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Effect of Sildenafil Citrate on Some Reproductive and Other Physiologic Parameters of Albino Rats (*Rattus norvegicus*)

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ABSTRACT

Sildenafil citrate (SC), a widely used oral medication for the treatment of erectile dysfunction, is frequently consumed without medical supervision due to its availability as an over-the-counter drug. This study investigated the effects of SC on selected reproductive and physiological parameters in albino rats (Rattus norvegicus) to assess potential systemic implications of its use. Acute toxicity testing revealed that SC is non-lethal at doses up to 2000 mg/kg body weight, suggesting a high safety margin. However, sub-acute administration resulted in notable physiological alterations. Rats administered SC showed increased serum testosterone levels and enhanced sexual activity, likely due to elevated luteinizing hormone (LH) levels and increased cholesterol utilization for steroidogenesis. These findings suggest SC may upregulate steroidogenic pathways. Despite these reproductive enhancements, there was a significant decline ($p<0.05$) in sperm count and motility in treated groups, indicating potential adverse effects on spermatogenesis following prolonged exposure. Haematological analysis revealed alterations in red blood cell morphology, haemoglobin distribution, and packed cell volume, while liver function tests indicated elevated AST/ALT ratios. Histopathological examination of liver tissue showed disorganized hepatic sinusoids and hepatocyte degeneration, confirming hepatotoxic effects at higher doses.In conclusion, although sildenafil citrate may enhance certain reproductive functions, chronic or high-dose usage can adversely affect sperm quality, haematological parameters, and liver integrity. These findings underscore the importance of regulated use and further investigations into the long-term safety and systemic impact of SC, particularly among non-prescribed users.

Keywords : Sildenafil citrate, *Rattus norvegicus, R*eproduction, Spermatogenesis

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Introduction

Penile erection involves a coordinated interplay of psychosomatic, neurological, and vascular mechanisms that elicit a physiological response in the penile vasculature [1]. Sexual dysfunction is a significant medical condition affecting both sexes, resulting from physiological, psychological, or pathological factors [2]. Erectile dysfunction (ED), a common form of sexual dysfunction, is defined as the persistent inability to achieve or maintain an erection sufficient for satisfactory sexual activity [3.4].

ED affects up to 50% of men aged 40 to 70 [5,6] and approximately 150 million men globally [7,8]. This number is projected to exceed 300 million by 2025 due to global population aging [9-11].

Numerous pharmacological and plant-derived agents with aphrodisiac properties have been reviewed for ED management [12-15]. According to the U.S. Food and Drug Administration, an aphrodisiac is any substance claiming to enhance sexual desire or performance. Aphrodisiacs are categorized into: (i) agents that enhance libido, (ii) agents that improve erectile function, and (iii) agents that enhance sexual pleasure [16,17]. Aphrodisiacs may be natural (plant or animal origin) or synthetic, such as sildenafil citrate—a widely used oral PDE5 inhibitor for ED treatment. Sildenafil enhances erectile function during sexual stimulation by increasing penile blood flow. As an over-the-counter medication in many regions, its accessibility has led to misuse, particularly among individuals without medical need.

While the drug's efficacy in ED treatment is well-established, its effects on reproductive health and systemic physiology remain insufficiently studied. This research aims to assess the broader physiological impacts of sildenafil citrate, focusing on reproductive and other organ systems using *Rattus norvegicus* as a model.

Although sildenafil citrate is effective in treating ED, its non-prescription availability has resulted in widespread and often indiscriminate use, including among individuals without erectile dysfunction. The potential systemic effects of chronic or inappropriate use remain unclear. The rising incidence of male infertility of unexplained origin, alongside concerns regarding the impact of sexual enhancers on major organs such as the testes, liver, kidneys, brain, and heart, necessitates further investigation. This study seeks to evaluate the effects of sildenafil citrate on selected reproductive and physiological parameters in albino rats (*Rattus norvegicus*).

MATERIALS AND METHOD

Study area

The study was conducted at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Abuja permanent site, Gwagwalada, Abuja, Nigeria. University of Abuja permanent site is in the capital city of Nigeria, lying between latitude 8°25' and 9°25' North of the equator and longitude 6°45' and 7°45' East of Greenwich. The university main campus is bounded in the north by Anagada village, on the west by Giri, on the east and south east by Kuje, and on the south-west by Gwagwalada town. University of Abuja permanent site has a hot, humid and tropical climate. Its major element has regimes that are intermediate from those of the Southern and Northern region of the country [18]. The temperature varies from 21–26.7°C yearly with total annual rainfall of approximately 1,650 mm. The month of July, August, and September records approximately 60% of the annual rain fall [18] . The maximum temperature ranges between 30°C and 35°C during the dry season months and is greatest during the daytime. During the wet seasons, the maximum temperature is between 25°C and 30°C.

Experimental Animals

The albino rats were sourced from Nsukka, Enugu state, Nigeria. Twenty Eight male and Ten female albino rats of 12-14 weeks of age weighing 130-250 grams were used for this study. Male and female rats were housed separately at the animal house in well ventilated metabolic cages. Male rats were randomly divided into four groups designated A, B, C and D of seven rats per group (n=7). The rats were acclimatized for two weeks. The environmental temperature of the animal house ranged between 25-30^{0}C. The animals were kept in standard rat cages with wood shaving bedding and provided with good clean water and feed *ad libitum.* The rats were maintained in a well-ventilated room with a 12-hour light and 12-hour dark cycle.

All the experimental procedures were performed according to the ethical guidelines for animal studies research established by the Ethical Committee of University of Abuja, Nigeria.

Methodology

Acute toxicity test

These test was performed to determine the clinical effects of sildenafil citrate (SC) following oral administration and to establish dose regimen for sub-acute toxicity (repeated dose study). The acute toxicity involved the revised UP-and-Down procedure [19]. Ten rats of both sexes (5 males and 5 females) were used for the test. The rats were administered with 2000 mg/kg body weight of SC. The rats were watched closely for 24 hours for signs of toxicity such as sedation, dullness, anxiety, wretching, cycling, and death.

General experimental design/protocol

The rats were randomly assigned to four groups (A, B, C and D) of 7 rats per group. Group A rats served as control and received distilled water while groups B, C and D rats were administered 100, 200 and 300 milligram per kilogram body weight (mg/kg bw) of sildenafil citrate by oral gavage. Administration of SC was done consecutively for 53 days before samples were collected.

Vaginal cytology

Vaginal swab was collected from female albino rats using a cotton tipped swab wetted with ambient temperature physiological saline and inserted into the vagina of the restrained rat. The swab was gently rotated and rolled against the vaginal wall and then gently removed. Cells were transferred to a clean dry glass slide by rolling the swab across the slide. The slide was air dried and then stained. The stage of the oestrous cycle was determined based on the presence or absence of leukocytes, cornified epithelial and nucleated epithelial cells. Females that were identified as in oestrus or proestrus with predominance of cornified cells were used as stimulus.

Sexual behavioural (SB) Study

Rats underwent a sexual behaviour test at the end of the 7 weeks of Sildenafil treatment; on day 51 of the experimental procedure, twenty minutes prior to the beginning of the test, the cages containing the experimental animals were moved to the study room. Males were exposed to an unfamiliar adult female in oestrous or pro-oestrous placed in new transparent cage (40 x 40 x 40 cm) without floor bedding to allow unrestricted observation and video recording of rats' behaviour. All experiments were carried out under red light

from 11:00 pm (5 hours after starting the dark phase) to 6:00 am. The data collection method consisted of a continuous sampling for the 30-minute period of the test [19-21]. After the study, the following behavioural parameters were registered from the recorded videos

1. **Mount latency:** Time from female entrance into the test cage until the first mount occurs.
2. **Mount frequency:** It is the number of mounts displayed without penile insertion These could be considered unsuccessful intromissions [20].
3. **Intromission latency:** Time from female entrance until the first intromission is achieved.
4. **Intromission frequency:** It is the number of intromissions achieved during the test duration [20]

Blood/serum collection and animal sacrifice

Twenty-four (24) hours after the last treatment and following withdrawal of feed in the night, the rats were sedated with ether anaesthesia and blood samples were collected via the retro-bulbar plexus of the median canthus of the eyes using heparinized capillary tube. About 0.5 ml of the blood was collected in an EDTA sample bottle for haematology while 3 ml of blood was collected in a plain sample bottle for biochemical analysis. The later was incubated at room temperature for 45 minutes and the serum separated by centrifugation at 3000 x g for 10 minutes. The serum was aliquoted into three portions and preserved at -20^{0}C until analyses.

Effects of sildenafil citrate on haematological parameters of male rats

Automated haemoanalyser (**Orphee mythic 18, Japan**) was used to determine the following haematological parameters of rats:

. Parked cell volume (PCV)

ii. Haemoglobin concentration (HbC)

iii. Erythrocytes count

iv. Total leucocytes cell (TLC)

v. Differential leucocyte count (DLC)

vi. **Erythrocytic indices**

   a. Mean corpuscular volume (MCV)

   b. Mean corpuscular haemoglobin (MCH)

   c. Mean corpuscular haemoglobin concentration (MCHC)

vii. Platelets

Effects of sildenafil citrate on some hormonal profile of male rats

The following reproductive hormones of male rats were assayed using commercial plate enzyme-linked immunosorbent assay (ELISA) kit (Monobind Inc., Lake Forest, USA) to determine the concentration of the following hormones according to manufacturer's instruction:

* Testosterone
* Fo*licle Stimulating Hormone (FSH)
* Luteinizing Hormone (LH)

Briefly, 50 µL of standard, sample serum, or control were dispensed into the well, followed by an addition of 100 µL of enzyme conjugate. After incubation for 1 hour at room temperature, wells were rinsed with diluted washing solution three times. Next, 150 µL of the substrate solution were added into the well and incubated for 15 minutes at room temperature. Measurement of the hormone (testosterone, LH and FSH) concentration was performed at 450 nm in ELISA reader

(Microplate Autoreader, BioRad Laboratory, Hercules, CA, USA) after adding the stop solution. All testing materials were triplicated for the assay. The sensitivity and intra- and inter-assay variation coefficients of the kit were 0 to 0.083 ng/mL, 3.28% to 4.16%, and 4.73% to 9.94%, respectively.

Effects of sildenafil citrate on some serum electrolytes, proteins and enzymes of male rats

Serum electrolytes were determined using Berckman Coulter Synchron CX9 PRO Automated Biochemistry analyser. Total protein, albumin and globulin and creatinine were also analyzed using the commercial kits [22].

The serum enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined using microplate reader based on spectrophotometric absorbances as described briefly below.

Aspartate aminotransferase (AST) assay

Procedure

The principle was based on the reaction of L-Aspartate and α-Ketoglutarate in the presence of GOT in the sample to yield oxaloacetate and L-glutamate. One hundred microliter (100 µL) of detection reagent A working solution was dispensed into each well and incubated for 1 hour at 37^{0}C after covering it with the Plate sealer. The solution was afterwards aspirated and the wells washed with 350µL of 1g Wash Solution to each well using a squirt bottle, multi-channel pipette and dispenser and allowed to sit for 1-2 minutes. Washing was carried out 3 times after this and the remaining liquid completely removed from the wells by blotting with an absorbent paper towel. Then, 100µL of Detection Reagent B working solution was dispensed to each well and incubated for 30 minutes at 37^{0}C after covering with plate sealer. Aspiration and washing were conducted afterwards and 90 µL of Substrate Solution was added into each well. It was then covered with a new plate sealer and incubated for 10-20 minutes at 37^{0}C. Afterwards, 50 µL of Stop Solution was later dispensed into each well and the plate tapped gently to ensure thorough mixing. Measurement at 450 nm was conducted using a microplate reader immediately.

Serum alanine aminotransferase (ALT) assay

Procedure

The principle was based on the reaction of L-Alanine and α-ketoglutarate in the presence of GPT in the sample to yield pyruvate and L-glutamate. The same procedure for AST was adopted for ALT

Serum alkaline phosphatase (ALP) assay

Procedure

The principle was based on the reaction of alkaline phosphatase in a sample hydrolysed by paranitrophenyl phosphate into paranitrophenol and phosphate in the presence of magnesium.

Sperm motility

These were determined according to the World Health Organization's (WHO) guidelines (2010). For sperm motility assessment, the left caudal epididymis was identified and blotted free of blood and thereafter punctured with a 21-gauge sterile needle and a drop of semen from the opening was collected on a clean prewarmed (37 ^{0}C) glass slide. The drop of

semen on the slide was later diluted with a drop of prewarmed normal saline and covered with a prewarmed (37 ^{0}C) cover slip and examined under ×40 magnification for spermatozoa percentage motility.

Organo-somatic indices (Relative organ weight)

Prior to animal sacrifice, the weight of each rat was obtained using a bench top sensitive scale before they were anaesthetized with intraperitoneal injection of ether.

The testicles, epididymides, liver, kidneys, brain, heart and spleen, were exteriorized and trimmed of extraneous tissue. The ratio of the weight of the organs to that of the animal was used in computing the organo-somatic indices (OSI) using the formula below;

$$OSI = \frac{Organ\ weight}{Weight\ of\ Animal} \text{ x } 100$$

The *ex situ* testicular morphometry was determined with the aid of Vernier calliper.

Gonadal (testicular) sperm count

The testes collected from the rats were macerated in a ceramic mortar with a ceramic pestle and 10 ml of normal saline used in homogenizing the macerated testes. It was then filtered through wire gauze after which 1 ml of the filtrate was aspirated out using a sterile syringe and was made up to 10 ml using 9 ml of white blood cells (WBCs) diluting fluid. A drop of eosin stain was then added to the homogenate. A drop of the homogenate was loaded onto either ends of the improved Neubauer haemocytometer. This was covered with a cover slip and the sperm cells counted using ×40 magnification and ×10 wide angle eyepiece of the light microscope. The sperm cells in the four large corner squares (measuring 1 mm^2 each) were counted and the total multiplied by a correction factor of 250,000 to give the number of sperm cells per ml of gonadal sperm.

The correction factor of 250,000 used in determining the gonadal sperm reserve was derived from the following calculations;

The cells were counted in four large squares of 1 mm^2 each. The total is 4 mm^2. The depth of the chamber was 0.1 mm. Therefore, the total volume of sperm in the chamber counted was = 4 mm^2 × 0.1 mm = 0.4 mm^3. Making it up to 1 mm^3 (1 µl) would need a multiplication by 2.5. But making it up to 1 ml (1 cm^3) would need a multiplication by 2.5 ×1000 = 2500.

The dilution factor used was 1:100. Therefore 2500 × 100 = 250,000.

Extra-gonadal (caudal epididymal) sperm count

The epididymis was exteriorized and after removal of extraneous tissues, the cauda was carefully dissected out of the remaining epididymis and weighed in a sensitive balance (Ohaus, USA). Thereafter, the same procedure as described for the testes was adopted for the assessment of the sperm reserve in this segment of the epididymis.

Feed intake, water consumption and weight gain of male rats

The feed consumption (FC) and water intake (WI) of albino rats were measured on daily basis using a digital weighing balance and graduated conical flask respectively. The measurements were done between 7:00am to 9:00am each day by noting the difference between the quantity given and the left over. The weight gain was measured on weekly basis using a digital weighing balance.

Histopathology of the testes (light microscopy)

Two rats from each group were perfused transcardially first with normal saline to wash out the blood cells and then with freshly prepared 4% paraformaldehyde (PFA) in 0.1M phosphate buffer till well fixed using stiffness of muscles and pallor of the liver as indicator for good fixation (Usende *et al.,* 2020). Following perfusion, testis and epididymis were quickly dissected out and bisected into two halves and used for light microscopy. Histopathological preparation of the rat testes from all the groups was done following standard procedure. The testes were rinsed in physiological saline and fixed by immersion in Bouin's fluid which is composed of 75 ml saturated aqueous solution of picric acid, 25 ml of 40% formaldehyde and few drops of acetic acid for 24 hours. The tissues were dehydrated in ascending grades of ethanol (70%, 80%, 90% and 100%) at an interval of 90 minutes each; cleared in xylene and embedded in paraffin wax. Serial sections (5-6um) were cut using rotary microtome. The tissue sections were mounted on glass slides coated with 20% albumin and kept on a dryer at a temperature of 45^0C. The tissues were deparaffinised twice using xylene for five minutes. Following this, the tissues were rehydrated by passing them through descending grades of alcohol (100, 95, 80 and 70%) and water for 10 minutes. Slides were finally stained with Haematoxylin (H) and Eosin (E) for light microscopy.

Statistical Analysis

The computer software, IBM statistical package for social sciences (SPSS) version 16.0 for windows (IBM Corp, Armonk, NY, USA) was used for the statistical analyses. Data obtained were statistically analysed using one-way analysis of variance (ANOVA). The means were separated using Duncan's New Multiple Range Test and differences in means less than probability value of 0.05 ($p<0.05$) were considered significant. The results were presented in tables and graphs as means ± standard errors of the mean

RESULTS

Acute toxicity study

Oral administration of rats with 2000 mg/kg of sildenafil citrate did not result in toxicity signs nor death of the treated rats. There was no adverse effect on the nervous system since the animals did not convulse. Similarly, no untoward changes such as sedation, dullness, anxiety, wretching, cycling, respiration, defecation, micturition and congestion of mucous membranes were noticed within the period. The LD_{50} was therefore, not obtained.

Sexual behavioural study result

Table I shows the results of sexual behavioural study. The mount latency (ML) was significantly lower ($p<0.05$) in treatment group D rats when compared with group A rats (control). However, there was no significant difference ($p>0.05$) among groups B, C and D rats even though group D had the least ML of 20.60± 12.10 seconds followed by group C and B rats. The mount frequency (MF) increased in a dose dependent manner with group D having significantly highest ($p<0.05$) MF of 16.20± 11.50 while group A had the least MF. The intromission latency was significantly shorter ($p<0.05$) in group D rats when compared with groups A, B and C rats. Group A rats had the highest intromission latency which was not significantly different ($p>0.05$) from group B but was significantly higher ($p<0.05$) than group C. Group D had significantly highest ($p<0.05$) intromission frequency (IF) when compared with the control even though it was not significantly different from groups B and C.

Table 1: Sexual behavioural study result of rats exposed to sildenafil citrate

PARAMETERS	A (Control)	B 100 mg/kgSC	C 200 mg/kgSC	D 300 mg/kgSC
ML (sec)	48.83 ± 26.47[a]	30.25 ± 16.03[b]	25.5 ± 10.31[b]	20.60± 12.10[b]
MF	3.67 ± 4.05 [a]	6.75 ± 2.25[b]	11.67 ± 10.64[c]	16.20 ± 11.50[d]
IL (sec)	459 ± 3.00[a]	450 ± 2.10[a]	314.17 ± 286.71[b]	61.20 ± 61.20[c]
IF	1.00 ± 0.00[a]	2.00 ± 0.00[b]	3.33 ± 2.62[b]	4.80 ± 4.80[b]

Different superscripts [a, c, b] in a row indicate significant ($p < 0.05$) differences between the means. **ML**= Mount latency, **MF**= Mount frequency, **IL**= Intromission latency and **IF**= Intromission frequency

Haematology Results

Table 2 shows the result of haematology. There was no significant difference (P>0.05) in the TLC values between treatment groups B, C, D rats and the control group A rats. However, group A had a higher TLC value of 19.08 ± 1.05 $x10^6/\mu L$ than the treatment groups B, C and D which had values of 14.48 ± 3.14, 17.00 ± 2.26 and 14.01 ± 2.29 $x10^6/\mu L$ respectively.

The red blood cells in group A were significantly higher (P<0.05) than group B but were not significantly different (P>0.05) from group C and D. Similarly, there was no significant difference (P>0.05) between treated groups B, C and D.

The Haemoglobin concentration in group A was significantly higher (P<0.05) than group B, but was not statistically different (P>0.05) from groups C and D. Similarly group B rats had no significant difference (P>0.05) from groups C and D rats.

The percentage of packed cell volume (PCV) in group A rats was significantly higher (P<0.05) than group B rats. The PCV in group A was however, not statistically different (P>0.05) from groups C and D. Group B also had no significant difference (P>0.05) between C and D in their PCV values. There was no significant difference (P>0.05) in the Mean corpuscular volume (MCV) and Mean corpuscular haemoglobin concentration (MCHC) of all groups. The Mean corpuscular haemoglobin concentration (MCV) in group B rats was significantly higher (P<0.05) than group D rats but were not significantly different (P>0.05) from group A and C. Similarly, there was no significant difference (P>0.05) between groups A, C and D rats. There was no significant difference (P>0.05) in the platelet values for all the groups.

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Neutrophil and Lymphocyte values in group B rats were significantly different (P<0.05) from group D but was not significantly different (P>0.05) from group A and C. Similarly, there was no significant difference (P>0.05) between groups A, C and D rats.

There was no significant difference (P>0.05) in the monocyte values for all the groups.

The level of eosinophil in group B rats was significantly different (P<0.05) from D rats. Group B eosinophil was however statistically similar (P>0.05) to A and C. Group D also had no significant difference (P>0.05) between A and C. There was also no significant difference (P>0.05) in the Basophil values for all the groups.

Table 2: Haematology results of rats exposed to sildenafil citrate

PARAMETERS	A (Control)	B 100 mg/kgSC	C 200 mg/kgSC	D 300 mg/kgSC
TLC (x10^6/µL)	19.08 ± 1.05	14.48 ± 3.14	17.00 ± 2.26	14.01 ± 2.29
RBC (x10^3/µL)	8.86 ± 0.63^a	7.10 ± 0.53^b	7.86 ± 0.55^{ab}	8.72 ± 0.35^{ab}
HB(g/dL)	15.55 ± 0.67 ^{a}	13.05 ± 0.93 ^{b}	13.73 ± 0.81 ^{ab}	14.53 ± 0.53 ^{ab}
PCV (%)	47.50 ± 1.66 ^{a}	38.25 ± 2.87 ^{b}	42.50 ± 3.28 ^{ab}	44.25 ± 1.38 ^{ab}
MCV (fL)	54.13 ± 2.21	53.63 ± 0.34	53.95 ± 1.01	50.90 ± 0.99
MCH (Pg)	17.65 ± 0.56^a	18.43 ± 0.54 ^{ab}	17.53 ± 0.33 ^{bd}	16.68 ± 0.30 ^{d}
MCHC (g/dL)	32.65 ± 0.34	34.38 ± 0.93	32.50 ± 0.80	32.78 ± 0.24
PLT (x10^3/µL)	452.00 ± 122.37	567.50 ± 178.10	574.50 ± 16.02	506.75 ± 47.88
NEU (x10^3/µL)	5.75 ± 1.31^a	16.25 ± 3.39 ^{b}	7.25 ± 3.79 ^{a}	4.75 ± 1.18^a
LYM (x10^3/µL)	85.75 ± 2.69^a	74.25 ± 3.90 ^{b}	86.50 ± 3.88^a	86.50 ± 0.50^a
MON (x10^3/µL)	7.00 ±1.96	6.50 ± 1.66	5.00 ± 1.08	8.75 ± 1.49
EOS (x10^3/µL)	1.00 ± 0.58^a	2.25 ± 0.75^b	0.75 ± 0.25^a	0.00 ± 0.00^c
BAS (x10^3/µL)	0.50 ± 0.29	0.75 ± 0.25	0.50 ± 0.29	0.00 ± 0.00

b

Different superscripts $^{a, c,}$ in a row indicate significant ($p < 0.05$) differences between the means **TLC**=Total leucocyte count, **PCV**=Packed cell volume, **Hb**=Haemoglobin, **RBC**=Red blood cells, **MCV**=Mean corpuscular volume, **MCH**=Mean corpuscular haemoglobin, **MCHC**=Mean corpuscular haemoglobin concentration. **PLT**=Platelets, **NEUT**=Neutrophil, **LYM**=Lymphocyte, **MONO**=Monocyte, **EOS**=Eosinophil, **BAS**=Basophil.

The results of hormonal profile following treatment with sildenafil citrate are as shown in table 3. Follicle stimulating hormone had no significant difference ($p>0.05$) between the treatment groups B, C, D rats and the control group A rats. Luteinizing hormone (LH) was significantly higher ($P<0.05$) in group D when compared with groups C, B and A. Similarly, testosterone was significantly highest ($p<0.05$) in group D when compared with groups B and A rats but was not significantly different ($p>0.05$) from group C rats. However, group C was significantly higher ($p<0.05$) than group B and A.

Table 3: Hormonal Profile result of rats exposed to sildenafil citrate

PARAMETERS	A (Control)	B 100 mg/kgSC	C 200 mg/kgSC	D 300 mg/kgSC
FSH (mIu/mL)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
LH (mIu/mL)	0.02 ± 0.00 ^{a}	0.03 ± 0.00 ^{a}	0.03 ± 0.00 ^{a}	0.50 ± 0.03^b
TESTOST(ng/mL)	2.66 ± 1.54^a	4.49 ± 4.17^b	7.59 ± 2.96^c	7.93 ± 0.82^c

Different superscripts $^{a, b, c}$ in a row indicate significant ($p< 0.05$) differences between the means **FSH** = Follicle stimulating hormone, **LH** = Luteinizing hormone., **TESTOST** = Testosterone.

Serum Biochemistry

Table 4 shows the result of serum biochemistry. There was no significant difference (P>0.05) in the sodium level among both the treatment groups B, C, D rats and the control group A rats. Potassium (K) level in group A was significantly higher (P<0.05) than that of group C and D, but had no significant difference (P>0.05) with group B. Group C on the other hand was significantly lower (P<0.05) than group A and B. The values gotten for Chloride (Cl) among all the groups had no significant difference (P>0.05). Although the value of bicarbonate (HCO) in group C was higher (19.50 ± 2.87) compared to groups D, B and A (18.50 ± 1.50, 12.00 ± 4.14 and 17.00 ± 4.06 respectively), there was no significant difference (P>0.05) between them. Urea was significantly highest (P<0.05) in the control group A and lowest in treatment group C that received 200mg (P<0.05), there was no difference (P>0.05) in the values in treatment groups B and D compared to A. there was also no difference (P>0.05) in the values of urea in between group C, B and D (treated groups). Although group D had the lowest values in Total bilirubin level (TBL) and Direct bilirubin levels (DBL), there was no significant difference (P>0.05) among them. The level of Alkaline phosphate (ALP) was significantly higher (P<0.05) in groups A and B compared to groups C and D, but were not statistically different (P>0.05) from each other in groups A Vs B; and C Vs D. Aspartate-aminotransferase (AST) levels were not significantly different (p>0.05) from each other in groups A and B but were significantly higher (P<0.05) than groups C and D. Group C however had the least level of AST which was significantly higher (P<0.05) than group D. Alanine aminotransferase (ALT) value was significantly higher (P<0.05) in group A when compared to treatment groups B, C and D. Treatment group B was also significantly higher (p<0.05) than groups C and D which were not significantly different (p>0.05) from each other. There was no statistical difference (P>0.05) between the control and treated groups for the values of total protein (TPRO), albumin (ALBUM), total cholesterol (TCHOLE) and high density lipoprotein (HDL)..

Table 4: Serum biochemistry result of rats exposed to sildenafil citrate

PARAMETERS	A (Control)	B 100 mg/kgSC	C 200 mg/kgSC	D 300 mg/kgSC
Na (mmol/L)	151.25 ± 2.29	147.50 ± 4.29	136.50 ± 7.84	146.75 ± 1.31
K (mmol/L)	6.38 ± 0.36[a]	6.40 ± 0.68 [a]	4.83 ± 0.17[b]	5.03 ± 0.38 [c]
Cl (mmol/L)	112.75 ± 2.56	112.50 ± 4.29	100.50 ± 6.84	110.25 ± 1.44
HCO (mmol/L)	17.00 ± 4.06	12.00 ± 4.14	19.50 ± 2.87	18.50 ± 1.50
UREA (mmol/L)	7.55 ± 0.21 [a]	7.33 ± 0.42 [ab]	6.28 ± 0.52[b]	6.48 ± 0.26 [b]
Cr (micromol/L)	25.50 ± 2.33	19.75 ± 2.84	19.00 ± 0.41	20.25 ± 1.38
TBL (micromol/L)	1.68 ± 0.13	1.78 ± 0.37	1.65 ± 0.16	1.30 ± 0.14
DBL (micromol/L)	1.20 ± 0.17	1.03 ± 0.21	1.30 ± 0.11	0.83 ± 0.05
ALP (IU/L)	730.25±105.92[a]	714.75±76.41 [a]	453.25±51.00 [b]	560.25±44.75[b]
AST (IU/L)	250.75±12.68[a]	290.00±45.67 [a]	170.25±17.61 [b]	202.25±17.83 [c]
ALT (IU/L)	99.25 ± 17.01[a]	73.25 ± 9.20[b]	49.25 ± 5.45 [c]	54.00 ± 2.68 [c]
TPROT (g/L)	78.50 ± 2.06	75.00 ± 5.61	68.50 ± 5.32	71.25 ± 2.72
ALB (g/L)	40.75 ± 2.43	41.00 ± 3.72	37.75 ± 2.02	39.25 ± 0.25
TCHOLE(mmol/L)	1.89 ± 0.23	1.68 ± 0.16	1.34 ± 0.21	1.47 ± 0.08
TRIG (mmol/L)	1.43 ± 0.25	2.31 ± 0.53	1.86 ± 0.24	1.90 ± 0.42
HDL (mmol/L)	1.31 ± 0.16	1.17 ± 0.06	0.98 ± 0.16	1.00 ± 0.04
LDL (mmol/L)	0.20 ± 0.10	0.20 ± 0.05	0.25 ± 0.14	0.10 ± 0.07

Different superscripts [a, b, c] in a row indicate significant ($p<0.05$) differences between the means **Na** = Sodium., **K** = Potassium., **Cl** = Chloride., **HCO** = Bicarbonate., **UREA** = Urea., **Cr** = Creatinine, **TBL** = Total bilirubin., **DBL** = Direct bilirubin., **ALP** = Alkaline phosphate **AST** = Aspartate aminotransferase., **ALT** = Alanine aminotransferase, **TPROT** = Total protein., **ALB** = Albumin., **TCHOLE** = Total cholesterol., **TRIG** = Triglycerol., **HDL** = High density lipoprotein., **LDL** = Low density lipoprotein.

Sperm motility and count results

Table 5 shows the result of sperm motility and sperm count. The sperm motility was significantly higher ($P<0.05$) in the control group A when compared to all the treatment groups B, C and D rats at the various time intervals. The extragonadal and gonadal sperm count were also significantly higher ($p<0.05$) in the control group A when compared with the treatment groups B, C and D rats which were not significantly ($p>0.05$) different from each other.

Table 5: Sperm motility and count result of rats exposed to sildenafil citrate

PARAMETERS	A (Control)	B 100 mg/kgSC	C 200 mg/kgSC	D 300 mg/kgSC
0 (min)	98.75 ± 0.25[a]	69.50 ± 23.60[b]	74.25 ± 24.75[b]	69.50 ± 23.60[b]
5 (min)	90.50 ± 0.87[a]	63. 75 ± 21.64[b]	70.50 ± 23.51[b]	52.50 ± 22.50[b]
10 (min)	83.00 ± 2.71[a]	56.25 ± 19.19[b]	64.25 ± 21.42[b]	45.50 ± 20.90[b]
15 (min)	68.00 ± 6.38[a]	45.00 ± 15.94[b]	55.00 ± 18.60[b]	37.50 ± 18.87[c]
20 (min)	51.25 ± 7.74[a]	30.50 ± 10.97[b]	36.25 ± 12.14[b]	31.50 ± 17.06[b]
25 (min)	35.00 ± 3.54[a]	21.25 ± 7.74[b]	23.75 ± 8.00[b]	24.00 ± 13.61[b]
30 (min)	21.25 ± 3.75	11.75 ± 4.25	15.00 ± 5.00	16.25 ± 9.44
35 (min)	10.50 ± 3.69	6.50 ± 2.22	10.00 ± 3.54	8.75 ± 5.15
40 (min)	6.00 ± 3.34[a]	1.75 ± 0.85[b]	4.25 ± 1.44[c]	4.00 ± 2.31[c]
45 (min)	3.00 ± 2.38[a]	0.50 ± 0.50 [b]	1.00 ± 0.41[b]	0.75 ± 0.48[b]
50 (min)	1.25 ± 1.25[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
55 (min)	0.50 ± 0.50[a]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
60 (min)	0.25 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
EGONAD($X10^6$/ml)	169.44± 4.42[a]	116.19±39.0[b]	131.81±44.58[b]	119.56±40.99[b]
GONAD ($X10^6$/ml)	289.50±52.16[a]	158.06±60.23[b]	189.38±68.02[b]	135.44±45.65[b]

Different superscripts [a, b, c] in a row indicate significant ($p<0.05$) differences between the means **EGONAD** – Extra gonadal sperm count; **GONAD** = Gonadal sperm count; min=minutes

Relative organ weight (ROW)

Table 6 shows the relative organ weights of the various organs measured. There was no significant difference ($P>0.05$) in the values of the relative organ weights of the various organs between the treatment groups B, C and D rats and the control group A rats. However, the kidney in the control group A had a significantly lower ($p<0.05$) relative organ weight when compared with the treatment groups B, C and D which were not significantly different ($p>0.05$) from each other.

Table 6: Relative organ weight result of rats exposed to sildenafil citrate

PARAMETERS	A (Control)	B 100 mg/kgSC	C 200 mg/kgSC	D 300 mg/kgSC
Brain (g)	0.66 ± 0.04	0.76 ± 0.04	0.73 ± 0.06	0.64 ± 0.03
Heart (g)	0.38 ± 0.02	0.40 ± 0.01	0.40 ± 0.01	0.41 ± 0.04
Lungs (g)	0.60 ± 0.07	0.58 ± 0.05	0.59 ± 0.07	0.45 ± 0.03
Liver (g)	3.97 ± 0.14	4.39 ± 0.48	3.82 ± 0.15	4.38 ± 0.18
Spleen (g)	0.40 ± 0.02	0.51 ± 0.10	0.42 ± 0.05	0.36 ± 0.04
Kidneys (g)	0.52 ± 0.06 [a]	0.70 ± 0.02 [b]	0.68 ± 0.01 [b]	0.65 ± 0.01 [b]
Epididymides (g)	0.27 ± 0.03	0.22 ± 0.06	0.22 ± 0.04	0.27 ± 0.05
Testes (g)	1.01 ± 0.07	0.97 ± 0.16	1.04 ± 0.13	1.03 ± 0.15

Different superscripts [a, c, b] in a row indicate significant ($p<0.05$) differences between the means

Weight gain/loss results

Table 7 shows the result for weekly and total weight differences. At week 1, there was a significant difference ($p<0.05$) in the weight gain among all the treatment groups with the highest weight gain recorded in control group A rats while treatment group D rats lost weight. At week 2, group A, C and D lost weight even though there was no significant difference ($p>0.05$) between them while group B gained weight. At week 3, there was a significantly ($p<0.05$) marked weight gain across all the groups with the highest gain recorded in group A which was followed by group B, D and C rats. At week 4 there was weight gain across all the groups with group C having a significantly ($p<0.05$) highest weight gain when compared with the control and treatment groups A and D rats which did not differ significantly ($p>0.05$). At week 5, group A and D rats lost weight while group B and C rats gained weight. There was no significant difference ($p>0.05$) in the weight gain between groups B and C rats but were significantly different ($p<0.05$) from the weight loss in groups A and D rats. At week 6, there was weight loss in group A rats while group B, C and D rats gained weight. The weight loss in group A rats was significantly different ($p<0.05$) from the weight gain in groups B, C and D rats which were not significantly different ($p>0.05$) from each other. At week 7, group B lost weight which was significantly different ($P<0.05$) from groups A, C and D rats that gained weight. The highest weight gain at week 7 was recorded by group D rats which was not significantly different ($p>0.05$) from group A rats but were significantly lower ($p<0.05$) than C rats. Summarily, there was an overall weight gain in all the groups with the highest value recorded in group B rats and the least weight gain recorded in group D rats.

Table 7: Weight gain/loss results of rats exposed to sildenafil citrate

PARAMETERS	A (Control)	B 100 mg/kgSC	C 200 mg/kgSC	D 300 mg/kgSC
WEEK 1 (g)	14.33 ± 4.06^{a}	1.33 ± 4.10^{b}	7.67 ± 1.98^{c}	-3.83 ± 1.96^{d}
WEEK 2 (g)	-4.83 ± 2.68	4.83 ± 4.25	-10.67 ± 9.68	-2.67 ± 4.21
WEEK 3 (g)	21.50 ± 1.50^{a}	16.83 ± 3.32^{b}	8.83 ± 5.88^{c}	10.00 ± 3.79^{c}
WEEK 4 (g)	13.17 ± 4.36^{a}	15.17 ± 3.63^{a}	22.00 ± 2.08^{b}	13.83 ± 2.07^{a}
WEEK 5 (g)	-1.00 ± 5.49^{a}	6.50 ± 1.88^{b}	5.83 ± 2.21^{b}	-1.00 ± 1.79^{a}
WEEK 6 (g)	-9.50 ± 3.22^{a}	4.33 ± 1.54^{c}	7.50 ± 1.73^{c}	5.50 ± 1.20^{c}
WEEK 7 (g)	11.17 ± 3.26^{a}	-3.17 ± 2.43^{b}	0.50 ± 2.54^{c}	13.00 ± 1.46^{a}
TOTAL (g)	44.51 ± 4.76	45.82 ± 3.22	41.66 ± 4.24	34.83 ± 2.54

Different superscripts $^{a, c, b}$ in a row indicate significant ($p<0.05$) differences between the means. Negative value indicate weight loss

Feed intake results

Table 8 shows the weekly and total feed intake. At week 2, group A rats consumed significantly higher ($p<0.05$) feed when compared with treatment groups B, C and D rats which did not differ ($p>0.05$) significantly. There was no significant difference ($p>0.05$) in the feed consumption in the remaining weeks of the experiment between the treatment groups and the control. The control group A rats consumed the highest total feed during the experiment. This was followed by group C, B and A rats.

Table 8: Feed intake results of rats exposed to sildenafil citrate

PARAMETERS	A (Control)	B 100 mg/kgSC	C 200 mg/kgSC	D 300 mg/kgSC
WEEK 2 (g)	117.29±2.97[a]	95.43±7.86[b]	87.71±9.97[b]	80.29±12.10[b]
WEEK 3 (g)	122.43±4.75	125.57±3.43	120.71±4.66	111.29±5.67
WEEK 4 (g)	123.43±11.44	120.29±5.89	129.71±8.53	119.14±4.60
WEEK 5 (g)	115.14±4.01	116.86±2.31	119.14±1.86	107.14±1.68
WEEK 6 (g)	109.71±5.85	114.29±3.02	127.14±1.55	117.29±1.78
WEEK 7 (g)	119.86±5.28	119.43±3.17	110.00±5.69	113.00±1.88
TOTAL (g)	707.86 ± 4.36	691.87 ± 3.14	694.41 ± 6.32	648.75 ± 2.54

Different superscripts [a, b] in a row indicate significant ($p<0.05$) differences between the mean

Water intake results of of rats exposed to sildenafil citrate

Table 9 shows the result for water intake. There was significant difference ($p<0.05$) in the water intake between the groups at week 3, 4 and 5. At week 3, group C rats had the highest water intake (259.14±8.54 ml) which was significantly different ($P<0.05$) from group D rats with the least value of 224.57±12.07ml. Groups C and D rats were however not different ($P>0.05$) from group B and A rats. At week 4, group C rats had the significantly highest value ($P>0.05$) of water intake with group D rats having the lowest value ($P<0.05$). At week 5, There was no significant difference ($P>0.05$) between group A and C, A and D, A and B. At week 5, group D rats had the significantly lowest ($p<0.05$) value of water intake which was followed by group A and B with group C having the significantly highest ($p<0.05$) value of water intake. At week 6 and 7, there was no significant difference in the water intake between the treatment groups and the control. The total water intake was significantly highest ($p<0.05$) in group C rats while group D rats hat the least total water intake.

Table 9: Water intake results of rats exposed to sildenafil citrate

PARAMETERS	A Control	B 100 mg/kgSC	C 200 mg/kgSC	D 300 mg/kgSC
WEEK 2	179.57±6.03	165.00±9.03	173.29±12.10	173.29±12.10
WEEK 3	255.00±8.83[a]	243.86±10.35[a]	259.14±8.54[a]	224.57±12.07[b]
WEEK 4	280.00±10.80[a]	259.57±10.19[b]	306.71±11.56[c]	239.71±6.02[d]
WEEK 5	281.00±10.80[a]	284.57±11.14[a]	325.70±7.11[b]	254.86±17.48[c]
WEEK 6	328.00±8.88	323.43±20.36	357.43±13.06	310.29±9.31
WEEK 7	314.00±24.67	311.71±20.43	365.43±12.08	306.43±16.64
TOTAL	**1637.57±12.87[a]**	**1588.14±11.76[a]**	**1787.70±14.11[b]**	**1509.15±12.44 [a]**

Different superscripts [a, c, b] in a row indicate significant ($p<0.05$) differences between the means

Histopathology

Figure 1 shows the photomicrograph of rat liver from the control group A rat and treated group D rat. The sinusoids of the liver are well arranged with the hepatocytes in their normal shape and sizes. The sinusoids in the treated group D are disorganised with the hepatocytes swollen indicating degenerative changes.

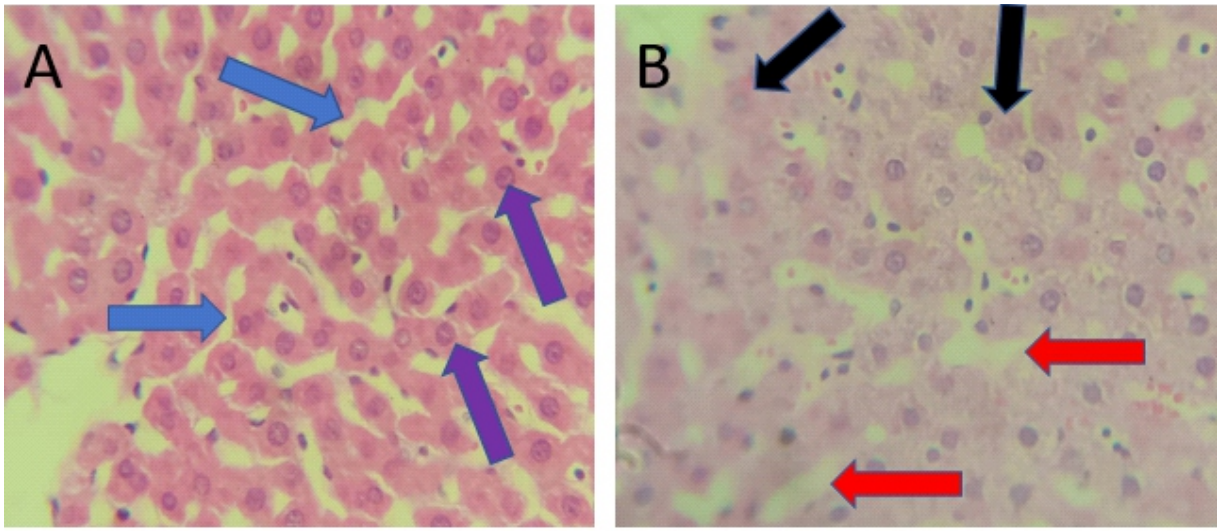

Figure 1: A=Rat liver with normal sinusoids arrangement (blue arrows) and hepatocytes sizes (purple arrows) from control group; **B=**Rat liver with disorganised sinusoids arrangement (red arrows) and swollen hepatocytes (black arrows) from sildenafil citrate treated group

DISCUSSION

The [22] findings of this study suggest that sildenafil citrate (SC) is not acutely toxic at a dose of 2000 mg/kg body weight, 24 hours post-administration. This low toxicity aligns with its classification as a non-prescription drug and over-the-counter availability. [23] reported that substances tolerated at doses up to 2000 mg/kg in animals exhibit a high safety margin. Similarly, the observed LD_{50} exceeding 2000 mg/kg in this study corroborates the earlier findings of [24], indicating that SC is relatively non-toxic at moderate doses.

Male fertility depends on the successful progression of spermatogenesis, a multi-phase process occurring in the seminiferous tubules. While various hormones contribute to this process, testosterone is indispensable [25]. One behavioral indicator of elevated testosterone is aggressiveness. In this study, SC-treated rats exhibited aggressive sexual behavior, demonstrated by reduced intromission and mount latencies (Table 1). Increased energy expenditure linked to this behavior may explain the weight loss observed in treated groups relative to controls.

Testosterone biosynthesis occurs in the Leydig cells of the testes, primarily from cholesterol [26,27]. The increased serum testosterone in SC-treated rats could be associated with the upregulation of steroidogenic genes such as *scavenger receptor class B type 1 (SRB1)*, *steroidogenic acute regulatory protein (StAR)*, and *cytochrome P450 side-chain cleavage enzyme (P450scc)*. These genes facilitate cholesterol transport and conversion during steroidogenesis. SRB1, in particular, mediates the uptake of cholesteryl esters from LDL into steroidogenic tissues [28, 29]. The significantly reduced serum cholesterol in treated groups (Table 4) may reflect increased utilization for steroidogenesis.

StAR mediates the rate-limiting step in testosterone synthesis by transporting cholesterol into mitochondria [30, 31]. Although gene expression was not directly assayed in this study, the marked decrease in serum cholesterol among treated groups suggests increased intracellular cholesterol use, likely facilitated by these regulatory genes. Elevated luteinizing hormone (LH) levels observed in treated rats may further stimulate StAR expression via LH-receptor binding, thereby enhancing testosterone production [32].

The capacity of SC to modulate steroidogenesis and influence gene expression in the testosterone biosynthetic pathway remains a largely unexplored aspect of reproductive physiology, meriting further investigation.

Haematological Effects

Haematological evaluations provide crucial insights into the physiological and pathological states of organisms. Alterations in haematological parameters can indicate systemic responses to stress, injury, or toxicity [33]. SC has been shown to influence angiogenesis, platelet activation, immune modulation, and cytokine production [34]. It also induces dose-dependent oxygen release from erythrocytes [35]. [34] further noted that SC overdose could alter red blood cell (RBC) morphology, including shape, size, and hemoglobin (Hb) distribution.

In this study, RBC distribution was irregular in SC-treated rats, with corresponding alterations in packed cell volume (PCV), Hb concentration, and erythrocyte indices. These findings suggest a disruption of normal haematopoiesis or RBC integrity. Such instability in haematological profiles supports the hypothesis of underlying pathophysiology induced by SC at high doses. Thus, high-dose SC usage warrants caution to prevent adverse hematological effects.

Liver Histopathology

Histological examination of the liver in treated rats revealed disorganized hepatic sinusoids and swollen hepatocytes, indicative of degenerative changes (Figure 1). The liver, being the primary site of xenobiotic metabolism, is often the first organ to exhibit toxicity following oral drug administration. Therefore, the observed hepatotoxicity, particularly at the high dose of 300 mg/kg body weight, is not unexpected. Notably, this dose exceeds the maximum therapeutic dose of 100 mg [37], suggesting that excessive SC administration may be deleterious to hepatic health.

Reproductive Effects

[38] reported reduced sperm count and motility in rats following chronic SC administration for 120 days, attributing these effects to testicular and epididymal toxicity. Long-term SC use also impairs Sertoli cell function and spermatogenesis [39], and may disrupt acrosome formation, thereby reducing fertilization capacity [40]. While short-term SC administration has been shown to enhance sperm motility and acrosomal activity [41, 42], prolonged use appears detrimental.

In this sub-acute study, SC-treated rats exhibited significant reductions ($P < 0.05$) in sperm count and motility relative to controls. These findings may reflect disruptions to spermatogenesis and support earlier reports regarding the reproductive toxicity of chronic SC exposure.

Body Weight and Liver Enzyme Ratios

Although variations in weekly weight 1changes were observed, cumulative weight gain was not significantly different across groups. This outcome aligns with findings [43], who reported no significant impact of SC on weight gain. However, an elevated AST/ALT ratio was recorded in the treated groups, suggestive of hepatic dysfunction. This biochemical indicator is supported by histological evidence of liver damage, further confirming the hepatotoxic potential of high-dose SC administration.

Conclusion

This study demonstrates that sildenafil citrate (SC), while exhibiting low acute toxicity at a dose of 2000 mg/kg body weight, has significant physiological effects on reproductive and systemic parameters in albino rats. The findings suggest that SC administration can elevate serum testosterone levels, likely through enhanced luteinizing hormone (LH) activity and increased utilization of cholesterol for steroidogenesis. These hormonal changes were reflected behaviorally in heightened sexual activity and physiologically in reduced serum cholesterol concentrations.

However, sub-acute exposure to high doses of SC negatively impacted sperm parameters, including significant reductions in sperm count and motility. Histopathological examination of the liver revealed structural disorganization and cellular degeneration, indicating hepatotoxicity at higher doses. Additionally, alterations in haematological indices and elevated AST/ALT ratios further support the potential systemic toxicity associated with unregulated SC use.

These results underscore the need for cautious use of sildenafil citrate, particularly in individuals without clinical indications for its use. While SC may offer short-term benefits in improving sexual function, chronic or high-dose usage could pose significant risks to reproductive health and systemic physiology. Further molecular studies are warranted to elucidate the precise mechanisms by which SC affects steroidogenic pathways and to evaluate its long-term safety profile.

Recommendation

From the findings of this study, further investigations is recommended to ascertain the exert effect of SC on the messenger ribonucleic acid expression of steroidogenic genes. Histology of all the vital organs of the body is recommended to evaluate the safety of SC in them. Chronic study is also recommended to evaluate the long term effect of SC.

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