World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

Histological Assessment and Quantification of Hypervitaminosis A-induced Fibrosis in Liver, Kidney and Testis of Albino Rats

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Received: 01-06-2017 / Revised Accepted: 29-07-2017 / Published: 01-08-2017

ABSTRACT

The purpose of this work is to clarify if hypervitaminosis-A may induce fibrosis, then quantification and grading of this possible induced fibrosis. The present study was included two groups of male rats. Group I: control group (10 rats). Group II: (20 rats) were injected with vitamin A (500 IU/Kg; interaperitoneally daily for 12 weeks). Livers, kidneys and testes were removed and stained with hematoxylin/eosin for histological alteration, Masson trichrome for collagen fibers staining and immunohistochemical stain for α-SMA. Fibrosis grading was done according to a scoring systems by knodell, Banff and Suskind. Collagen IV in serum and 4-hydroxyproline in tissues were assessed. Hypervitaminosis A induced fibrosis and significant increase ($P < 0.05$) of collagen fibers in liver, kidney and testis. Scoring of fibrosis revealed that hepatic fibrosis established in 90% of animals, renal fibrosis was found in 80% of sections, while Testicular fibrosis was established in 70% of sections. Hypervitaminosis A induced significant increase ($P < 0.05$) in α-SMA expression, serum collagen IV and 4-hydroxyproline in tissues. In conclusion**,** Hypervitaminosis A induced fibrosis in liver kidney and testis.

Keywords: Collagen; Fibrosis; Hypervitaminosis A; kidney; liver; testis; α-SMA.

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How to Cite this Article: Rania A. Ahmed. Histological Assessment and Quantification of Hypervitaminosis A-induced Fibrosis in Liver, Kidney and Testis of Albino Rats. World J Pharm Sci 2017; 5(8): 209-220.

INTRODUCTION

Vitamin A is a fat-soluble vitamin, essential micronutrient that cannot be synthesized de novo neither by man nor animals. Vitamin A has two forms: inactive (provitamin A carotenoids) which is obtained from plant sources and the second type , active form (preformed vitamin A retinol) which can be supplied from animal sources, such as liver, kidney, and fish oil ; or from fortified formulas and pharmaceutical supplements [1,2]. Vitamin A and its derivative retinoic acid are involved in many biological processes as embryogenesis, vision, reproduction, skeletal development, and neurodevelopment, growth maintenance of epithelial tissues, and cellular proliferation and differentiation. Vitamin A is stored primarily in the liver and transported in plasma combined with a specific retinol-binding protein (RBP) [3,4].

Vitamin A toxicity or Hypervitaminosis A is resulted from excessive consumption of the active (preformed) vitamin A, while hypervitaminosis with provitamin A is largely impossible. Hypervitaminosis A may be acute or chronic. Acute hypervitaminosis A is rare and has many symptoms such as; vomiting, diarrhea and headaches [2&5]. Chronic hypervitaminosis A is more common and frequent problem worldwide. Chronic vitamin A toxicity leads to hepatotoxicity, nephrotoxicity, hypercalcemia, hyperglycemia, hyperostosis, hypercholesterolemia and increased cerebrospinal fluid pressure [4,5].

Fibrosis is the excessive accumulation of extracellular matrix (ECM) and replacement of normal tissues with functionless scar tissue, and then progressively leads to organ malfunction and death [6]. Fibrosis or scarring process is mainly driven by chronic inflammation and might lead to cirrhosis representing the end-stage of fibrosis. Collagen is the major insoluble fibrous protein in the ECM and in connective tissue, and normally occurs in various organs. Fibrosis affects nearly every tissue in the body including liver, lung, testis, kidney, heart and pancreas [6-8].
The purpose of this work is to investigate if hypervitaminosis A may induce fibrosis, then quantification and grading of this possible induced fibrosis.

MATERIALS AND METHODS

Animals and Experimental Design: The present study was carried out on two groups of adult male Sprague-Dawley rats weighing 150±15 g. **Group I:** This group contained 10 rats, and was kept as control. **Group II**: Animals of this group (20 rats) were injected with vitamin A at a dose level of (500 IU/Kg/ daily; intraperitoneally for 12 weeks). Rats were housed in plastic cages at normal atmospheric temperature (25 ± 3°C) and normal 12-h light/dark cycle. Rodent diet and water were provided *ad libitum*. To exclude any infection and acclimatization, rats were left under observation for seven days before the experimentation. By the end of experiment , all animals (10 rats) in control group still alive and healthy , while the second group exhibited a number of deaths (6 out of 20) , then we randomly selected 10 rats for the biological investigations ; thus the final number of animals in each group is 10 rats ; (*n=10*).
Animal protocols were used according to the international standard protocols for the use of laboratory animals [9]. All investigations were approved by the Animal Care and Bioethics Committee, Menoufia University, Egypt. (Approval No**.** MNSH178**).**

Methods:
Histogical Examinations: At the end of the experiment, all animals were sacrificed by cervical dislocation, dissected out and their livers, kidneys and testes were removed, then fixed in 10% neutral formalin, dehydrated, cleared and embedded in paraffin wax.
a- For histological examination : Paraffin sections of 5 microns thickness were prepared and stained with routine haematoxylin and eosin stain according to Drury and Wallington [10].
b- For visualization of collagen fibers: Paraffin sections of 5 microns thickness were prepared and stained with Masson trichrome stain according to Masson [11].

Histological Grading and Scoring of fibrosis: Slides stained with Hematoxylin/Eosin and Masson trichrome were used to examine the fibrotic changes and scoring in each of liver, kidney and testis.
i- Liver: Hepatic fibrosis intensity was evaluated according to the modified knodell scoring system according to Ishak *et al*. [12].
0 – No fibrosis
1 – Fibrous in some portal areas, with or without short septa
2 –Fibrous in most of the portal areas, with or without short septa.
3 – Fibrous in most portal areas with portal to portal (PP) bridges.
4 – Fibrous in portal areas, with PP and portal-center (PC) bridges.
5 – Pronounced PP and/or bridges with occasional nodules
6 - Cirrhosis.

ii- Kidney: Renal fibrosis was graded according to the Banff quantitative criteria for interstitial

fibrosis [13]. Images of slides stained with Masson trichrome was analyzed by ImageJ software through determining the percentage of colored stained area. Fibrosis were graded as score 0, 1, 2 and 3.

0 – No fibrosis - fibrosis in up to 5% of cortical area.
1 - Mild - fibrosis in 6-25% of cortical area.
2 - Moderate - fibrosis in 26 - 50% of cortical area.
3 - Severe - fibrosis more than 50% of cortical area.

iii- Testis: Testicular fibrosis was evaluated according to Suskind et al. [14]. Briefly, they were stained with Masson's trichrome was analyzed by ImageJ software through determining the percentage of colored stained area. Testicular fibrosis was graded, as follows:
(0): no fibrosis in interstitial space up to (<5%)
(1): mild fibrosis in interstitial connective tissue (6%–25%)
(2): moderate fibrosis in interstitial connective tissue (26%–50%)
(3): severe fibrosis in interstitial connective tissue (>50%).

Immunohistochemical demonstration of α-SMA: For immunolocalization of alpha Smooth muscle actin (α-SMA), paraffin sections of 5 microns thickness were stained utilizing avidin-biotin peroxidase method. Sections were deparaffinized, endogenous peroxidase activity was blocked by H2O2 in methanol, then heated for 20 minutes in 0.01 mol/l citrate buffer using microwave pressure cooker. Sections were left to reach room temperature and nonspecific binding was blocked with normal horse serum for 20 minutes. α-SMA immune-staining was achieved by polyclonal rabbit anti-human (A3533 I g fraction; DAKO, Glostrup, Denmark). Counterstaining was done using Mayer's hematoxylin (Cat. No. 94585, BioGenex, Menarini Diagnostics, Antony, France).

Image analysis: Analysis of the digital images was obtained via semi-quantitative scoring system (Image J software, Java based application for analyzing images). The greenish blue- stained areas with Masson trichrome and brown-stained immunohistochemical expressions of α-SMA were analyzed through determining the percentage of colored stained area / field area in five randomly high power fields at magnification of 400X.

Biochemical investigation:

i – Determination of 4-hydroxyproline content in tissues: 4-Hydroxyproline (4-Hyp) is a major component of collagen and widely used as a factor to estimate the collagen content in biological specimens. 4-hydroxyproline content was quantitated colorimetrically in liver, kidney and testis using the chloramine T method according to Lee et al. [15] with minor modifications by Shaaban et al. [16].

In brief, 50 mg of tissues specimens were weighed in Eppendorf tubes and hydrolyzed in 0.5 ml of 2 N NaOH at 100° C for 2 hr. The hydrolysate was cooled and then centrifuged at 8000g for 10 min. Two hundred microliters of the supernatant was added to 0.125 ml of the prepared chloramine T solution and then was incubated at room temperature for 10 min. Chloramine T solution was prepared by dissolving 1 part of 7% chloramine T to 4 parts of citrate/acetate buffer (pH 6.0, 57 g sodium acetate, 37.5 g trisodium citrate, 5.5 g citric acid, 385 ml of isopropanol, and dissolved in H2O to a final volume of 1 L)]. Thereafter, 0.75 ml of Ehrlich's solution was added. The Ehrlich's solution was prepared by dissolving 2 g of P-dimethylamino-benzaldehyde in 3 ml of 60% HClO4 and then mixed with 9 ml of isopropanol. The final mixture was incubated at 60 ºC for 35 min, then at room temperature for 10 min. The absorbance of mixture was determined at 560 nm. Standard solutions containing authentic 4-hydroxy-L-proline were treated likewise.

ii - Collagen IV in serum: To determine the serum activity of collagen IV in serum, blood samples were collected from the retro-orbital venous plexus before sacrificing rats by using capillary tubes inserted in the medial canthus. Blood samples were left at room temperature to clot, and then centrifuged at 3000 rpm for 15 minutes to separate serum. Sera were stored at -20°C until used. Collagen IV was determined by ELISA (Biotrin International LTD, Ireland), using a commercial kit in accordance with manufacturers' instructions (Uscn Life Science Technology, Wuhan, China) [17].

Statistical analysis: Data were analyzed using student's *t-tes*t by a computer statistical package (SPSS program version 16; SPSS Inc., Chicago, USA). Results were presented as mean ± SD. A P value ($P < 0.05$) considered significant.

RESULTS

Histological Results:

i. Liver

Histological examination of control group showed that hepatic parenchyma consisted of several hepatic lobules separated from each other by very delicate connective tissue septa. Each hepatic lobule contained a thin walled central vein surrounded by radiating hepatic cords. No fibrosis or cirrhosis or was detected in this group (Fig 1a). On the other hand, in liver sections of animals with hypervitaminosis A, there was a thickness in portal

vein wall and sent fibrous septa from portal area to the hepatic parenchyma in the form of bridging fibrosis; bile duct proliferation was observed (Fig 1b). Moreover, some slides exhibited more advanced degree of fibrosis as fibrous septa extended to the surrounding parenchyma and bridging with adjacent portal tract or with the central vein forming porto-central fibrous link. Multiple centro-central fibrotic septa incompletely compassed and divided hepatic lobules, without disorganization of the lobular architecture. (Fig 1c).

Concerning collagen fibers; Section in the liver of the control rat stained with Masson trichrome stain revealed a normal distribution of collagen fibers as a greenish blue colour, as collagen occurred as wavy fibrils which were either singly or clustered together in dense bundles surrounding sinusoids, central and portal vein (Fig 1d).

In rats treated with hypervitaminosis A, the collagen fibers were increased in between hepatocytes, around the central vein and in the portal veins. High proliferation of collagen fibers that appeared in the form of thick bundles distributed in many portions of the tissues with advanced fibrous septa of non-functional scar (Figs 1e , 1f & 1g).

ii. Kidney:

Microscopic examination of kidney sections of control animals showed normal structure of renal structure with normal corpuscles , glomeruli and tubules **(Figure 2a)**. Chronic administration with vitamin A induced marked histological abnormality, as tubulointerstitial area was damaged , glomeruli were atrophied , in addition to observance of sever hemorrhage (Fig 2b),

Concerning collagen fibers, examination of slides stained with Masson trichrome stain revealed that; Kidney of control rats showed a normal distribution of the collagen fibers surrounding Malpighian corpuscles, in the brush border of the proximal tubule and in walls of blood vessels (Fig 2c). However, microscopic examination of kidney sections of Hypervitaminosis A group showed a marked increase of extracellular matrix deposition. Fibrosis was mainly interstitial and perivascular as the collagen fibers were increased in the glomerular tuft and between the renal tubules (Fig 2d), or as a thick bundles surround blood vessels.

iii. Testis

Histological examination of sections of control rats testes showed the typical testicular architecture with normal seminiferous tubules which include normal spermatogenic cells, Sertoli cells, and intertubular connective tissue (Fig 3a). Animals with hypervitaminosis A, showed marked abnormalities of interstitial leydig connective tissue as massive proliferation and hemorrhage (Fig 3b).

Microscopic examination of Masson trichrome stained slides of control rats showed the normal distribution of collagen fibers in interstitial leydig connective tissue, tunica albuginea, basement membrane of seminiferous tubules and around the blood vessels (Fig 3c). After being treated with vitamin A, the distribution of collagen fibers increased in the interstitial leydig connective tissue, tunica albuginea (fig 3d) or as thick bundles around the blood vessels fig 3e).

Assessment of Collagen Content: Data obtained from image analysis of liver, kidney and testis, denoted a significant statistical increase ($P<0.05$) of the percentage of collagen fibers content in hypervitaminosis A group when compared with control group (table 1).

Histological Grading and Staging of Fibrosis: Microscopic evaluation and grading of liver, kidney and testis sections showed that chronic administration of vitamin A resulted in fibrosis in the three organs but in different stages (table 2). In liver, fibrosis established in the most of animals (9 out of 10). However, grade 3 predominated (5 out of 10) while no hepatic cirrhosis was detected. Evaluation of fibrosis grading of kidney sections revealed that fibrosis was found in 80% of sections (8 out of 10). Grade II fibrosis was more common (5 out of 10). Concerning testicular fibrosis grading, microscopic scoring revealed that fibrosis was established in 70% of sections (7 out of 10) with different grades (Grade I 3 out of 10 ; Grade II 4 out of 10) , but Grade III wasn't evaluated of any animals.

Immunohistochemical Results: In sections of control liver, α-SMA-positive cells were observed in the wall of blood vessels in the portal areas and in sinusoids (fig 4a). While in hypervitaminosis A group, strong expression and numerous α-SMA-positive cells were observed in periportal area and around the proliferating bile ducts. Also, strong perisinusoidal α-SMA expression and early stage portal bridging was shown (fig 4b).

In normal kidney, minimal positivity of α-SMA is shown in the walls of renal arterioles and in tubulointerstitial area (fig 4c). On the other hand, rats of hypervitaminosis A group showed a strong expression of α-SMA in the wall of renal tubules (fig 4d). Concerning testis sections, α-SMA was detectable weakly in the peritubular cells that surround the seminiferous tubules and in blood vessels walls (fig 4e). Sections of rats with hypervitaminosis A showed strong expression of α-

SMA in the interstitial tissue and in the seminiferous peritubular boundary tissues (fig 4f).

Semi-Quantitative immunohistochemical Scoring of α-SMA: As shown in table (3) ; Data obtained from image analysis of liver , renal cortex and , testis declared a statistically significant increase ($P < 0.05$) in mean % area of α-SMA in hypervitaminosis A group when compared with control group.

Biochemical results: Data in table (4) showed that chronic administration with vitamin A induced significant increase of 4-Hydroxyproline values in liver, kidney and testis when compared with control group. Furthermore, there was a significant difference in serum collagen IV level between control group and hypervitaminosis A group (table 5).

DISCUSSION

The current study showed that, hypervitaminosis A induced fibrosis in each of liver, kidney and testis of albino rat. Concerning hepatic fibrosis, histological results showed a thickness in portal vein wall and sent fibrous septa from portal area to the hepatic parenchyma in the form of bridging fibrosis; bile duct proliferation was observed fibrosis. Collagen fibers was significantly increased and appeared as thick bundles of collagen fibers surrounding sinusoids, central and portal vein. Histological grading recorded that fibrosis established in the most of animals (9 out of 10; grade 3 predominated). Immunohistochemical observations showed a significant increase in the expression of α-SMA around portal vein and proliferating bile ducts. Furthermore, hepatic 4-hydroxyproline was increased significantly.

Many authors recorded the hepatotoxicity and histological alterations induced by hypervitaminosis A. Ibrahim and Okdah [4] reported that hypervitaminosis A caused blood vessels congestion and dilation , fatty and hydropic degeneration , depletion of glycogen and increased of Bcl-2 and PCNA expression , increased levels of AST , ALT and alkaline phosphatase (ALP) were observed . Leo et al. [18] suggested that vitamin A caused necrosis in which the necrotic materials may exhibits a chemotactic properties leading to infiltration of lymphocytes and macrophage in the portal area . Lane [19] recorded that, hypervitaminosis A induces vaculation of hepatocytes, which can be attributed to mitochondrial swelling and endoplasmic reticulum proliferation. The pathogenesis of hypervitaminosis A-induced hepatic fibrosis remains unknown because there are a limited number of studies on the direct effect of high amounts of the vitamin A on the fibrotic reaction. Nollevaux et al. [20] investigated liver biopsies of 9 patients intoxicated with vitamin A. Histological examination revealed that one out of nine specimens showed normal hepatic structure , while four specimens out of 9 underlined a perisinusoidal fibrosis .

Vitamin A is stored in the normal liver, as fat droplets in the space of Disse by hepatic Stellate cells (HSC). Excessive accumulation of vitamin A induces stellate cells activation which induce a myofibroblast-like cells surrounded by newly formed collagen fibrils. Activated HSC results in expression the smooth muscle actin (SMA) gene, then increases the secretion of extra-cellular matrix (ECM) components and matrix-degrading enzymes [20]. Thus, the vitamin A-induced fibrosis is resulted from the retinol toxicity and the release of inflammatory cytokines and fibrogenic materials by injured hepatic cells [20,21].

Concerning renal fibrosis, hypervitaminosis A induced a significant increase of collagen fibers and the expression of α-SMA immunostain in smooth muscle of renal arterioles and in tubulointerstitial area . Evaluation of fibrosis grading revealed that fibrosis was found in 80% of sections. Moreover there was significant increase in renal 4-hydroxyproline. Our results are in accordance with Daher et al. [5], as they investigated a series of 16 patients with chronic kidney disease (CKD) resulting from prolonged injection of ADE vitamins supplements containing high doses of vitamin A, D and E vitamins . Using KDIGO scoring system, acute kidney injuries (AKI) was diagnosed in 13 out of 16 cases. The injection of ADE resulted in a significant increase of serum urea, creatinine, and calcium levels. Kidney functions were restored in some of cases by early treatment, while partial recovery in patients with chronic hypercalcaemia exhibited tubular degeneration, nephrocalcinosis and fibrosis.

Hypercalcaemia is the increased serum calcium levels in serum, and it is one of the common syndromes of hypervitaminosis A [3]. Hypercalcemia results in granulomatous reaction and reduction of glomerular filtration by renal vasoconstriction of the arterioles as it affects the vascular smooth muscle [22]. Hypercalcemia and deposition of calcium in the renal parenchyma induces histological alterations as glomerular hyalinization, thickening of Bowman's capsule, tubular atrophy and leucocytic infiltration [23]. Chronic calcification of convoluted tubules cells inducing Randall's plaques which are formed in the

basement membranes of the inner medullary interstitium in the loop of Henle. These plaques can extend into the surrounding interstitial tissue, causing nephrocalcinosis, intratubular stones, nephrolithiasis and obstructive uropathy, which leads to tubular epithelium degeneration, accumulation of granular material in and around the collecting tubules, which results in mild to moderate renal interstitial fibrosis [22-26].

The present work showed that chronic administration with vitamin A induced testicular fibrosis, as there was a significant increase of collagen fibers and the expression of α-SMA in the interstitial connective tissue. Evaluation of fibrosis grading revealed that testicular fibrosis was found in 70% of sections. Moreover there was a significant increase in testicular 4-hydroxyproline. Our findings are supported by some studies that discussed the histopathological effects induced by hypervitaminosis A on testis. Hypervitaminosis A induced degenerative changes and necrotic lesions in the testes. The germinal epithelium was completely sloughed away, the spermatids nuclei were vacuolated and formed giant cells composed of dead and/or degenerating spermatids. The spermatocytes reduced in numbers, their nuclei were clumped and their chromatins was lost [27,28].

Hypervitaminosis A induced a significant reduction in testis weight, volume and consistency. Furthermore, many histological alterations were observed such as, marked alterations of connective tissue in tunica albuginea and tunica vascularis, as well as degeneration in Lydig cells and somniferous tubes [29]. Vitamin A intoxication induced significant decrease in the size of seminal vesicles and appeared empty of fluid, moreover, the testes exhibited necrosis in all layers except the spermatogonial layer [30]. Some investigations suggested that there was an association between excess intake of beta carotene (one of the plant sources of vitamin A), and fertility disorders in human males [31].

Hypervitaminosis A was proved to induce oxidative stress **[32]**. Fluctuations induced during Leydig cell steroidogenesis and Sertoli cell population are the main sources of the generation of free radical in the testicular tissue. Sertoli cell population induced Reactive oxygen species (ROS) is stimulated by retinoic acid (RA) , a derivative of vitamin A and works as a cofactor for spermatogenesis , which leads to activation of ROS generation , lipids peroxidation and necrosis [33,34]. The relation between ROS generation and fibrosis was explained by findings showing that generation of ROS plays an important role in the common fibrotic pathway. ROS activates of fibroblasts and induces metabolic homeostasis and chronic inflammation; also ROS induce the release of profibrotic metabolites, all of which play important roles in fibrosis development and persistence. ROS activate/induce transforming growth factor beta (TGF-β1) and mediate many of TGF-β's fibrogenic effects. TGF-β is considered to be the most important pro-fibrogenic cytokine and increased significantly in all of fibrotic diseases [35]. Nevertheless, the free radical generation caused vitamin A and its derivates retinoids, in the testes may explain the testicular fibrosis.

CONCLUSION

The obtained findings revealed that, chronic consumption or injection with excessive amounts of vitamin A induces hepatic, renal and testicular fibrosis. Further studies are required to explain the mechanism of hypervitaminosis A- induced renal and testicular fibrosis.

Conflict of interest: The authors declare that no conflict of interests existed in the organization, results, presentation and the finance of the article.

Table (1) : Hypervitaminosis A induced alterations in the percentage (%) of collagen content in liver , kidney and testis.

Liver		Kidney		Testis	
Control	**H. vit A.**	**Control**	**H. vit A**	**Control**	**H. vit A**
6.45±0.48	**27.71 ± 1.91***	**2.76 ± 1.09**	**22.20±12.66 ***	**2.29 ± 0.50**	**20.80 ± 13.23***

(*) Significant increase at $P < 0.05$ compared with control group ; (Mean ± SD , *n=10*)

Table 2 : Scoring and Grading of hypervitaminosis A induced fibrosis in liver , kidney and testis according to a scoring systems by knodell , Banff and Suskind respectively.

Organs & Groups / Animals	Liver Graded (0-6)		Kidney Graded (0-3)		Testis Graded (0-3)	
	Con. G.	**H. Vit. A G.**	**Con. G.**	**H. Vit. A G.**	**Con. G.**	**H. Vit. A G.**
1	0	3	0	2	0	2
2	0	2	0	1	0	0
3	0	1	0	0	0	0
4	0	3	0	2	0	1
5	0	0	0	0	0	0
6	0	3	0	2	0	2
7	0	1	0	1	0	1
8	0	3	0	1	0	1
9	0	4	0	2	0	2
10	0	3	0	2	0	2
Mean±SD	**0±0**	**2.30±1.25***	**0±0**	**1.30±0.82***	**0±0**	**1.10±0.87***
P value	**P < 0.05**					

(*) Significant increase at P < 0.05 compared with control group ; (Mean ± SD , *n=10*)

Table 3 : Mean % area occupied by α-SMA in liver , kidney in and testis .

Organ / Groups	Liver	Kidney	Testis
Control group	2.2 ± 0.60	1.86 ± 0.23	1.36 ± 0.32
Hyp. Vit A. group	15.56 ± 3.78 *	23.42 ± 6.25 *	11.71 ± 2.26 *

(*) Significant increase at P < 0.05 compared with control group ; (Mean ± SD , *n=10*)

Table (4): Hypervitaminosis A induced alterations in 4-Hydroxyproline content in liver, kidney and testis (µg/mg wet tissue)

liver		kidney		Testis	
Control	**Hyp. vit. A**	**Control**	**Hyp. vit. A**	**Control**	**Hyp. vit. A**
210.20 ± 10.08	**624.70± 12.85***	**10.48±2.20**	**51.88±5.02***	**2.01±0.23**	**4.84±0.56 ***

(*) Significant increase at P < 0.05 compared with control group ; (Mean ± SD , *n=10*)

Table (5) : Hypervitaminosis A induced alterations in – Collagen IV in serum (ng/ ml)

Groups	Control G.	Hypervitaminosis A
Col. IV values	**24.60 ± 4.08**	**114.80 ± 7.65 ***

(*) Significant increase at P < 0.05 compared with control group ; (Mean ± SD , *n=10*)

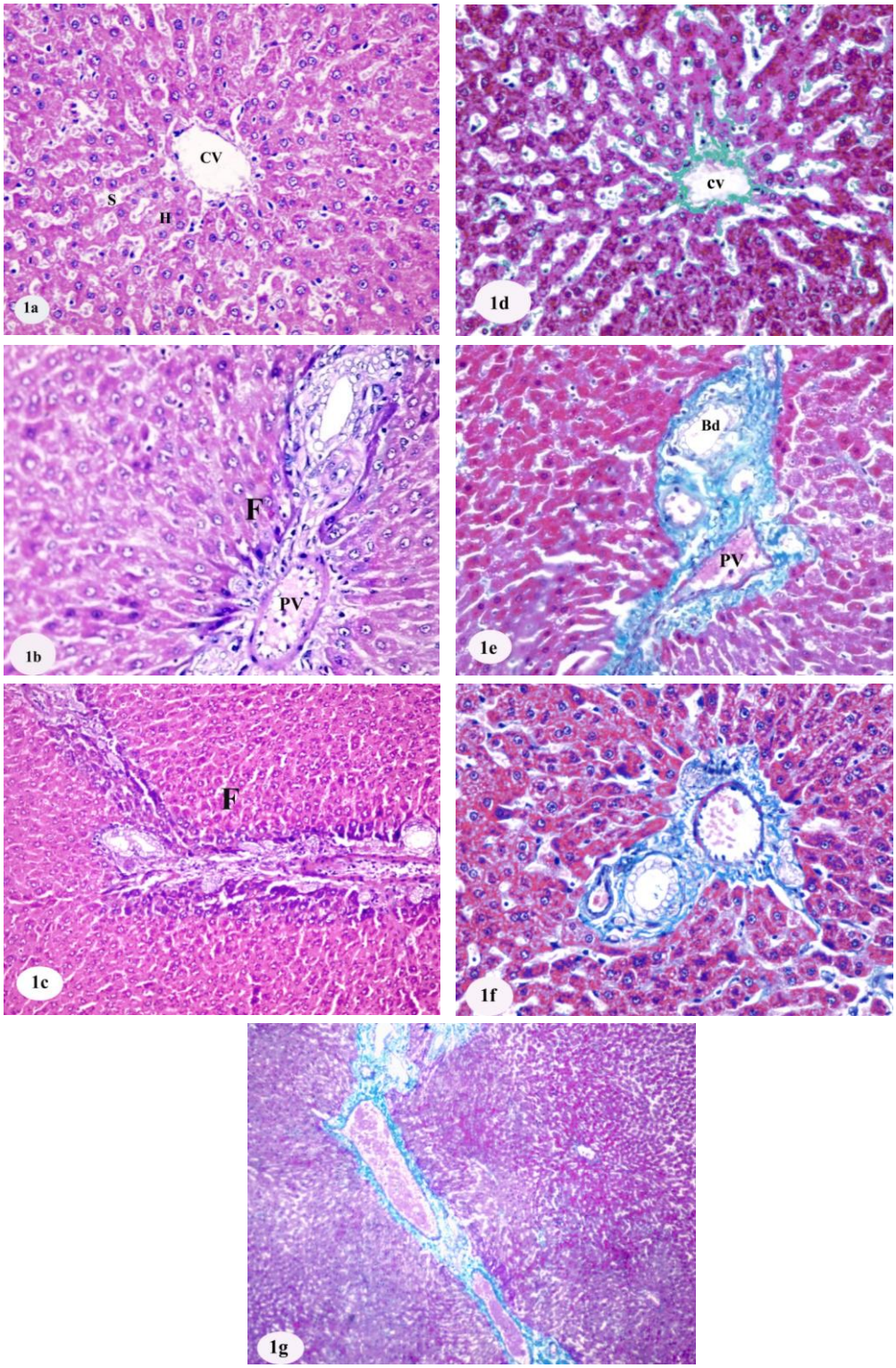
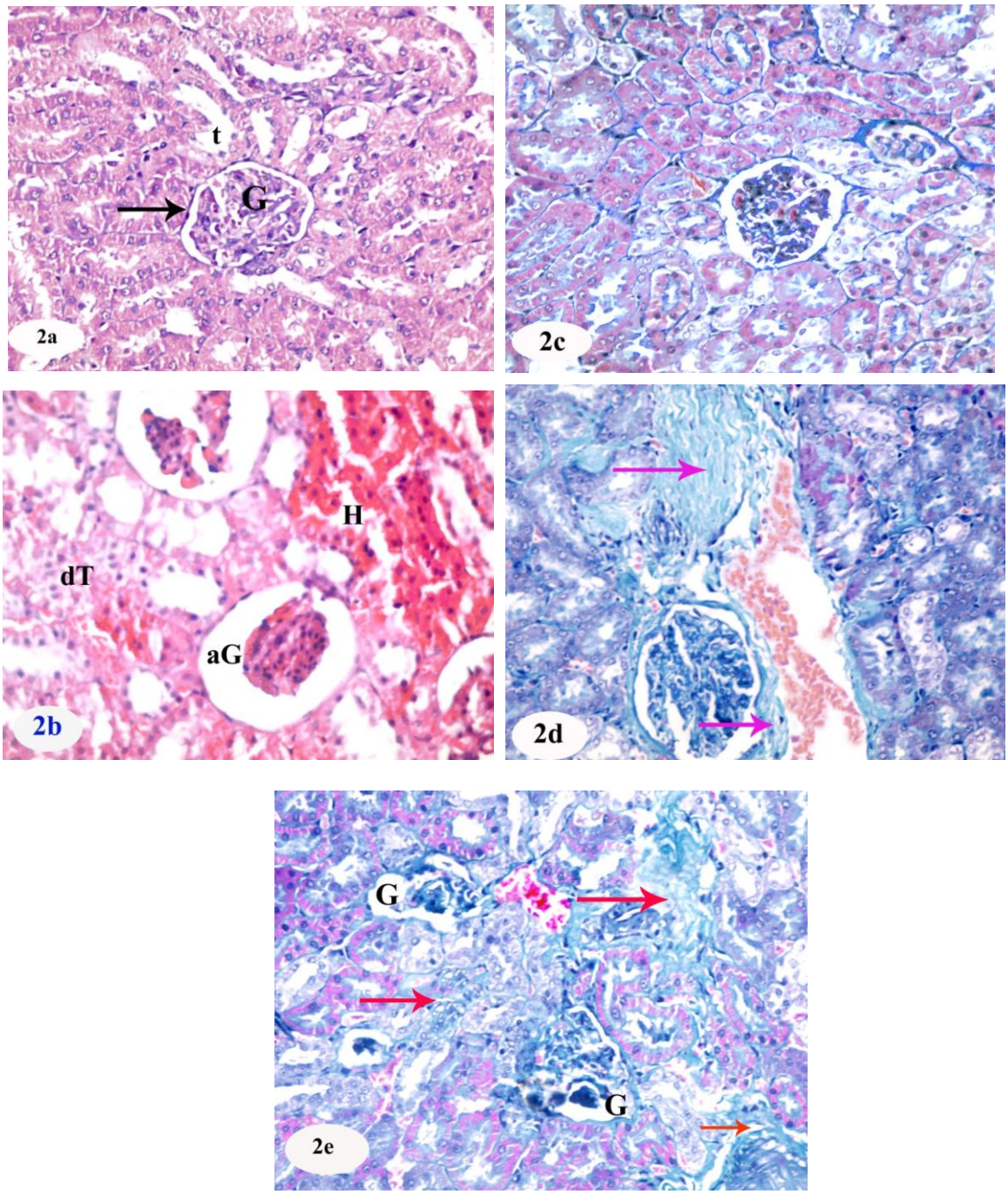


Figure 1: (a) control rat showing normal hepatic tissue with normal central vein (CV), hepatocytes (H), sinusoids (s) (H&E, X400). (b) Rat from hypervitaminosis A group showing fibrous septa extended from portal vein to the hepatic parenchyma and bile duct proliferation (H&E, X400). (c) Another rat with vitamin A toxicity showing fibrous septa interconnecting between centrilobular areas, and portal area (F) (H&E, X200). (d) Control rat showing normal distribution of collagen fibers, stained greenish blue around the central vein and sinusoids (Masson trichrome, X400). (e & f) Rats with hypervitaminosis A Shows marked increase in collagen fibers around the blood vessels in the portal vein (g) Marked collagenous septa dividing the hepatic lobule (Masson trichrome, X200).



Figiure (2): (a) Section from control group showing normal renal tissue architecture with normal Bowman's capsule (arrow), glomeruli (G) and normal tubules (t). (b). Rat from hypervitaminosis A group showing atrophied glomeruli (aG), degenerated tubules (dT) and severe hemorrhage (H). (H&E, X400). (c). Normal collagen distribution in kidney of control rat. (d & e). Sections from hypervitaminosis A group showing thick bundles of collagen surrounds congested vessel and extends in the tubulointerstitial area. (Masson trichrome X400).

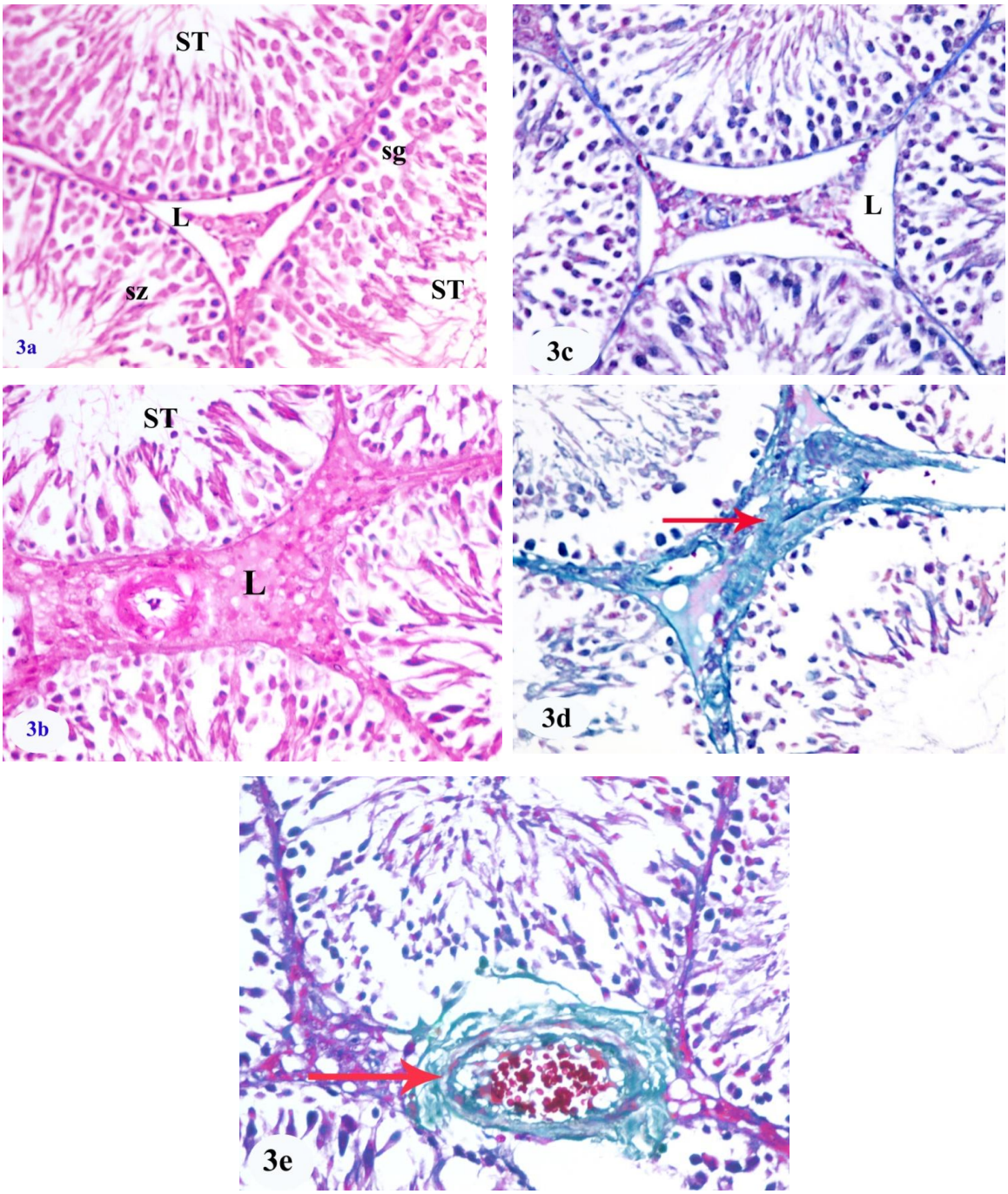


Fig (3) : (a).Section in testis of a control rat showing normal seminiferous tubules (ST) , spermatogonia (sg) ; spermatozoa (sz) , interstitial leydig cells (L) (b) Section in testis of a rat with hypervitaminosis A showing abnormal interstitial leydig (L) (H&E , X400). (c). Control rat showing normal distribution of collagen fibbers in leydig tissue and tunica albuginea. (d) Rat with hypervitaminosis A showing marked increase of collagen in leydig tissue (arrow). (e) Another rat with hypervitaminosis A showing thick bundles of collagen surrounding congested blood vessel (arrow). (Masson trichrome stain , X400).

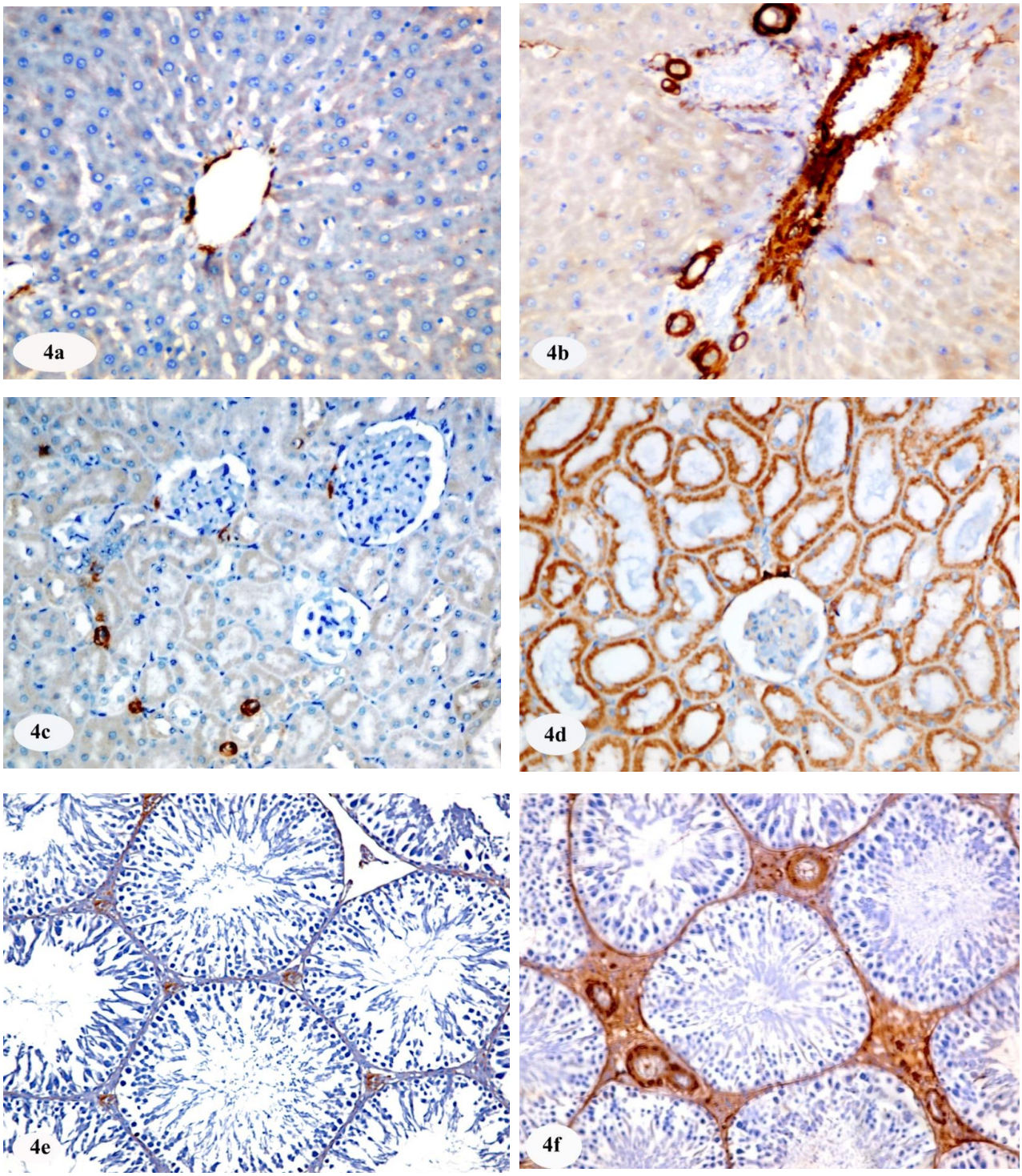


Fig (4) (a) liver section of control rat showing weak expression of α-SMA. (b) liver section of rat from hypervitaminosis A group showing strong reaction of α-SMA in periportal area and around the proliferating bile ducts. (c) Kidney section of control rat showing minimal expression of α-SMA in renal arterioles. (d) kidney section of rat from hypervitaminosis A group , showing strong expression of α-SMA in the tubulointerstitial area. (e) Testis section of control rat showing minimal expression of α-SMA in the peritubular cells. (f) Testis section of rat from hypervitaminosis A group showing high expression of α-SMA in the interstitial tissue and in the seminiferous peritubular boundary tissues. (α-SMA immunostain , a-d X400 ; e&f X200).

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