Journal of Investigational Biochemistry
www.scopemed.org
DOI: xx.xxxx/xxx.xxxxxxxxxxxxxx

Original Research

GESDAV

Toxicological effects of aqueous extract of *Cnestis ferruginea* (Vahl ex DC) root in paroxetine-induced sexually impaired male rats

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Received: July 05, 2013
Accepted: September 30, 2013
Published: August 25, 2014

ABSTRACT

Aim: The effects of aqueous extract of *Cnestis ferruginea* (Vahl ex DC) root on some function indices and histology of the liver and kidney of sexually impaired male rats were evaluated. **Materials and Methods:** Male rats (156.24 ± 3.22 g) were randomized into six Groups (A-F) of five animals each. Rats in Group A (negative control) received orally 0.5 ml of distilled water while animals in Groups B, C, D, E, and F were induced with sexual dysfunction (p.o 10 mg/kg of the paroxetine hydrochloride suspension in Tween-80) and in addition received orally 0.5 ml each of distilled water and the same volume corresponding to 7.14 mg/kg body weight of PowmaxM (reference herbal drug) and 13, 26, and 52 mg/kg body weight of the extract respectively once, for 5 days. **Results:** The extract and PowmaxM significantly ($P < 0.05$) reduced the levels of serum albumin, globulins, conjugated bilirubin, blood urea nitrogen, and activity of alkaline phosphatase (ALP) in the liver, kidney, and serum, kidney and serum acid phosphatase (ACP) and liver alanine aminotransferase (ALT) of the animals. The levels of serum total bilirubin, urea, uric acid, creatinine, potassium, chloride, bicarbonate, liver aspartate aminotransferase (AST) activity and AST/ALT ratio increased in all the treatment groups while serum sodium ions and AST activity were not significantly ($P > 0.05$) altered. There were dose specific alterations on liver ACP activity. The decrease in the activity of liver AST was accompanied by corresponding increase in the serum enzyme. There was no treatment-related histo-architectural changes in the organs of the animals. **Conclusion:** Overall, the extract aggravated (by synergism) the functional toxicity in the animals with no corresponding structural toxicity and, therefore, not completely "safe" as an oral remedy for sexual inadequacies in male rats.

KEY WORDS: Antagonism, *Cnestis ferruginea*, connaraceae, functional toxicity, structural toxicity, synergism

INTRODUCTION

The use of plants as medicine to cure or prevent illness which dates back to antiquity is found in every society, irrespective of its level of development and sophistication [1]. The general acceptability of phytomedicines is, however, limited by lack of dose regimen and adequate toxicity or safety. The search for herbs with aphrodisiac activity has fostered research not only on the efficacy for the management of sexual inadequacies but also on the toxicity risk/safety. Studies have reported that herbs with aphrodisiac activity could also adversely affect the cellular systems of experimental animals. For example, aqueous extract of *Fadogia agrestis* stem reported to enhance sexual behavior in male rats has also been implicated to adversely alter the normal functioning of the liver and kidney of male rats [2-4]. Therefore, toxicological evaluation of these botanicals with aphrodisiac activity to ascertain their safety as oral remedies in normal experimental animals and those induced with the disease condition is thus, needed to increase their acceptability.

Cnestis ferruginea (Vahl ex DC) (Connaraceae), also known locally as Gboyin gboyin or Omu aja (Yoruba), Fura amarya (Hausa), Amu nkita (Igbo), Ukpo-ibieka (Edo), and Usiere ebua (Efik), is a perennial shrub found mainly in the savannah region of tropical West Africa. The plant is about 3.0-3.6 m high with densely, rusty brown, pubescent branches, indecidous leaves with more or less alternate or sometimes opposite, ovate to narrowly oblong leaflets, and orange-red fruits [5]. Various parts of the plant have been claimed in folk medicine to be used in the management of several diseases. For example, the roots of *C. ferruginea* have been explored in relieving constipation, sinusitis, skin infection, small-pox, toothache, snakebite, migraines, and gynecological problems such as abortion, sexual dysfunction,

urethral discharge, and ovarian troubles [6-9]. The aqueous root extract has been reported to contain alkaloids (28.6 mg/L), tannins (0.1 mg/L), saponins (4.6 mg/L), flavonoids (14.6 mg/L), and anthraquinones (0.3 mg/L) and possess anit-stress and laxative activities [10,11]. The same extract at the doses of 13, 26, and 52 mg/kg body weight have also been reported to exhibit aphrodisiac activities in sexually impaired male rats by progressively reversing the reduced mount frequency (MF), intromission frequency (IF), and ejaculation frequency (EF) as well as the increased mount latency (ML), intromission latency (IL), ejaculation latency (EL), and post-ejaculatory interval (PEI) associated with the administration of 10 mg/kg of the paroxetine suspension in a manner that compared favorably with the reference drug, PowmaxM [12]. Furthermore, the extract has also been shown to elevate serum testosterone content of the paroxetine-induced sexually sluggish animals [12]. In addition, the methanolic root extract has been reported to exhibit analgesics and anti-inflammatory activity[13] while the toxicological implications of the crude alkaloidal fraction from *C. ferruginea* root on the liver function indices of normal male rats have also been reported [14].

Despite all these studies, there has not been any report, in the open scientific literature that has addressed the toxicity of the aqueous extract of *C. ferruginea* root in sexually impaired male rats. Therefore, the present study was aimed at investigating the safety/toxicity risk of aqueous extract of *C. ferruginea* root in paroxetine-induced sexually dysfunction in male rats. This study is furtherance to the efficacious study of the *C. ferruginea* root as an aphrodisiac previously reported by Yakubu and Nurudeen [12].

MATERIALS AND METHODS

Plant Material

Fresh roots of *C. ferruginea* free from a fungal infection or other contaminants, obtained from a farmland through the help of a herb seller at the market (Oja tuntun) in Ilorin, Nigeria, were authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. A voucher specimen (UIH No. 007) was deposited in the Departmental Herbarium.

Animals

Thirty, healthy, in-bred, male Wistar rats (156.24 ± 3.22 g) obtained from the animal holding unit of the Department of Biochemistry, the University of Ilorin, Ilorin, Nigeria were housed in clean aluminum cages contained in well-ventilated animal house (temperature: 22 ± 3°C; photoperiod: 12 h; humidity: 45-50%) [15].

Drugs, Assay Kits, and Chemicals

Paroxetine hydrochloride and PowmaxM were products of S.C. Europharm, Brasov and Beijing Kowloon Pharmaceuticals Co., Limited, Beijing, China, respectively. The assay kits for albumin, total and conjugated bilirubin, urea, uric acid, creatinine, sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were products of Randox Laboratory Limited, Co-atrim, UK, while those of alkaline phosphatase (ALP) and acid phosphatase (ACP) were from Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany. All other reagents used were from Sigma Chemicals, St. Louis, USA.

Preparation of Extract

C. ferruginea roots (300 g) was washed under tap water, sliced, oven-dried at 40°C, and pulverized in a blender (Mikachi Blender, Model MK-1830, China). A known weight of the resulting powder (50 g) was extracted in 200 ml of distilled water at room temperature for 48 h. The filtrate was concentrated on steam bath to give a yield of 5.24 g (percentage yield of 10.48%). This was reconstituted in distilled water to give the doses of 13, 26, 52 mg/kg body weight. The dose of 26 mg/kg body weight was the frequently mentioned dose during the ethnobotanical survey on the plant which correspond to "a teaspoonful" of the plant powder estimated to be consumed as a remedy for sexual dysfunction by an adult man of 70 kg while the doses of 13 and 52 mg/kg body weight were half and twice the calculated dose of 26 mg/kg body weight. The doses (13, 26, and 52 mg/kg body weight) adopted in this study were also the same that were used in the efficacious study of the plant as sexual invigorator in male rats that were sexually impaired with paroxetine [12]. Since these were the doses used in folk medicine of Nigeria as sex enhancer, it seems logical to adopt same in the present study and not following the guidelines of toxicity testing [15].

Induction of Sexual Dysfunction and Assessment of Mating Behavior Indices in Male Rats

Twenty-five male rats were induced with sexual dysfunction by oral administration of 10 mg/kg of the paroxetine suspension (prepared daily in Tween-80 [BDH Chemicals, Limited, Poole, England], suspended in 0.9% saline solution) using a metal oropharyngeal cannula [16,17]. Healthy female rats were made receptive by sequential subcutaneous administration of oestradiol benzoate (10 μg/100 g body weight) and progesterone (0.5 mg/100 g body weight), 48 and 4 h, respectively, prior to pairing [18]. Oestrus phase in female rats was confirmed by vaginal smear examinations according to OECD-106 guidelines [19]. The oestrous female rats were then introduced into the male rats in their respective cages and observed for 30 min for mating behavior of MF (number of mounts without intromission from the time of introduction of the female until ejaculation), IF (number of intromissions from the time of introduction of the female until ejaculation), EF (number of ejaculations made during the observatory period), ML (time interval between the introduction of the female and the first mount by the male), IL (time interval between the introduction of the female to the first intromission by the male, usually characterized by pelvic thrusting and springing dismounts), EL (time interval between the first intromission and ejaculation, usually characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of inactivity or reduced activity),

and PEI (time interval between ejaculation and erection of the male copulatory organ for the next phase). Male rats which showed minimum of 25% reduction in MF, IF, and EF as well as a minimum increase of 25% in ML, IL, and PEI were considered as sexually impaired and were used for the subsequent study [12,17]. Therefore, the sexual dysfunction being referred to in the present study included the testosterone-dependent behavior of mating, copulation, and ejaculation. This same set of rats were the animals earlier used in our previous study and were indeed sexually impaired as evidenced in minimum of 25% reduction in MF, IF, and EF and same percentage increase in ML, IL, and PEI [12].

Animal Grouping and Extract Administration

A total of 30 male rats that was acclimatized for 2 weeks were assigned into six Groups (A-F) in a complete randomized design, with each group comprising five animals as follows:

Group A: Normal rats that received distilled water.

Group B: Sexual dysfunction rats administered distilled water.

Group C: Sexual dysfunction rats administered 7.14 mg/kg body weight of PowmaxM (a reference male sexual stimulant and energy enhancing polyherbal drug made up of *Panax ginseng*, *Camelia sinensis*, *Cnidium monnier*, *Epimedium brevicornum*, *Songaria cynomorium*, *Gingko biloba*, *Dahurian angelica*, *Salvia miltiorrhiza* root, L-arginine hydrochloride, and gamma aminobutyric acid).

Groups D, E, and F: Sexual dysfunction rats administered 13, 26, and 52 mg/kg body weight of the extract, respectively.

The various groups of animals were orally administered 0.5 ml each of distilled water, PowmaxM and the extract, once daily (08:00-08:45 h) for 5 days, with the aid of a metal oropharyngeal cannula. The animals were maintained on unrestricted access to rat pellets (Premier Feeds, Ibadan, Nigeria) and tap water. The study was conducted following an ethical clearance from the Ethical Committee on the care and use of laboratory animals of the Department of Biochemistry, the University of Ilorin, Ilorin, Nigeria. The guidelines on the use of experimental animals by the European Convention and other scientific purposes-ETS-123 were also strictly adhered to [20].

Preparation of Serum and Tissue Supernatants

The procedure described by Yakubu *et al.*[21] was adopted in the preparation of serum and tissue supernatants. 24 h after five daily doses of the distilled water, extract. and PowmaxM to the experimental animals, the rats were weighed and anesthetized in diethyl ether fumes. When they became unconscious, the jugular veins were cut, and 5 ml of the blood was collected into centrifuge tubes, allowed to clot for 10 min at room temperature and centrifuged at 503 ×*g* for 10 min. The serum was collected with the aid of Pasteur pipette and used within 12 h of preparation. The dead animals were then quickly dissected, and the liver and kidneys removed and blotted, the kidney decapsulated, after which the organs were weighed, homogenized separately in ice-cold 0.25 M sucrose solution (1:4 w/v) and centrifuged at 1398 × *g* for 15 min. The supernatants were frozen and used within 24 h of preparation.

Determination of Biochemical Parameters

The concentrations of albumin, globulin, total and conjugated bilirubin, urea, uric acid, creatinine, K^+, Cl^-, HCO_3^-, ALP, ACP, AST, and ALT were determined by adopting standard procedures [22]. The blood urea nitrogen (BUN): Creatinine and AST: ALT was computed as the ratio of serum urea to creatinine and AST to ALT, respectively.

Histopathological Examination

The organs of interest (liver and kidney) were fixed in 10% (v/v) formaldehyde and processed as described by Krause [23], while tissue sectioning was done according to the procedure described by Drury and Wallington[24] and stained with hematoxylin/eosin (H and E). Photomicrographs of the liver and kidney were captured at ×400 with a light microscope connected to a computer with Presto! Image Folio package software (Newsoft Technology Corporation, USA).

Statistical Analysis

Data were expressed as the mean ± standard error mean of five determinations and subjected to statistical analysis using Duncan multiple range test with the aid of Statistical Package for Social Sciences, version 16.0 (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at $P < 0.05$.

RESULTS

All the doses of the extract significantly ($P < 0.05$) reduced the levels of serum albumins, globulins, conjugated bilirubin, and computed BUNs: Creatinine of the sexually impaired animals [Table 1]. This trend of reduction was obtained in the sexually impaired rats treated separately with distilled water and the reference drug. In contrast, the extract increased ($P < 0.05$) the levels of total bilirubin, urea, uric acid, creatinine, K^+, Cl^-, and HCO_3^- in the serum of the sexually dysfunction animals in a manner similar to the animals in the control groups [Table 1]. Furthermore, the serum Na^+ was not significantly ($P > 0.05$) altered in all the treatment groups when compared to the nonsexually impaired animals administered distilled water only [Table 1].

The extract significantly reduced the activity of ALP in the liver, kidney, and serum as well as ACP activity in the kidney and serum of the sexually impaired male rats [Table 2]. This pattern of decrease in the activity of the enzyme was also similar to the sexual dysfunction rats administered distilled water and reference drug [Table 2]. In contrast, administration of the extract at 13 and 26 mg/kg body weight did not significantly alter the liver ACP whereas the 52 mg/kg body weight and PowmaxM increased the liver ACP activity in the sexually impaired rats similar to the paroxetine-treated animals [Table 2].

The activity of liver AST was increased by the extract whereas the serum enzyme was not significantly altered in the sexually impaired animals. These trends were similar to the sexually impaired animals administered distilled water and PowmaxM [Table 3]. The decrease in the activity of liver ALT in all the treatment groups except those treated with distilled water and PowmaxM was accompanied by corresponding increase in the serum [Table 3]. Furthermore, the extract increased the ratio of serum AST/ALT of the animals whereas the AST:ALT of sexually impaired animals treated with distilled water and PowmaxM were similar to the non-sexually impaired rats administered distilled water [Table 3].

The medullary and cortical architecture in the kidney of all the treated animals were preserved with no evidence of distortion on the proximal and distal convoluted tubules [Plates 1a-1d]. In addition, the lobular architecture, hepatocytes, central vein, and portal tracts were within normal physiology with no evidence of adhesion, inflammation, degenerative changes, and necrosis in the liver of all the experimental animals [Plates 2a-2d].

DISCUSSION

The toxicological evaluation of C. *ferruginea* root in sexually impaired rats has provided additional information on its adverse effects on the liver and kidney of the animals. The importance

Table 1: Effect of aqueous extract of *Cnestis ferruginea* root on some liver and kidney function parameters of sexually impaired rats

Serum parameters	Sexually impaired			*C. ferruginea* (mg/kg body weight)		
	Control	Paroxetine	PowmaxM	13	26	52
Albumin (g/L)	20.42±0.16^{a}	17.54±1.04^{b}	14.51±0.20^{c}	15.72±0.54^{d}	13.33±0.12^{c}	14.23±0.19^{c}
Globulin (g/L)	27.34±0.87^{a}	24.53±0.44^{b}	22.84±1.02^{c}	14.76±0.53^{d}	18.29±0.96^{e}	23.58±0.61^{c}
Total bilirubin (μmol/L)	15.38±0.54^{a}	22.84±0.50^{b}	18.84±0.36^{c}	17.35±1.19^{d}	20.59±0.78^{e}	19.77±0.83^{f}
Conjugated bilirubin (μmol/L)	24.52±0.11^{a}	14.47±0.43^{b}	16.56±0.73^{c}	18.38±0.62^{d}	17.54±0.87^{d}	16.22±0.42^{c}
Urea (mmol/L)	6.54±0.64^{a}	12.72±0.29^{b}	8.22±0.49^{c}	8.87±0.39^{c}	10.28±0.22^{d}	9.53±0.35^{d}
Creatinine (μmol/L)	39.43±1.32^{a}	38.39±0.76^{a}	46.55±0.65^{b}	49.34±0.59^{c}	54.58±0.74^{d}	53.22±0.86^{d}
BUN: creatinine	1:6.03	1:3.02	1:5.66	1:5.56	1:5.31	1:5.58
Uric acid (μmol/L)	3.22±0.35^{a}	9.87±0.63^{b}	7.46±0.22^{c}	5.56±0.65^{d}	6.42±0.61^{d}	8.47±0.11^{e}
Na^{+} (mmol/L)	5.65±0.22^{a}	5.54±0.19^{a}	4.98±0.34^{a}	5.24±0.37^{a}	5.11±0.08^{a}	5.32±0.20^{a}
K^{+} (mmol/L)	1.72±0.11^{a}	3.57±0.77^{b}	2.23±0.51^{c}	2.21±0.19^{c}	2.89±0.15^{d}	2.09±0.21^{a}
Cl^{-} (mmol/L)	52.21±0.57^{a}	71.00±0.02^{b}	63.04±0.35^{c}	61.39±0.30^{c}	58.95±0.69^{d}	67.54±0.54^{e}
HCO_3 (mmol/L)	10.10±0.07^{a}	11.01±0.02^{a}	21.40±0.50^{b}	25.41±0.76^{c}	15.35±1.12^{d}	17.20±0.76^{e}

Data are mean±standard error mean of five determinations. Test values carrying superscripts different from the control for each parameter are significantly different ($P<0.05$). BUN: Blood urea nitrogen, K^{+}: Potassium ions, Cl^{-}: Chloride ions, Na^{+}: Sodium ions, HCO_3: Bicarbonate ions, *Cnestis ferruginea: C. ferruginea*

Table 2: Effect of aqueous extract of *C. ferruginea* root on phosphatase activity of the liver, kidney, and serum of sexually dysfunction rat

Parameters	Sexually impaired			C. ferruginea (mg/kg body weight)		
	Control	Paroxetine	PowmaxM	13	26	52
Liver ALP	8.32±0.31^{a}	3.12±0.20^{b}	6.21±0.36^{c}	5.97±0.12^{c}	4.89±0.42^{d}	5.93±0.31^{c}
Kidney ALP	36.64±1.10^{a}	23.54±0.28^{b}	34.12±0.96^{c}	29.21±0.71^{d}	32.85±0.56^{e}	22.89±0.48^{b}
Serum ALP	1.53±0.21^{a}	0.84±0.13^{b}	0.63±0.17^{c}	0.46±0.05^{d}	0.23±0.04^{e}	0.53±0.01^{f}
Liver ACP	13.67±1.32^{a}	35.65±0.91^{b}	39.23±0.43^{c}	13.84±0.69^{a}	14.17±0.28^{a}	31.38±0.96^{d}
Kidney ACP	27.65±0.12^{a}	12.34±0.26^{b}	14.22±0.47^{c}	13.96±0.22^{c}	15.37±0.46^{d}	15.22±0.38^{d}
Serum ACP	3.32±0.11^{a}	1.41±0.09^{b}	2.74±0.07^{c}	1.43±0.05^{d}	1.12±0.04^{d}	1.63±0.04^{c}

Data are mean±standard error mean of five determinations; Enzyme activity is expressed in nM/min/mg protein; Test values carrying superscripts different from the control for each parameter are significantly different ($P<0.05$), ALP: Alkaline phosphatase, ACP: Acid phosphatase, *Cnestis ferruginea: C. ferruginea*

Table 3: Effect of aqueous extract of *C. ferruginea* root on the aminotransferase activity in the liver and serum of sexually dysfunction rats

Parameters	Sexually impaired			*C. ferruginea* (mg/kg body weight)		
	Control	Paroxetine	PowmaxM	13	26	52
Liver AST	17.35±0.12^{a}	36.46±2.17^{b}	26.17±0.26^{c}	22.75±0.40^{d}	29.76±0.24^{e}	25.88±0.11^{c}
Serum AST	9.22±0.18^{a}	9.16±0.10^{a}	9.10±0.13^{a}	8.99±0.09^{a}	8.98±0.16^{a}	9.12±0.14^{a}
Liver ALT	32.46±2.03^{a}	13.56±1.84^{b}	28.54±0.53^{c}	29.17±0.75^{c}	23.56±0.35^{d}	21.98±0.27^{e}
Serum ALT	5.57±0.16^{a}	5.49±0.14^{a}	5.77±0.27^{a}	8.44±0.21^{b}	8.27±0.11^{b}	7.99±0.20^{c}
Serum AST: ALT	0.60	0.61	0.63	0.94	0.92	0.87

Data are mean±standard error mean of five determinations; Enzyme activity is expressed in U/L; Test values carrying superscripts different from the control for each parameter are significantly different ($P<0.05$). ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, *Cnestis ferruginea: C. ferruginea*

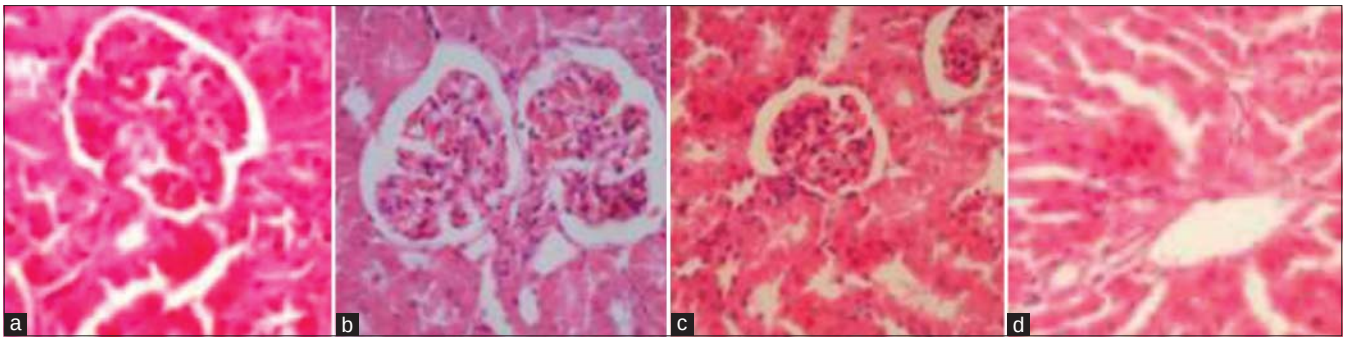

Plate 1: (a) Photomicrograph of the cross section of the kidney of distilled water treated control rat (H and E, ×400). (b) Photomicrograph of the cross section of the kidney of distilled water treated sexually impaired rat (H and E, ×400). (c) Photomicrograph of the cross section of the kidney of PowmaxM-treated sexually impaired rat (H and E, ×400). (d) Photomicrograph of the cross section of the kidney of rat administered 52 mg/kg body weight of aqueous extract of *Cnestis ferruginea* root (H and E, ×400)

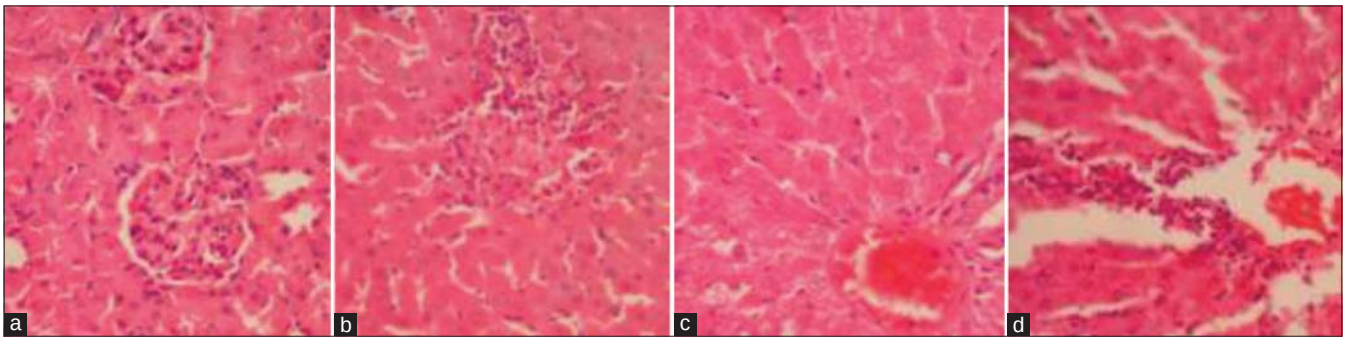

Plate 2: (a) Photomicrograph of the cross section of liver of distilled water treated control rat (H and E, ×400). (b) Photomicrograph of the cross section of liver of PowmaxM-treated sexually impaired rat (H and E, ×400). (c) Photomicrograph of the cross section of liver of sexually impaired rat administered distilled water (H and E, ×400). (d) Photomicrograph of the cross section of liver of sexually impaired rat administered 52 mg/kg body weight of aqueous extract of *Cnestis ferruginea* root (H and E, ×400)

of evaluating the levels of albumin, globulin, and bilirubin (total and conjugated) in the serum of animals following the administration of chemical compounds including this plant extract cannot be overemphasized as they are useful criteria for assessing not only the secretory ability and or functional capacity of the liver but also the types of liver damage [25]. Therefore, the reduction in the levels of albumin, globulin, and conjugated bilirubin in all the paroxtine, PowmaxM and extract-treated animals suggest that the normal functioning of the liver with respect to these compounds have been compromised. Specifically, reduced levels of albumin, globulin and conjugated bilirubin indicates diminished synthetic function of the liver probably arising from impaired hepatocellular function, continuous loss of the metabolites, infection, and or liver damage [22,25,26]. In contrast, the increased serum total bilirubin might be a consequence of blockage of bile duct, leading to the buildup of total or direct bilirubin and subsequent escape from the liver to manifest as an increase in the blood. All these will adversely affect the normal functioning of the liver of the animals. From this study, the administration of the extract further aggravated by the way of synergy the altered levels of these biomolecules by paroxetine. This might be an indication that the extract also has potential toxicity when used as a remedy in sexually dysfunction experimental animals.

There were also treatment-related effects on the function indices of the kidney with most pronounced synergistic effects in the sexually impaired animals treated with the extract. Renal function and physiologic reserve involve the ability of the kidney not only to filter and clear unwanted substances but also reabsorption of essential nutrients. Therefore, the increase in serum creatinine levels which was most pronounced (synergistic effect) in the extract-treated sexually impaired animals indicate deficiency in the process of filtration at the glomerulus. The reductions in glomerular filtration rate (glomerular dysfunction) by the extract and other treatments in the present study were further corroborated by the increase in the serum contents of urea and uric acid. Increase in serum uric acid content of the animals could also imply reduced excretion of the chemical compound by the kidney. High concentration of uric acid can lead to formation of crystals in a joint which may progress into kidney stone and eventually damage the kidney. Since K^+, Cl^-, and HCO_3^- are reabsorbed at the distal tubules, the elevated levels of these electrolytes suggest damage to the renal tubules. All these alterations corroborate renal dysfunction arising probably from interference with the metabolic process of the metabolite, inefficient filtration by the kidney and obstruction of the lower urinary tract, impaired glomerular and tubular reabsorption, or excretion of these ions [27]. The serum BUN: Creatinine ratio measures the amount of nitrogen in the blood, and can also be used to distinguish which of the liver or kidney is affected since urea is produced by the liver and excreted by the kidney. Therefore, a decrease in the computed BUN: Creatinine suggests that the elevated urea in the serum of the animals

was a consequence of liver dysfunction [27]. Furthermore, the more pronounced alteration in the kidney function indices by the extract implies synergistic effects and thus, suggests that the extracts are producing an additive toxicological effect. This may imply that the PowmaxM and the extract of *C. feruginea* roots might have a similar mechanism of toxicity with respect to the liver and kidney function indices. This assumption will, however, await further studies since the doses of the extract and the selective serotonin reuptake inhibitor, paroxetine, were not the same in the present study. However, the absence of an effect on the Na^+ ion in the serum of the animals treated with the distilled water, PowmaxM and extract is not immediately known but may not be unconnected with a selective effect of the extract. Similarly, absence of an effect on K^+ by the 52 mg/kg body weight (highest dose used in the present study) may also suggest dose specific effect of the extract.

A significant reduction in both the liver and kidney ALP, as well as kidney ACP activities by the extract and PowmaxM, without a corresponding increase in the activity of the enzyme in the serum, could be attributed to either inhibition of the enzyme activity at the cellular/molecular level [28], or inactivation of the enzyme molecules *in situ* [29]. The absence of an effect on the activity of ACP in the liver of the extract-treated animals at lower doses (13 and 26 mg/kg body weight) and PowmaxM-treated sexually impaired animals as well as an increase in the 52 mg/kg body weight of the extract further corroborate the dose-specific effect. The increase in liver AST without a corresponding change in the activity of the serum enzyme suggests that the *De novo* synthesis of the enzyme molecule was enhanced probably as a consequence of increased functional activity of the organ. The reduction in the activity of liver ALT which was accompanied by increase in the serum enzyme may not be attributed to permeability changes since the pattern of ALP in both the liver and the serum did not indicate disruption of the ordered lipid-bilayer of the membrane. However, the reduction in the liver ALT may be due to inactivation of the enzyme molecule while the increase in the serum enzyme may be due to the contribution probably from other organs not investigated in the present study. Computed AST: ALT is sometimes useful in differentiating between causes of liver damage [30,31]. Therefore, the computed AST:ALT which was not up to the unit value suggest that the effects on the liver which was not due to cirrhosis or other diseases associated with the organ, was a consequence of treatment-related effects which did not manifest in any structural defect in the hepatocytes.

The extract exhibited both antagonistic and synergistic effects on the biochemical parameters of liver and kidney function evaluated with the effects being slightly more of synergistic (39.13%) than antagonistic (30.43%).

The absence of gross abnormality or histopathological changes following the administration of the reference aphrodisiac, PowmaxM, and the aqueous extract of *C. ferruginea* root to paroxetine-induced sexual dysfunction animals suggests that there was no treatment-related structural toxicity. The findings in this study are similar to the reports by Tagliati *et al.*[32] and Lakmichi *et al.*[33] where phytopharmaceutical product, lerobina, and administration of aqueous ethanolic root extract of *Corrigiola telephifolia* Pourr. to rodents, respectively, despite altering biochemical parameters showed no morphological disturbance.

Overall, the aqueous extract of *C. ferruginea* root aggravated the toxicity profile induced by paroxetine in a manner similar to PowmaxM. The additive toxic effect which manifested only on the biochemical parameters of liver and kidney function implied that there was only functional toxicity and no structural toxicity.

CONCLUSION

The aqueous extract of *C. ferruginea* root has adversely affected the normal functioning of the liver and kidney of the sexually impaired rats. Therefore, the extract is not completely safe as an oral remedy for sexual dysfunction in male rats. The seemingly more deleterious effect produced by the extract when compared with the PowmaxM and the actual mechanism of toxicity of the extract on the liver and kidney of sexually impaired animals will have to await the outcome of further studies since the same doses were not used in the present study.

REFERENCES

1. Odugbemi TA. Textbook of Medicinal Plants from Nigeria. Lagos, Nigeria: University of Lagos Press; 2008. p. 542-612.
2. Yakubu MT, Akanji MA, Oladiji AT. Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. Asian J Androl 2005;7:399-404.
3. Yakubu MT, Akanji MA, Oladiji AT. Evaluation of selected biochemical parameters of male albino rat liver following administration of aqueous extract of *Fadogia agrestis*. Recent Prog Med Plants 2006;12:393-402.
4. Yakubu MT, Akanji MA, Oladiji AT. Effect of chronic administration of aqueous extract of *Fadogia agrestis* stem on male rat kidney function indices. Recent Prog Med Plants 2008;19:359-67.
5. Garon D, Chosson E, Rioult JP, Eldin de Pecoulas P, Brasseur P, Vérité P. Poisoning by *Cnestis ferruginea* in Casamance (Senegal): An etiological approach. Toxicon 2007;50:189-95.
6. Irvine FR. Woody Plants of Ghana. Oxford, UK: Oxford University Press; 1961. p. 146-7.
7. Malcolm SA, Sofowora EA. Antimicrobial activity of selected Nigerian folk remedies and their constituent plants. Lloydia 1969;32:512-7.
8. Kerharo J, Adam JG. La Pharmacopee Senegalaise Traditionnelle: Plantes Medicinales et Toxiques. Paris, France: Vigot Frere; 1974. p. 362.
9. Gill LS. Ethnomedical Uses of Plants in Nigeria. Benin, Nigeria: Uniben Press; 1992. p. 77.
10. Yakubu MT, Adams DM, Akanji MA, Oladiji AT. Laxative activity of aqueous root extract of C*nestis ferruginea* (Vahl ex DC) in loperamide-induced constipated rats. Nig J Gastroenterol Hepatol 2011;3:13-21.
11. Ishola IO, Ashorobi RB. The anti-stress potential of the aqueous root extract of *Cnestis ferruginea.* Int J Pharm 2007;3:295-8.
12. Yakubu MT, Nurudeen QO. Effects of aqueous extract of *Cnestis ferruginea* (Vahl ex De Cantolle) root on paroxetine-induced sexual dysfunction in male rats. Asian Pac J Reprod 2012;2:118-23.
13. Ishola IO, Akindele AJ, Adeyemi OO. Analgesic and anti-inflammatory activities of *Cnestis ferruginea* Vahl ex DC (Connaraceae) methanolic root extract. J Ethnopharmacol 2011;135:55-62.
14. Atere TG, Ajao AT. Toxicological implications of crude alkaloidal fraction from *Cnestis ferruginea* D.C root on liver function indices of male Wistar rats. Int J Biomed Health Sci 2009;5:145-56.
15. Organization for Economic Co-operation and Development. Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies. (OECD Guidelines for the Testing of Chemicals: Series on Testing and Assessment, No. 35). Final Consultation's Proposal. Available from: http://www.oils.oecd.org/

oils/2002doc.nsf/LinkTo/env-jmmono (2002) 19. [Last retrieved on 2010 Jan 18].
16. Chan JS, Waldinger MD, Olivier B, Oosting RS. Drug-induced sexual dysfunction in rats. Curr Protoc Neurosci 2010;Chapter 9:Unit 9.34.
17. Malviya N, Jain S, Gupta VB, Vyas S. Effect of garlic bulb on paroxetine-induced sexual dysfunction in male rats. Asian J Pharm Biol Res 2011;1:218-21.
18. Amin KM, Khan MN, Rahman SZ, Khan NA. Sexual function improving effect of *Mucuna pruriens* in sexually normal male rats. Fitoterapia 1996;67:53-8.
19. OECD. Guidance or Genetic Document Histological Evaluation of Endocrine and Reproductive Tests in Rodents. Preparation, Reading and Reporting of Vaginal Smears; 2009.
20. Council of Europe. European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasbourg: European Treaty Series; 2005. Available from: http://www.conventions.coe.int/treaty/en/treaties/html/123.htm. [Last accessed on 2005 Dec 02]
21. Yakubu MT, Oladiji AT, Akanji MA. Mode of cellular toxicity of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male rat liver and kidney. Hum Exp Toxicol 2009;28:469-78.
22. Tietz NW, Pruden EL, Siggaard-Anderson O. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. London: W. B. Saunders Company; 1994. p. 1354-74.
23. Krause WJ. The Art of Examining and Interpreting Histologic Preparations. A Student Handbook. UK: Partheton Publishing Group; 2001. p. 9-10.
24. Drury RA, Wallington EA. In: Carleton's Histological Techniques. 4th ed. NY: Oxford University Press; 1973. p. 58.
25. Yakubu MT, Bilbis LS, Lawal M, Akanji MA. Effect of repeated administration of sildenafil citrate on selected enzyme activities of liver and kidney of male albino rats. Nig J Pure Appl Sci 2003;18:395-400.
26. Moudgil KD, Narang BS. The liver and biliary system. In: Talwar GP, Srivastava LM, Moudgil KD, editors. Textbook of Biochemistry and Human Biology. 2nd ed. New Delhi, India: Prentice-Hall of India Private Ltd.; 1989. p. 271-3.
27. Yakubu MT, Adeshina AO, Ibrahim OO. Toxicological evaluation of aqueous leaf extract of *Senna alata* in pregnant Wistar rats. Int J Curr Res Rev 2012;4:89-109.
28. Akanji MA, Olagoke OA, Oloyede OB. Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system. Toxicol 1993;81:173-9.
29. Umezawa H, Hooper IR. Amino-Glycoside Antibiotic. Berlin: Springer-Verlag; 1982.
30. Olsson R. High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drug. Alcohol 2004;39:336-9.
31. Nyblom H, Bjornsson E, Simren MA, Idenberg F, Almer S, Olsson R. The AST/ALT ratio as an indicator of cirrhosis in patients with PBC. Liver Int 2006;26:840-5.
32. Tagliati CA, Silva RP, Feres CA, Jorge RM, Rocha OA, Barga FC. Acute and chronic toxicological studies of the Brazilian phytopharmaceutical product lerobina. Braz J Pharmacogn 2008;18 Suppl: 676-82.
33. Lakmichi H, Bakhtaoui FZ, Gadhi CA, Ezoubeiri A, El-Jahiri Y, El-Mansouri A, *et al.* Toxicity profile of aqueous ethanol root extract of *Corrigiola telephiifolia* Pourr.(Caryophyllaceae) in rodents. Evid Based Complement Alternat Med 2011;2011(Article ID 317090):10, doi:10.1155/2011/317090.

Source of Support: Nil, Conflict of Interest: None declared.