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The Possible Protective Effect of Vitamin C Against Sildenafil Citrate Affected Liver of Adult and Senile Male Albino Rats (Light and Electron Microscopic Study)

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The Possible Protective Effect of Vitamin C against Sildenafil Citrate Affected Liver of Adult and Senile Male Albino Rats (Light and Electron Microscopic Study)

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ABSTRACT

Background: Erectile dysfunction (ED) affects a growing number of men in the USA and abroad. Phosphodiesterase 5 inhibitors as sildenafil citrate (SC) are used to treat erectile dysfunction and pulmonary hypertension. SC is mainly metabolized in the liver. Vitamin C is an antioxidant that has hepatoprotective effects.
Aim of the study: To reveal the histological alterations in liver of adult and senile rats treated with SC, and to explore the impact of vitamin C co-treatment.
Materials and Methods: Study was done at the House of Animals, Pharmacy Faculty, Al-Azhar University between July, and September 2021. Sixty male albino rats were used (divided in equal pattern into 5 groups); Each one was subdivided to two equal subgroups: adult and senile rats. They received drugs through oro-gastric feeding tube. Group I: received water 10 ml/ kg/ d for 30 days; group II: received 10 mg /kg /d of SC for 30 days; group III: received 26mg /d of vitamin C one hour prior to 10 mg /kg /d of SC for 30 days; group IV: received 26mg/d of vitamin C for 30 days; group V: received 10 mg /kg /d of SC for 30 days, then left one month. Then, the animals were anesthetized, and tissues of liver were proceeded and microscopically examined.
Results: Histological changes were more severe in senile rats and significantly improved with vitamin C co-treatment and minimally improved in withdrawal group.
Conclusion: This study revealed that vitamin C might have a protective effect against sildenafil citrate induced hepatic injury.

Keywords: *Vitamin C; sildenafil citrate; hepatic injury; liver; rats..*

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Authorship: *The author has a substantial contribution to the article.*

INTRODUCTION

Sildenafil citrate (SC) was originally discovered in 1991 and used for treatment of angina. SC was introduced as the name Viagra and got the approval of food and drug administration (FDA) in March 1998[1]. It is the first oral medication accepted for erectile dysfunction (ED) in the European Union (EU).[2]

SC is an inhibitor of Phosphodiesterase 5 enzyme that metabolizes cyclic guanosine monophosphate (cGMP) in the penile tissues and in the pulmonary artery smooth muscle causing relaxation of the smooth muscles and vasodilatation of penis blood vessels. According to this action, sildenafil promotes and prolongs the duration of erection of penis and treat pulmonary hypertension.[3]

The great part of SC metabolized in the liver, so there are precautions when giving sildenafil to children, and patients with liver diseases[4]. The process of aging predisposes to hepatic functional, metabolic, and structural changes[5]. As about 2–5% of the world's population has chronic liver disease, there is a need to investigate the effect of long-term administration of SC on the structure of the liver.[4]

Vitamin C is an antioxidant with an anti-inflammatory action. It eradicates reactive oxygen molecules, that promote pro-inflammatory cytokines in inflammatory diseases and hepatic fibrosis. Therefore, vitamin C supplementation improves liver function and reduces hepatic injury of liver tissues in experimental studies induce liver injury in rats.[6]

Therefore, this study aimed to detect the possible changes in the structure of the tissues of liver after experimental administration of sildenafil citrate with

age respect and the reversibility of SC effect after discontinuation. It also concerned to evaluate the effect of vitamin C against the changes in the structure of liver tissues in senile and adult rats following administration of sildenafil.

MATERIALS AND METHODS

Animals

We used sixty male albino rats. Study was done at the House of Animals, Pharmacy Faculty, Al-Azhar University between July, and September 2021.

We divided the rats to five groups (twelve in each group). Each group was subdivided into two subgroups. Adult group aging 3-6 months and weighting 150-200 that is nearly equal to 20 years in human and senile group aging 18-24 months and weighting 300-350 gm that is nearly equal to 60 years in human[7].

Group I (control group): rats were given 10 ml/kg distilled water orally by oro-gastric feeding tube every day for 30 days[8,9,10]. Group II (sildenafil treated group): rats were given 10 mg/kg/d of sildenafil citrate for 30 days [8,9,10]. Group III (vitamin c and sildenafil treated group): rats were given 26mg/d of vitamin C orally[11] one hour prior to administration of 10 mg/kg/d of SC for 30 days[8,9,10]. Group IV (vitamin C treated group): rats were given of vitamin C 26mg/d for 30 days[11]. Group V (withdrawal group): rats were given 10 mg/kg/d of SC for 30 days, then left one month for observation from the last dose[8,9,10].

Chemicals

The chemicals used in this work were: Sildenafil citrate 50mg tablets, were purchased from Pfizer Egypt Pharmaceutical Company, and was dissolved in 100 cubic cm of distilled water; Vitamin C 500mg tablets, were purchased from Memphis Co- for Pharmaceutical and chemical industries in Egypt and was dissolved in 50 ml distilled water.

Methods

General examination of the liver and mortality recording:

The external appearance of the liver was examined by naked eye. Also, the mortality rate was recoded in each group.

Measurements (weight of the liver, body, and liver/body weight ratio): was recorded at the 1st, 10th and 30th days. in withdrawal group was recorded at the 60nd day.

The rats in all groups were collected by the end of the 30th day, except the rats in the withdrawal group, were collected by the end of the 60nd day. We anaesthetized rats of each group, and liver specimens were collected[8,9,10].

Light and electron microscopic processing:

We cut the specimens into small pieces 1mm^3 and immersed and fixed in cold 3 % glutaraldehyde, then fixed with 1% osmium tetra-oxide at 4°C, processed and embedded in epoxy resin. Semithin sections (1µm thick) stained with toluidine blue stain that were examined by light microscopic devices. Sections of Ultrathin specimens (50 - 70 nm thick) were cut and put on the copper grids. After that, tissues were stained by uranyl acetic acid and lead citric acid for examination by transmission electron microscopic (TEM)[12].

Then, the liver specimens were preserved in 10 % formalin and paraffin sections were processed then stained by eosin and hematoxylin for light microscopic examination[12].

Statistical analysis

Data was examined and tested with SPSS23 using ANOVA test then least significant difference (LSD) post hoc test. Data was interpreted as mean ± standard deviation (SD). So, values are considered significant when P <0.05, highly significant when P <0.001 and non-significant when P >0.05.

RESULTS

External appearance and mortality:

Examination of the external appearance of livers showed that there was no difference between the livers excised from all groups.

Regarding mortality, all groups showed no death except senile sildenafil treated group and withdrawal group that showed a low mortality rate (16% mortality rate as one rat died in each group. (Figure 1).

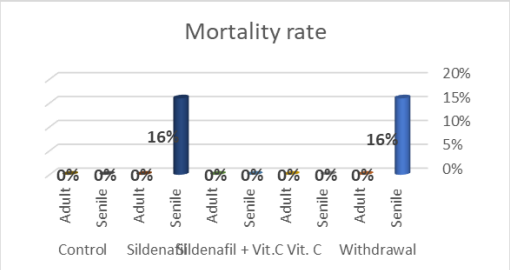

Fig.1: Comparison between all groups regarding mortality rate

Liver weight, body weight and liver to body weight ratio

We noticed a significant difference between the studied groups by using one way ANOVA test (P< 0.001). Also, least significant difference (LSD) test comparing between the studied groups showed that the values of weight and ratio in SC and withdrawal groups were decreased significantly compared to other groups.

This indicated decreased rate of weight gain in adult sildenafil treated group and adult withdrawal group and good weight in control and vitamin C treated group with minimal effect on weight gain in adult sildenafil with vitamin C treated group.

There was a significant loss of body weight in senile sildenafil treated group and senile withdrawal group and stable weight in control and vitamin C treated group with minimal effect on weight loss in adult sildenafil with vitamin C treated group.

Effect of sildenafil on liver histology

Light microscopic results:

Group I (control group) and group IV (vitamin C-treated group): H&E sections showed classical lobules with radiating and anastomosing cords of hepatocytes arising from the central vein. The cords of hepatocytes were separated by hepatic sinusoids which were lined by von-kupffer cells. The portal triad showed a clear branch of portal vein, hepatic artery, and bile duct. (Fig. 2-A, D and Fig. 3-A,D).

Group IIA (adult sildenafil treated group): This group showed mild changes of the hepatic lobular architecture. The central vein, hepatic artery and portal veins were congested. There was moderate inflammatory cell infiltration (lymphocytes and neutrophils) around the portal vein. Areas of intercellular vacuolization were observed in liver parenchyma. The bile ducts showed moderate hyperplasia. Most hepatocytes exhibited vacuolated cytoplasm and pyknotic nuclei, with few intercellular areas of hemorrhage and necrosis (Fig. 2-B).

Group IIB (senile sildenafil treated group): This group showed marked changes in the hepatic lobular architecture. It showed marked congestion in both portal and central veins and increased number of von-kupffer cells. Areas of hemorrhage and necrosis were observed between hepatocytes and around central vein. Most hepatocytes degenerated with reduction of number. Focal intercellular vacuolization was observed in liver parenchyma. The portal areas revealed massive peri-portal cellular infiltration, dilated congested portal vein and hepatic artery branches and proliferation of bile duct. (Fig. 3-B).

Group IIIA (adult vitamin C and sildenafil treated group): showed restoration of hepatic architecture; hepatocytes were arranged in cords and some of them appear healthy, but the others showed few cytoplasmic vaculations. Few congested blood sinusoids are seen. Average portal vein with few or no inflammatory cell infiltration around it and mildly congested hepatic artery. The bile duct showed no hyperplasia. Most of hepatocytes showed normal cytoplasm and average nuclei (Fig. 2-C).

Group IIIB (senile vitamin C and sildenafil treated group): This group showed less degenerative changes, compared to senile sildenafil treated group IIB. It showed average hepatocytes with average nuclei and intercellular Kupffer cells. Moderate congesion of portal tract with few inflammatory cell infiltrations around the portal vein and mildly congested hepatic artery. The bile duct showed normal appearance with no hyperplasia. Few areas of cytoplasmic vacuolization were noticed in liver parenchyma. Few intercellular areas of hemorrhage and necrosis (Fig. 3-C).

Group 5 (sildenafil withdrawal group): H & E-stained sections and toluidine blue stained sections obtained from the liver of withdrawal group showed a picture of liver injury induced by sildenafil treated group, but less severe (Fig. 2-E,3-E).

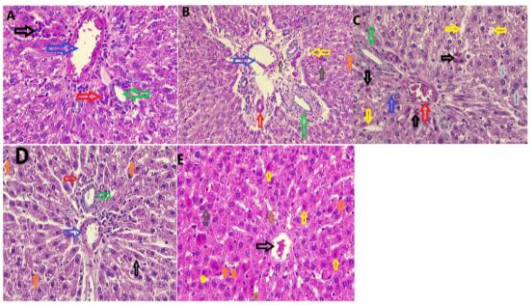

Fig. 2: A photomicrograph of liver sections of: A) Adult control group showing hepatocytes are arranged in cords separated by blood sinusoids containing Von Kupffer cells (black arrows), portal tract shows hepatic artery (red arrow) bile duct (green arrow) and portal vein (blue arrow). B) Adult sildenafil treated group showing congested portal vein (blue arrow), inflammatory infiltrations around it (black arrow), congested hepatic artery (rad arrow). The bile duct shows severe hyperplasia (green arrow). Areas of intercellular vacuolization (yellow arrow). Pyknotic nuclei (orange arrow), with areas of hemorrhage and necrosis (grey arrow). C) Adult vitamin C and sildenafil treated group (IIIA) showing the mild congestion of portal tract with no inflammatory cell infiltration around the portal vein (blue arrow), congested, dilated hepatic artery (rad arrow). The bile duct shows normal appearance with no hyperplasia (green arrow). Hepatocytes shows vacuolated cytoplasm (yellow arrows) and pyknotic nuclei (grey arrows), with few areas of necrosis and hemorrhage (black arrows). D) adult vitamin C treated group showing hepatic classical lobule with radiating and anastomosing cords of hepatocytes (black arrow). The portal triad shows a clear branch of portal vein (blue arrow), hepatic artery (red arrow) and bile duct (green arrow). The cords of hepatocytes are separated by hepatic sinusoids (black arrow), which are lined by von-Kupffer cells (orange arrow). E) adult sildenafil withdrawal group showing mild dilatation of central vein (black arrow). Hepatocytes were arranged in cords or plates radiating from the central vein. Hepatocytes show vacuolated cytoplasm (brown arrows) and average nuclei (grey arrow). Hepatocytes cords are separated by hepatic sinusoids lined by von-Kupffer cells (orange arrow). There are also few apoptotic hepatocytes (yellow arrow) (H & E, X 400).

Transmission electron microscopic (TEM) results:

Group I (control group) and group IV (vitamin C-treated group): TEM sections obtained from the liver of the control group and vitamin C-treated adult and

senile groups showed similar results. They showed hepatocytes with intact cell membrane Hepatocytes had rounded euchromatic nuclei with prominent nucleoli and intact regular nuclear envelop. Some hepatocytes are binuclear. Well organized cytoplasm contained numerous mitochondria with prominent cristae, stacks of rough endoplasmic reticulum, cisternae of smooth endoplasmic reticulum, glycogen granules, average bile canaliculus, average blood sinusoids and average endothelial cells (Figs. 4-A,D and 5-A,D).

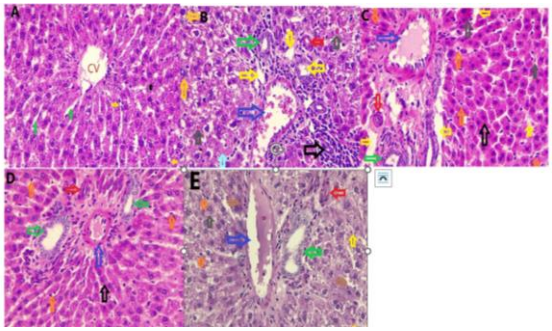

Fig. 3: A photomicrograph of a section of the liver of: A) Senile control group showing central vein (CV) at the center of hepatic lobule and cords of hepatocytes (black arrow) with rounded vesicular nuclei radiating from it. The hepatic cords are separated by blood sinusoids (green arrow) lined by Kupffer cells (yellow arrow). B) senile sildenafil treated group showing markedly congested portal vein (blue arrow), congested hepatic artery (red arrow) areas of necrosis and hemorrhage (grey arrows). Most hepatocytes are degenerated with reduction of number (aqua arrow). Cytoplasmic vacuolization (yellow arrows) in liver parenchyma with increased number of von-Kupffer cells (black arrow) were seen. C) senile vitamin C and sildenafil treated group showing the moderate congestion of portal tract with few inflammatory cell infiltrations around the portal vein (blue arrow), mildly congested hepatic artery (rad arrow). The bile duct shows normal appearance with no hyperplasia (green arrow). Areas of intercellular vacuolization (yellow arrows). Average hepatocytes with intercellular Kupffer cells (orange arrows) and few intercellular areas of hemorrhage and necrosis (grey arrows). D) senile vitamin C treated group (IVA) showing radiating and anastomosing cords of hepatocytes (black arrow). The portal triad shows a clear branch of portal vein (blue arrow), hepatic artery (red arrow) and bile duct (green arrow). The cords of hepatocytes are separated by hepatic sinusoid lined by von-Kupffer cells. E) senile sildenafil withdrawal group (VB) showing the mild congestion of portal tract with few inflammatory cell infiltrations around the portal vein (blue arrow), mildly congested hepatic artery (rad arrow). The bile duct shows normal appearance with no hyperplasia (green arrow). Areas of intercellular vacuolization (brown arrows). Average hepatocytes with intercellular Kupffer cells (orange arrows) and few areas of necrosis and hemorrhage (yellow arrows).

Group IIA (adult sildenafil treated group): It showed abnormal hepatocytes with indented nuclei, clumped chromatin, prominent nucleoli and discontinuous nuclear envelope (black arrow). The cytoplasm showed numerous clumped mitochondria, few rough endoplasmic reticula, many lysosomes, lipid droplets, dilated bile canaliculus and large electron dense granule of large Kupffer cell indicating its activity (fig. 4D).

Group IIA (senile sildenafil treated group): It showed abnormal hepatocytes with destructed cell membranes and small nuclei showing clumped chromatin and irregular nuclear membrane. The cytoplasm showed numerous swollen clumped mitochondria with unclear cristae, vacuoles, few dilated rough endoplasmic reticulum, dilated blood sinusoids, glycogen, lipid and secretory droplets, many large cytoplasmic vacules, and wide areas of rarefied cytoplasm (fig 5B).

Group IIIA (adult vitamin C and sildenafil treated group): It showed mild changes of hepatocytes with preserved hepatic architecture. The hepatocytes showed vesicular euchromatic nuclei, prominent nucleolus, and intact, regular nuclear envelope. The cytoplasm showed dispersed small or clumped mitochondria, rough endoplasmic reticulum, few small vacules, scattered secretory granules, average bile canaliculi and average blood sinusoids with average endothelial cells (fig. 4C).

Group IIIB (senile vitamin C and sildenafil treated group): It showed mild changes of hepatocytes with preserved hepatic architecture. The hepatocytes showed vesicular euchromatic nuclei with intact nuclear envelope. The cytoplasm is well organized and showed dispersed small ovoid mitochondria, few rough endoplasmic reticulum, few small vacules, scattered secretory granules, large electron dense granules and average endothelial cells (fig. 5C).

Group 5 (sildenafil withdrawal group): It showed a picture of liver injury induced by sildenafil treated adult and senile groups, but less severe (fig 4E and 5E).

DISCUSSION

Numerous studies have shown that conservative treatment of both-bone forearm fractures in older children, results in problems. This is because acquiring and sustaining a reduction in cast is challenging. As the edema subsides and the muscles spasm, a subsequent fracture displacement

As liver has the sole role in detoxification and excretion of drugs, administration of injurious materials, it is the first organ to be affected through the portal circulation. Sildenafil is mainly metabolized in the liver, and there are major considerations when prescribing sildenafil dose to neonates, children, and patients with hepatic impairment. [4]

Female sex is known to be less susceptible to oxidative stress owing to estrogen's protection by decreasing oxidative stress and increasing antioxidant defenses. Male animals were favored as they have relatively constant hormone levels which help to pass over the role that could such hormones

play in many inflammatory conditions, so it excluded effects of hormonal changes of estrous cycles in female animals[13].

In the present work, the mortality among the rats, all groups showed no death except senile sildenafil treated group that showed a low mortality rate as one rat only died (16% mortality rate) and senile sildenafil withdrawal group that showed a low mortality rate as one rat only died (16% mortality rate) which is not significant. These results were in agreement with another study[14].

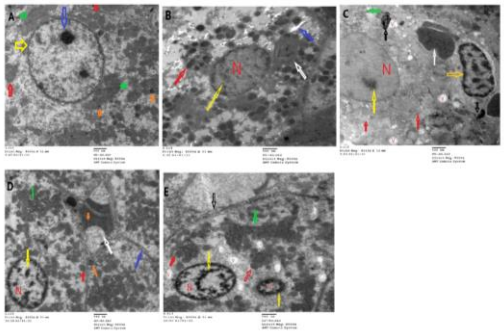

Fig. 4: An electron photomicrograph of sections of the liver of: A) adult control group showed hepatocyte with rounded vesicular euchromatic nucleus with regular intact nuclear membrane (yellow arrow) and prominent nucleoli (blue arrow). The cytoplasm contains many mitochondria with prominent cristae (red arrows), rough Endoplasmic Reticulum (green arrows) and lipid droplets (orange arrows). B) adult sildenafil treated group showed hepatocyte with small nucleus showing clumped chromatin (yellow arrow) and discontinuous nuclear envelope (black arrow). The cytoplasm showed numerous clumped mitochondria (red arrow), small vacuoles (blue arrow) and large electron dense granule (white arrow). C) adult vitamin C and sildenafil treated group showed a hepatocyte with a vesicular euchromatic nucleus (N), condensed chromatin (yellow arrow) and intact nuclear envelope. The cytoplasm showed clumped mitochondria (red arrows), few rough endoplasmic reticula (green arrow), few small vacuoles (V), scattered secretory granules (black arrows), and average blood sinusoids (white arrows) with average endothelial cells (orange arrow). D) adult vitamin C treated group (VA) showed hepatocytes with vesicular euchromatic nuclei regular intact nuclear membrane (N), condensed chromatin, prominent nucleoli (yellow arrow) and intact cell membrane (blue arrow). Average cytoplasm showing dispersed ovoid mitochondria (red arrow), rough Endoplasmic Reticulum (green arrow), glycogen granules (orange arrow) and average blood sinusoids (white arrow). E) adult sildenafil withdrawal group (VA) showed abnormal binuclear hepatocyte with small nuclei (N) with clumped chromatin (yellow arrow) specially at the periphery of the nucleus and intact cell membrane with average junction complex (black arrow). The cytoplasm showed numerous clumped mitochondria (red arrows), few small vacuoles (V), and compressed areas of rough endoplasmic reticulum (green arrow) (TEM, X 15000).

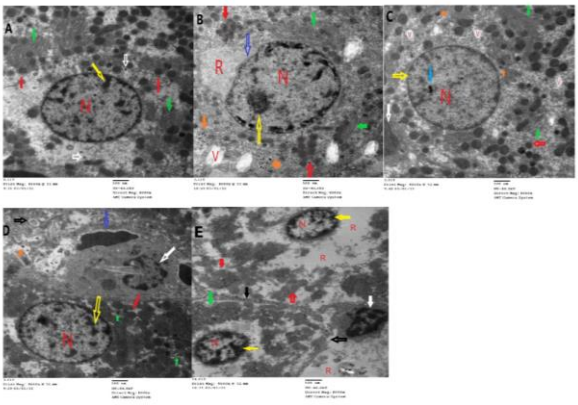

Fig. 5: An electron photomicrograph of sections of the liver of: A) adult control group showed the hepatocyte with rounded vesicular euchromatic nucleus with regular intact nuclear membrane (yellow arrow) and prominent nucleoli (blue arrow). The cytoplasm contains many mitochondria with prominent cristae (red arrows), rough Endoplasmic Reticulum (green arrows) and lipid droplets (orange arrows). B) adult sildenafil treated group showed hepatocyte with small nucleus showing clumped chromatin (yellow arrow) and discontinuous nuclear envelope (black arrow). The cytoplasm showed numerous clumped mitochondria (red arrow), small vacuoles (blue arrow) and large electron dense granule (white arrow). C) adult vitamin C and sildenafil treated group showed a hepatocyte with a vesicular euchromatic nucleus (N), condensed chromatin (yellow arrow) and intact nuclear envelope. The cytoplasm showed clumped mitochondria (red arrows), few rough endoplasmic reticulum (green arrow), few small vacuoles (V), scattered secretory granules (black arrows), and average blood sinusoids (white arrows) with average endothelial cells (orange arrow). D) adult vitamin C treated group (VA) showed hepatocytes with vesicular euchromatic nuclei regular intact nuclear membrane (N), condensed chromatin, prominent nucleoli (yellow arrow) and intact cell membrane (blue arrow). Average cytoplasm showing dispersed ovoid mitochondria (red arrow), rough Endoplasmic Reticulum (green arrow), glycogen granules (orange arrow) and average blood sinusoids (white arrow). E) adult sildenafil withdrawal group (VA) showed abnormal binuclear hepatocyte with small nuclei (N) with clumped chromatin (yellow arrow) specially at the periphery of the nucleus and intact cell membrane with average junction complex (black arrow). The cytoplasm showed numerous clumped mitochondria (red arrows), few small vacuoles (V), and compressed areas of rough endoplasmic reticulum (green arrow).

These results were in agreement with Abbott et al., 2004) that showed the separation between exposure to sildenafil citrate of animals at large nontoxic doses and the much smaller human therapeutic exposure. This profile highlights the very low risk of human toxicity for sildenafil.[13]

Regarding body weight the results of our study agreed with Abbott et al., that showed that body

weight was decreased in high-dose rats compared to the controls.[15].

Another study reported that SC may have the effect of reducing the rate of body weight loss in diabetic rats receiving insulin treatment.[16]

The results of our study showed that sildenafil citrate induced progressive hepatic injury with the increase of age of albino rats which is similar to the previous study.[10]

The current study showed that the intake of sildenafil citrate in adult rats caused congestion of the portal veins, central vein and hepatic artery and severe hyperplasia around bile ducts. Some hepatocytes showed vacuolated cytoplasm and areas of hemorrhage and necrosis.

TEM showed abnormal hepatocytes with indented nuclei, clumped chromatin, prominent nucleoli and discontinuous nuclear envelope (black arrow). The cytoplasm showed numerous clumped mitochondria, few rough endoplasmic reticulum, many lysosomes, lipid droplets, dilated bile canaliculus, large electron dense granule and large Kupffer cell indicating its activity.

These results are similar to the previous study that provided evidence that SC drug may cause serious effect on the liver[17], which becomes worse with advance in age.[10]

Our results were similar to the study performed injection of sildenafil (1µg/g body wt.) in adult rats for 2 weeks, in the form of congestion of central vein and vacuolization of the cytoplasm.[18]

Cytoplasmic vacuolization is one of the primary responses to any cellular injury. It occurs because of increased permeability of cell membranes leading to increase water content inside cells, lysosomal enzymes leakage and cytoplasmic degeneration.[19]

Nuclear changes as chromatin condensation were observed in sildenafil overdoses while karyolysis resulted from chromatin matter dissolution of a necrotic cells.[20]

Our study showed that the treated senile rats showed marked congestion in both portal and central veins, increased number of von-Kupffer cells and Areas of hemorrhage and necrosis. Most hepatocytes were degenerated with reduction in number. Marked intercellular vacuolizations. These results are similar to the previous study.[14]

The advanced liver changes in senile rats may be explained by the fact that there is a reduction in drugs clearance with age.[21] Studies in humans showed that aging is associated with reduction in hepatic drug metabolism. Cytochrome P450 in human decreases 30% after the age of 70 years. Reduced drug metabolism may be also due to reduction of liver volume and the blood flow.[22]

The administration of Vitamin C to SC treated albino rats in our study decreases the hepatic effect of SC that in agreement with other studies[23,24]. Ascorbic acid protected against acetaminophen hepatotoxicity as it inhibits oxidative stress and markedly decreased the hepatocyte cell death and liver fibrosis caused by bile acid[25]. Moreover, it has been reported that ascorbic acid decreased hepatic fibrosis by suppression of oxidative stress. It decreased protein peroxidation and lipid products.[26]

Many of the mentioned hepatic changes in adult and senile sildenafil treated groups were reduced but not reversed completely after discontinuation of SC, suggesting improved structure of the liver, even if control levels were not restored. Similar findings were mentioned by.[9]

CONCLUSION

This study proved histologically that SC has a hepatic injury effect on the liver of adult and senile male albino rats, even after stoppage of the drug and the effect was more severe in senile than adult rats.

Also, this study showed that vit C has an evident protective effect on liver injury induced by SC. Therefore, concomitant use of vit C with SD is suggested in patients treated with SC.

Conflict of interest : none

REFERENCES

1. Ghofrani HA, Osterloh IH and Grimminger F. Sildenafil from angina to erectile dysfunction to pulmonary hypertension and beyond. *Nat Rev Drug Discov*. 2006; 5:689–702.
2. McMurray GJ, Feldman AR, Auerbach MS, et al. Long-term safety and effectiveness of sildenafil citrate in men with erectile dysfunction. *Ther Clin Risk Manag.* 2007; 3(6): 975–81.
3. Adinoyi SS. Sildenafil citrate in healthy and diseased hearts. *J Cardiol Cardiovasc Med*. 2021; 6: 33-9.
4. Simsek T, Ersoy OF, Ozsoy Z, et al. Effect of sildenafil citrate on the liver structure and function in obstructive jaundice: An experimental study. *Turk J Surg*. 2018; 34: 111-6.
5. Baiocchi L, Glaser S, Francis H, et al. Impact of aging on liver cells and liver disease: focus on the biliary and vascular compartments. *Hepatology Communications.* 2021;5(7) 1125-37.
6. Huseyin S, Guclu O, Yuksel V, et al. Avoiding liver injury with papaverine and ascorbic acid due to infrarenal cross-clamping: an experimental study. Braz. *J. Cardiovasc. Surg*. 2017; 32(3):197-201.
7. Pallav S. The Laboratory Rat: Relating Its Age with Human's. *Int J Prev Med*. 2013; 4(6): 624–30.
8. Eweka AO and Eweka AB. The effects of sildenafil citrate on the superior colliculus of adult Wistar rat a histological study. *Biology and Medicine.* 2010; 2(1): 24-9.

9. Abdel-Hafez AM and Othman MA. Effect of sildenafil citrate on the structure of rat liver: a histological, histochemical and immunohistochemical study. *The Egyptian Journal of Histology Egypt J Histol*. 2013; 36: 991-1003.

10. Hashish H. Effect of age on the sildenafil impact on the histological and ultra-structure of the liver in male albino rat. *Herbal open access journal (HOAG) Journal of Histology & Histopathology*. 2016;3 (5): 2055-91.

11. Padayatty JS, Wang Y, Riordan DH, et al. Vitamin C pharmacokinetics: implications for oral and intravenous use. *Ann Intern Med*. 2004 6;140(7): 533-7.

12. Suvarna SK, Layton C and Bancroft JD. Bancroft's theory and practice of histological techniques, 8th ed, Elsevier, London, Edinburgh, New York, Philadelphia, St Louis, *Sydney and Toronto;* 2019; 40-61, 126-37, 165, 66 and 434-75.

13. Hegazy AA, Abd Al Hameed EA, El-Wafaey DI, et al. Potential role of Moringa Oleifera in alleviating paracetamol-induced nephrotoxicity in rat. Eur. J. Anat. 2020;24(3):179-91.

14. Zaghlol D, Ouis S, Gad W, et al. Effects of Administration of Sildenafil Citrate on Histological Structure of the Testis and the Possible Protective Role of Selenium in Adult Albino Rat by Histological and Immuno-Histological *Method. Med. J. Cairo Univ.* 2020;88(5): 2329-36.

15. Abbott D, Comby P, Charuel C, et al. Preclinical safety profile of sildenafil. *International Journal of Impotence Research.* 2004; 16: 498–04.

16. Ngulde SI, Umaru B, Mahre MB, et al. Effect of sildenafil citrate on the body weight, blood glucose and white blood cell count during wound healing process in diabetic rats. *Kanem Journal of Medical Sciences.* 2016; (10)1: 13-20.

17. Jarrar BM and Almansour MI. Hepatic histological alterations and biochemical changes induced by sildenafil overdoses. *Pak. J. Pharm*. Sci. 2015;28(6):2119-27.

18. Suriyakumari K, Dayakumar R and Panneerselvam A. Sildenafil citrate induced histological changes in liver of albino mice. *International J. of Healthcare and Biomedical Research.* 2015; 3:94-8.

19. Shubin AV, Demidyuk IV, Komissarov AA, et al. Cytoplasmic vacuolization in cell death and survival. *Oncotarget J.* 2016; 7, (34) 55863-89.

20. Pandey G, Srivastava DN and Madhuri SA standard hepatotoxic model produced by paracetamol in rat. *Toxicology international*. 2008; 15:69- 70.

21. Kim H, Kisseleva T and Brennerb D. Aging and liver disease. *Curr Opin Gastroenterol*. 2015 May; 31(3): 184–91.

22. Mangoni A and Jackson S. Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. *Br J Clin Pharmacol.* 2004 Jan; 57(1): 6–14.

23. Attar M. Hepatoprotective Influence of Vitamin c on Thioacetamide Induce Liver Cirrhosis in Wistar Male Rats. *Journal of Pharmacology and Toxicology.* 2011 6(3): 218-33.

24. Al-Bideri AW. Histopathological study on the effect of antioxidants (vitamin E and selenium) in hepatotoxicity induced by lead acetate in rats. *Q. M. J.* 2011; 7(12): 142-55.

25. Kurahashi T, Lee J, Nabeshima A, et al. Ascorbic acid prevents acetaminophen-induced hepatotoxicity in mice by ameliorating glutathione recovery and autophagy. Arch. Biochem. *Biophys*. 2016; 604:36-46.

26. Prathibha P, Rejitha S, Harikrishnan R, et al. Additive effect of alpha-tocopherol and ascorbic acid in combating ethanol-induced hepatic fibrosis. *Redox Rep*. 2013; 18(1): 36- 46.