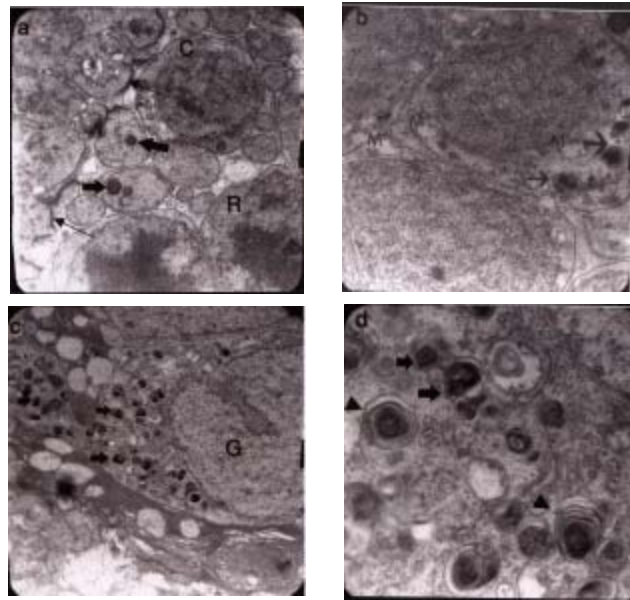


Fig. 19: Photomicrographs of ultrathin sections of retinae of rats receiving chloroquine for 1 month showing in: (a) Outer nuclear layer with nuclei of rods (R) and cones (C). Note the electron dense inclusions (thick arrow) in the cytoplasm of cones. The outer limiting membrane (thin arrow) can also be seen. X 6000; (b) Bipolar cells with membrane-bound electron-dense cytoplasmic inclusion bodies (arrow) and mitochondria (m) with broken cristae. X 6000; (c) A ganglion cell (G) with many inclusion bodies (arrow). X 4000; (d) A higher magnification of the previous photomicrograph showing many membrane-bound inclusion bodies with heterogeneous electron-dense content (arrow). Sometimes they appear as multilamellar myelin figure-like bodies (arrow head). X 15000

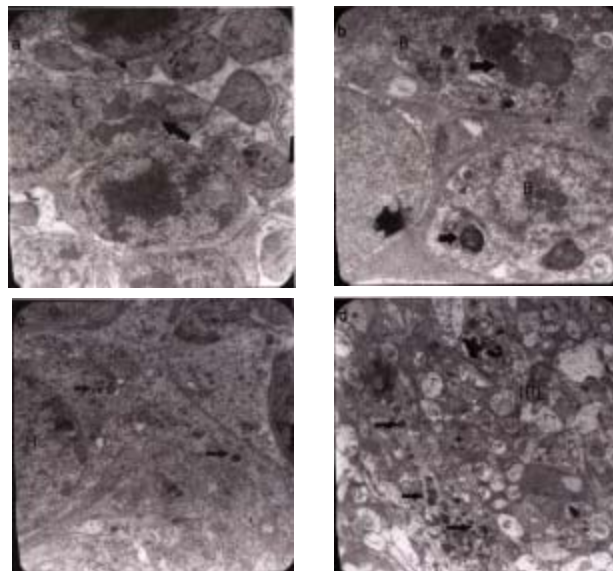


Fig. 20: Photomicrographs of ultrathin sections of retinae of rats receiving chloroquine for 4 months showing in: (a) Inclusion bodies (arrow) in the inner segment of a cone cell (C). X 6000; (b) Large membrane bound cytoplasmic inclusions (arrow) in the cytoplasm of two bipolar cells (B). X 6000; (c) Inclusion bodies (arrow) in the cell body and cytoplasmic processes of a horizontal cell (H). X 6000 (d) Large amount of inclusion bodies (arrow) in the cytoplasmic processes of Muller cells extending through the inner reticular layer (IRL). X 4000

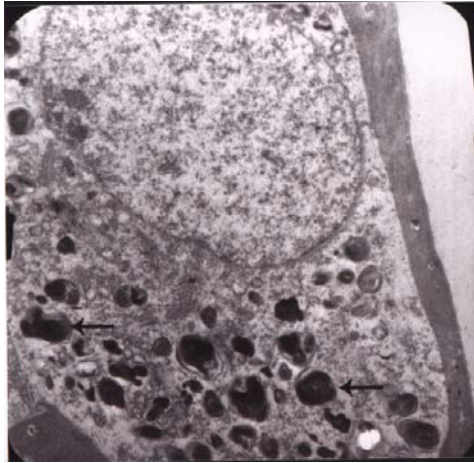


Fig. 21: Photomicrographs of ultrathin sections of retinae of rats receiving chloroquine for 4 months showing in another ganglion cell with multiple membrane bound inclusion bodies (arrow). X 4000

Large amount of inclusion bodies were similarly detected in the cytoplasmic processes of Muller cells that extended through the IRL (Fig. 20d). Multiple membrane-bound inclusion bodies involved the ganglion cells as well (Fig. 21).

DISCUSSION

The present study was carried out to determine the alterations in the retinal cells chronically exposed to Chloroquine. Such alterations were detected both immunohistochemically and ultrastructurally. A definite duration effect relationship was detectable as well. The interest in monitoring the affection following variable durations of treatments arose as rat retinal changes appeared to be directly proportional to the duration of Chloroquine administration (Dunker *et al.*, 1995; Yoshida *et al.*, 1997). This could be ascertained by the findings of Vu *et al.* (1999) and So *et al.* (2003), in early disease, since they observed clear physiological retinal defects including ring scotoma, electroretinogram abnormalities and development of colour vision deficiencies. While, prolonged chloroquine exposure was reported by Mahon *et al.* (2004) to induce severe retinopathy which might be indistinguishable from retinitis pigmentosa being characterized by outer retinal neurodegeneration.

In the present study, histological examination of H and E stained sections revealed significant progressive

thinning of ORL as well as INL. Such thinning became markedly evident after four months of treatment to the extent that there was fusion between ONL and INL in some areas. This thinning might be due to the suggested loss of the affected neuroretinal cells and hence the ultimate lack of their processes which would justify the perceived decrease in the ORL. Finally, ganglion cells were clearly decreasing in number with chronicity of treatment. This was concomitant with the results of Yoshida *et al.* (1997), since they found that by 20-24 weeks of chloroquine administration, ORL has virtually disappeared with merging of INL and ONL. They added that the more severely affected retinae showed ganglion cell loss and nuclear pyknosis in the ganglion cell layer.

Meanwhile, the immunostained sections revealed positive APP immunoreaction involving the ganglion cells as well as some cells within the INL as early as one month after treatment. Later on, it involved more cells within the INL and extended within the IRL by the fourth month of treatment. These findings were concomitant with those reported by Yoshida *et al.* (1997). They added that increased APP immunoreactivity was also reported in retinal cells of old aged persons, in retinitis pigmentosa and in age-related macular degeneration. They concluded that this indicates a relationship between this protein and retinal cells aging and degeneration.

Results of the semithin sections examination revealed evident granules within the ganglion cells, involving the INL cells and extending into the IRL as well as the ORL. These observations were further supported by the ultrastructural examination which revealed involvement of almost all layers of the retina. Several membrane-bound multilamellar inclusions were detected in the ganglion cells, the bipolar cells and cones as early as one month after treatment. These electron-dense cytoplasmic bodies became more prevalent by the fourth month involving the cytoplasm of the horizontal cells as well as the cytoplasmic processes of Muller cells within the IRL.

The detection of these membranous cytoplasmic bodies was previously reported by Abraham and Hendy (1970), in their studies of retinae following chloroquine administration. This also agreed with Mahon *et al.* (2004) who found large accumulations of membranous cytoplasmic inclusions in the ganglion cells, while smaller number was observed in the bipolar, amacrine and horizontal as well as in cone cells.

These membranous inclusions were similar in structure to the so called cytosomes which were described by (Johannessen, 1980) as concentrically arranged membranous layers. They stated that they were detectable in neuronal lysosomal storage diseases as well

as in chloroquine toxicity. They attributed their occurrence to lysosomal dysfunction and added that chloroquine induces a general deficiency of lysosomal enzymes, in contrast to the individual lysosomal enzyme defects present in the hereditary storage diseases.

The early perception of histological alterations in this current work disagreed with Hodgkinson and Kolb (1970) who reported some of these changes but only with excess of three months. However, it partially agreed with Rosenthal *et al.* (1978) who did observe early appearance of multilamellated cytoplasmic inclusion bodies after only one week of treatment, but were limited to the ganglion cells only.

The detected involvement of cones and sparing of rods in this present study was also supported by the observations of Mahon *et al.* (2004). These results could justify the occurrence of color vision defects, following prolonged use of chloroquine, reported by So *et al.* (2003). However, the present study could not explain the affection of cones and not rods. It could only explain the clinical observation of color vision affection.

In the present study, the increase in prevalence of cytoplasmic multilamellar inclusion bodies with chronicity of treatment coincided with that of APP immunostaining. Both showed first involvement of ganglion cells and those of INL and later on affection of cones. Even the positive APP immunostaining detected within the IRL met an equivalent presence of inclusions within the longitudinal processes traversing the same layer in ultrathin sections which were most probably the processes of Muller cells.

This observed similarity in distribution of APP and multilamellated inclusion bodies agreed with what was previously reported by Yoshida *et al.* (1997). They demonstrated a striking similarity in distribution of APP, in chloroquine retinopathy, together with cathepsin D, which is a lysosomal enzyme.

Accordingly, it could be speculated that the exposure to chloroquine resulted in accumulation of APP inside lysosomes. This could be based upon what was previously reported by Caporaso *et al.* (1992). They mentioned that the lysomotropic drug Chloroquine exerted an inhibitory effect on the lysosomal degradation of the mature APP. They explained that chloroquine, which was a weak base, neutralized the acidic organelles such as lysosomes. This results in elevation of its pH with subsequent inhibition of their acid dependent hydrolases. Such explanation could also be supported by Mahon *et al.* (2004) who reported that all their observed changes were all related to lysosomal system dysfunction. They added that it could be attributed to the lysomotropic

property of Chloroquine, due to its capacity to alter the lysosomal pH deoptimising the conditions of degradative enzymes.

Finally, it would be appropriate to deduce that the defective degradative capacity of APP and its subsequent accumulation in various cells of the retina could have an impact on their function. This proceeds further resulting in their depletion over time as evidenced by progressive decrease in ganglion cell count and thinning of INL with increasing the duration of drug consumption. This would clearly explain why no recovery could be expected after long term treatment with chloroquine, as the depleted cells of the neuroretina will never be regained. However, this draws to attention that chloroquine treatment should be discontinuous. This suggestion could be built upon what was formerly stated by Rosenthal *et al.* (1978). They reported that chloroquine was highly concentrated in pigmented ocular tissues such as retinal pigment epithelium, where it binds to melanin and remains there for long periods of time even after cessation of therapy.

On the other hand, the use of a less ocular toxic drug which is hydroxychloroquine (Finbloom *et al.*, 1985) could also be recommended. The addition of a hydroxyl group in hydroxychloroquine would, according to Yam and Kwok (2006), limit its ability to cross the blood-retinal barrier, which reduces its ocular toxicity.

Furthermore, some screening approaches should be established, as cases of definitive retinopathy had been reported in patients receiving even less than the recommended dosage of chloroquine (Easterbrook, 2002). The dilemma of whether to screen for retinopathy or not, lied in its rare occurrence despite its seriousness and irreversibility (Yam and Kwok, 2006).

However, the irreversible degenerative loss of retinal cells the first of which is the ganglion cells is still alarming. It should provoke marked effort for reaching a reliable screening method for antimalarial drug retinopathy. This concept of continuous ophthalmological follow up should be adopted as well by dermatologists and rheumatologists as any risk of visual loss should be unacceptable.

REFERENCES

- Abraham, R. and R.J. Hendy, 1970. Irreversible lysosomal damage induced by chloroquine in the retinae of pigmented and albino rats. *Exp. Mol. Pathol.*, 12: 185-200.
- Bancroft, J.D. and M. Gamble, 2002. *Theory and Practice of Histological Techniques*. 5th Edn. Churchill Livingstone, London UK, pp: 126-130.

- Caporaso, G.L., S.E. Gandy, J.D. Buxbaum and P. Greengard, 1992. Chloroquine inhibits intracellular degradation but not secretion of Alzheimer B/A4 amyloid precursor protein. *Proc. Natl. Acad. Sci.*, 89: 2252-2256.
- Dawes, C.J., 1980. *Biological Techniques for Transmission Electron Microscopy*. 1st Edn. Fadd Research Industries Pub. Inc., pp: 1-16.
- Dunker, G., M. Schmiederer and T. Bredehorn, 1995. Chloroquine-induced lipidoses in the rat retina: A functional and morphological study. *Ophthalmologica*, 209: 79-83.
- Easterbrook, M., 2002. Screening for antimalarial toxicity: Current concepts. *Can. J. Ophthalmol.*, 37: 325-328.
- Finbloom, D.S., K. Silver, D.A. Neosome and R. Gunkel, 1985. Comparison of hydroxchloroquine and chloroquine use and the development of retinal toxicity. *J. Rheumatol.*, 12: 692-694.
- Hodgkinson, B.J. and H. Kolb, 1970. A preliminary study of the effect of chloroquine on the rat retina. *Arch. Ophthalmol.*, 84: 509-515.
- Ingster-Moati, I., E. Bui Quoc, M. Crochet, C. Orssaud, J.L. Dufier and O. Roche, 2006. Severe chloroquine- and hydroxychloroquine-induced retinopathy. *J. Fr. Ophthalmol.*, 29: 642-650.
- Johannessen, J.V., 1980. Cellular Pathobiology, Metabolic and Storage Diseases. In: *Electron Microscopy in Human Medicine*. 1st Edn. McGraw-Hill International Book Co., New York, USA., pp: 62.
- Maenpaa, H., T. Toimela, M. Mannerstrom, P. Saransaari, and H. Tahti, 2004. Toxicity of selected cationic drugs in retinoblastomal cultures and in cocultures of retinoblastomal and retinal pigment epithelial cell lines. *Neurochem. Res.*, 29: 305-311.
- Mahon, G.J., H.R. Anderson, T.A. Gardiner, S. McFarlane, and A.W. Stitt, 2004. Chloroquine causes lysosomal dysfunction in neural retina and RPE: Implications for retinopathy. *Curr. Eye Res.*, 28: 277-284.
- Petrie, A. and C. Sabin, 2005. *Medical Statistics at a Glance*. 2nd Edn. Sugden M. and K. Moore (Eds.)/ Blackwell Publishing Ltd., USA., pp: 55.
- Rosenthal, R.A., H. Kolb, D. Bergsma, D. Huxsoll and J.L. Hopkins, 1978. Chloroquine retinopathy in the rhesus monkey. *Invest. Ophthalmol. Vis. Sci.*, 17: 1158-1175.
- So, S.C., T.R. Hedges, J.S. Schuman and M.L. Quireza, 2003. Evaluation of hydroxychloroquine retinopathy in multifocal electroretinography. *Ophthalmic Surg. Lasers Imaging*, 34: 251-258.
- Tripp, J.M. and H.I. Maibach, 2006. Hydroxychloroquine-induced retinopathy: A dermatologic perspective. *Am. J. Clin. Dermatol.*, 7: 171-175.
- Tzekov, R., 2005. Ocular toxicity due to chloroquine and hydroxychloroquine: Electrophysiological and visual function correlates. *Doc. Ophthalmol.*, 110: 111-120.
- Vu, B.L., M. Easterbrook and J.K. Hovis, 1999. Detection of color vision defects in chloroquine retinopathy. *Ophthalmology*, 106: 1799-1803.
- Yam, J.C.S. and A.K.H. Kwok, 2006. Ocular toxicity of hydroxychloroquine. *Hong Kong Med. J.*, 12: 294-304.
- Yoshida, T., R. Fukatsu, K. Tsuzuki, Y. Aizawa and N. Takahata, 1997. Amyloid precursor protein, A β and amyloid-associated proteins involved in chloroquine retinopathy in rats-immunopathological studies. *Brain Res.*, 764: 283-288.