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Effects of Anti-erectile Dysfunction Drug on some Key Tissues in Healthy Male Rats

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ABSTRACT

Erectile Dysfunction (ED) occurs in folk men which poses great threat to couples awaiting children. The liver is one of the most vital organs involved in drug detoxification process. However, the concomitant toxic effects of Viagra on liver with other tissues are unknown. The present study was aimed to elucidate the effects of therapeutic doses of orally administered Viagra on the antioxidant defense status of liver, kidney, spleen and lung tissues in healthy rats. Wistar healthy albino Rats were orally administered with various therapeutic doses (20 mg kg⁻¹ b.wt.) of Sildenafil citrate (viagra) for 4 weeks exhibited mild hepatocyte necrosis, periportal cellular infiltration by mononuclear cells and no visible lesions in kidney cells. This is accompanied with non-significant elevated plasma alanine amino transaminase (ALT) and aspartic amino transaminase (AST) levels, decreased hepatic MDA level and increased levels of MDA in kidney, spleen and lung. GSH was increased in liver, kidney, spleen and decreased in lung. CAT and SOD activity were elevated in liver, kidney and spleen. Lung showed high activity of SOD and low activity of CAT. Hence, administration of Viagra potentiates both enzymatic and non-enzymatic antioxidants in liver, kidney and spleen while it may promote inflammatory response in male rats. The present study provides evidence for stabilizing antioxidant defense systems in liver, kidney, spleen and may be a contributory factor for lung inflammatory responses.

Key words: Sildenafil citrate, antioxidants, key tissues, lung, inflammation

INTRODUCTION

Sildenafil citrate is a white to off-white crystalline powder with a solubility of 3.5 mg mL⁻¹ in water. Viagra has the molecular weight of 666.7 mg. It is formulated as blue, film-coated rounded-diamond shaped tablet (Fig. 1). In addition to the active ingredients, each tablet contains some inactive ingredients such as microcrystalline cellulose, anhydrous dibasic calcium phosphate,

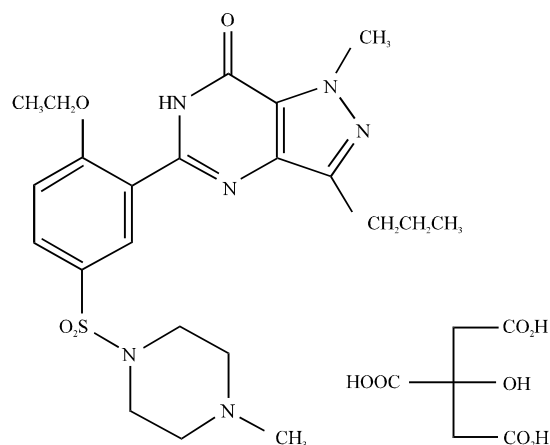


Fig. 1: Sildenafil citrate is a white to offwhite crystalline powder with a solubility of 3.5 mg mL^{-1} in water, Viagra has the molecular weight of 666.7 mg, It is formulated as blue, filmcoated rounded diamond shaped tablet, In addition to the active ingredients, each tablet contains some inactive ingredients such as microcrystalline cellulose, anhydrous dibasic calcium phosphate, croscarmellose sodium, magnesium stearate, hypromellose, titanium dioxide, lactose, triacetin and aluminium lake

croscarmellose sodium, magnesium stearate, hypromellose, titanium dioxide, lactose, triacetin and Aluminium Lake (Sivasankaran *et al.*, 2007). Erectile Dysfunction (ED) is a life gruesome disorder that always does not manifest itself until it becomes an awful threat particularly to couples awaiting children. The treatment of ED has changed significantly in the past few years (Shabsigh and Anastasiadis, 2003). Sildenafil citrate tablets became one of the most prescribed medicine worldwide (McCullough, 2002). The patients noticed that the drug showed an erectogenic effect (Boolell *et al.*, 1996) when it was first used. It is now well established that the drug (Viagra) competitively inhibits phosphodiesterase, type 5 (PDE 5) enzyme activity, due to the analogy of its molecular structures (Uckert *et al.*, 2000).

The inhibition of PDE 5 activity increases the availability of cyclic Guanosine Mono-Phosphate (cGMP) in the penile and corpus cavernosal muscles. This leads to relaxation of the muscles and increased blood flow and consequently promotes erection (Dey and Shepherd, 2002; Gragasin *et al.*, 2004).

Oxygen is one of the most abundant elements in our world, constituting 21% of the air (Cantin, 1999; Valleyathan and Shi, 1997). It is essential for the oxidation of organic compounds by which mammalian cells generate the energy needed to sustain life. However, excess oxygen (pro-oxidant) may also damage the lung and other tissues (Fig. 5). Inhaled ozone and nitric oxide may induce toxic processes that impair lung function (Cantin, 1999; Doelman and Bast, 1990; Tanswell and Freeman, 1995; Meyer, 1994; Valleyathan and Shi, 1997). Under normal conditions, potentially toxic oxygen metabolites are generated at a low level in lung cells by the transfer of a single electron during aerobic metabolism (Clark and Lambertsen, 1971; Crystal, 1991; Freeman and Crapo, 1982). The resulting reactive oxygen species (ROS), which include hydroxyl radicals (OH^\bullet), superoxide (O_2^-) and hydrogen peroxide (H_2O_2), play an integral role in the modulation of several physiological functions but can also be destructive if produced in excessive

amounts (Conner and Grisham, 1996; Doelman and Bast, 1990; Meyer, 1994; Rahman and MacNee, 1999; Repine *et al.*, 1997; Schraufstatter, 1996). Similarly, reactive nitrogen species (RNS) such as nitric oxide, nitrite and peroxynitrite (ONOO₂) are both physiologically necessary and potentially destructive.

Another oxygen-mediated mechanism of damage is inflammation, during which leukocytes, macrophages and mast cells release mediators that may cause broncho-constriction and edema as observed during an asthmatic reaction (Calhoun *et al.*, 1992; Doelman and Bast, 1990; Kingsley *et al.*, 1998). Lung tissue can also be destroyed during reperfusion after an ischemic period such as produced by surgery (Erzurum *et al.*, 1993; Pietarinen-Runtti *et al.*, 1998; Meyer, 1994; Rahman and MacNee, 1999; Repine *et al.*, 1997; Wright *et al.*, 1994). All these mechanisms have one thing in common; damage mediated by oxidants and nitrogen species (Fridovich, 1978; Heffner and Repine, 1989). The resulting excess oxygen species can damage major cellular components, including membrane lipids, protein, carbohydrates and DNA. The pathophysiological consequences of the injury are inflammation and widespread tissue damage (Freeman and Crapo, 1982). In the light of the above findings; the concomitant toxic effects of Viagra on liver with other tissues are largely unknown. However, in this study, we elucidate the effects of therapeutic dose of orally administered Sildenafil citrate (Viagra) on the antioxidant defense status of liver, kidney, spleen and lung tissues in male healthy rats. Also, its toxicity mechanism of action in inflammatory response was expounded.

MATERIALS AND METHODS

Chemicals: Sildenafil citrate (Viagra), hydrogen peroxide and thiobarbituric acid (TBA) were purchased from Sigma (St Louis, MO, USA). All chemicals and reagents used in the present study were of analytical grade and were obtained from Sigma Chemical Company, Saint Louis, USA.

Animals protocol: Twenty-five healthy adult male wistar rats weighing approximately 200-220 g obtained from the Department of Physiology, University of Ibadan, Nigeria were randomly assigned into 5 groups of 5 animals per group. They were housed in a plastic suspended cage placed in a well ventilated rat house, provided rat pellets and water *ad libitum* and subjected to a natural photoperiod of 12 h light and 12 h dark cycle, 50% humidity and at 30±2°C. Rats in Group I served as control and were administered distilled water. Animals in Groups II, III, IV and V were orally administered at the different dose levels of 20 mg kg⁻¹ b.wt. per 30 days in four divided doses of 50, 100, 150 and 200 mg kg⁻¹ b.wt. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health.

Necropsy: The animals were fasted overnight, weighed and sacrificed by decapitation 24 h after the last treatment. Liver, kidney, spleen and lung were removed and cleared of adhering tissues, washed in ice cold 1.15% potassium chloride and dried with blotting paper. The blood was allowed to clot and centrifuged at low speed (3000 g) at room temperature for 15 min. The supernatant (serum) was removed and used for biochemical analysis.

Enzymatic and non-enzymatic antioxidant assays: The liver, kidney spleen and lung were homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% KCl and the homogenate was

centrifuged at 10,000 g for 15 min at 4°C. The supernatant was collected for the estimation of CAT activity using hydrogen peroxide as substrate according to the method of Sinha (1972). SOD activity was determined by measuring the inhibition of autoxidation of epinephrine at pH 10.2 at 30°C according to Misra and Fridovich (1972). Protein concentration was determined by the method of Lowry *et al.* (1951). Reduced GSH was determined at 412 nm using the method described by Ellman (1959).

Lipid peroxidation assay: Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Ohkawa and expressed as mmol g⁻¹ tissue.

Toxicological analysis: Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured by the method of Reitman and Frankel (1957).

Histological analysis: The liver and kidney were collected and cut into two pieces, fixed in Bouin's fixative for 6 h, then transferred to formalin, sectioned and stained routinely with hematoxylin and eosin for histopathologic examination. Permanent photomicrograph was obtained using light microscope.

Statistical analysis: All the results obtained are expressed as Mean±SD of five rats in each group. Statistical evaluation was done by using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The statistical significance was at a p<0.05.

RESULTS

The toxic effects of therapeutic doses of Viagra on the antioxidant defense status of liver, kidney, spleen and lung tissues in healthy rats were investigated. The results revealed that the LPO (MDA) levels of the liver were significantly (p<0.05) decreased in a non-dose dependent manner by 12.78, 30.33, 28.32 and 43.36%, respectively compared with the control group (Table 1). Conversely, the LPO (MDA) levels in kidney tissue increased significantly (p<0.05) in dose dependent fashion by 14.57, 43.71, 74.29 and 142.29%, respectively against the corresponding control (Table 2). Similarly, there was a significant (p<0.05) marked elevation of spleen LPO (MDA) levels in a dose dependent manner by 24.81, 96.74, 144.36 and 197.74%, respectively against the corresponding control (Table 3). In addition, the levels of MDA in the lung of Viagra treated-rats was significantly (p<0.05) reduced by 6.78, 35.93, 50.17 and 68.14%, respectively against the corresponding control group (Table 4). However, the spleen was highly susceptible to lipid damage than kidney and the lung (Spleen>Kidney>Lung).

Table 1: Effects of antiaerectile dysfunction drug (Viagra) on lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase and total protein in the liver of healthy wistar albino rats after 30 days of administration

Dose (mg)	LPO	GSH GSH	SOD	Catalase	Total protein
0	3.99±0.56	21.75±2.11	2.43±0.63	19.31±5.11	1.0±0.18
50	3.49±0.41*	24.43±1.44*	3.24±1.03*	22.50±0.46*	1.0±0.33
100	2.78±0.66*	27.70±4.96*	3.32±1.02*	23.59±5.42*	1.1±0.24
150	2.86±0.17*	28.82±3.26*	3.89±0.88*	24.11±6.08*	1.1±0.11
200	2.26±0.48*	35.78±6.28*	4.33±0.41*	28.42±3.27*	1.2±0.12

Significantly different (*p<0.05) (ANOVA) against control, values are expressed in Mean±SD of five rats per group, LPO (unit mg⁻¹ protein) GSH (mmoles min⁻¹ mg⁻¹ protein), SOD (U mg⁻¹ protein), CaT (mmoles min⁻¹ mg⁻¹ protein), Total protein (mg protein)

Table 2: Effect of antiaerectile dysfunction drug (Viagra) on lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase and total protein in the kidney of healthy wistar albino rats after 30 days of administration

Dose (mg)	LPO	GSH	SOD	Catalase	Total protein
0	3.50±0.35	27.29±3.50	7.44±1.75	44.37±5.72	0.278±0.02
50	4.01±0.13*	29.72±4.96*	8.61±2.27*	46.12±3.69*	0.29±0.030
100	5.03±0.54*	31.83±2.60*	9.56±3.20*	48.15±4.58*	0.34±0.040
150	6.11±2.13*	35.20±0.81*	10.66±1.18*	49.99±1.732*	0.38±0.140
200	8.48±2.17*	37.47±3.33*	11.72±2.12*	51.88±9.48*	0.41±0.140

Significantly different (*p<0.05) (ANOVA) against control, values are expressed in Mean±SD of five rats per group, LPO (unit mg⁻¹ protein) GSH (mmoles min⁻¹ mg⁻¹ protein), SOD (U mg⁻¹ protein), CaT (mmoles min⁻¹ mg⁻¹ protein), Total protein (mg protein)

Table 3: Effects of antiaerectile dysfunction drug (Viagra) on lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase and total protein in the spleen of healthy wistar albino rats after 30 days of administration

Dose (mg)	LPO	GSH	SOD	Catalase	Total protein
0	3.99±0.26	617.51±45.660	6.24±1.32	25.56±3.57	0.51±0.09
50	4.98±0.84*	902.26±51.340*	9.96±1.46*	31.03±2.57*	0.34±0.06
100	7.85±1.14*	1534.55±95.710*	13.57±1.66*	32.67±2.63*	0.18±0.05
150	9.75±2.59*	2418.80±287.49*	17.25±5.89*	49.63±3.36*	0.08±0.02
200	11.88±1.04*	2494.37±160.06*	20.92±3.44*	62.76±2.34*	0.02±0.01

Significantly different (*p<0.05) (ANOVA) against control, values are expressed in Mean±SD of five rats per group, LPO (unit mg⁻¹ protein) GSH (mmoles min⁻¹ mg⁻¹ protein), SOD (U mg⁻¹ protein), CaT (mmoles min⁻¹ mg⁻¹ protein), Total protein (mg protein)

Table 4: Effect of antiaerectile dysfunction drug (Viagra) on lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase and total protein in the lung of healthy wistar albino rats after 30 days of administration

Dose (mg)	LPO	GSH	SOD	Catalase	Total protein
0	2.95±0.25	878.42±53.53	9.68±2.00	35.73±7.67	0.38±0.06
50	3.15±1.09*	828.59±83.23*	12.13±1.02*	33.31±2.08*	0.38±0.03
100	4.01±0.55*	647.13±73.10*	12.40±1.88*	28.57±4.74*	0.41±0.07
150	4.43±0.75*	498.57±108.76*	14.12±2.07*	23.43±1.78*	0.68±0.17
200	4.96±0.31*	382.93±53.64*	15.48±1.38*	20.37±0.96*	0.73±0.17

Significantly different (*p<0.05) (ANOVA) against control, values are expressed in Mean±SD of five rats per group, LPO (unit mg⁻¹ protein) GSH (mmoles min⁻¹ mg⁻¹ protein), SOD (U mg⁻¹ protein), CaT (mmoles min⁻¹ mg⁻¹ protein), Total protein (mg protein)

The reduced liver GSH levels were significantly (p<0.05) elevated in a dose-dependent manner by 12.32, 27.37, 32.51 and 64.51%, respectively (Table 1) against the control group. Similarly, the kidney GSH levels of treated-rats with the anti-erectile dysfunction drug (Viagra) increased significantly (p<0.05) in dose dependent manner by 8.90, 16.64, 28.98 and 37.30%, respectively compared to the control group (Table 2). There was a significant (p<0.05) marked elevation of spleen reduced GSH levels in a dose-dependent manner by 46.11, 148.51, 291.70 and 303.94%, respectively compared to the control (Table 3). Conversely, the reduced GSH levels in the lung of treated rats decreased significantly (p<0.05) in dose dependent fashion by 5.67, 26.33, 43.24 and 56.41%, respectively against the corresponding control (Table 4).

Sub-chronic treated-rats with Viagra caused a significant (p<0.05) increased in the activity of superoxide dismutase (SOD) in all the treated tissues (Table 1-4). Their percentage increase against the corresponding control are given as thus: Liver increased significantly (p<0.05) by 33.33, 36.63, 60.08 and 78.60%, respectively (Table 1), kidney increased significantly (p<0.05) by 15.73, 28.49, 43.28 and 57.53%, respectively (Table 2), spleen increased significantly (p<0.05) by 59.87, 117.82, 176.89 and 235.79%, respectively (Table 3) and the lung increased significantly (p<0.05) by 25.31, 28.10, 25.31 and 59.92%, respectively (Table 4).

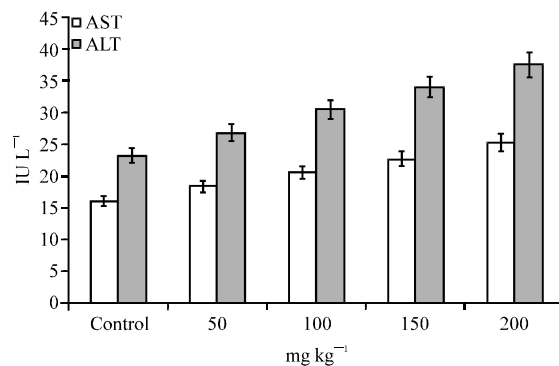


Fig. 2: Effects of antiaerectile dysfunction drug on plasma ALT and AST level, Data are expressed as the Mean±SD, (*p<0.05) compared with the control group

Furthermore, liver catalase (CAT) activity was significantly ($p < 0.05$) elevated in a dose-dependent manner by 16.52, 22.16, 24.86 and 47.18%, respectively (Table 1) against the control group. Similarly, the kidney CAT activity of treated-rats with the drug (Viagra) increased significantly ($p < 0.05$) in dose dependent manner by 3.94, 8.52, 12.67 and 16.93%, respectively compared to the control group (Table 2). Also, the activity of CAT in the spleen significantly ($p < 0.05$) higher than the control group by 21.40, 27.82, 94.17 and 145.54%, respectively (Table 3). Conversely, catalase (CAT) activity in lung tissue decreased significantly ($p < 0.05$) in dose dependent fashion by 6.77, 20.04, 34.42 and 42.99%, respectively against the corresponding control (Table 4). The result then suggests that anti-erectile dysfunction drug (Viagra) promotes inflammatory response in the lung.

Administration of therapeutic dose of anti-erectile dysfunction drug (Viagra) in rats showed non-significantly ($p > 0.05$) higher plasma levels of alanine aminotransferase (ALT) and aspartic transaminase (AST) compared with the control group, indicating mild hepatic injury (Fig. 2).

Additionally, the histopathological examination revealed that therapeutic doses of Viagra showed mild hepatic necrosis, periportal cellular infiltration by mononuclear cells (Fig. 3) while no visible lesions in the kidney cells of rats (Fig. 4).

DISCUSSION

Sildenafil citrate (Viagra) is widely used as an effective and safe oral treatment for erectile dysfunction of various etiologies (Goldstein *et al.*, 1998). It is a potent and selective inhibitor of phosphodiesterase type 5 enzymes that breaks down cyclic guanosine monophosphate (cGMP) (Boolell *et al.*, 1996). Accumulation of cGMP inhibits the degradation of nitric oxide that is responsible for smooth muscle relaxation in the corpora cavernosa. Nitric oxide is released by intra-cavernous non-adrenergic, non-cholinergic nerve terminals. This does not only follow a central or local erectogenic stimulus but also during Rapid Eye Movement (REM) sleep (Burnett, 1997). Psychogenic Erectile Dysfunction (ED) patients are excellent candidates for sildenafil citrate as therapy (Eardley *et al.*, 2001). The drug has been reported to be effective in about 78% of patients with psychogenic ED (McMahon *et al.*, 2000; Farooq *et al.*, 2008). The preclinical and limited clinical data suggest that sildenafil citrate may have therapeutic potential in selected neurological disorders (Rosen, 2001). Similarly, sildenafil citrate shows some promise as a therapeutic agent in neurological disorders by preventing brain oxidative stress.

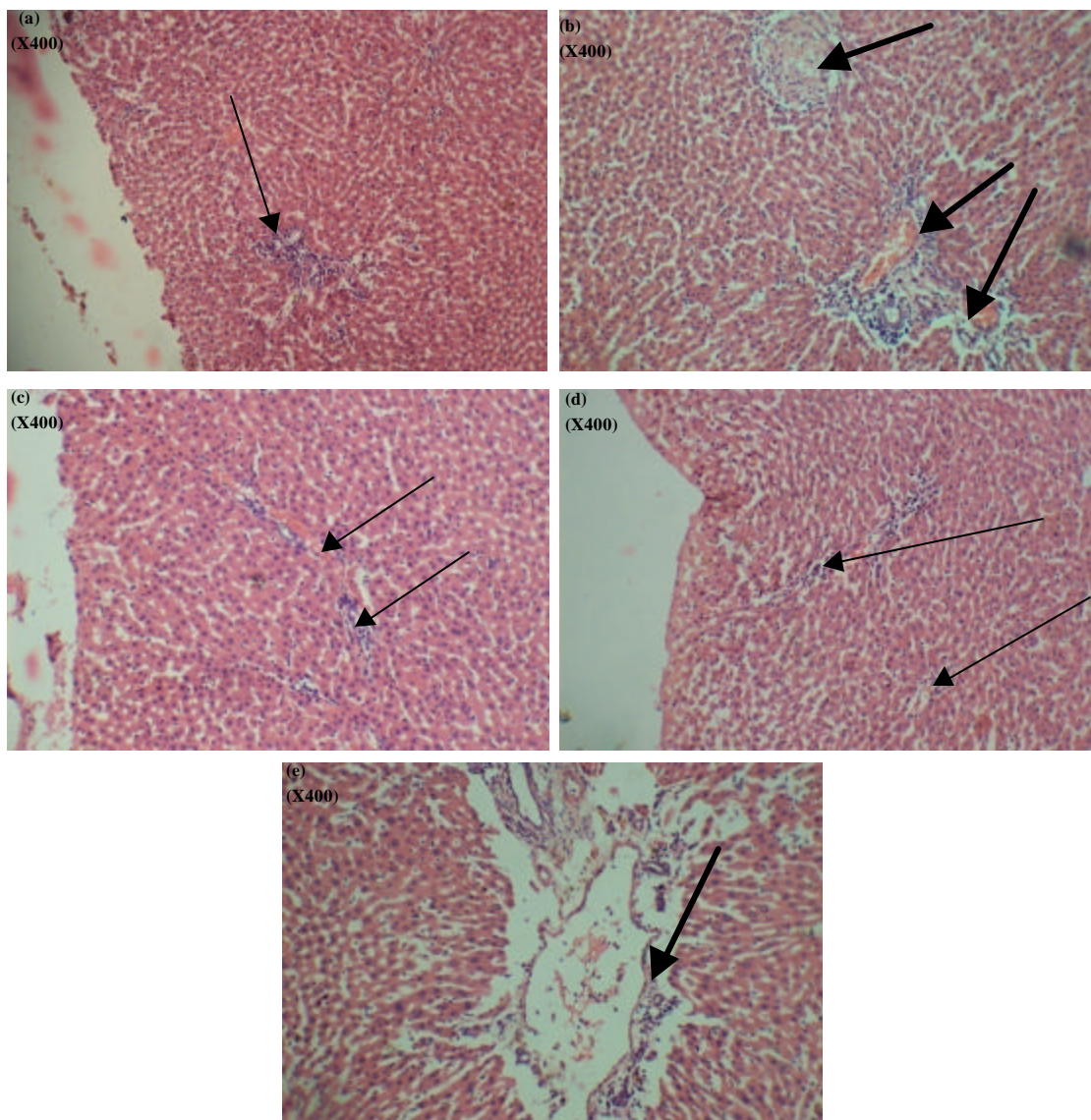


Fig. 3(a-e): Changes of liver histopathology in rats under various therapeutic doses treatment conditions, (a) Hematoxylin and eosin stained liver sections from rat's liver (Original magnification, X400), (b) Shows few multiple foci of hepatic necrosis and severe periportal cellular infiltration by mononuclear cells as depicted by the arrows, (c) Shows mild periportal cellular infiltration by mononuclear cells as shown by the arrows, (d) Shows mild periportal cellular infiltration by mononuclear cells as depicted by the arrows and (e) Shows periportal hepatic necrosis, with severe periportal cellular infiltration by mononuclear cells as revealed by the arrow

Our present study elucidated the possible toxic effects of Viagra on the antioxidant status of liver, kidney, spleen and the lung. It was observed that the Viagra significantly induced lipid peroxidation in the kidney, spleen and lung of the experimental animals in a dose dependent

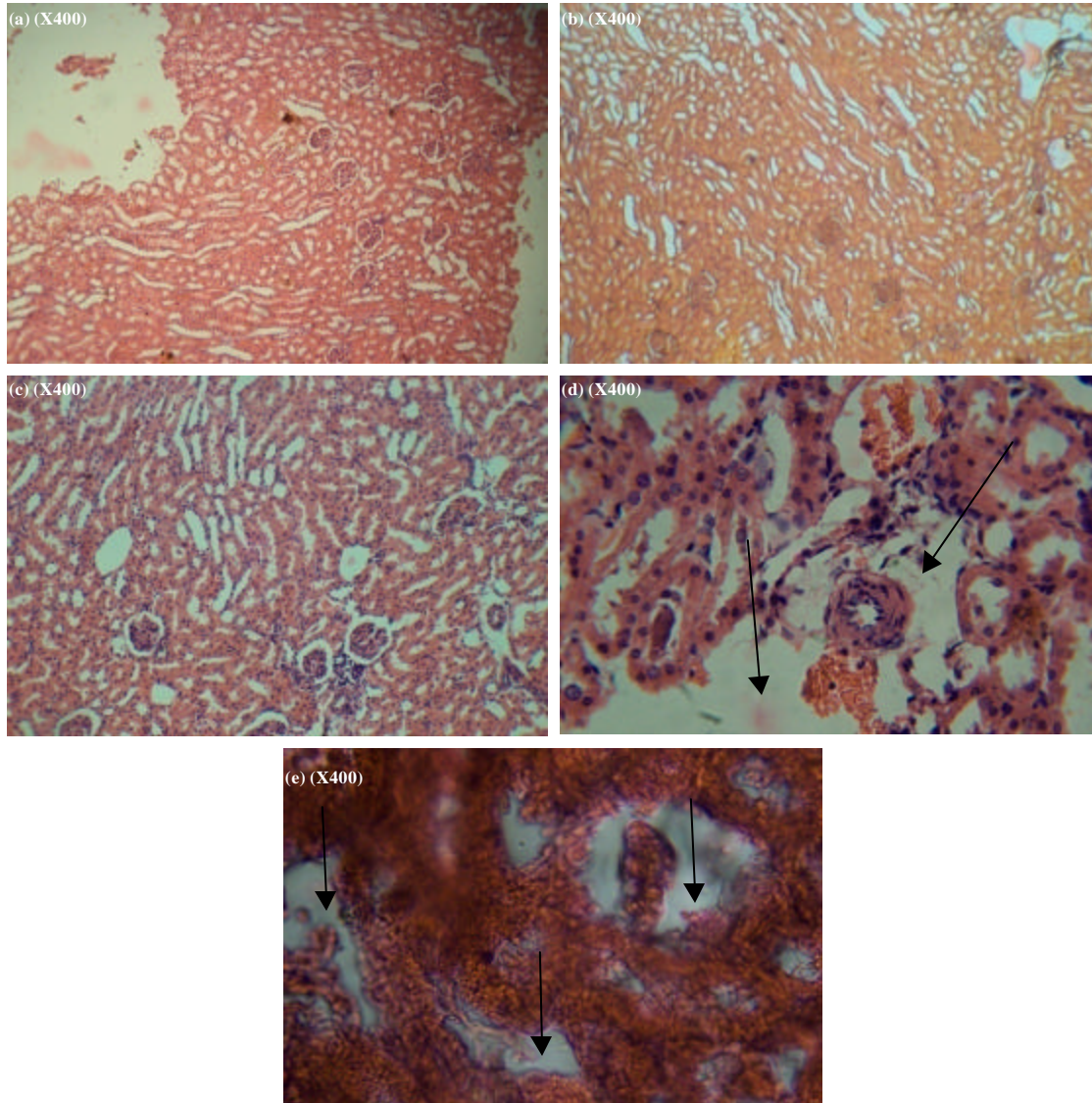


Fig. 4(a-e): Changes of kidney histopathology in rats under various therapeutic doses treatment conditions, (a) Hematoxylin and eosin stained kidney sections from rat's kidney, It shows numerous protein casts in the tubular lumen of renal tubules, (Original magnification, X400), (b) Shows no visible lesion (c) Shows no visible lesion, (d) Shows few protein casts in the tubular lumen of renal tubules as depicted by the arrows and (e) Shows few protein casts in the tubular lumen of renal tubules as depicted by the arrows

manner compared to the control group. The MDA of liver tissue decreased significantly in a dose dependent fashion in rats administered with Viagra. This observation corroborates with the findings of Abdel-Hamid *et al.* (2008) who reported that SC down-regulated TBARS in liver (Fig. 5 reaction 21, 22 and 23) tissues of diabetic rats. This reduction in hepatic TBARS

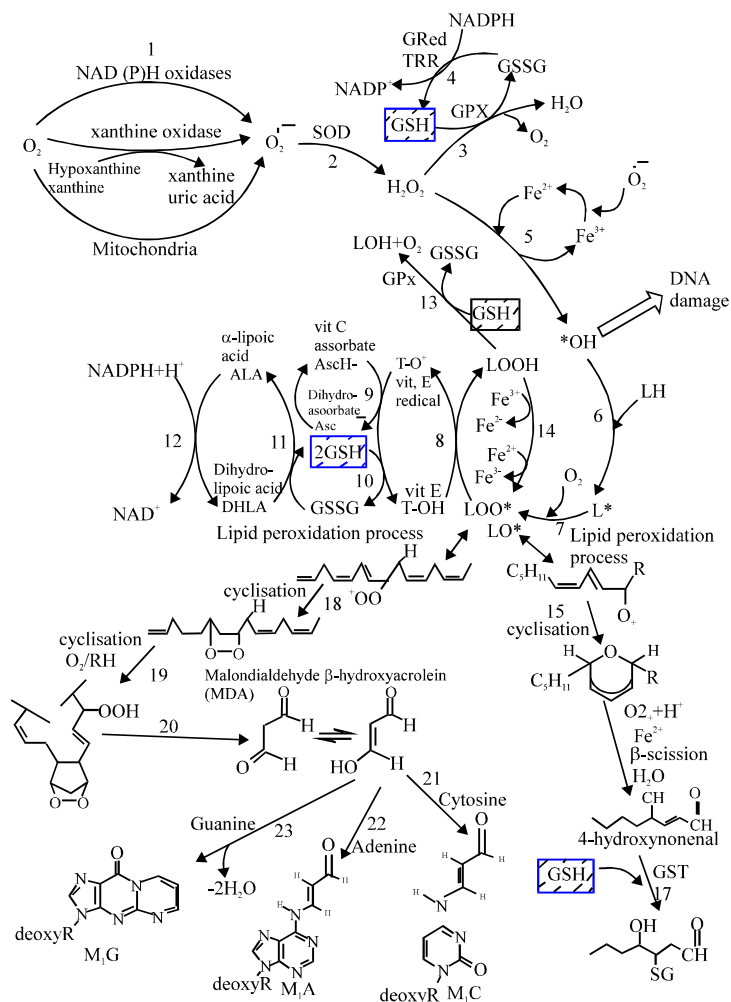


Fig. 5: Pathways of ROS formation, the lipid peroxidation process and the role of glutathione (GSH) and other antioxidant (Viagra) in the management of oxidative stress

Reaction 1: The superoxide anion radical is formed by the process of reduction of molecular oxygen mediated by NaD(P)H oxidases and xanthine oxidase or nonenzymatically by redoxreactive compounds such as the semiaubiquinone compound of the mitochondrial electron transport chain, Reaction 2: Superoxide radical is dismutated by the superoxide dismutase (SOD) to hydrogen peroxide. Reaction 3: Hydrogen peroxide is most efficiently scavenged by the enzyme glutathione peroxidase (GPx) which requires GSH as the electron donor (it occurs in liver, kidney and spleen following Viagra administration). Reaction 4: The oxidised glutathione (GSSG) is reduced back to GSH by the enzyme glutathione reductase (Gred) which uses NaDPH as the electron donor (it occurs in liver, kidney and spleen following Viagra administration), Reaction 5: Some transition metals (e.g. Fe^{2+} , Cu^+ and others) can breakdown hydrogen peroxide to the reactive hydroxyl radical (Fenton reaction; this occurs in the lung following Viagra administration). Reaction 6: The hydroxyl radical can abstract an electron from polyunsaturated fatty acid (LH) to give rise to a

carboncentred lipid radical ($L\bullet$) (occurs mostly in the lung following Viagra administration), Reaction 7: The lipid radical ($L\bullet$) can further interact with molecular oxygen to give a lipid peroxy radical ($LOO\bullet$) (occurs mostly in the lung following Viagra administration). If the resulting lipid peroxy radical $LOO\bullet$ is not reduced by antioxidants, the lipid peroxidation process occurs (reactions 18-23 and 15-17), Reaction 8: The lipid peroxy radical ($LOO\bullet$) is reduced within the membrane by the reduced form of Viagra ($VaOH$) or reduced form of Vitamin E ($TaOH$) resulting in the formation of a lipid hydroperoxide and a radical of Viagra ($VaO\bullet$) or a radical of Vitamin E ($TaO\bullet$), Reaction 9: The regeneration of Vitamin E or Viagra by Vitamin C: The Viagra radical ($VaO\bullet$) is reduced back to Viagra ($VaOH$) by ascorbic acid (the physiological form of ascorbate is ascorbate monoanion, $ascHa$) leaving behind the ascorbyl radical ($asc\bullet a$), Reaction 10: The regeneration of Viagra by GSH: The oxidised Viagra radical ($VaO\bullet$) is reduced by GSH (occurs mostly in liver, kidney and spleen), Reaction 11: The oxidised glutathione (GSSG) and the ascorbyl radical ($asc\bullet a$) are reduced back to GSH and ascorbate monoanion, $ascHa$, respectively, by the dihydrolipoic acid (DHLA) which is itself converted to α lipoic acid (αLa), Reaction 12: The regeneration of DHLA from αLa using NADPH, Reaction 13: Lipid hydroperoxides are reduced to alcohols and dioxygen by GPx using GSH as the electron donor, Reaction 14: Lipid hydroperoxides can react fast with Fe^{2+} to form lipid alkoxyl radicals ($LO\bullet$), or much slower with Fe^{3+} to form lipid peroxy radicals ($LOO\bullet$), Reaction 15: Lipid alkoxyl radical ($LO\bullet$) derived for example from arachidonic acid undergoes cyclisation reaction to form a sixmembered ring hydroperoxide, Reaction 16: Sixmembered ring hydroperoxide undergoes further reactions (involving α scission) to form 4-hydroxynonenal, Reaction 17: 4-hydroxynonenal is rendered into an innocuous glutathionyl adduct (GST, glutathione S-transferase), Reaction 18: a peroxy radical located in the internal position of the fatty acid can react by cyclisation to produce cyclic peroxide adjacent to a carboncentred radical, Reaction 19: This radical can then either be reduced to form a hydroperoxide (reaction not shown) or it can undergo a second cyclisation to form bicyclic peroxide which after coupling to dioxygen and reduction yields a molecule structurally analogous to the endoperoxide, Reaction 20: Formed compound is an intermediate product for the production of malondialdehyde, Reactions 21, 22, 23: Malondialdehyde can react with DNA bases Cytosine, adenine and Guanine to form adducts M1C, M1a and M1G, respectively (Valko *et al.*, 2007).

TBARS might be due to maintaining nitric oxide production within physiological level which acts as intrinsic antioxidant (Tooby *et al.*, 2004). Our finding suggests that the drug does not have any deleterious effects on the liver. This may be linked to the presence of active compounds incorporated into the drug especially the presence of phenolic component -OH group. Conversely, the increased MDA levels in kidney, spleen and the lung may be attributed to the free radical generation from nitric oxide reacting with metabolic oxygen. This consequently initiates lipid peroxidation as there is imbalance between endogenous antioxidants (Fig. 5) and reacting oxygen species of the tissues. Together, our present result confirms that the use of the drug (Viagra) prevents oxidative stress in the tissue (liver) which is sensitive to oxidative damage due to high consumption of foods containing pro-oxidants and high unsaturated fatty acid contents (Halliwell, 1996).

Most organisms are endowed with an armory of antioxidants defense molecules (Fig. 5). This is to prevent and neutralize the effects of free radical induced damage (Bandyopadhyay *et al.*, 1999).

Our present data depicted that reduced glutathione (GSH) levels in the liver, kidney and the spleen were significantly up-regulated with Viagra treated-rats. But there is a significant marked reduction level of reduced glutathione (GSH) in the lung of the treated rats. It also proffers protection by preserving the structural integrity of the hepatocellular membrane (Fig. 5 reaction 3 and 4). This result also agrees with Ahmed *et al.* (2012) who studied the ameliorative effects of Sildenafil citrate in acetic acid-induced Chronic Colitis in rats. This suggests that the therapeutic dose of the drug (Viagra) is not deleterious when used for erectile dysfunction but may reveal some pathological conditions in the lung if taken overdose.

Moreover, Superoxide dismutase is catalyzed to scavenge excess superoxide anions and convert them to H_2O_2 . It is acting as the first-line of antioxidant defense in biological systems (Husain and Somani, 1997; Bondy, 1992). In the present study, rats administered with Viagra showed significant marked elevation of SOD activity in all the examined tissues. Increase of SOD activity in these tissues may be adduced to increased denovo synthesis of SOD protein or irreversible activation of enzyme proteins in response to either decreased or increased free radical production (Fig. 5 reaction 2) resulting from oxygen metabolism in the mitochondrion (Santiard *et al.*, 1995; Bondy, 1992). Hence, our data showed increased SOD activity suggesting quick elimination of superoxide anions from the body.

Additionally, Sildenafil citrate significantly increased CAT activity in liver, kidney and spleen tissues but reduced in the lung of treated rats. The primary role of CAT is to scavenge H_2O_2 to H_2O and O_2 that has been generated by free radicals or by SOD during removal of superoxide anions (Ribiere *et al.*, 1992). The present study suggests that the increase in tissues CAT activity may be related to excess H_2O_2 production resulting from other intermediary metabolism or induction of lipid peroxidation in liver, kidney and spleen. A reduced CAT activity in the lung indicates prolonged accumulation of reacting oxygen species (H_2O_2) which implicates inflammation responses (Irfan *et al.*, 2005). In addition, free radical reactions have been suggested to play contributory role through inflammatory responses a inflammatory lung diseases (Choi and alam, 1996).

Mechanistically, it is observed from the present study that Reactive Oxygen Species (ROS) such as O^{2-} and $OH\bullet$, generated and released by a ctivated immune and inflamma tory cells are highly reactive and when generated close to cells membranes oxidizes membrane phospholipids (lipid peroxidation) which initiatesachain reaction. Thus, a single $OH\bullet$ can result in the formation of many molecules of lipid hydroperoxides in the cell membrane. The peroxidative breakdown of polyunsaturated fatty acids impair membrane function and inactivate membraneabound receptors and enzymes, increase tissue permeability which have been implicated in the pathogenesis of many forms of lung injury (Rahman, 2003). Excess ROS induced by Viagra are released by lung epithelial cells and stimulate inflammatory cells directly thereby, amplifying lung inflammatory and oxidant events (Fig. 6). ROS in addition act on certain amino acids in proteins (e.g., enzymes, kinases) such as methionine, tyrosine and cysteine, profoundly altering the function of these proteins in inflammatory lung diseases.

The present finding elucidates that free radicals and lipid peroxidation aldehydes which are stable, diffuse within or escape from the cell and attack lung tissues far from the site of original free radical generation. This aldehyde (MDa) generated endogenously during the process of lipid peroxidation caused by administration of viagra are involved in pathological effects especially inflammation associated with oxidative stress in cells and tissues of the lung.

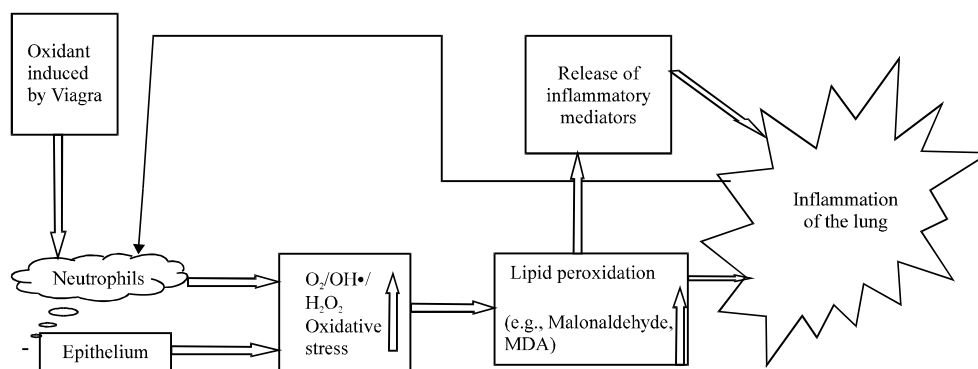


Fig. 6: Mechanism of ROS mediated lung inflammation, inflammatory response is mediated by oxidant induced by Viagra and released by the activated neutrophils, eosinophils and epithelial cells leading to production of ROS and membrane lipid peroxidation, Thus, increased release of proinflammatory mediators e.g., MDA involved in the inflammatory responses in the lung

AST and ALT estimation in serum is a useful quantitative marker to indicate hepatocellular damage (Hwang and Wang, 2001; Singh *et al.*, 2001). Administration of sildenafil citrate (Viagra) nonsignificantly increased the levels of AST and ALT, suggesting that it offers mild damage to hepatic cells at abused doses. The histological analysis showed little or no visible damage on the treated tissues with the therapeutic dose of Viagra.

CONCLUSION

Antioxidants are the major *in vivo* and *in situ* defence mechanisms of the cells against oxidative stress. aberrations in oxidantaantioxidant balance can lead to a variety of pathological conditions. Viagra may potentiate both enzymatic and nonaenzymatic antioxidants in liver, kidney and spleen while promoting lung inflammatory responses by the activated neutrophils, eosinophils and epithelial cells leading to production of ROS and membrane lipid peroxidation in male rats. Therefore, the abuse and/or overdose use of the antiaerectile dysfunction drug (Viagra) without consulting physicians should be avoided as this could cause nephritis, spleen damage and inflammation of the lung. In view of the importance of this drug in clinical practice, the relevance of our study to humans merits further investigation on the key marker (cytokines) of inflammation before making a general recommendation.

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