



Comparative Effects of Sildenafil Citrate and Revive Capsule on Some Liver and Kidney Parameters in Male Albino Rats

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Abstract

Revive capsule is a polyherbal drug that has been reportedly used for the treatment of erectile dysfunction or enhancement of libido in men. There are speculations that herbal drugs are relatively safer and more potent than western drugs; this may be true owing to the phytochemicals present in herbs. Contrarily, it is believed that herbal medicines are formulated on a relatively lesser scientific basis compared with the western drugs; hence, this study was aimed at evaluating the comparative effects of Viagra (Sildenafil citrate) and Revive (polyherbal) capsule on some liver and kidney parameters in male albino rats. A total of 21 male albino rats were used for the study, and were divided into three (3) groups of 7 rats each. They were allowed to acclimatize for two (2) weeks by maintaining 12-hour light and dark cycles daily, with access to standard feed and water ad libitum. Group I (Negative Control) rats were administered with distilled water once daily, while groups II and III rats were administered once daily with Sildenafil citrate and Revive capsule respectively for 4 weeks. The rat doses administered were extrapolated from the human dose using the formula by Paget and Barnes in 1964. Group II (Viagra group) rats were administered with 4.5mg/kg of Sildenafil citrate, while group III rats were administered with 72mg/kg of Revive capsule. At the end of the treatment period, the rats were allowed to fast overnight, followed by their anaesthetization using chloroform, and blood sample collection via jugular vein puncture. Also, the liver and kidneys were excised and examined histologically. Rat-specific test kit with ELISA method was used to analyze serum Cystatin C, while Randox test kits with colorimetric method were used to analyze AST, ALT, ALP and GGT. The results showed that after 4 weeks of treatment, there were significantly lower serum levels of AST and ALT only in Revive group, and a significantly lower serum ALP level in both Revive and Viagra groups compared to the negative control. Also, photomicrographs of the histologically examined tissues of the

treatment groups appeared indifferent from that of the negative control. Based on the rat model used in this study, these findings may suggest that treatment with either Sildenafil citrate or Revive capsule for 4 weeks at their appropriate dosages did not pose hepatotoxicity or nephrotoxicity, but that Revive capsule may rather exert a protective or curative effect on the liver.

Keywords: Revive, Viagra, Sildenafil citrate, Erectile dysfunction, Polyherbal, libido, Phytochemicals

1. Introduction

The administration of drugs to living organisms is geared towards the exertion of pharmacological effects which are beneficial to the organisms. However, for some drugs, some adverse effects may be produced which may be harmful to the organisms. Also, some drugs may produce beneficial effects other than the ones they were intended; for example, Sildenafil citrate was originally administered for the treatment of pulmonary arterial hypertension, but was later discovered to be very effective in the treatment of erectile dysfunction and enhancement of libido in male individuals.

Viagra (also called Sildenafil citrate) was the first phosphodiesterase type-5 inhibitor launched in 1998. It is administered in 25 mg, 50 mg and 100 mg doses; however, the recommended dose is 50 mg, and can be adjusted due to the patients' response and side effects. It is effective from 30-60 minutes after administration, and the efficacy may be maintained for about 12 hours. However, its action is lessened after a meal composed of heavy fat, possibly due to prolonged absorption, and is metabolized primarily by the CYP3A4 pathway, and converted to an active metabolite known as N-desmethyl; this metabolite as well as the parent drug (sildenafil) has a half-life of approximately 4 hours. It is excreted mainly as metabolites in the feces and, to a lesser extent, in the urine.^[4]

Revive capsule (also called Kedi Revive) is a polyherbal formulation manufactured by Kedi Healthcare Company Limited in Hong Kong, China. It is composed of several herbs known to possess aphrodisiac properties, and is used for the treatment of erectile dysfunction and enhancement of libido in men. Its effects are exerted 4 hours after administration via the oral route.^[12] Each capsule of the drug contains 400mg of the constituent herbs which include; 80mg of Herb Epimedii (mainly *Epimedium sagittatum*), 80mg of Radix ginseng,

40mg of *Cordyceps militaris*, 80mg of *Tribulus terrestris*, 80mg of *Radix polygوني multiflori*, and 40 mg of *Eucommia ulmoides*. The dosage for an adult man is two capsules (800 mg) to be taken once daily for a minimum of 28 days (to expect significant improvement when used to treat erectile dysfunction).^[12]

The kidneys are paired, bean-shaped organs situated behind the peritonium on either side of the spinal column. Each kidney contains about one million nephrons (functional units of the kidney), and each nephron is made up of five main segments which include the glomeruli, proximal convoluted tubule, loop of Henle, distal convoluted tubule and collecting ducts. The kidneys eliminate drugs and other toxic wastes from the blood, with the resultant formation of urine (through which these toxic substances are eliminated) which migrates to the bladder via the ureter.^[13]

The liver is the largest internal organ in humans.⁸ It is the most complicated and functional organ, situated below the diaphragm at the upper right of the abdomen, and has a weight of about 1.2-1.5kg in healthy adults. Among other functions, the liver detoxifies and rapidly excretes drugs. Every substance absorbed from the gut into the blood must first pass through the liver, a process called First Pass.¹ By this process, it prevents the entry of toxic substances and allows the entry of only non-toxic substances in the blood.^[3]

2. Materials and Methods

2.1 The experimental animals

A total of twenty one (21) male albino rats weighing between 130 to 220g were used for this study. The rats were divided into three (3) groups of seven (7) rats each. Each group of the rats were put into well-labeled cages, and were allowed to acclimatize for two (2) weeks by maintaining light and dark cycles for 12 hours daily, with access to standard rat feeds and water ad libitum.

2.2 The drugs used for the study

The drugs used for this study were Viagra (Sildenafil citrate) and Revive capsule. Viagra is a synthetic drug manufactured by Pfizer Pharmaceuticals, Ireland. It was used in the study as a standard or reference drug, while Revive capsule is a polyherbal formulation produced by Kedi Healthcare Company Limited, Hong Kong in China and sold in Nigeria. However, the phytochemicals in Revive capsule were determined qualitatively using a classical method, and quantitatively using a spectrophotometric method.^[7]

2.3 Acute Toxicity Study

This was done to determine the LD50 of Revive capsule, using the Fixed Dose Procedure,¹⁵ in which three (3) male albino rats were put together in a cage, and allowed to fast overnight, after which each of the rats was administered with Revive capsule at a dose of 2000mg/kg body weight. Then, they were physically observed for 72 hours (3 days) for signs of toxicity or death. However, no sign of toxicity or fatality was physically recorded; this may imply that the herbal formulation Revive was safe at a single dose even at a dosage of 2000mg/kg.

2.4 Calculation of Doses

The doses administered were extrapolated from the human dose using the formula by Paget and Barnes in 1964, which is represented as rat dose = human dose x 0.018 x 5. The rats in each group were weighed, and the average of their weights taken. Based on the average weight taken, the appropriate dose (expressed mg/kg body weight of the rats) of the drug for each group was determined, as well as the appropriate volume of distilled water used as diluent for the drugs following the OECD guidelines;^[16] this preparation was done weekly throughout the period of the drug administration.

For Viagra (Sildenafil citrate):

Each tablet is 50mg, and the adult human dosage (approximately 70kg adult) is 50mg (one tablet) to be taken once daily. Therefore, the rat dose (mg/kg) = human dose (50mg) x 0.018 x 5, which is equal to 4.5mg/kg (4.5mg/1000g) body weight of each rat (for a rat weighing 200g).

For Revive:

Each capsule is 400mg, and the adult human dosage is 800mg (two capsules) to be taken once daily. Therefore, the rat dose (mg/kg) = human dose (800mg) x 0.018 x 5, which is equal to 72mg/kg (72mg/1000g) body weight of each rat (for a rat weighing 200g).

2.5 Study Design

The rats were administered with the drugs through oral gavage using a metal oropharyngeal cannula.

Group I (Negative Control group) was administered with distilled water once daily, group II (Viagra group) was treated with Viagra (Sildenafil citrate) at a dosage of 4.5mg/kg once daily for four (4) weeks, while group III was treated with Revive capsule at a dosage of 72mg/kg once daily for 4 weeks.

At the end of each treatment period, the rats were allowed to fast overnight, and were anaesthetized using chloroform. Then 5ml of whole blood specimen was collected (using a sterile syringe and needle) into sterile sample containers (plain bottles) through jugular vein puncture, followed by surgical removal of the kidneys and liver which were immediately preserved in 10% formal saline for histological analysis.

The serum was used to analyze liver enzymes (AST, ALT, ALP and GGT) and using standard laboratory assay methods, while the kidney marker (Cystatin C) was analyzed using rat-specific ELISA test kits manufactured at the Bioassay Technology Laboratory in Shanghai, China.¹¹ The histological analysis of the rats' liver and kidney was done through tissue processing, and staining using the "Haematoxylin and Eosin" staining technique.^[10]

2.6 Statistical Analysis

The data generated from the analysis were expressed as Mean \pm Standard deviation, and analyzed using the Statistical Package for Social Sciences (SPSS) version 23. Comparisons of mean and standard deviation values were made for the various parameters for tests and control using the one-way ANOVA and Tukey tests. Results were considered statistically significant at 95% confidence interval ($p < 0.05$).

3. Results

3.1 Results of the phytochemical analysis of Revive capsule

Details of this are shown in Table 1 below. It shows the presence of the phytochemicals flavonoid,

cardiac glycoside, tannins, polyphenols, alkaloids and quinones in Revive capsule with mean concentrations of 20.48 ± 1.18 , 2.76 ± 0.15 , 1.43 ± 0.43 , 25.4 ± 0.19 , 23.87 ± 0.44 and 0.76 ± 0.01 respectively.

Table 1: Results of the Qualitative and Quantitative Phytochemical Analysis of Revive Capsule

Components	Qualitative	Quantitative
Flavonoid	+	20.48±1.18
Cardiac glycoside	+	2.76±0.15
Tannins	+	1.43±0.43
Polyphenols	++	25.4±0.19
Alkaloids	++	23.87±0.44
Quinones	+	0.76±0.01

KEY: + = present

3.2 Comparison between the Mean Levels of AST, ALT, ALP and GGT of Group I, II and VI

Details of this are shown in Table 2 below. It shows that the mean AST and ALT levels of group VI were significantly lower ($p < 0.05$) compared to those of group I and group II, but no significant difference was noted between group I and II. Also,

the mean ALP level of group VI was significantly lower compared to that of group I and group II, and the mean ALP level of group II was significantly lower compared to that of group I. Finally, the mean GGT level of group VI was significantly higher compared to that of group II; but there was no significant difference between groups I and II, and between groups I and VI.

Table 2: Results of AST, ALT, ALP and GGT of Group I, II and VI compared

N=7	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)
Group I	69.13±15.15 ^c	100.95±22.54 ^c	211.11±16.89 ^{bc}	31.70±5.21
Group II (Viagra)	56.04±11.07 ^c	91.71±13.77 ^c	175.63±9.75 ^{ac}	29.12±3.92 ^c
Group VI (Re-vive)	30.04±8.60 ^{ab}	32.59±8.31 ^{ab}	156.30±14.05 ^{ab}	44.53±15.06 ^b
F-value	19.509	37.669	28.104	5.314
P-value	<0.001*	<0.001*	<0.001*	0.015*

KEY:* = statistically significant

n= number of samples

^a= significantly different from the negative control

^b= significantly different from the Viagra group

^c= significantly different from the Re-vive group

3.3 Comparison between the Mean Levels of Cystatin C of Group I, II and VI

Details of this are shown in Table 3 below. It shows that there was no significant difference in the

mean Cystatin C level when groups I, II and VI were compared.

Table 3: Results of Cystatin C of Group I, II and VI compared

N=7	Cystatin C
Group I	2.01±0.06
Group II (Viagra)	1.97±0.05
Group VI (Revive)	2.42±0.72
F-value	2.465
P-value	0.113

KEY: * = statistically significant

n= number of samples

^a = significantly different from the negative control

^b = significantly different from the Viagra group

^c = significantly different from the Re-vive group

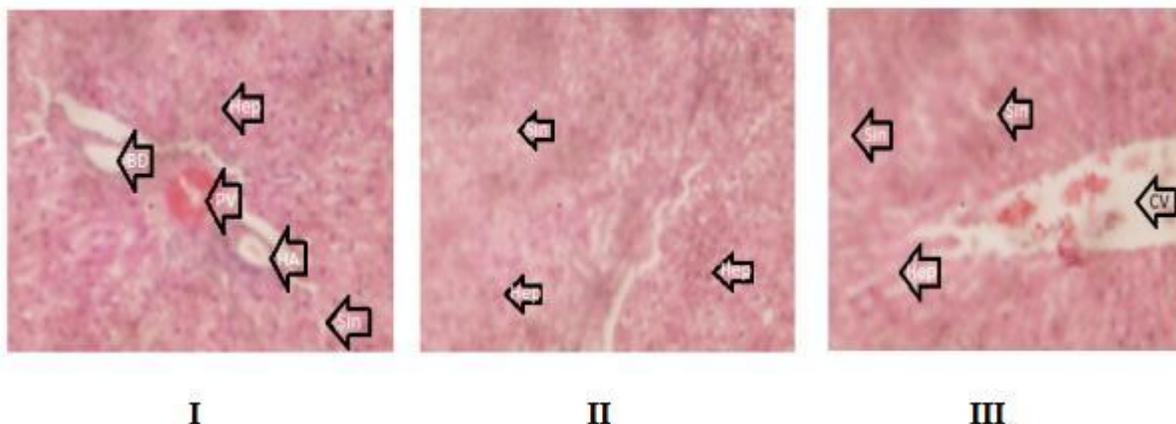


Figure 1: I, II and III: Photomicrograph (X 400) H & E stained histologic sections of the liver of the rats. The negative control (group I) shows histologically normal liver cells presenting normal hepatocyte (Hep), congested portal vein (PV), patent bile duct (BD), patent hepatic artery (HA) and sinusoids (Sin) containing kupffer cells. The other groups also show histologically normal liver cells

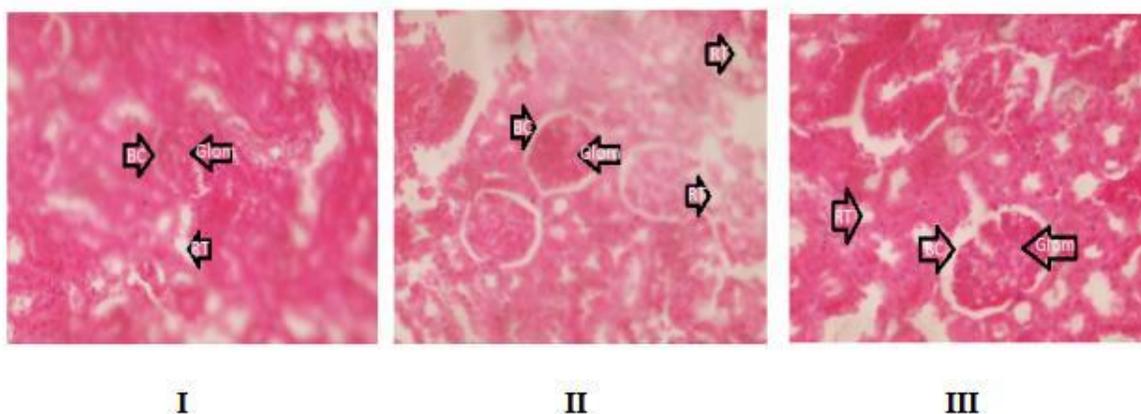


Figure 2: I, II and III: Photomicrograph (X 400) H & E stained histologic sections of the kidney of the rats. The negative control (group I) shows histologically normal kidney presenting glomerular tuft (Glom), bowman's capsule space (BC) surrounding the glomerular tuft and renal tubules (RT) lined by simple epithelial cells. The other groups also show histologically normal kidney cells

4.0 Discussion

Phytochemical analysis of the polyherbal drug Revive showed the presence of flavonoid, cardiac glycosides, tannins, phenols, alkaloids, and quinones in different quantities. Herbs or herbal products contain several bioactive phytochemicals which, apart from possessing nutritive value, may also exert pharmacological responses by influencing

metabolic pathways, and are hence useful in medicine.^[13]

Results from this study showed that after four (4) weeks of treatment with Viagra and Revive capsule, there was a significant decrease in AST and ALT in group VI (Revive group) compared to group I (negative control) and II (Viagra group), however no significant difference between group I and II was noted. The enzymes AST and ALT are produced in hepatocytes into the cytoplasm, where

they react to membrane stress, and are as such, used together in the diagnosis of hepatocellular damage; in this condition, AST and ALT leaks into the blood circulation, resulting in a rise in their plasma concentrations.^[20] Contrarily, a decrease in AST and ALT levels is an implication of normal integrity of the liver cells. Thus, the decreased serum AST and ALT levels obtained from this study may suggest that the polyherbal drug Revive may possess the potential of maintaining the normal integrity of the liver cells, which may be attributed to the phytochemicals present in Revive capsule; tannin (a phytochemical in Revive capsule) was reported to ameliorate liver disease by potentiating a decrease in serum AST and ALT levels in carbon tetrachloride-induced liver toxicity in mice.^[5] Also the effects exerted by Revive capsule may be attributed to the individual herbs (containing phytochemicals) used in the formulation of the Revive capsule; from a research carried out by Eagappan et al,^[6] it was reported that the cooked fruit extract of the plant *Tribulus terrestris* (one of the herbs present in Revive capsule) protects the liver from lead-induced hepatotoxicity by inducing a decrease in serum AST and ALT in Sprague dawley rats. Also, from a research carried out by Hung et al, ^[9] it was reported that the water extract of the leaves of the plant *Eucommia ulmoides* (one of the herbs present in Revive capsule) possesses a hepatoprotective potential in carbon tetrachloride-induced chronic hepatotoxicity in rats by inducing a decrease in serum AST and ALT, and that this hepatoprotective potential may be attributed to the presence of polyphenols in the plant. Shehab et al reported that polyphenols including flavonoids possess an increased antioxidant capacity, and play a protective function in liver diseases; ^[18] interestingly, polyphenols are one of the phytochemicals present in Revive capsule.

The Revive-treated group had a significantly lower serum ALP level when compared with the Viagra-treated group and negative control; also the Viagra-treated group had a significantly lower ALP level when compared to the negative control. ALP is synthesized by the sinusoidal surface of hepatocytes, and is usually found in the microvilli of bile canaliculi in the liver. ALP usually flows out through the bile duct into the small intestine; however in cholestasis, ALP regurgitates into the

blood stream resulting in a rise in its concentration in the plasma. The decreased serum ALP level obtained from the Revive-treated group may be attributed to the contained phytochemical components, which may have played a role in the maintenance of the normal excretory capacity of the liver.

Additionally, the Revive-treated group had a significantly increased serum GGT level when compared to the Viagra-treated group II; however no significant difference was found between each of the treatment groups and the negative control. The enzyme GGT is found in many tissues, including the liver, where it plays an essential role in glutathione metabolism, and plays a major role in assisting the liver in the metabolism of drugs and other toxins.^[21] Increased serum levels occur mainly in alcoholic cirrhosis, and also in neoplasms, or due to certain medications.^[14] The non-significant difference obtained from this study may be an implication of the inability of the drug to induce hepatotoxicity that could result in the elevation of serum GGT levels.

There was no significant difference in cystatin C levels when all three groups were compared. Cystatin C is synthesized by the liver, secreted into the blood stream at a steady rate, and eliminated from the circulation by the kidneys via a process of glomerular filtration.^[15] Its level in the blood rises as renal function and glomerular filtration decreases.^[19] However, the report from this study suggests that both drugs had no effect on the renal function.

5.0 Conclusion

After 4 weeks (28 days) of treatment with Viagra and Revive capsule, there was no form of hepatotoxicity or nephrotoxicity as revealed by the results of the biochemical analysis and photomicrographs of the histologically examined liver and kidney tissues. Rather, there were significantly decreased levels of the hepatic enzymes, which could be an implication of a protective or curative potential of the Revive capsule.

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