From Zimbabwean Indigenous Knowledge: Scientific Substantiation of the Aphrodisiac Efficacy and Safety of *Pittosporum viridiflorum* in Male Rat Models

ABSTRACT

Introduction: *Pittosporum viridiflorum*, the multi habitat thriving plant species is widely believed to have been the source of sexual prowess for the polygamous kings and elites of the mighty medieval empire known as 'Great Zimbabwe'. Despite the lack of systematic scientific validation, the macerated roots fractions continue to be the aphrodisiacs of choice among Zimbabweans.
Aims: To scientifically validate the aphrodisiac claims using male rats' sexual performance investigation protocols. To evaluate the toxicity profile and to identify the pharmacologically active metabolites present in the lyophilized hydro-ethanolic crude extracts.
Methodology: Four concentrations of prepared *Pittosporum viridiflorum* root extract doses of 200, 400, 800 and 2000mg/kg as well as a commercial 5 mg/kg sildenafil citrate (standard) and distilled water (control) dosages were gavaged to male rats (n = 5 animals per group) for 21 days. The crude extract was subjected to screening protocols for various secondary metabolites of pharmacological relevance as well as acute oral toxicity profiling using OECD 425 TG. The sexual behaviour, mating parameters and hormonal changes in the male rats were evaluated accordingly.
Observation and Results: Compared with the control, a significant increase in mating behaviour, mating performance and the serum hormonal levels were observed in test animals which showed a dose-dependent bias. The phytoscreening evaluation confirmed the presence of saponins, phytosterols, flavonoids, di-terpenoids, tannins, and polyphenols. The Extract was toxicological safe with an LD50 above 2000mg/kg body weight.
Conclusion: *Pittosporum viridiflorum* has excellent aphrodisiac activity on rats and is toxicologically safe for oral use. The abundant phytochemicals found are known to be pharmacologically active with regards to modulation of sexual hormones as well as sexual enhancement. It was further concluded that the traditional belief is valid, the plant can indeed be used as an alternative to modern medicines for various sexual dysfunctions and sexual performance enhancement.

Keywords: Pittosporum viridiflorum; sexual enhancement; phytoscreening; sexual dysfunction.

1. INTRODUCTION

1.1 Sex and the Ancient City of Great Zimbabwe

The modern day nation of Zimbabwe is named after a medieval African city known for its large granite circular walls and conical stone towers called the Great Zimbabwe [1]. The ancient city was home to over 20 000 inhabitants at its greatest, which was at that time more populous than the European City of London [2]. This famed ancient capital of the Shona people was the epicentre of an immensely wealthy, gold and other commodities, regional and intercontinental trading empire that controlled the entire breadth of the lower half of the East African (Indian Ocean) coast from the 11th to the 15th centuries C.E [3]. 15th Century Portuguese and Arabic traders and writers did not only confirm the same but also alluded to the fact that the elite of the empire controlled much of the interior including parts of present day Mozambique, Zambia and Botswana from their domain in the Southern part of Zimbabwe. Great Zimbabwe, which means "great houses of stone" is the largest ancient archaeological monument south of the Egyptian pyramids [1]. The walls of the ancient city which was designated a UNESCO World Heritage site in 1986 are on average higher than 9m in places and were built expertly without mortar from precisely cut stones that shape and curve the walls. As testament to the wide trading tentacles of the ancient empire, archaeologist's unearthed

Chinese and Persian pottery as well as Arabian coins from the site of the ruins [4]. Many artefacts of religious function including the soapstone Zimbabwe birds that appear on the flag of modern day Zimbabwe as the national symbol were unearthed within the perimeter of the ruins [4].

At a time when polygamy and fertility were the only guarantee to bloodline posterity and ultimate power from numbers, according to folklore, the potentate of the kingdom was a King with a harem of more than 250 sexually satisfied wives [2,5]. Members of his elite circles were believed also to have had staggering numbers of wives and concubines which were also said to have been sexually fulfilled by their men [6]. To the Shona people, the secret of the King and his henchmen's insatiable sexual appetite and prowess has been passed down by oral tradition, and the evidence still thrives along the narrow passages and steep rise towards the King's kopje residence among the ruins also referred to by archaeologists as the Hill Complex [2,7]. It is an indigenous tree known locally as *Muchemedzambuya*, literally translated to mean 'can make elderly women moan (with sexual pleasure)'. These trees that are abundant on and around the mystical hill are believed to be the 'secret' immensely potent aphrodisiacs that kept the King's strong contingent of women sexually satisfied. The widespread tree has been taxonomically confirmed to be *Pittosporum viridiflorum* or the cheesewood tree in common English nomenclature [8]. The mighty city may now lie in ruins with its Kings, wives and elites all departed now, but the legend of *Muchemedzambuya* (*Pittosporum viridiflorum)* did not depart from the modern day Zimbabweans (Fig. 1). The legacy of the herb reputed for properties that favour sexual performance lives on as one of the favourite and most culturally important plants for aphrodisiac use in Zimbabwe [6].

Among the Shona men, sexual conquest has always been a hallmark of masculinity and no pride compares with that of a Zimbabwean man's need to sexually satisfy his partner. For many Shona men, the legend of the Great Zimbabwe Empire is synonymous with the story of the sex boosting tree, used by their ancestors [8]. It is therefore not surprising, that tens of thousands of Zimbabwean men still throng the Great Zimbabwe area and the nearby town of Masvingo frequently not only to see the ruins but to source the original ancient Zimbabwean Kings sexual remedy: *Pittosporum viridiflorum*.

1.2 *Pittosporum viridiflorum*

The *Pittosporaceae* family is found throughout tropical, subtropical and warm temperate regions of the world. It is comprised of 9 known genera from over 200 species that are found in most parts of the old world [9]. It is prevalent in most habitats in Africa, Australia, New Zealand as well as Arabia and India. The name *Pittosporum* loosely translates to 'resinous seed', driven from *pittos* (resin) and *sporum* (seed).The Zimbabwean species name *viridiflorum* is in reference to the heavily scented green flowers and the deep green foliage [10]. The indigenous *Pittosporum viridiflorum,* commonly known as cheesewood (due to the white soft textured trunk wood) is the only known member of the genus in tropical Africa (Fig. 2) [10]. The capsule shaped fruit are distinctive and the deep green leafy branches provide beautiful shade from the roundish crown. These attributes and the delightful fragrant are a favourite to tropical insects making the tree quite popular with insectivorous birds as well as seed eating birds when the scarlet resinous seeds appear [9,10]. Apart from the aphrodisiac claims the cheesewood is also reputed to have numerous other medicinal attributes. The leaves and bark infusions are used for stomach ailments, as an emetic and for chest ailments and other fevers. Its leaf uses as an antimalarial herb have been reported in many studies [11]. However it is the claimed aphrodisiac attributes from Shona indigenous knowledge that has prompted this study. From Zimbabwean traditional knowledge, only the roots are used as aphrodisiacs, the leaves are not used for such purposes but for other ailments listed above. The soft cheesewood bark has never been reported in oral tradition as an ingredient for concoctions related to sexual performance enhancement. Therefore, the present study only focussed on the roots and this investigation was carried out to screen for and identify the crude root extract's pharmacological and phytochemical constituents, the acute and sub-acute toxicity profiles as well as to validate the claimed aphrodisiac effects *in vivo* using male laboratory rats with a view to reconcile the great Zimbabwe traditional basis of its usage with systematic scientific evaluations.

Fig. 1. *Pittosporum viridiflorum* and *Aloe excelsa* plants among the abundant flora found within the Great Zimbabwe ancient city ruins (credit: Desmont Kwande/AFP via Getty Images)

Fig. 2. *Pittosporum viridiflorum* leaves and fruits
(Source: Hyde et al. Flora of Zimbabwe: http://www.zimbabweflora.co.zw, 2016)

1.3 Aphrodisiacs and Male Sexuality

Sexual feelings and sexual conquests are a hallmark of the animal kingdom [12]. Whilst the primeval and instinctive drive prompting sex and sexual desires is progeny and the continuation of life guaranteeing the endurance of the human species [12], to the average Shona male of breeding age, sex and the ability to pleasure a woman to a climax is perhaps an indispensable, intimately integral part of the confirmation of real manhood and a source of personal achievement, pleasure and contentment [13,14 and 15]. Failure to consummate, flawless sexual delivery for a man which is commonly referred to as impotence is linked to infertility in a culture that values posterity and thereby making it is a source of anguish and distress. To the Shona people such problems have for generations traditionally prompted the intervention and assistance from the elders [2, 14]. Traditionally, aphrodisiacs from plant sources are the primary dosages which have always been used to increase sexual activity and help in fertility and sexual recreation. In traditional Zimbabwe knowledge impotence includes erectile dysfunction, premature ejaculation as well as male infertility [7, 8]. Such is the importance of male virility in Shona culture that for a good reason, treatment of male impotence is usually

prophylactic and ongoing during the entire course of an adult male's life [7]. This rich heritage of traditional sexual enhancement practices is largely threatened by modern trends and changing social aspects of Shona way of life characterised by migrations and young men growing up in communities without the elders for guidance. There is pervasive belief especially among traditionalists that young men are progressively getting sexually weaker in contemporary Zimbabwean society [7,8]. It is believed that there is a lot of ignorance, misinformation, apprehension as far as sexuality and male sexual prowess is concerned. Myths and misconceptions with regards to sexual enhancement herbs are rampant within the current generation of men [7,16]. These weaknesses have led men to use imported prescription sexual dysfunction drugs to enhance sexual activity ahead of traditional remedies due to the lack of systematic scientific studies regarding their activity, safety and mechanism of action [8]. This study therefore primarily focusses on bridging that information gap and restore the use of traditional Shona aphrodisiacs as the mainstay of male sexual enhancement and sexual dysfunction treatment amid the various addiction and safety concerns shrouding prescription drugs. Reported side effects from prescription aphrodisiacs include headaches, light headedness, disorientation, upset stomachs, photophobia, blurred vision, poor cognition, balance and fainting [17]. We report here the results of an investigation to validate the aphrodisiac claims associated with *Pittosporum viridiflorum*, as well as to determine the phytoconstituents and toxicological profiles of the purported sexual enhancement herb.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh whole plant material was donated by the botanical gardens in Harare, Zimbabwe. The plants were taxonomically authenticated by the same national herbarium. The fresh roots were cut off and washed with running water and left to air dry for 20 days. The semi dried roots were grated into smaller pieces using a mechanical cutter and the pieces were further air dried for 10 days and then ground into a fine powder using a coffee grinder. The fully dried plant material powder, was subjected to crude hydro-ethanolic extraction methods by cold maceration.

2.2 Preparation of the Hydro-Ethanolic Extract

To a 2000 ml round bottom flask on a stand, 400g of *Pittosporum viridiflorum* root powder were immersed in a 30:70 hydro-ethanolic solution (70% ethanol) and was subsequently cold extracted for 5 days with daily 3 minute shakings. After 5 days, the round bottomed flask contents were shaken one last time and immediately filtered using filter paper through a Buchner funnel. The obtained clear filtrate was evaporated under low pressure (Rotavapor® R-200, Buchi, Switzerland), followed by lyophilization (Lyovapor l-200, Buchi, Switzerland) under 120Pa pressure and -22 °C. Finally, the lyophilized extract was kept in a refrigerator at 4°Celcius to be used for further studies

2.3 Qualitative Phytochemical Analysis

The lyophilized hydro-ethanolic extracts of *Pittosporum viridiflorum* were subjected to various phyto-analysis techniques to screen for the presence of specific phytoconstituents of relevance that signal potential pharmacological activity. The qualitative tests conducted included tests for the presence of alkaloids, tannins, phenolics, saponins, flavonoids, terpenes and steroids.

2.4 Tests for Alkaloids

The Mayer's test as described by N Kancherla, 2019 [17], was used to determine the presence of alkaloids. In this assay, to 2 ml of the lyophilized *Pittosporum viridiflorum* extract, two drops of Mayer's reagent were slowly added along the sides of the test tube. The presence of alkaloids would then be identified by the appearance of a white creamy precipitate.

2.5 Tests for Tannins and Phenolics

The ferric chloride test as described by MS Auwal, 2014 [18], was used to determine the presence of tannins in the lyophilized sample. In a test tube, 1 ml of the hydro-ethanol extract was added to 2 ml of distilled water. Followed by 2-3 drops of ferric chloride. The test sample was checked for the development of a green-blue colour which indicates the presence of catechic tannins and blue-black indicated the presence of Gallic tannins.

2.6 Test for Flavonoids

Flavonoids were detected by means of the alkaline reagent test [19]. To 2ml of the lyophilized root extract, 2 to 3 drops of sodium hydroxide were added. The Initial formation of a a deep yellow which gradually fades to colourless after adding a few drops of dilute HCL, indicates the presence of flavonoids.

2.7 Test for Terpenoids

The test for terpenoids was done by dissolving 2 or 3 granules of tin metal in 2 ml thionyl chloride solution and then, adding 1 ml of the extract into the test tube. The formation of a pink colour indicates the presence of terpenoids [19].

2.8 Tests for Steroids

The test for steroids was confirmed by adding 5 ml of chloroform to 2 ml of the *Pittosporum viridiflorum* extract followed by the addition of 1 ml of concentrated H_2SO4. The emergence of a reddish brown colour confirms the presence of sterols in the test samples [17].

2.9 Test for Saponins

The simplified foam test as described by Rahman Gul *et al* 2017, was used to determine the presence of saponins. In this assay 2 ml of the *Pittosporum viridiflorum* extract was added to 20ml distilled water, the mixture was shaken in a graduated cylinder for 15 minutes. The presence of saponins would be confirmed by the formation of form with at least a head height of 1cm [20].

2.10 *In-vivo* Animal Experiments

In the whole study both female and male laboratory albino rats aged between 8 to 10 weeks old and weighing around plus ±225g were used. The rats acclimatised to the standard housing conditions for 7 days before the commencement of the experiments. The rats were fed a standard pellet diet from Agrifoods ® and water *ad libitum.*

2.11 Acute Oral Toxicity Evaluation and Treatment Schedule

The acute oral toxicity evaluation of *Pittosporum viridiflorum* was done using a modified OECD technical guideline 425 (The up and down method) methodology [21,22]. The test consisted of single ordered dose progressions in which animals were dosed, in sequence, at 48 hour intervals. The first animal received a dose below a randomly selected estimated LD50. When animals survived the dose, the next animal received an increased dose subject to our observations on the determined condition of the previous animal over 48 hours [22]. In this instance we decided to double subsequent doses, up to the limit of 2000mg/kg body weight. In the present toxicity assay, 12 female rats were used. The female rats were used because literature indicates that in conventional toxicity profile evaluations there is usually very small notable differences in observed sensitivity between animal sexes and in the instances where significant differences were noted, it was observed, that female rats were slightly more sensitive to toxicity than males. So, it was therefore decided to use a worst case scenario. The selected animals were marked so as to facilitate individual identification. The rats were kept in the test facilities for 7 days prior to dosing. The female rats were fasted for 18 hours with water [21]. The *Pittosporum viridiflorum* was orally gavaged in a water solution in 4 different doses of: 200, 400, 800, and 2000 mg/kg body weight, The female rats were observed by a veterinary specialist for mortality and in the absence of mortality they were observed for any observed changes and clinical signs and symptoms of toxicity every 1 hour up to 12 hours on day 1 and thereafter, everyday up to 14 days [21, 22].

2.12 Aphrodisiac Activity of *Pittosporum viridiflorum*

For the aphrodisiac activity, the experiments were partly guided by methods reported by Sahoo H D *et al*. (2014). For our study 30 male rats were selected after confirmation by a trained veterinary specialist for sexual maturity. The doses chosen for the aphrodisiac activity were 200, 400, and 800 mg/kg body weight. The volume of oral administration was 1 ml/100 g of rat weight. The Animals were put into five groups with each group having 5 animals. The first group of animals was treated with normal water only; the second, third and fourth groups were orally gavaged with the 3 different suspensions of *Pittosporum viridiflorum* at doses of 200, 400, and 800 mg/kg, respectively; the fifth and last group was used as a positive control and the all-male rats were treated with 5mg/kg body weight commercial Sildenafil citrate. Dosing was repeated every 48 hours and the experiments were observed for 3 weeks [23].

2.13 Mating Behaviour Study

The mating behaviour of the male rats was observed under the guidance of a trained animal

behaviour expert and qualified veterinary practitioner from the University of Zimbabwe, Faculty of veterinary Sciences on-campus teaching hospital. The observations were done in a confined experimental room under dim reddish light. Both the male and female animals were checked for general good health. The female animals were checked for regular oestrus cycles patterns and the males were selected after observations of sexual interest over a 7 day observation period. The mating scene was a transparent glass chamber in which the male selected rats were placed 15 minutes before the introduction of a primed female to acclimatise with the changed environment. Food and water were available in the glass compartment [22]. The primed female was then introduced into the chamber with one female to one male ratio and the mating behaviours observed for three weeks after commencement of the *Pittosporum viridiflorum* treatment dosing. The veterinary expert recorded the following mating behaviours in (Table 1) [23,24].

The values of the observed parameters were measured over the entire 3 weeks of *Pittosporum viridiflorum* gavaging and compared with the Sildenafil citrate control.

2.14 Hormonal Analysis

Soon after the termination of the experimental observations, blood samples were collected from all the experimental animals and taken to an independent clinical laboratory for hormonal analysis. The clinical laboratory measured the concentration of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone [24].

2.15 Statistical Analysis

Results were reported as M ± SE. The data were subjected to one-way analysis of variance (ANOVA) using Dunnet's test [25] for determining statistical significance. Significance level was set at P = .05 and confidence level at 95%. Statistical analysis was carried out using IBM-SPSS (Statistical Package for the Social Sciences).

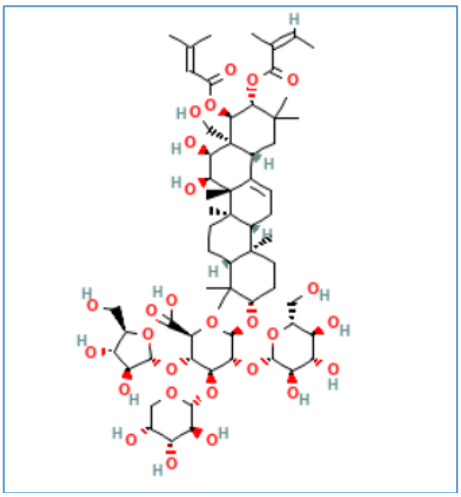
3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Table 2 shows the results from the various qualitative phytochemical screening protocols to identify the secondary pharmacologically active metabolites from the crude extract.

Table 1. Observed Mating Behaviour parameters descriptions [24]

Parameter	Abbreviation	Description
Mount frequency	MF	The number of mounts without inserting the penis in the vagina (intromission) from female introduction until ejaculation.
Intromission frequency	IF	The number of penis insertions from female introduction until ejaculation
Mount latency	ML	The time taken by the male rat to engage in the first mounting attempt with or without intromission after introduction of the female
Intromission latency	IL	The time taken from introduction of the female to the first penis insertion by the male and engagement of sexual motions and quick dismount
Ejaculation latency	EL	The time taken between the first penis insertion and ejaculation and engagement of deeper sexual motions thrusting and slowed dismount and subsequent inactivity and sexual disinterest
Post-ejaculatory interval	PEI	The time taken between ejaculation and dismounting and the next intromission .The experiments were terminated on the male rat resumption of mounting after post ejaculation inactivity



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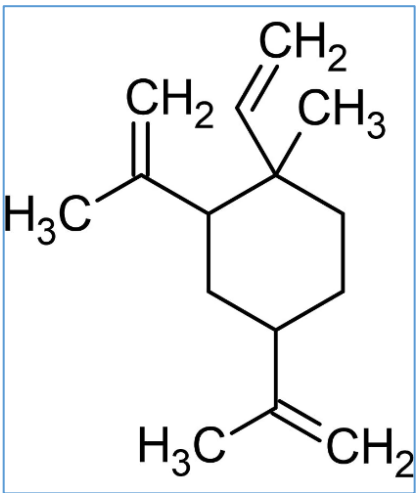

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Fig. 3. Common compounds isolated from various studies of Pittosporum viridiflorum (a) pittoviridoside (b) Beta elemene

Table 2. Qualitative Phytochemical analysis of *Pittosporum viridiflorum* root extracts

Phytochemicals	Hydro-ethanolic extract	Hydro extract
Carbohydrates	-ve	-ve
Glycosides	-ve	-ve
Saponins	+++	+
Phytosterols	+++	+
Phenols	++++	+
Tannins	++	++
Flavonoids	+++	+
Amino acids	-ve	-ve
Diterpenes	+++	+
Alkaloids	++	+

(+): Indicates the presence of chemical constituents; (+++): Indicates strong presence of chemical constituents (-ve): Indicates absence of chemical constituents

The pharmacological, properties of plant species used as herbal remedies are directly related to the secondary metabolite phytoconstituents [20,27]. In order to correlate plant ingredients and the potential medicinal use, a comprehensive phytochemical screening is required. Most of the *Pittosporum* species studies have confirmed the presence of several secondary metabolites with potential use in pharmacology [9,11]. Most of the studies, have centred on leaf and bark extracts excluding extracts from the roots. However, since the roots also have a bark, it is assumed that the roots will have most of the phytoconstituents found in bark extracts as well as extra phytoconstituents [18,19]. Thus the study confirmed the abundance of saponins, phytosterols, flavonoids, di-terpenoids, tannins, and polyphenols shown in Table 2.

In both the hydro-ethanolic as well as the aqueous fractions of *P. viridiflorum*, there was an absence of carbohydrates, glycosides and amino acids despite the fact that other studies have identified these compounds in *P. viridiflorum [*10,11]. These discrepancies could be consequent of the plant geographical location and seasonal changes in plant metabolites which is a phenomenon reported in numerous studies. The time and season of plant collection has been shown to affect the availability or absence of specific secondary plant metabolites. Chemotype variations and soil types can also contribute to these discrepancies [11]. The presence of phytoconstituents including flavonoids, polyphenols, saponins, and phytosterols indicate the potential of plants to produce anti-microbial, anti-oxidant, and anti-inflammatory activities which can correlate with its use in alleviating various ailments in traditional medicine. The major constituents of leaf and bark extracts reported in most papers are α-cadinol, and δ-cadinene, sabinene and β-elemene (Fig 3). From these, the most widely studied chemical isolated

from *P. viridiflorum* is β-elemene, various studies have confirmed its therapeutic use in kidney ailments as well as in retarding cancerous growths [10,27,28]. A new triterpenoid saponin, *pittoviridoside*, identified through spectral and GC analysis is also believed to possess extensive pharmacological effects [11]. The presence of phytosterols is the most likely source of the aphrodisiac activity. Phytosterols have been shown to be bioactive agents that can increase testosterone levels and enhance male sexuality by raising androgen levels [23].

3.2 Acute oral Toxicity Evaluation Results

Behavioural pattern and LD50.

The observations from the acute oral toxicity studies showed that the hydro-ethanolic root extract as well as the aqueous extracts of *Pittosporum viridiflorum* appeared to be safe up to the dose of 2000mg/kg body weight. Observed parameters tested for, including restlessness, touch response, pain response, urination, skin colour, fur erection, and food and water intake were assessed by a trained veterinary surgeon (Table 3). The only adverse observations noted were slight drowsiness at doses of 2000mg/kg. Apart from the isolated case of drowsiness in 2 individual rats under observation from the whole experiments, the study revealed the absence of signs of toxicity for the rest of the parameters under observation and the absence of any deaths recorded up to the last day of the experiments. Therefore, the LD50 of the extracts is considered to be greater than 2000mg/kg which deems it safe by all standard pharmacological interpretation [26].

Table 3. Observations for behaviour and appearance of rats during studies

Observed parameter	Dose of *Pittosporum viridiflorum* in mg/kg body weight			
	200mg	**400mg**	**800mg**	**Control**
Food intake	Normal	Normal	Normal	Normal
Water intake	Normal	Normal	Normal	Normal
Death	Alive	Alive	Alive	Alive
Breathing	Normal	Normal	Normal	Normal
Diarrhoea	Not observed	Not observed	Not observed	Not observed
Urination	Normal	Normal	Normal	Normal
Skin colour	Normal	Normal	Normal	Normal
Drowsiness	Not observed	Not observed	present	Not observed
Erection of Fur	Not observed	Not observed	Not observed	Not observed

The lack of systematic scientific evaluation of the toxicological profiles of herbal remedies from indigenous knowledge systems based traditional medicines is one of their major drawbacks from inclusion into mainstream medicine [29]. It is therefore imperative that prior to any efficacy evaluations, the most logical preliminary step is the evaluation of safety for use in mammals. The toxicity profiling is also the basis for the dosages that can be safely used in the efficacy studies.

The OECD technical guideline 425 (up-and-down testing approach) used here is based on the proposal by Bruce 1985 and adopted by ASTM in 1987 as a suitable determination of acute toxicity of chemicals [22]. As per the guideline, only healthy adult animals of breeding age were used in the study. The females chosen for the study were nulliparous and non-pregnant. The rats were specifically bred for such studies and the ages which were already known were between 8-12 weeks as required by the guidelines [21,22]. The animals were all fasted before dosing overnight with only water provided for them. Before commencement of dosing, the animals were weighed and checked for any adverse health indications. The up and down study of the *Pittosporum viridiflorum* extracts was carried out using rat models at doses of 200, 400, 800 and 2000mg/kg body weight. The rats were continuously monitored during the experiments for changes in body weight and other observable indicators of poor health effects.

No animals died during the studies and no animals were withdrawn from the study due to adverse health symptoms from the extracts. All animals were normal at the termination of studies with regards to all physical parameters under observation. There were no significant changes observed in all rats for all categories except for 2 isolated cases of drowsiness that were observed in the 2000m/kg body weight category in week 3 (Table3). The drowsiness sign was resolved within 2 hours of dosing in the animals that exhibited the signs. The experiment confirmed that the extracts were toxicologically safe at 2000mg/kg body weight and therefore LD50 is concluded to be beyond 2000mg/kg body weight. With reference to the Hodge and sterner classification for toxicity, the hydro-ethanolic root

extract of *Pittosporum viridiflorum* is classified as a practically nontoxic herbal medicine [30].

Bodyweight observations.

The weekly body weights of the five groups of animals under study were measured on the initial day and on the 7th and 14th and 21st days, as illustrated in Fig. 4. In all recorded weights, all treated groups did not exhibit statistically relevant or significant aberrations in body weight in comparison with the control group.

Progressive Weight loss of animals is usually indicative of stress, failure to feed or a response to observed or underlying adverse health conditions [31]. The body weights of all the rats were noted weekly over the course of the experiments for all the 3 dosage concentration groups as well as the control. The initial weights for all the rats selected for the study were within specifications for 8 to 10 week old laboratory rats. The observations showed an expected gradual progressive increase in weight over a three week period expected of the animals [31]. The body weight changes among the groups and the control were not statistically significant. Bodyweight changes coupled with continued feeding can be used as a confirmation of the absence of any toxicity effects on the rats from *Pittosporum viridiflorum* and correlates well with the absence of any deaths from any of the animals under the tests [31,32]. This gives confidence that the roots of *Pittosporum viridiflorum* do not interfere at any level with the normal metabolism of the animals as well as their appetite.

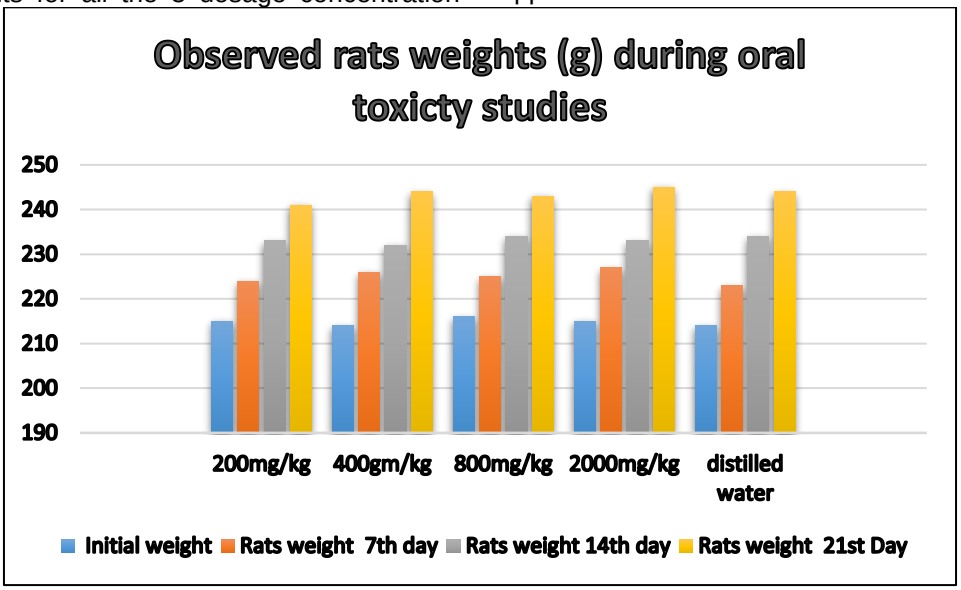

Fig. 4. Observations for rat's weights over the experimental period

3.3 Effects of *Pittosporum viridiflorum* Doses on the Mating Behaviour of Rats

From the mating behaviour studies it was observed that after continuous gavaging with all doses of *Pittosporum viridiflorum* for 3 weeks, all the doses were able to reduce the time intervals between introduction of the female and the time taken for mounting, intromission, ejaculation and post ejaculatory inactivity before the next series of mounts compared to the control. The observations noted a significant increase in mounting frequency and intromission frequency as compared to the frequencies for the control and were closely related to the standard Sildenafil citrate. It was also observed that there was a marked increase in pre-coital mounting and intromission behaviours related to sexual arousal including chasing and genital sniffing, which were observed in all *Pittosporum viridiflorum* dosed groups and the standard. The increase in frequencies and shortening of time lapses observed were all dose depended and increased with increasing doses. There was therefore a confirmed statistically significant increase in observed sexual behaviour parameters in *Pittosporum viridiflorum* treated groups as noted from the results for MF, IF and EF as well as a statistically significant decrease in ML, IL, EL and PEI. The results confirm the observations that there was an overall increase in the sexual behaviour parameters in *Pittosporum viridiflorum* treated groups of rats as reflected in MF, IF and EF, and reduction in ML, IL, EL, and PEI. These results were also statistically significant as shown in Table 4.

The investigation aimed to establish among other things the sexual competence enhancement effect of *Pittosporum viridiflorum* on laboratory rats. The most commercially available and publicly appraised male sexual impotence drug, sildenafil citrate was used as a positive standard reference for comparison purposes [33]. Our prior literature review could not establish reference to any similar systematic study to confirm the sexual enhancement effect of *Pittosporum viridiflorum* done on animals. Available literature mostly reported on the plant leaves' anti-bacterial effects. The present study provides scientific evidence and confirms that *Pittosporum viridiflorum* indeed imparts sexual performance advantages in male rat models as both a sexual arousal stimulator and performance enhancement supplement. From the mating behaviour studies it was observed that *Pittosporum viridiflorum* at all investigated dosage levels significantly increased mount frequency and Intromission frequency compared to the control group. The effect for the 800mg/kg body weight estimated very closely to the standard sildenafil citrate. The 3 investigated doses significantly reduced Mount latency and intromission latency in comparison with the control dosage group, the standard again had reduced the ML and IL further than the crude extract dosages as shown in Table 4. Lower Mount latency and intromission latency are indicative of animal arousal and higher Mount

Table 4. Averaged effects of *Pittosporum viridiflorum* on mating behaviour variables over a three week period

Mating behaviour variable	21 DAY TESTING PERIOD AVERAGED FREQUENCIES				
	Control	**200mg/kg**	**400mg kg**	**800mg/kg**	**Sildenafil 5mg/kg**
ML	10.3± 0.76	7.8±1.12	5.4±0.6	3.8±0.06	1.97±0.08
IL	10.6±1.54	6.4±1.,23	4.2±0.94	2.85±1.45	1.82±1.34
EL	244±0.87	380±2.16	566±1.44	970±2.34	1298±1.87
NI	5.4±0.94	6.1±0.88	6.4±0.74	6.5±0.34	6.94±1.06
III	15.8±2.32	14.54± 1.46	12.01±1.88	10.36±1.86	7.18±1.22
NM	5.43±0.65	6.56±0.69	6.74±0.74	6.88±0.82	6.94±0.34
MF	72.42±3.24	80.4±0.94	98.5±2.88	142±1.28	195±1.23
IF	77.4±3.20	95.4±3.6	124.5±4,24	166.76±2.43	193±2.04

Values are expressed as Mean ±SD (n=5);

ML=Mounting Latency, IL=Intromission latency, EL=Ejaculation latency, PEI=Post-ejaculation Interval, NI=Number of intromission, III=Inter-Intromission interval, NM=Number of mount, MF=Mounting frequency and IF=Intromission frequency

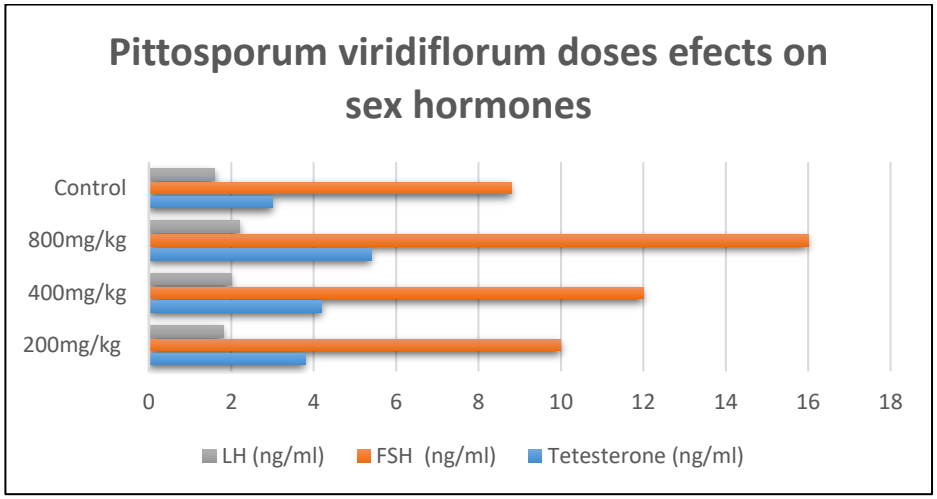

Fig. 5. Hydro-ethanolic doses effects on sex hormones

frequency and intromission frequency are indicative of libido and sexual potency which are measures of sexual prowess [33]. This therefore confirms that overall male sexual execution and performance were enhanced by the hydro-ethanolic root extract of *Pittosporum viridiflorum*. Evaluation observations noted the extended sexual activity duration as indicated by the prolonged Ejaculation Latency (EL).

There was a notably fast rate of recovery of the rats after ejaculation before the next series of mountings as indicated by the Post Ejaculatory Interval (PEI), a sign of enhanced sexual potency [33], [34]. The above observations confirm the potential of *Pittosporum viridiflorum* in enhancing sexual performance in male rats.

The standard used in all the experiments, sildenafil citrate is perhaps the most common modern medication for erectile dysfunction as well as recreational sexual performance enhancement [35]. The most popular commercial product containing Sildenafil citrate is perhaps Viagra ®, this oral prescription medication has received mixed reviews. Studies have confirmed that a lot of men are not compatible with the drug and Sildenafil citrate has been reported to work only in 70% of male users of differing aetiologies with a host of side effects including headaches, drowsiness, disorientation, as well as upset stomachs [35,36]. The availability of Viagra as a prescription only drug makes it inaccessible to most men who do not have health insurance, because they then need to get a prescription from a medical practitioner before purchase of the aphrodisiac. The International federation of pharmacists (IFP) in its latest Global Pharmacy Workforce and Migration Report (2023) estimates that in some developing countries the average number of pharmacies per 100 000 people can be as low as five [37]. This entails that a drug like Viagra® or any drug containing Sildenafil Citrate is not readily available at all in most developing countries settings and communities. Its retail price of an average USD$20 per 4 tablets is also out of reach of the majority of potential male users [38]. In some countries, oral testosterone is also available on prescription to treat erectile dysfunction, however it' is reported to be ineffective in boosting sexual performance and it has also been linked to potential liver damage [39]. A number of even more expensive erectile dysfunction drugs are also available for administration under a specialist medical doctor including Tadalafil, aprostadil, Yohimbine, phentoamine and papaverine hydrochloride which also have widely reported adverse side effects and infectivity in huge sections of the male populations [39,40].

3.4 Effects of *Pittosporum viridiflorum* on the Serum Testosterone, LH and FSH in Male Rats

For this study, the blood serum was used and not the 'whole blood' sample. The blood serum is essentially blood without the cellular components as well as the clotting factors. To the whole blood, no anticoagulants were added and clotting was not prevented because it is within the blood serum that we find composites, such as the hormones, proteins and the antibodies which are the focus of this study. The effect of the Pittosporum viridiflorum extract on the blood serum concentration of the hormones under investigation (testosterone, LH, and FSH) was significant compared to the control group as shown in Fig. 5. Hormonal levels increased gradually in alignment with increases in dosages. The highest increase observed for the 800mg/kg body weight dose was very close to the standard sildenafil citrate.

The 3 doses of *Pittosporum viridiflorum,* increased the serum hormonal levels of testosterone and LH over the period under review. Testosterone levels are a hallmark of masculinity associated with both physical and sexual stamina and endurance [41]. Testosterone levels are also linked to erectile quality, increase in coital desire and duration as well as semen levels. Testosterone levels have been reported to facilitate dopamine release and stimulation of the hypothalamic–pituitary gonadal axis [39, 41]. The increased sexual performance, elongation of the EL and shortening of the PEI after the administration of *Pittosporum viridiflorum* can directly be linked to the increased serum testosterone levels and these observations may also directly confirm an increase in the male rat's semen levels. This observation can also be adequate validation of the traditional Zimbabwean belief that *Pittosporum viridiflorum* can resolve premature ejaculation in weak men [2,3].

4. CONCLUSION

The study informs, confirms, validates and substantiates the Zimbabwe indigenous knowledge claim that doses of *Pittosporum viridiflorum* may enhance overall sexual function and performance in males whilst also increasing the levels of testosterone, FSH and LH. The availability of phytochemicals of known

pharmacological relevance also confirms the potential availability of efficacy in sexual enhancement. The absence of toxicity is also an added bonus. The overall approximation of the male sexual performance enhancement of 800m/kg body weight dose of *Pittosporum viridiflorum* on male rats to that of standard prescription sildenafil citrate with negligible toxicity profiles as confirmed by the above studies not only confirms the use of the plant as a formidable aphrodisiac, but invites for further translational science processes to rethink the use of unsafe modern prescription sexual performance enhancement drugs while safer, more affordable and equally efficacious alternatives exist in traditional medicine.

ETHICAL APPROVAL

Prior to the investigations, animal use and research ethics approvals were obtained from the Joint Parirenyatwa Research Ethics Committee (JREC) which is the local research Institutional Review board for the University of Zimbabwe.

ACKNOWLEDGEMENTS

The authors wish to thank the national herbarium in Zimbabwe, in particular the Director, Dr Chapano for provision and authentication of the plant species specimens. We also wish to acknowledge assistance in observation and interpretation of animal study protocols and results by the University of Zimbabwe faculty of veterinary sciences teaching hospital staff.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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