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Effect of Revive Capsule on Cardiovascular Risk Indices in Male Albino Rats in Relation to the Duration of Administration

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Abstract

Revive capsule is a polyherbal formulation commonly used to treat erectile dysfunction or enhance libido in men. This drug has similar mechanism of action with phosphodiesterase type-5 (PDE5) inhibitors. However, there have been speculations about the relationship between PDE5 inhibitors and cardiovascular disease, hence the need for this study, which was aimed at evaluating the effects of Revive capsule on cardiovascular risk indices in male albino rats in relation to the duration of administration. A total of 42 male albino rats were used for the study, and were divided into six (6) groups of seven (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 group) was administered with distilled water once daily, while groups II, III, IV, V and VI were treated with Revive capsule at a dosage of 72mg/kg once daily for 1 week, 2 weeks, 3 weeks, 4 weeks and 6 weeks respectively. The rat dose administered was extrapolated from the human dose using the formula by Paget and Barnes. At the end of each treatment week, the rats were allowed to fast overnight, followed by their anaesthetization using chloroform, and blood sample collection via jugular vein puncture. Also, the heart was excised and examined histologically. Fasting lipid profile total cholesterol, triglycerides and high density lipoprotein was analyzed using Randox test kits with enzymatic method; the low density lipoprotein was calculated using Friedewald's equation; then, the cardiovascular (atherogenic) risk indices were calculated using the various lipid parameters. The results

showed a significant increase ($p < 0.05$) in serum high density lipoprotein level, and a decrease in serum low density lipoprotein level and cardiovascular risk indices (atherogenic index of plasma, castelli's risk index-I, castelli's risk index-II and atherogenic coefficient) in the treatment groups, with the maximum effect attained after 6 weeks of treatment. Also, photomicrographs of histologically examined cardiac muscle of the treatment groups appeared indifferent from that of the negative control. These findings may suggest that treatment with Revive capsule at the appropriate dosage for 6 weeks did not pose any cardiovascular risk or cardiotoxicty, but may rather be helpful in the amelioration of cardiovascular diseases in a rat model.

Keywords: Revive, Cardiovascular, Atherogenic, Cardiac, Histological, Administration

1. Introduction

Revive capsule (also called Kedi Revive) is a polyherbal drug formulated by Kedi Healthcare Company Limited in Hong Kong, China. It is made up of different herbs known to possess aphrodisiac properties, and is used to treat erectile dysfunction and enhance libido in men. The effects of this drug are exerted 4 hours after administration through the oral route. 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 polygoni 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 a significant improvement when used to treat erectile dysfunction) [9].

This drug has a similar mechanism of action with that of Sildnafil citrate, vardenafil and Tadalafil, and are all referred to as phosphodiesterase type-5 inhibitors; these drugs are mildly vasoactive in nature and exert their pharmacological effects by selectively inhibiting an enzyme called phosphodiesterase type-5 (PDE-5). PDE-5 promotes the hydrolysis of cyclic guanosine monophosphate (cGMP) responsible for the relaxation of the smooth muscle in the cavernosum tissue of the penis; the cGMP is also a second messenger of nitric oxide [16]. This inhibition of PDE-5 increases arterial blood flow, induces a rise in cGMP levels, and decreases intracellular calcium (Ca^{2+}) which promotes the smooth muscle to relax, the blood vessels to dilate and the penis to become erect [1].

Cardiovascular disease (CVD) refers to diseases that affect the circulatory system, such as the blood vessels and the heart. It is a group of diseases and injuries affecting the heart and its blood vessels (cardiovascular system), and also affecting the blood vessels of the brain. It is the largest single cause of mortality worldwide. Generally, cardiovascular diseases affect individuals in the later stages of life such that, at age 35, most individuals at risk of developing cardiovascular disease already have the onset of the disease [13]. There are different types of cardiovascular diseases, some of which include coronary artery disease, angina and stroke. Lipid profile results are helpful in the diagnosis or rule out of a cardiovascular disease; Jappesen et al. reported that decreased amounts of high density lipoprotein cholesterol (HDL-c), increased amounts of triglycerides (TG), and increased amounts of low density lipoprotein cholesterol (LDL-c) have been associated with elevated incidence of coronary artery disease [10]. However, Bhardwaj et al. reported that the absence of an abnormal lipid profile does not rule out the possibility of coronary artery disease, and that, even if the lipid profile is normal, the various combinations of the of the lipid parameters may be useful in identifying patients with risk of cardiovascular disease [3]; these combinations are referred to as atherogenic indices, and include Atherogenic Index of Plasma (AIP), Castelli Risk Index I (CRI-I) and II (CRI-II) and Atherogenic Coefficient (AC). The formulas for their calculations are stated below:

Atherogenic Index of Plasma (AIP) calculated as Log (TG/HDL), Castelli's Risk Index-I (CRI-I) calculated as Total cholesterol/HDL-c, Castelli's Risk Index-II (CRI-II) calculated as (LDL-c/HDL-c) and Atherogenic Coefficient (AC) calculated as (Total cholesterol- HDL-c)/HDL-c or (TG/HDL-c) [4].

Some of the individual herbs used in the formulation of this drug have been known scientifically to affect various biochemical components of the human body; hence the need for this study, which evaluated the effect of Revive capsule on cardiovascular risk indices in male albino, rats in relation to the duration of administration.

2. Methodology

2.1 The experimental animals

A total of forty-two (42) male albino rats weighing between 130 to 220g were used for this study. The rats were divided into six (6) 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 drug used for this study was Revive capsule. The drug 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 [6].

2.3 Acute Toxicity Study

This was done to determine the LD50 of Revive capsule, using the Fixed Dose Procedure [14], 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 [15], 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 guideline [14]; this preparation was done weekly throughout the period of the drug administration.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).

Group I (Negative Control group) was administered with distilled water once daily.

Groups II, III, IV, V and VI were treated with Revive capsule at a dosage of 72mg/kg once daily for 1 week, 2 weeks, 3 weeks, 4 weeks and 6 weeks respectively.

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 to obtain the serum) through jugular vein puncture, followed by surgical removal of the heart which was immediately preserved in 10% formal saline for histological analysis.

The serum sample was used to analyze fasting lipid profile (total cholesterol, triglycerides and HDL) using Randox test kits with enzymatic method; the LDL was calculated using Friedewal's equation; then, the cardiovascular (atherogenic) risk indices were calculated using the various lipid parameters. The histological analysis of the rats' heart was done through tissue processing, and staining using the "Haematoxylin and Eosin" staining technique [8].

2.5 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 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 Serum Levels of Total cholesterol (T.chol), Triglycerides (TG), HDL and LDL of Groups 1-VI

Details of this are shown in Table 2 below. It shows that the mean levels of T.chol of groups II and IV were significantly higher when compared with those of groups I and III; then groups V and VI were significantly lower than groups II and IV. However, no significant difference occurred between groups I, III, V and VI when compared. The mean levels of TG of group II was significantly higher when compared with that of group I; groups III, IV, V and VI were significantly lower when compared with group II. However, there was no significant difference between groups I, III, IV, V and VI when compared. The mean level of HDL of group IV was significantly higher when compared with that of group III; group was significantly higher when compared with other groups; however, there was no significant difference noted between groups when compared. The mean level of LDL of group VI was significantly lower when compared with those of other groups, and no significant difference was noted between groups I, II, III, IV and V when compared.

Table 2: Results of T.chol, TG, HDL and LDL of Groups I-VI compared

N=7	T.chol (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Group I (NC)	2.18±0.08bd	1.12±0.14b	0.57±0.07f	1.10±0.08f
Group II	2.38±0.10acef	1.60±0.23acdef	0.65±0.06f	1.25±0.33f
Group III	2.15±0.05bd	1.10±0.08b	0.52±0.07df	1.14±0.06f
Group IV	2.40±0.20acef	0.93±0.13b	0.72±0.10cf	1.27±0.23f
Group V	2.11±0.10bd	0.93±0.10b	0.60±0.10f	1.11±0.11f
Group VI	2.14±0.15bd	0.97±0.06b	1.10±0.20abcde	0.60±0.15abcde
F-value	7.528	24.23	25.655	11.960
P-value	<0.001*	<0.001*	0.002*	<0.001*

KEY
* = statistically significant
n= number of samples
a = significantly different from group I
b= significantly different from group III
c= significantly different from group IV
d=significantly different from group V
e= significantly different from group VI
f= significantly different from group VII

3.3 Comparison between the Atherogenic Index of Plasma (AIP), Castelli's Risk Index-I (CRI-I), Castelli's Risk Index-II (CRI-II) and Atherogenic Coefficient (AC) of Groups 1-VI

Details of this are shown in Table 3 below. It shows that the mean level of AIP of group IV was significantly lower when compared with those of groups I, II and III; the mean level was significantly lower in group V when compared with those of groups II and III; however, group VI was significantly lower when compared to all other groups. The mean level of CRI-I of group IV was significantly lower when compared with that of group III; however, group VI was significantly lower when compared with all other groups. The mean level of CRI-II of group VI was significantly lower when compared with all other groups. The mean level of AC of group IV was significantly lower when compared with group III; however, group VI was significantly lower when compared with all other groups.

Table 3: Results of AIP, CRI-I, CRI-II and AC of Groups I-VI compared

N=7	AIP	CRI-I	CRI-II	AC
Group I (NC)	0.30 ± 0.05^{df}	3.89 ± 0.43^{f}	1.98 ± 0.37^{f}	2.89 ± 0.43^{f}
Group II	0.39 ± 0.04^{def}	3.67 ± 0.19^{f}	1.91 ± 0.45^{f}	2.67 ± 0.19^{f}
Group III	0.33 ± 0.07^{def}	$4.20\pm0.50d^{f}$	2.23 ± 0.35^{f}	3.20 ± 0.50^{df}
Group IV	0.12 ± 0.09^{abcf}	3.42 ± 0.59^{cf}	1.82 ± 0.51^{f}	2.42 ± 0.59^{cf}
Group V	0.19 ± 0.06^{bcf}	3.58 ± 0.45^{f}	1.89 ± 0.39^{f}	2.58 ± 0.45^{f}
Group VI	-0.05 ± 0.10^{abcde}	1.99 ± 0.32^{abcde}	0.58 ± 0.24^{abcde}	0.99 ± 0.32^{abcde}
F-value	33.298	22.077	15.426	22.077
P-value	<0.001*	<0.001*	<0.001*	<0.001*

KEY
* = statistically significant
n= number of samples
[a] = significantly different from group I
[b]= significantly different from group III
[c]= significantly different from group IV
[d]=significantly different from group V
[e]= significantly different from group VI
[f]= significantly different from group VII

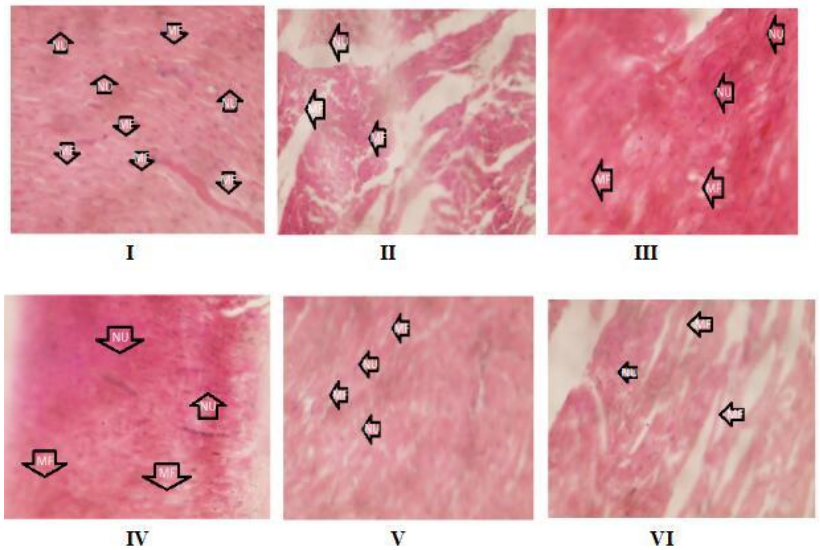

Figure 1: I, II, III, IV, V and VI: Photomicrograph (X 400) H & E stained histologic sections of the cardiac muscles of the rats. The negative control (group I) shows histologically normal cardiac muscle showing centrally located nucleus (NU), and cardiac myofibrils (MF) that are branched and reunited forming a network called cyncythium. The other groups also show histologically normal testes

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 [12].

Results from this study showed that the mean total cholesterol levels significantly increased after 1 week and 3 weeks of treatment (groups II and IV) compared to the negative control; however, it decreased significantly after 4 weeks and 6 weeks of treatment (groups V and VI respectively) compared to groups II and IV. There was no significant difference when the rats were treated for 2, 4 and 6 weeks compared with the negative control. The triglyceride level after 1 week of treatment increased significantly compared to the negative control; and further decreased after 2, 3, 4 and 6 weeks of treatment, however, there was no significant difference in the triglyceride level when these treatment groups were compared with the negative control. The HDL level after 3 weeks of treatment was significantly higher when compared with that after 2 weeks of treatment; however, the HDL level increased maximally after 6 weeks of treatment compared with other every other group including the negative control. On the other hand, the LDL level after 6 weeks of treatment was significantly lower when compared with every other group including the negative control. Following the various reports from the different lipid parameters, the treatment with Revive capsule induced an increase in the serum HDL level and a decrease in the serum LDL level, but unable to induce significant effects on the serum total cholesterol and triglyceride levels. Some of the various herbs used in formulating the Revive capsule have also been proven scientifically to produce results that conforms with the reports from this study; the herb Cordyceps militaris (which is an herbal component of Revive capsule) known as cordycepin or 3`-Deoxyadenosine induced a fall in the concentration of LDL levels in rats (which were made hyperlipidemic by placing them on high-fat diet) [7]. Also, the aqueous fruit extract of Tribulus terrestris (an herbal component of Revive capsule) was reported to posses hypolipidemic potential owing to a research carried out by Sailaja et al. in which an intra-peritoneal injection of isoproterenol induced elevated levels of total cholesterol, triglyceride and LDL, and a decreased level of HDL, however pre-treatment with the aqueous fruit extract of Tribulus terrestris induced a decrease in the total cholesterol, triglyceride and LDL levels, and an increase in HDL level when compared to the isoproterenol control group [16]. Furthermore, the reports from this study may be attributed to the contained phytochemicals; tannin (which is a phytochemical component of Revive capsule) possesses anti-hyperlipidemic potential; Yugarani et al. carried out a study which reported that an intraperitoneal injection of tannic acid in spontaneously hypertensive rats induced a decrease in the serum total cholesterol, triglyceride and LDL levels [18]. Bao et al. reported that treatment of albino rats with flavonoid induced a decrease in serum triglyceride and total cholesterol levels, and induced an increase in HDL levels [2]. Furthermore, Kata et al. reported that after treatment of hypercholesterolemic rabbits with low and high doses of alkaloid extracts, both doses induced a significant decrease in total cholesterol, triglycerides, LDL and VLDL, and an elevation in HDL [11].

The mean AIP value of group after 3 weeks of treatment (group IV) was significantly lower when compared with that of groups I, II and III. The value continued to decrease significantly after 4 weeks of treatment, with the highest significant decrease noted after 6 weeks of treatment compared to all other groups including the negative control. The mean CRI-I and CRI-II values after 6 weeks of treatment (group VI) was significantly lower when compared with those of the other groups including the negative control. Similarly, a significant decrease in the mean AC value was noted after 6 weeks of treatment compared to the other groups. AIP, CRI-I, CRI-II and AC are atherogenic indices, which are made up of several combinations of the lipid parameters, and are used to discover patients at risk of cardiovascular disease even though the lipid profile is normal [3]; elevated levels of these atherogenic indices suggests a risk of cardiovascular disease, while a decreased levels suggests “no risk

of cardiovascular disease". However, the report from this study may suggest that the drug had no potential of posing any risk of cardiovascular disease. The reports from this study may be attributed to the phytochemicals present in the drug. Some herbs used in the formulation of the Revive capsule have also been proven scientifically to produce reports that agree with those from this study; the bioactive component of the herb Cordyceps militaris (which is a component of Revive capsule) known as cordycepin or 3`-Deoxyadenosine induced a decrease in the CRI-I and CRI-II levels in rats (which were made hyperlipidemic by placing them on high-fat diet) [7]. Additionally, one of the phytochemicals in Revive capsule known as cardiac glycosides has been said to be used in the treatment of congestive heart failure and cardiomyopathy [5].

5.0 Conclusion

This study evaluated the effect of Revive capsule on cardiovascular risk indices in male albino rats in relation to the duration of administration. Treatment with Revive capsule for different durations (1-6 weeks) gave rise to different effects, with the maximum effect attained after 6 weeks of treatment; the drug induced a decrease in LDL, and an increase in HDL. Also, it induced a decrease in the cardiovascular (atherogenic) risk indices, which may be suggestive or indicative of a cardioprotective potential of the drug. Furthermore, there was no distortion in the heart as revealed by photomicrograph of the histologically examined heart tissue. Therefore, after 6 weeks of treatment with Revive capsule, no pathological effect was recorded in the albino rats, as such the drug is considered safe when administered at the appropriate dosage for a period of 6 weeks.

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