Effect of concomitant administration of sildenafil citrate and selected herbal bitters on sex hormones and some biochemical indices in Wistar rats

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ABSTRACT

Background: The increase in prevalence of male sexual dysfunction particularly erectile dysfunction has resulted in males consuming various types of products to improve their sexual life.

Objective: This study was designed to determine the effect of simultaneous administration of sildenafil citrate and herbal bitters on LH and testosterone, and a few biochemical markers (urea, creatinine, ALP, AST, and ALT) in Wistar rats.

Methods: Forty (40) male Wistar rats with weight range of 115 g to 125 g were divided into 8 groups. Group A was orally given distilled water and rats' feed as control, Group B was orally administered water, rats' feed and sildenafil citrate, Group C was orally administered water, rats' feed and orijin bitters, Group D was orally administered water, rats' feed and Action bitters, Group E was orally administered water, rats' feed and Coco Samba bitters, Group F was orally administered water, rats' feed and sildenafil citrate plus orijin bitters, Group G was orally administered water, rats' feed and sildenafil citrate plus action bitters and Group H was orally administered water, rats' feed and sildenafil citrate and Coco Samba bitters for 42 days. Blood samples (5 mL each) were collected from various rats and used for biochemical analysis.

Results: A statistically significant increase in AST, ALT, Urea, LH and testosterone was seen across all experimental groups in comparison with the control group ($p < 0.05$). The result also showed a statistically significant increase in creatinine in coco samba bitters administered Wistar rats and rats given coco samba butters concurrently with sildenafil citrate ($p < 0.05$).

Conclusion: Results obtained from this study revealed significant benefit of concomitant administration of herbal bitters and sildenafil citrate, causing an increase in LH and testosterone which can have a positive impact in men experiencing sexual disorders like erectile dysfunction. There is also potential deleterious effect of concomitant administration of sildenafil citrate and herbal bitter on male Wistar rats with elevation of serum level of urea, creatinine, AST, and ALT.

Keywords: Erectile dysfunction, Herbal bitters, Sildenafil citrate, Concomitant Administration

Effet de l'administration concomitante de citrate de sildénafil et de certaines plantes amères (bitters) sur les hormones sexuelles et certains paramètres biochimiques chez le rat Wistar

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RÉSUMÉ

Contexte: L'augmentation de la prévalence des dysfonctions sexuelles masculines, en particulier des troubles de l'érection, a conduit les hommes à consommer divers types de produits pour améliorer leur vie sexuelle.

Objectif: Cette étude a été conçue pour déterminer l'effet de l'administration simultanée de citrate de sildénafil et d'herbes amères sur l'hormone lutéinisante (LH) et la testostérone, ainsi que sur quelques paramètres biochimiques (urée, créatinine, ALP, AST et ALT) chez les rats Wistar.

Méthodes: Quarante (40) rats mâles Wistar, pesant entre 115 g et 125 g, ont été répartis en 8 groupes. Le groupe A a reçu par voie orale de l'eau distillée et de la nourriture pour rats (groupe témoin). Le groupe B a reçu par voie orale de l'eau, de la nourriture pour rats et du citrate de sildénafil. Le groupe C a reçu par voie orale de l'eau, de l'aliment pour rats et Orijin bitters. Le groupe D a reçu par voie orale de l'eau, de la nourriture pour rats et Action bitters. Le groupe E a reçu par voie orale de l'eau, de la nourriture pour rats et Coco Samba bitters. Le groupe F a reçu par voie orale de l'eau, de la nourriture pour rats, du citrate de sildénafil et Orijin bitters. Le groupe G a reçu par voie orale de l'eau, de la nourriture pour rats, du citrate de sildénafil et Action bitters. Enfin, le groupe H a reçu par voie orale de l'eau, de la nourriture pour rats, du citrate de sildénafil et Coco Samba bitters. L'expérience a duré 42 jours. Des échantillons de sang (5 mL chacun) ont été prélevés chez différents rats et utilisés pour des analyses biochimiques.

Résultats: Une augmentation statistiquement significative des taux d'AST, d'ALT, d'urée, de LH et de testostérone a été observée dans tous les groupes expérimentaux par rapport au groupe témoin ($p < 0.05$). Les résultats ont également montré une augmentation statistiquement significative de la créatinine chez les rats Wistar ayant reçu Coco Samba bitters et chez les rats ayant reçu simultanément Coco Samba bitters et du citrate de sildénafil ($p < 0.05$).

Conclusion: Les résultats de cette étude révèlent un bénéfice notable de l'administration concomitante de bitters à base de plantes et de citrate de sildénafil, entraînant une augmentation de LH et de testostérone, ce qui peut avoir un effet positif chez les hommes souffrant de troubles sexuels tels que la dysfonction érectile. Cependant, cette administration concomitante pourrait avoir un effet délétère chez le rat Wistar mâle, se traduisant par une élévation des taux sériques d'urée, de créatinine, d'AST et d'ALT.

Mots-clés: Dysfonction érectile, bitters à base de plantes, citrate de sildénafil, administration concomitante

INTRODUCTION

Both genders can experience sexual dysfunction, with likelihood of doing so rising with age.[1] Erectile dysfunction (ED) in males has been the most common sexual dysfunction in males. ED prevalence is generally acknowledged to be quite close with coronary heart diseases, high blood sugar, lipid disorder, hypertension, and related diseases. The similar link in patients with these co-morbidities is dysfunction.[2] Since many patients do not see a doctor and unwillingness of doctors to inquire about their patients' sexual well-being, it is challenging to collect accurate statistics on real prevalence of erectile dysfunction. Oral phosphodiesterase 5 inhibitors, (PDE-5 inhibitors) which include sildenafil and taldalafil, are used as first-line treatments for erectile dysfunction. It has been reported that PDE-5 inhibitors are incredibly successful,[3] with success rates as high as 76 %.

Worldwide, applications of herbal remedies increased over the years, and developed nations are increasingly using them to supplement their current conventional medical systems.[4] Eighty percent of the people in low income nations rely on local plants as drug for their primary healthcare.[5] According to a previous report,[6] herbal medicines use is usually self-prescribed, and these herbal preparations are frequently taken concurrently with prescribed medications. A large percentage of patients rarely tell their physicians about their consumption of herbal medicines.[7]

Polyherbal formulations like Orijin have a large number of consumers despite initial concerns about the product's composition and level of hygiene.[8] Customers continue to patronize polyherbal drinks in greater numbers due to the assertion that it restores and boosts sexual energy.[9]

Approximately 5000 years ago, native peoples, Egyptians, Greeks, Chinese, Romans, and Syrians began using herbal medicines for therapeutic purposes.[10] These people supposedly discovered transformation took place when grains, seeds, with other plant parts were combined in water and exposed to the sun. The end result of this was the first meads which are arguably the oldest alcoholic beverages that humans have ever consumed.[10]

Herbal medicines are only those traditional medicines in which medicinal plant preparations are mainly used for therapeutic purposes.[10] Traditional medicine, which includes herbal remedies, has been crucial in the past and its use will continue to be relevant over the years.[11]

Today's drinkers favour local herbal blends that mimic alcoholic beer of international standard for bitterness. There is shift in consumption of beers to alcoholic bitters. This is because bitter users believe the plant contains substances that cleanse the body, are anti-malarial, boost male virility, are hypolipidemic, are anti-diabetic, and are non-renal toxic.[8,11]

Traditional herbal bitters which are rich blend of rich medicinal roots and herbs have been used all over Nigeria due to its various acclaimed benefits. Erectile dysfunction has also been seen to be on the increase in young men in Nigeria. Sildenafil citrate is one of the approved orthodox medication for the treatment of erectile dysfunction. Most herbal alcoholic bitters are advertised with the promise of increased sexual performance and hard rock erection. With the alcoholic bitters advertised with various benefits and treating erectile dysfunction there are limited studies on its safety and its claim of improving erection.

With wide acceptance of alcoholic bitters for its numerous benefits and aphrodisiac claim, men with erectile dysfunction may combine the use of alcoholic herbal bitters with sildenafil citrate. The study on the effect of the concomitant use of sildenafil citrate and alcoholic herbal bitters is not available in the environment of study, thus necessitating the need to know the consequences of concomitant oral dosing of sildenafil citrate and herbal bitters on male sex hormones and some biochemical indices in Wistar rats.

MATERIALS AND METHODS

Materials and equipment used

Materials used included propylene oral gavage tubes, electronic weighing balance manufactured by WANT Balance Instrument Co., Ltd, China, and is Model WT6000GT. Equipments and kits used included Axiom biochemistry semi-auto anlayser, Randox ALP, AST, ALT, Urea and creatinine reagent kits produced by Randox Laboratories, Crumlin United Kingdom and Accubind LH and testosterone ELISA kits.

Procurement and care of animals

Forty (40) healthy adult male wistar rats weighing an average of 115 g to 125 g were procured from the animal house of biological departments, of Federal College of Education, Oyo, Oyo state. They were housed (Five rats per cage) in the animal house of the Department of Biology of the College of Education. The rats were kept in a

cage made of polycarbonate plastic that had a solid bottom and a stainless steel grid top with openings on the lids for water and food bottles for the rodents.

Cages were properly aerated rat cages with a consistent temperature, unrestricted access to food (grower chicken marsh (Local feeds) which were purchased from the market, and free access to water. Handling of the rats conformed to the National Research Ethics Committees Guidelines.[12]

Experimental design

The study is a longitudinal study involving a total of forty adult wistar rats. After acclimatization for five days, the rats were randomly divided into 8 groups (group A-H), with 5 rats in each. Before the experimental study began, the rats were weighted and then again after receiving sildenafil and alcoholic bitters for 42 days. The control group (A) received 2.68 mls of distilled water and rats' feed only. The experimental group B received 5.625mg/kg body weight of sildenafil citrate which was dissolved in water and given orally via gavage tube.[13]

The experimental groups C-E received 2.68 mg/kg body weight of herbal bitters once daily orally via gavage tube.[14] The other three groups F-H received a combination of sildenafil citrate and herbal bitters. The herbal bitters used include Orijin bitters produced by Diageo/Guinness Nigeria Plc, Action bitters produced by intercontinental distillers limited, Coco Samba bitters produced by Bullion Go-neat Global Limited.

Research protocol and administration schedules

Table 1 shows the treatment given to the control group (A) and the various experimental groups (B -H).

Table 1: Research Protocol and Administration Schedules

GROUP	Treatment Administered to each Group
Group A	Normal rats' feeds and 2.68mls Distilled water only
Group B	Rats' feed and tap water+ 5.625mg/kg body weight of sildenafil citrate
Group C	Rats' feed and tap water + 2.68mg/kg body weight of Orijin Bitters
Group D	Rats' feed and tap water + 2.68mg/kg body weight of Action Bitters
Group E	Rats' feed and tap water + 2.68mg/kg body weight of Coco Samba Bitters
Group F	Rats' feed and tap water + 5.625 mg/kg body weight of sildenafil + 2.68mg/kg body weight of Orijin Bitters
Group G	Rats' feed and tap water + 5.625 mg/kg body weight of sildenafil + 2.68mg/kg body weight of Action Bitters
Group H	Rats' feed and tap water + 5.625mg/kg between o f sildenafil + 2.68mg/kg body weight of Coco Samba Bitters

Note:

- Each group has 5 rats
- Group A rats served as control
- Group B – H rats served as experimental groups
- In each group the treatment administered were given orally daily for 42 days

Blood sample collection, storage and preparation

On the morning of the Forty-second (42nd) day, the final dose of sildenafil citrate, alcoholic bitters, and alcoholic bitters plus sildenafil citrate was given and all meals were stopped by seven o'clock for an overnight fasting. A 5 ml hypodermic syringe was used to draw blood samples from the animals (rats) the next day by cardiac puncture. Chloroform was used to induce anesthesia in the rats, and before the heart stopped beating entirely, 5 ml of blood was drawn through a cardiac puncture and placed in plain bottles with labels. Serum was then extracted from the blood samples by centrifuging. The collected sera were kept at - 4 ^{o}C and later analysed for the biochemical parameters that were considered in this study.

Biochemical parameters

Serum levels of Urea, Creatinine, ALP, AST and ALT were measured spectro-photometrically using reagent kits purchased from Randox Laboratories, Crumlin United Kingdom strictly following the manufacturer's protocol. ELISA kits purchased from Accubind, USA was used to determine the serum levels of Testosterone and Luteinizing hormone following the manufacturer's protocol.

Data analysis

The statistical package for social scientists (SPSS version 21.0) was used to conduct the statistical analysis, with the results expressed as mean ± SD. One-way analysis of variance was used to determine the differences between the experimental (Group B-H) and control (Group A) groups. This was followed by Duncan analysis to test for difference between individual groups. Values were considered statistically significant at $p < 0.05$.

RESULTS

The results of the biochemical parameters observed in this study are presented in tables 2 to 4. The mean of the serum enzymes (AST, ALP and ALT) in control and experimental groups showed (Table 2) that there is a difference between the mean AST in the experimental groups compared to the control and this is statistically significant ($F = 11.425$; $P = 0.000$). The difference between the means of the various experimental groups is shown by the use of superscript alphabets on the groups. Groups with the same superscript alphabets (C^C, D^C, F^C, G^C, H^C; C^d, E^d, F^d, G^d, H^d) have no significant difference ($p > 0.05$), while those with a difference have different superscript alphabets ($p < 0.05$). Similarly the difference between the mean ALT in the control and experimental groups was statistically significant ($F = 16.750$; $P = 0.000$).The difference between the means of the various experimental groups is also explained by the use of superscript alphabets, where groups with same superscript alphabets were not statistically significant. The difference between the mean ALP in the group was not statistically significant.

Table 2: Comparison of mean ± SD of serum level of AST, ALP and ALT in control and experimental groups

Serum Enzymes	GROUPS								F test	P value
	A	B	C	D	E	F	G	H		
AST	18.0 ± 1.87^{a}	25.0 ± 2.83^{b}	32.4 ± 2.79^{c,d}	29.6 ± 4.45^{c}	34.8 ± 2.86^{d}	32.4 ± 5.37^{c,d}	30.4 ± 2.70^{c,d}	31.40 ± 4.16^{c,d}	11.425	0.000 *
ALP	231.4 ± 3.71^{a}	235.8 ±2.39^{a}	234.8 ± 2.59^{a}	234.6 ± 3.97^{a}	234.8 ±4.76^{a}	233.0 ± 3.39^{a}	235.2 ± 3.96^{a}	234.2 ± 2.77^{a}	0.787	0.603
ALT	12.2 ± 1.92^{a}	17.6 ± 2.41^{b}	24.4 ± 2.88^{c,d}	22.0 ± 3.16^{c}	26.2 ± 1.92^{d}	22.0 ± 3.87^{c}	25.6 ± 2.07^{c,d}	25.0 ± 2.00^{c,d}	16.750	0.000 *

Values are expressed as mean ± SD and are statistically significant at $p < 0.05$.
Differences between the means of the various groups were expressed by superscript alphabets, where the difference between groups with the same superscript alphabets was not statistically significant at $p > 0.05$

The mean of the serum level of urea and creatinine in control and experimental groups showed (Table 3) that there is a difference between the mean Urea in the experimental groups compared to the control and this is statistically significant (F = 18.878; P = 0.000). The difference between the means of the various experimental groups is shown by the use of superscript alphabets on the groups. Groups with the same superscript alphabets (C^C, D^C, F^C, G^C, H^C; C^d, E^d, G^d, H^d) have no significant difference (p > 0.05). For creatinine there is only a statistically significant difference between the control group and the group fed with 2.68 mg/kg body weight of coco samba bitters alone or 2.68 mg/kg body weight of coco samba bitter and sildenafil citrate concurrently.

Table 3: Comparison of Mean ± SD of Serum Levels of Urea and Creatinine in Control and Experimental Groups

VARIABLES (mg/dl)	GROUPS								F- test	P value
	A	B	C	D	E	F	G	H		
Urea	22.6 ± 5.92^{a}	30.7 ± 2.66^{b,c}	31.8 ± 1.89^{c,d}	35.4 ± 2.76^{d,e}	40.5 ± 1.57^{f}	26.9 ± 3.56^{b}	34.5 ± 1.8^{c,d}	39.2 ± 2.2^{e,f}	18.878	0.000
Creatinine	0.64 ± 0.11^{a}	0.66 ± 0.09^{a}	0.66 ± 0.09^{a}	0.62 ± 0.08^{a}	1.10 ± 0.19^{b}	0.70 ± 0.07^{a}	0.60 ± 0.10^{a}	0.96 ± 0.11^{b}	13.642	0.000

Values are expressed as mean ± SD and are statistically significant at p < 0.05.
Differences between the means of the various groups were expressed by superscript alphabets, where the difference between groups with the same superscript alphabets was not statistically significant at p > 0.05

Looking at the result on hormone assay (Table 4) the mean of the serum Testosterone in experimental groups when compared with the control group showed a statistically significant difference (F = 95.945; P = 0.000). The difference between the means of the various experimental groups are explained with the use of superscript alphabets, where those with the same superscript alphabets have no statistically significant (P<0.05). Lastly the difference between the mean of LH in the control and the various experimental groups was statistically significant (F=13.114; P=0.000). The difference between the mean of the various groups are explained by the use of superscript alphabets, where groups with same superscript alphabet have no statistically significant difference (P > 0.05).

Table 4: Comparison of Mean ± SD of Serum Levels of Testosterone and Luteinizing Hormone in Control and Experimental Groups

Serum Hormones	GROUPS								F- test	P value
	A	B	C	D	E	F	G	H		
Testosteron	2.60 ±	2.61 ±	3.45 ±	3.55 ±	3.57 ±	3.50 ±	3.48 ±	3.46 ±	95.94	0.000
e(ng/ml)	0.061^{a}	0.038^{a}	0.149^{b}	0.126^{b}	0.095^{b}	0.098^{b}	0.647^{b}	0.077^{b}	5	
LH (pg/ml)	0.62 ±	0.60 ±	1.09 ±	0.95 ±	1.03 ±	0.89 ±	0.88 ±	0.81	13.11	0.000
	0.030^{a}	0.042^{a}	0.176^{d}	0.160^{b,c,d}	0.175^{c,d}	0.044^{b,c}	0.033^{b}	±0.056^{b}	4	

Values are expressed as mean ± SD and are statistically significant at p < 0.05.
Differences between the means of the various groups were expressed by superscript alphabets, where the difference between groups with the same superscript alphabets was not statistically significant at p > 0.05

DISCUSSION

The inability to obtain or sustain a penile erection strong enough to provide sexual satisfaction is known as erectile dysfunction (ED).[15] Men might experience sexual dysfunction for a variety of reasons, each with a unique set of risk factors and therapies. Low sexual desire includes not wanting to think about or involve in copulation, whether alone or masturbation.[15] Since many men do not self-report their symptoms of erectile dysfunction, doctors must inquire about sexual function and health in order to make a diagnosis.[16]

Some reports have mentioned identified medical causes of erectile dysfunction. This include Neurogenic cause,[17] Diabetes mellitus due to hyperglycaemia[18] and Oxidative stress,[19] psychogenic cause[20] and Vasculogenic cause due to poor low blood flow as in atherosclerosis of major vessels.

Polyherbal formulation contains varieties of plant phytochemicals which are extracted from various plant parts for treatment of various diseases. Their popularity stemmed from the belief that they are harmless and more effective than orthodox medications.

The findings in this study (Table 2) showed an elevation of serum levels of liver aminotransferases (AST and ALT) in Wistar rats administered with sildenafil citrate, herbal bitters and those concomitantly administered with herbal bitters and sildenafil citrate. This study corroborates a previous study which also reported a rise in AST and ALT level after administration of tetrapleura tetraptera a major constituents of action bitters.[8] This observation implies injury to the liver by the herbal bitters, this is supported by a report that liver injury could be caused by drugs, supplements and herbal medicines.[21] Another report in this respect was that a sub-chronic administration of sildenafil overdose causes significant biochemical and structural changes in the hepatic tissues that may adversely affect liver function. According to a previous study, antidepressants, antihypertensive medications, androgens, diuretics, opioids, sympathetic blockers, and antiparkinsonian drugs can all cause sexual dysfunction.[22] According to a study, Erectile dysfunction's main treatments includes diet and habit modifications, counselling, medication, physical exercise, and surgery.[23]

Another finding in this study is the effect of the bitters on kidney function. When the experimental groups and the control group were compared there was a statistically significant difference in the serum urea level with a significant increase in the experimental groups (Table 3). The difference in the urea level between the experimental groups is expressed using superscripts alphabets on the groups, where groups with the same superscript alphabets have no statistically significant difference. However, the study showed that statistically significant difference in creatinine when compared with the control was only in group fed with coco samba bitter alone or coco samba bitters and Sildenafil citrate concurrently. The increase in urea in this study is an indication of dysfunctional reabsorption and elevated creatinine observed in the coco samba bitters group indicates a reduced glomerular filtration rate in the experimental groups which is a reflection of impairment of kidney function. This finding agrees with a previous finding.[24] Increased urea level is likely due to dehydration, alcohol ingestion.[25] This study has revealed that coco samba bitters have a more potential nephrotoxic effect than the other Polyherbal formilations. Furthermore, this study also showed the anti-oxidant and anti-inflammatory characteristics of herbal formulations causing release of free radicals resulting in oxidative stress on the liver and kidney this is supported by a previous study.[28]

On hormonal evaluation of the effect of the herbal bitters in the Wistar rats (Table 4). The findings of this study revealed a significant increase in LH and testosterone in Wistar rats administered with sildenafil citrate. These findings are in accordance with a previous study[27] who reported a significant increase in FSH, LH and Testosterone in Wistar rats administered sildenafil citrate. Preclinical studies have proven that sildenafil citrate has a direct effect on leydig cell steroidogenesis.

A significant rise in LH and testosterone was seen in groups administered with herbal bitters. This is in agreement with a previous study[28] who reported a significant increase in LH and testosterone in Wistar rat administered various brands of herbal bitters. Increased levels of the sex hormones LH and testosterone shows the ability of the bitters to enhance sexual function and fertility. Testosterone is the major sex hormone that enhances and maintains sex drive. The elevation of LH observed in this study will further increase the production of testosterone by the interstitial cells as previously reported.[29] The combined action of phytonutrient composition of herbal bitters and the direct effect of sildenafil citrate contributes to the increase serum level of LH and testosterone in Wistar rats concomitantly administered herbal bitters and sildenafil citrate.

CONCLUSION

This study revealed significant benefits of concomitant administration of herbal bitters and sildenafil citrate, causing an increase in LH and testosterone which can have a positive impact in men experiencing sexual disorders like erectile dysfunction. Also there are potential deleterious effects of concomitant administration of sildenafil citrate and herbal bitter on individuals making use of herbal bitters to improve their sexual disorders, as it was observed in rats in this study with rise in serum concentration of urea, creatinine, AST, and ALT which implies a likely adverse effect on both the renal and hepatic functions.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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