MORPHOLOGICAL ALTERATIONS OF RED BLOOD CELLS CAUSED BY OVERDOSES OF SILDENAFIL

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ABSTRACT

Objective: The objective of this study was to investigate whether the overdose of sildenafil (Viagra) causes red blood cytotoxicity in rats by seeing the morphological alterations.

Methods: Fifteen rats which were randomly divided into four groups of rats were received intraperitoneal injections of sildenafil. The sildenafil was dissolved in sterile saline 45% for three groups except the control group. They were injected for 5 days in three doses as followed (0.3 mg/rat for G1, 0.5 mg/rat for G2, and 1 mg/rat for G3). Blood smears were made to test the alterations in the blood cells after injection.

Results: The result indicated the presence of a relationship between sildenafil overdosing and red blood cell morphology. Mild-to-moderate alterations of hematological morphology were observed. The hematologic morphology variation was observed in size, shape, and distribution of hemoglobin using light microscope and confirmed by the scanned electronic microscope.

Conclusion: Possible hemotoxicity effects of sildenafil overdosing might be existing, which might be proportional to the duration of usage. However, further studies are recommended properly illustrate the effect of usage duration on sildenafil overdosing toxicity on red blood cells.

Keywords: Sildenafil, Overdosing, Red blood cells, morphology alterations.

INTRODUCTION

Sildenafil is a medication used to treat cavernous dysfunction, widely known as Viagra [1]. Sildenafil overdoses are mainly seen in men suffering from erectile dysfunction where they and their partners are surrounded by the stigma of this disease [2]. Sildenafil is an effective drug in elderly men clinically but has a lower efficacy rate with age [3,4,7]. In addition, sildenafil is increasingly popular with young adults because of drug may improve sexual performance. Research studies also show that sildenafil is widely used by bodybuilders and athletes and is currently legal in the sports world [5,7]. On the other hand, the fatality of sildenafil overdoses in some countries is on the rise and has reached crisis levels [6,7]. In case studies, epistaxis, facial flushing, hearing impairment, hypotension, nose stuffiness, chest pain, priapism, arrhythmia, and tachycardia are some of the possible effects of sildenafil overdoses [8-11]. It was also reported that overdoses of sildenafil have the potential to cause death to men with potential arrhythmia [12]. In some experimental studies, male rabbits were exposed to overdoses of sildenafil, which had caused tubular and interstitial testicular histological alterations, including spermatocyte's degeneration, arrest of spermatogenesis, and spermatocytes karyokinesis [7,13,14]. Overdoses of Sildenafil may induce hematological abilities that may disturb the normal functioning abilities of blood cells. Red cell's alterations induced by sildenafil overdosing toxicity are not yet well demonstrated. This study attempted to demonstrate the possible red cells morphology alterations caused by overdoses of sildenafil in rats.

METHODS

The study was performed after it was approved by the College of Medicine, King Faisal University Ethical Committee guidelines. Fifteen healthy male Wistar rats (*Rattus norvegicus*) of similar age (4 weeks) initially weighting 21–48 g were used throughout the study. All rats were obtained from the animal house of college of medicine, King Faisal University and maintained at constant room temperature of 22°C ± 1°C with a 12-h dark/light cycle. All animals were provided food and mineral water and given 1 week of acclimatization before pharmacologic intervention. Sildenafil (Wafi®) was obtained from a pharmacy as 50 mg tablets, and sterile saline 0.45% (pH = 4.35–4.5) was prepared according to previous data [15]. The rats were randomly allocated in four groups, control (G1) and three test groups (G2, G3, and G4.) The control group was consisted of three members; the other three groups were consisted of four members. In the 1st, 3rd, and 5th day of the experiment, the control group was injected with saline 0.45%. The three test groups were exposed to intraperitoneal injections of sildenafil that was immediately dissolved before administration in aseptic saline at concentrations (0.3 mg/rat for G2, 0.5 mg/rat for G3, and 1 mg/rat for G4). The routes of administration and doses were determined based on the collected data from previous researches and experimental protocol [13,16,17]. At the end of the 9th day of the experiment, all the rats were euthanized by ether. Fresh blood was collected from two rats from each group. The blood was smeared on a glass slide and then stained using Giemsa stain to observe blood differential of cell's morphology under the oil immersion lens (×1000). Scanning electron microscopy (Hitachi H300 equipped with an H-3010 scanning attachment) was used to confirm the morphological alteration or red cells. The statistical software SPSS 17 for windows was used for data analysis. Statistics are provided as mean ± standard deviation differences between groups were analyzed through one-way ANOVA test for more than one comparison, with a probability value $p<0.05$ appeared as statistically significant.

RESULTS AND DISCUSSION

Table 1 represents some red cell's parameters. In the 1st day of experimental, the mean corpuscular hemoglobin concentration (MCHC) level was significantly changed in G2 and G3 (p=0.0026 and p=0.0.280, respectively). Significant similar correlations were also

observed in the MCHC in the 3rd day among all groups (G2, G3, and G4; p=0.0028, p=0.0087, and p=0.0027, respectively) besides Hb level in G3 and G4 (p.0049 and 0.0045, respectively). In the 5th day, there was significantly changed in the levels of Hb and mean corpuscular volume (MCV); the Hb level was slightly changed in G3 only (p=0.0391), but MCV was significantly associated with G2, G3, and G4 (p=0.0115, p=0.0072, and p=0.0064, respectively) compared to control group (G1). Hematological examination under the light and electron microscopes showed variation in the sizes of red blood cells (RBCs) (anisocytosis). Some cells were found to be microcytic and others were normocytic (Figs. 1 and 2). Hemoglobin distribution abnormalities were also well noticed which included lesser coloration of RBCs (hypochromia) (Figs. 2 and 3) and periodic immature of RBCs (polychromatophilic cells/reticulocytes) (Figs. 1 and 2) were present. As regard to the shape variation, irregular, smaller, denser, and sphere-shaped RBCs
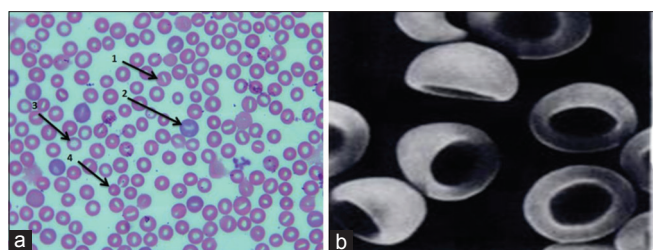

Fig. 1: (a) Arrow 1 shows stomatocyte. Arrow 2 shows polychromatophilic cell (reticulocyte.) Arrow 3 shows hypochromic cell. Arrow 4 shows microcytic cell. Figure shows clear variation of the size of the cells (Anisocytosis.). (b) Scanning electron micrographs of red cells showing stomatocyte
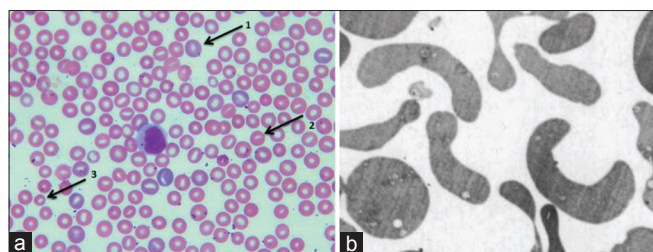

Fig. 2: (a) Arrow 1 shows polychromatophilic cell (reticulocyte.) Arrow 2 shows stomatocyte. Arrow 3 shows a microcytic cell. Overall, there were an increased in hypochromic cells. (b) An electron micrograph of blood freshly showing reticulocytes

(spherocytes) (Fig. 4) and RBCs with slit-like central pallor were detected (stomatocytes) (Figs. 1 and 3).

Sildenafil citrate is considered a vital drug because of its widespread action on many disorders. Vaginal application of sildenafil will increase the mucosa thickness in women with a history of repeated miscarriage, and it has been reported to decrease natural killer cell activity in such women [18,19]. The most necessary action is to enhance performance throughout a sexual issue. Zuk *et al.* in 2011 [20] posted a report at the look of the effect of some selected tablets used in asthma treatment of the morphology and elasticity of RBCs. Greater sizes of cells were observed during the treatment with aminophylline at a base awareness (8.3 ± 0.1 in diameter) while no longer a lot alternate can be observed within the case of methylprednisolone treatment at a low base. However, the stiffness of RBCs incubated with each tablet turned into determined to be growing in time, which may additionally bring about a reduced functionality of the cells to hold oxygen molecules. Sildenafil enters blood circulate after being absorbed inside the intestine. The drug molecules might attain the site of movement through adhering to the erythrocytes. The binding interaction between the erythrocytes and the drug molecules may have become as well in positive adjustments in surface features. This look at was evaluated the hemolytic action of sildenafil citrate on ordinary rats' erythrocytes at high doses and to decide morphological changes both before and after drug administrations. The result of this experiment confirmed that sildenafil overdoses can set off a few changes inside the RBC morphology, inclusive of version in length, shape, and distribution of hemoglobin. This result shows the presence of a dating among sildenafil overdosing and RBCs which become agreed with the examiner finished by Guha *et al*. All determined that the hemolytic action of sildenafil citrate on everyday erythrocytes by way of experiment can be observed from the cell morphology [19]. Our evaluation changed into supported the observations derived from AFM images that showed a decrease in surface roughness may be associated with the action of sildenafil citrate at the cytoskeleton community of the cell [19-21]. Sildenafil citrate would possibly interact within the outer layer of the protein-lipid bilayer and inlayer, as a result. The proteins contribute much less to the floor roughness. A decrease in floor roughness can be related to the action of sildenafil citrate at the cytoskeleton of the cellular composed became confirmed with the aid of the self-controlled of abnormalities in the present study (Hb, MCHC, and MCV). Although sildenafil citrate has been determined to cause hemolysis of erythrocytes, the direct extrapolation of our outcomes as contraindications limited because our investigations are being achieved at an excessive concentration of medication, its use (repeated and high dose) for recreation reasons boosting sexual overall performance without prescription would

Table 1: The alteration on red cells parameters after sildenafil administration during the experimental days in rats

Red cells parameters	Mean±SD			
	Control (G1)	G2	G3	G4
1st day				
Hb (g/dl)	12.14±0.63	10.36±0.27 (0.0108)	11.22±0.29 (0.0832)	11.13±1.21 (0.2690)
PCV (%)	41.41±1.20	40.25±0.91 (0.2531)	41.22±1.11 (0.8503)	40.25±0.91 (0.2531)
MCHC (g/dl)	31.33±1.12	27.31±0.23 (0.0026)*	28.22±1.21 (0.0280)*	27.31±0.23 (0.0026)
MCV (fl)	66.30±3.10	66.41±3.13 (0.9764)	67.18±2.10 (0.7142)	67.41±3.63 (0.7151)
3rd day				
Hb (g/dl)	12.14±0.63	9.77±1.50 (0.0651)	8.95±0.57 (0.0049)*	9.32±0.57 (0.0045)*
PCV (%)	41.41±1.20	42.03±1.21 (0.5688)	40.73±1.81 (0.6164)	41.83±1.01 (0.6669)
MCHC (g/dl)	31.33±1.12	24.81±1.31 (0.0028)*	26.32±1.42 (0.0087)*	23.74±1.65 (0.0027)*
MCV (fl)	66.30±3.10	61.75±1.73 (0.0906)	63.72±1.43 (0.2607)	62.45±3.13 (0.2047)
5th day				
Hb (g/dl)	12.14±0.63	11.31±1.22 (0.3542)	10.93±0.29 (0.0391)*	11.21±2.31 (0.5380)
PCV (%)	41.41±1.20	41.49±1.31 (0.9416)	41.66±1.51 (0.8334)	41.61±1.84 (0.8823)
MCHC (g/dl)	31.33±1.12	29.52±1.71 (0.1999)	30.02±2.11 (0.3960)	29.12±1.82 (0.1477)
MCV (fl)	66.30±3.10	77.63±3.17 (0.0115)*	77.32±2.16 (0.0072)*	77.81±2.23 (0.0064)*

The rats were subdivided into four groups; G1 setting as control group and the other three groups (G2, G3, and G4) were the tested for red cell alteration after administration of sildenafil. *p<0.05 is statistically significant. Hb: Hemoglobin, PCV: Packed cell volume, MVHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume, G: Group, SD: Standard deviation
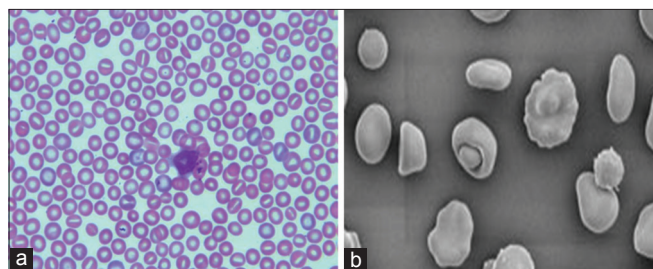
Fig. 3: (a) There was overall increased hypochromia. Variation in the size of cells was also obvious (anisocytosis); stomatocytes were also present in large numbers. A considerable number of polychromatophilic cells (reticulocytes) were also detected. (b) Scanning electron micrograph documenting anisocytosis

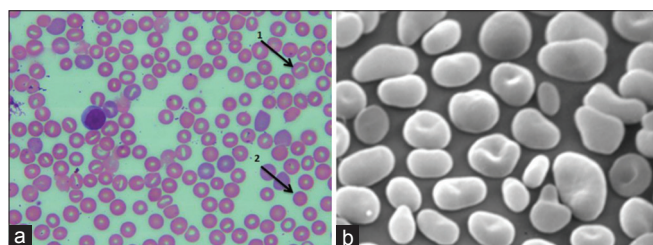**Fig. 4: (a) Arrow 1 shows stomatocyte. Arrow 2 shows a spherocyte. Overall, there was an increased in spherocytes. (b) Scanning electron micrograph documenting spherocyte**

possibly pose threats to clients suffering from nutritional-deficiency anemia or any sort of hemoglobinopathy. Our observations reported here are of general scientific importance, which can find relevance to further research regarding the ultrastructure of cell membranes. Moreover, the nature of this study design did not clarify this relationship accurately because of the short duration and the low numbers of the given doses. Therefore, we recommend further investigation to be done to define this relationship more properly.

CONCLUSION

The findings of this study have a look at advice that sildenafil overdosing has moderate hemotoxic results on red cells related to morphological alteration that was probably proportional to the duration of usage. However, addition experiments are recommended to more illustrating the impact of utilization length on sildenafil overdosing toxicity on the cell morphology. We also encourage extra researches to be achieved at the hemotoxicity of sildenafil and the scientific effect of those findings at the blood cell morphology.

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AUTHORS' CONTRIBUTIONS

Author 1 was involved in data interpretation, concept, and design. Author 2 carried out the experimental part of the work and performed data collection, analysis, and drafted. The data interpretation, concept, revision of the work, and correction of the manuscript were done by the corresponding author. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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