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Hepatic and Renal Histological Alterations Induced by Topical Hydroquinone Administration

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Abstract: Problem statement: Although the regulatory agencies of the United States and Europe banned hydroquinone in cosmetics skin lightening but it is still used in most of Middle East countries, including Saudi Arabia. Few studies were carried out on the histological alterations of hydroquinone on kidney and liver. **Approach:** The present study was carried out to investigate the histological alterations in liver and kidney caused by hydroquinone topical administration. **Results:** The induced histological alterations in the liver were mainly hydropic degeneration, bile duct hyperplasia and glycogen depletion. The kidney of treated rabbits showed hydropic degeneration, hyaline casts, congestion, perivascular edema and fibrosis together with lymphocytic aggregation. The skin showed hyperkeratosis, lymphocytic and eosinophilic infiltration together with congestion of dermal blood vessels. **Conclusion:** Taken together, the histological findings of this study indicate that chronic exposure to hydroquinone produce significant histological alterations that might affect the liver and kidney.

Key words: Hydroquinone, eldoquin, histological alterations, topical administration, hepatic adenoma

INTRODUCTION

Hydroquinone has been used for decades in treatment of dyschromia specially hyperpigmentation such as melasma (Zawar and Mhaskar, 2004; Makropoulos and Alexopoulos, 2006). Also, it functions in cosmetics as a antioxidant or polymerization agent in a number of topical skin creams (Boyle and Kennedy, 1986; Draelos, 2007; Costa *et al*., 2010; Anderson *et al*., 2010).

Studies have been reported hydroquinone to produce renal tubule hyperplasia, adenomas and chronic progressive nephropathy by enhancing renal cell proliferation (Boyle and Kennedy, 1985; Hard *et al*, 1997; Karamagi *et al*., 2001). Other reports indicated that hydroquinone to increase the incidence of leukemia and to inhibit apoptosis of neoplastic cells in experimental animals while genotoxicity and mutagenicity studies on this drug were almost negative (Hazel *et al*., 1996; McGregor, 2007; Anderson *et al*., 2010). Also, it has the potential of exogenous ochronosis and affects the host defense in infectious diseases by lowering the level of neutrophils (Westerhof and Kooyers, 2005; Ribeiro *et al*., 2011). Studies revealed that the potential hazards of topical application of hydroquinone is more than oral exposure (Kooyers and Westerhof, 2006). However high doses of oral administration of hydroquinone showed that it affects the feed consumption and/or body weight and the function of the central nervous system (Devillers *et al*., 1990; Murphy *et al*., 1992; DeCaprio, 1999; Joseph *et al*., 1998).

The usage of hydroquinone in cosmetics skin lightening in Europe has been banned since 2001 due to its effects (Kooyers and Westerhof, 2006; Makropoulos and Alexopoulos, 2006). Also regulatory agencies of the United States banned hydroquinone on the base of reports confirmed the induction of exogenous ochronosis in human, hepatic adenoma and renal adenoma in experimental animals over extended time period (Draelos, 2007; Levitt, 2007). However, it is still used in most of Middle East countries, including Saudi Arabia and is available in the pharmacy as topical formula, in concentrations of 2 and 4% (W/W).

There are a few studies about the histological alterations of hydroquinone on kidney and liver (Boatman, 1996; Topping *et al*., 2007). Also, most of these studies used oral or intra-peritoneal routes of

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administration which are not clinically administrated (Boatman, 1996; McGregor, 2007). So, the present study was carried out to investigate the histological changes in liver and kidney caused by topical administration of hydroquinone.

MATERIALS AND METHODS

Experimental animals: A total of 24 adult female rabbits (weight 400-1500 gm) were kept in animal house cages where each contained one animal and divided into four groups (6 animals each). The temperature and the relative humidity were monitored daily during the period of experimental course where the temperature in the experimental laboratory was $25 \pm 2.06°C$ and relative humidity of 47.44 ± 9.29. Further, animals were maintained under natural lighting of 12 h daylight and 12 h darkness.

The animals were subjected to eldoquin (hydroquinone 2% w/w) topical administration for 6 weeks as follows:

Group I: Control group: the members of this group were not subjected to hyroquinone administration.
Group II: Hyroquinone (2%,w/w) cream was applied on the total outer surface of the right ear of each member.
Group III: Hyroquinone (2%,w/w) cream was applied on the total outer surface of both ears of each member.
Group IV: Hyroquinone (2%,w/w) cream was applied on the abdomen (2 gm/cm^2) after hair shaving of each member.

Animals were euthanized and fresh biopsies were taken from the kidney, liver, abdominal skin and both ears of each rabbit. Tissue specimens were fixed in 10% neutral buffer formalin and then dehydrated in ascending grades of ethanol (70, 80, 90 and 100%). Dehydration was followed by clearing the tissue samples in 2 changes of xylene before being impregnated with 2 changes of melted paraffin wax, embedded and blocked out. Tissue sections (4-5µm) were stained with hematoxylin eosin, Mallory trichrome and Periodic Acid-Schiff (PAS) according to the methods described by Taib *et al*. (2004); Pearse (1985); Carleton *et al*. (1980) and Bancroft and Stevens (1990) for the conventional histological procedures.

RESULTS AND DISCUSSION

Exposure to 2% (w/w) hydroquinone cream has produced the following histological alterations in the liver of treated rabbits.

Diffuses and hydropic degeneration: The hepatocytes of hydroquinone treated rabbits were swollen and showed clear cytoplasm. This alteration was seen in all hydroquinone treated rabbits (Fig. 1a).

Bile duct hyperplasia: Marked bile duct hyperplasia was seen in the treated rabbits specially those subjected to hydroquinone on both ear auricle (Fig. 1b and c).

Glycogen depletion: Periodic Acid-Schiff (PAS) stained showed evident partial glycogen depletion in the hepatocytes of treated rabbits in comparison with the control liver. Hydroquinone exposure had has produced significant reduction in the liver glycogen in a heterogeneous pattern. The reduction was mainly in the hepatocytes adjacent to the peripheral zone while the pericentral and the midzone hepatocytes were less affected (Fig. 1d).

Also exposure to 2% (w/w) hydroquinone has produced the following histological alterations in the kidney.

Hydropic degeneration: Renal tubules showed hydropic degeneration with deteriorated cytoplasm (Fig. 2a). This alteration was more prominent in rabbits exposed to hydroquinone in both ears.

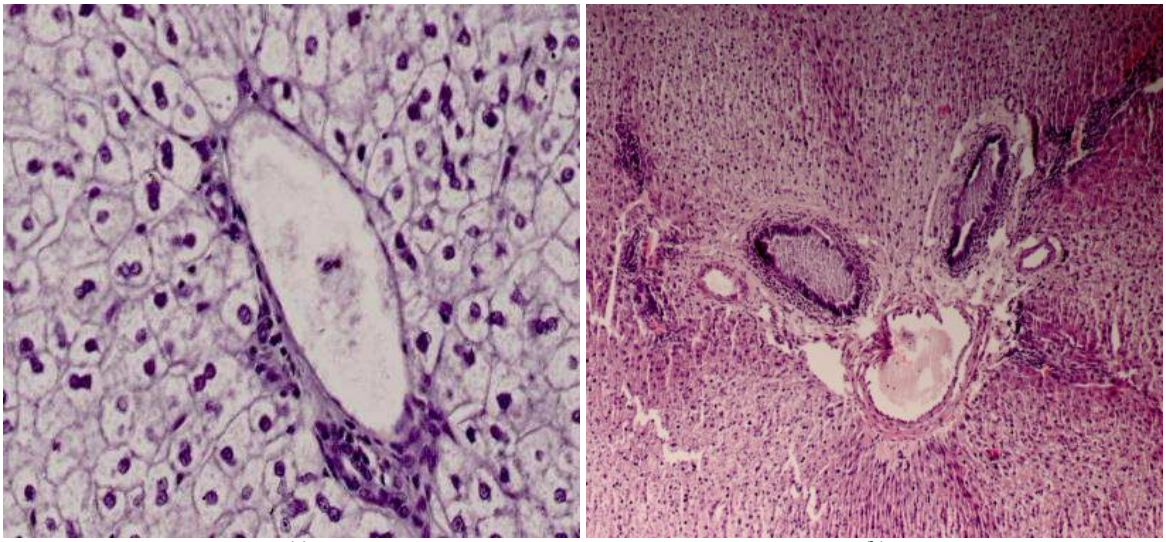
Hyaline casts: The lumen of some renal tubules showed hyaline casts (Fig. 2b).

Congestion: The kidney of treated rabbits showed dilatation and congestion of intertubular blood capillaries in comparison with the control ones (Fig. 2c). Rabbits of group 4 were more affected than other treated groups.

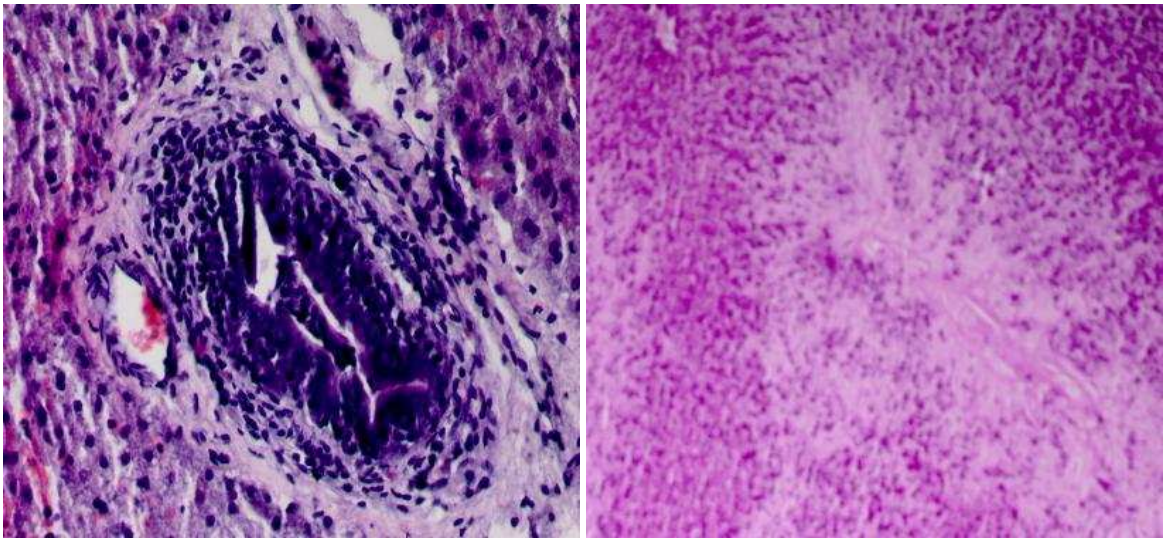
Perivascular edema: The kidney of hydroquinone treated rabbits showed occasional perivascular edema (Fig. 2d). Connective tissues surrounding renal blood vessels became widely separated from the adventia of these vessels due to accumulation of the edematous fluid. This change was more prominent in rabbits exposed to hydroquinone in both ears in comparison to the animals of other treated groups.

Perivascular fibrosis: Interstitial perivascular fibrosis was noted in renal tissue of the hydroquinone treated rabbits (Fig. 2e). This change was seen in the kidney of all treated rabbits.

Perivascular lymphocytic aggregation: An occasionally aggregation of mononuclear inflammatory cells mainly lymphocytes were seen next to the renal veins (Fig. 2f).



(a) (b)



(c) (d)

Fig. 1: Light micrograph section in the liver of rabbit exposed to eldoquin (hydroquinone, 2%, w/w) showing (a) Diffuses and hydropic degeneration. H&E stain; (b) Bile duct hyperplasia. H&E stain; (c) Thickening bile duct epithelium. Trichrome stain; (d) Glycogen depletion mainly in the periportal space . PAS stain

The following histological alterations in the skin subjected to the drug were also seen.

Hyperkeratosis: Parakeratosis was observed in the skin covers the ear of all members of both groups 2 and 3 (Fig. 3a).

Inflammatory cells infiltration: Skin epidermis of hydroquinone treated rabbits showed inflammatory cells infiltration mainly lymphocytes and eosinophils (Fig. 3b).

Severe congestion: Dermal blood vessels exhibited severe congestion. This alteration was observed mainly in the papillary layer.

Melanocytes alterations: Melanocytes were less predominant in the skin of the rabbits exposed to hydroquinon in the abdomen in comparison with the control ones. Also melanin granules of both basal and reticular layers were less concentrated in the melanocytes of hydroquinone treated animals in comparison to the control ones. Melanocytes in the drug exposed skin showed some shrinkage.

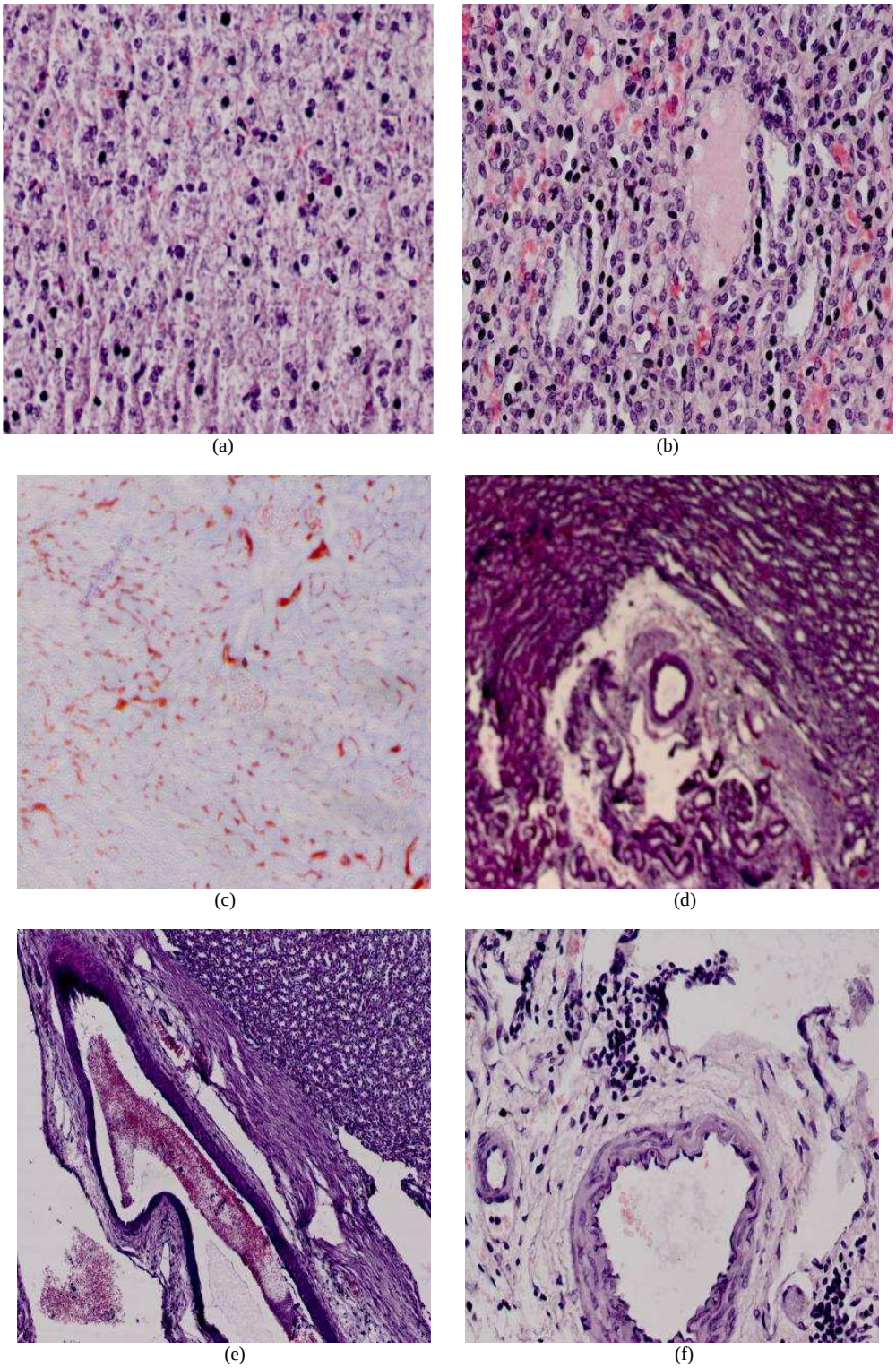
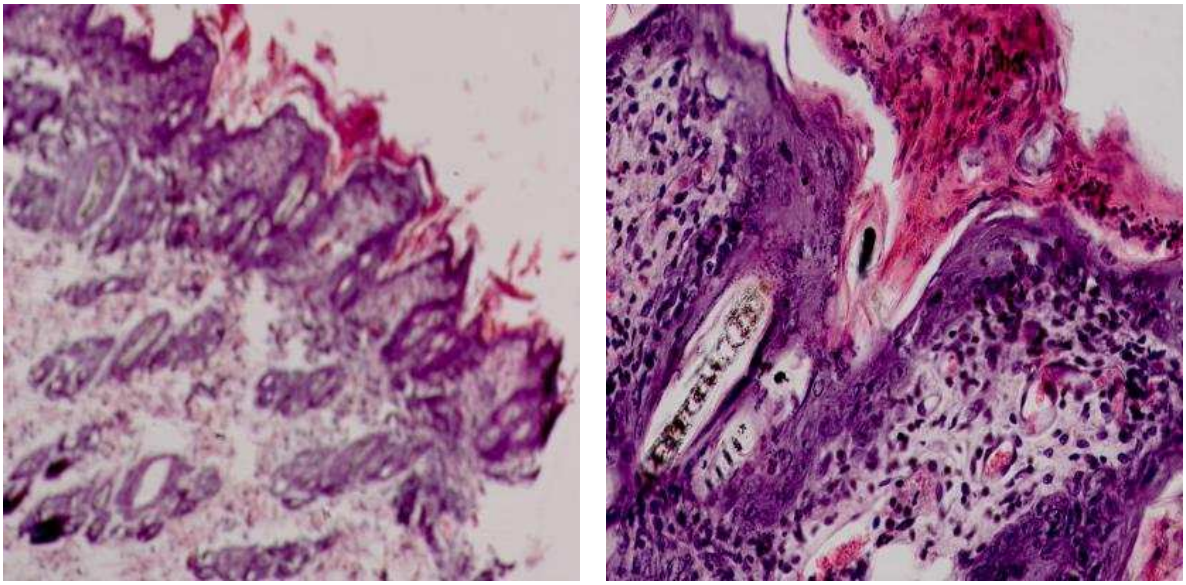


Fig. 2: Light micrograph section in the kidney of rabbit exposed to eldoquin (hydroquinone, 2%, w/w) showing (a) Hydropic degeneration. H&E stain; (b) Hyaline casts. H&E stain; (c) Renal congestion; (d) Interstitial perivascular edema; (e) Perivascular fibrosis. Trichrome stain; (f) Perivascular lymphocytic aggregation. H&E



(a) (b)

Fig. 3: Light micrograph section in the skin of rabbit exposed to eldoquin (hydroquinone, 2%, w/w) showing (a) Hyperkeratosis H&E stain; (b) Lymphocytic infiltration H&E stain

The results of the present study indicate that hyroquinone caused several histological alterations in the liver mainly hydropic degeneration, bile duct hyperplasia and glycogen depletion. Some investigations indicated the liver to plays an important role in the detoxification of hydroquinone (Kooyers and Westerhof, 2006; Levitt, 2007). This drug is absorbed through the skin and metabolized to benzoquinone and multiple glutathione conjugates, that retain the ability to generate reactive oxygen species and detoxified subsequently via mercapturic acid formation (Barber *et al*., 1995; Wester *et al*., 1998; Poet *et al*., 2004; Lau *et al*., 2010).

The induced hydropic degeneration on the hepatocytes by hyroquinone might indicate toxic autolytic injury while the detected bile duct hyperplasia could be attributed to its irritation effect on the epithelium of the bile duct. The induced reduction in hepatocytes glycogen content by hydroquinone as seen in the results of the present study might be due to the effect of this drug on glucose absorption or on the enzymes involved in the process of glycogenesis or/and glycolysis. Hepatocytes of the periportal zones as seen in the present work were more affected than the perivenous hepatocytes. Accordingly, glycogenesis was more affected than glycolysis in which glucose is metabolized by the perivenous cells that contain higher levels of glucokinase and pyruvate. Hepatocytes in the area surrounding the terminal afferent are mainly gluconeogenic, while those ones surrounding the terminal efferent venule are mainly glycolytic and lipolytic and are involved in biotransformation as general detoxification mechanism (Al-Mansour *et al*., 2009). Other studies showed that murine hepatic adenomas were induced by hydroquinone exposure (Levitt, 2007).

The findings of the present investigation showed that hydroquinone might cause congestion in renal blood vessels together with other alterations in the renal interstitial tissue mainly perivascular edema, perivascular fibrosis and perivascular lymphocytic aggregation. These alterations could be resulted from injury of renal blood vessels endothelia induced by hydroquinone. More over, the induced hydropic degeneration together with the formation of hyaline casts in the lumen of some renal tubules of treated rabbits might indicate an insult of the kidney functions. Hydroquinone was reported to produce renal tubule adenomas and chronic progressive nephropathy (Hard *et al*, 1997; Levitt, 2007; Anderson *et al*., 2010).

The data of the present work showed that the cortex is more affected than medulla by hyroquinone treatment. This could be partly due to uneven distribution of the hyroquinone in the tissue of the kidney where about 90% of the total renal blood flow enters the cortex via the blood stream (Jarrar, 2003). This may indicate that more hyroquinone might reach the cortex via the blood stream than that would enter the medulla. Also, the findings showed that the insult by hydroquinone was more prominent in the proximal

convoluted tubules than the distal ones. This could be due to the fact that the proximal tubules are primary sites of reabsorption and active transport leading to higher concentration of hyroquinone in the epithelial lining of these tubules. These alterations might be a result of hydrolic changes in the renal tissue and suggest that hyroquinone intoxication is an injurious stimulus that yields to a partial failure in the ion pump transport of tubules cells which in turn produce tubular degeneration of proximal tubules cells.

The presence of hyaline casts in the lumen of the damaged tubules by hydroquinone is an indication of glomerulnephritis and represents partial failure of tubular reabsorption. The kidney is rich in acid glycosidase and proteolytic enzymes located in lysosomes and other subcellular components (Kirschbaum *et al*., 1973). Oral administration of hydroquinone caused significant hyperplasia of the renal pelvic transitional epithelium and induced renal cortical casts (Kari *et al*., 1992). However, some studies did not record any significant change in the kidney after dermal administration of hydroquinone in rats which might indicate species specific toxicity of hydroquinone (David *et al*., 1998; O'Donoghue, 2006; Topping *et al*., 2007).

CONCLUSION

The findings of the present study indicate that dermal application of the hyroquinone caused hyperkeratosis and dermal congestion together with inflammatory cellular infiltration mainly lymphocytes and eosinophils. This may indicate an allergic effect of this drug or its metabolites that can cause dermatitis. Hydroquinone inhibits tyrosinase impairing partly the conversion of tyrosin to melanin via dopaquinone (Nordlund, 2007). This might explain the reduction of melanin granules in the melanocytes of hydroquinone treated rabbits in comparison to the control ones. Electron microscopic studies indicated that hydroquinone topical application could decrease the formation of melanosomes and caused melanocytes necrosis (Jimbow *et al*., 1974).

The findings of the present work indicate that alterations were more prominent in rabbits subjected to hydroquinone on both auricles on comparison to the members of other treated groups. This might indicate faster and more rapid distribution of the drug via the ear auricle than the abdominal skin. Further investigations are needed to elaborate the histological, histochemical and ultrastructural alterations induced by hydroquinone. In addition, it is recommended to study the effect of hydroquinone topical administration among patients with liver and kidney diseases and to test the safety of using hydroquinone among elderly people, where liver and kidney functions are significantly decreased (Klotz, 2009).

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