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Moderate doses of *Mucuna pruriens* seed powder is safe and improves sperm count and motility

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ABSTRACT

Background: Conventional remediation techniques involving male fertility include hormonal therapy, *in vitro* fertilization and surgery. However, the use of natural products continues to be a popular option. Emerging new products that have not been well investigated is the use of *Mucuna pruriens* seed powder.
Aim: This study aimed at determining the efficacy and safety of *Mucuna pruriens* (MP) seed powder on the male fertility using normal animal models.
Methodology: Four groups of seven (7) male Sprague-Dawley rats were used. Groups comprised Control (distilled water), Low dose (500 mg/kg b.wt MP), Medium dose (1000 mg/kg b.wt MP) and high dose (2000 mg/kg b.wt MP). Test groups were administered aqueous crude extract of MP by gavage over 90 days. Upon sacrifice, the following assays were performed: FSH, testosterone, oestrogen, PSA, semen analyses, histology of reproductive organs, and general haematological and biochemical analyses.
Results: FSH increased, whilst oestrogen decreased, across groups: however, it was not statistically significant between groups. Although testosterone increases were not statistically significant, increases were dose-dependant. Sperm count increased significantly between the Control and Medium dose groups ($p<0.001$). Motility significantly increased with the Medium and High dose groups compared to Control group ($p = 0.022$, $p = 0.029$, respectively). Additionally, immotility significantly decreased in all treatment groups compared to control group ($p = 0.013$). No abnormality was observed in biochemical, haematological and histological analyses.
Conclusion: This study demonstrates a margin of safety and improved spermogram pattern between the low and medium dose administration of *Mucuna pruriens* seed powder.

Introduction

Infertility is conventionally diagnosed following unprotective sexual intercourse by a couple over a period of one year or longer, without conception. According to WHO, about 72.4 million people are affected and further suggests that half of 9% of couples struggling to conceive are attributable to men (Boivin et al., 2007).

The causes of male infertility are numerous but can be categorized into abnormality with spermogram of which the aetiology may not be identified (65–80%), followed by a situation of normal spermogram in which causes remain unknown (10–20%). Hormonal and sperm transport may account for another 10% (Winters, and Walsh, 2014). Lifestyle factors such as obesity which imbalances the testosterone and oestrogen ratio is also responsible for male infertility (Bieniek et al., 2016). Smoking, abuse of drugs and illicit substances also have a role to play, though a causal association has not yet been established (Yu et al., al., 2018).

Other lifestyle factors such as diet, use of caffeine and the level of

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activity also contribute to infertility. Lifestyle changes with a departure from junk food, smoking and excessive use of alcohol, departure from excessive red meat, consumption of more vegetable have been reported to improve sperm quality (Gameiro et al., 2016), (Cairo Consensus Workshop Group, 2020). Environmental factors such as pesticides and cell phones have also been implicated in infertility (Gameiro et al., 2016), (Bieniek et al., 2016). The role of stress has been recognized (Gollenberg et al., 2010) considering that physical or economic pressures affect hormonal levels and subsequently, spermatogenesis and semen quality (Anderson et al., 2010). The role of oxidative stress and mutational damage to sperm DNA seem to suggest that dietary vitamin supplement with antioxidant capacity have the potential of improving male fertility (Suresh et al., 2013). The scrotum and the temperature of the sac have its impact on spermatogenesis through hyperthermia; scrotal skin temperature affects sperm output whilst scrotal hyperthermia results in impairment of spermatogenesis (Jung and Schuppe, 2007).

Treatment modalities employed in infertility treatment include surgical techniques aimed at improving sperm production, sperm delivery, correcting retrograde ejaculation or retrieving sperms for *in vitro* fertilization (Lopushnyan, 2012). The use of stem cell therapy for male infertility treatment is still in the evolutionary stage of inducing pluripotent stem cells and mesenchymal stem cells to counter azoospermia (Saha et al., 2021).

Herbal extracts such as *Epimedii Herba* and *Angelicae Gigantis Radix* are known to reduce oxidative stress and subsequently increase the production of sperm (Park et al., 2017). Other medicinal plants highly used in the Palestine and the West Bank rural areas include *Ferula hermonis* roots (96.08%), *Phlomis brachyodon leaves* (88.24%) and *Phoenix dactylifera* pollen grains (86.27%) (Jaradat and Zaid, 2019). Ashwagandha (*Withania somnifera*) an Indian herb, is known for its aphrodisiac properties. Furthermore, studies have shown that the root extract of *Withania somnifera* is said to treat oligospermia (Ambiye et al., 2013) as well as improve concentrations of lactate, citrate and essential amino acids in semen (Gupta et al., 2013) and thus, consequently improve overall semen quality. Additionally, it has been reported to reduce oxidative stress and improve testosterone, Luteinizing Hormone and Follicle Stimulating Hormone, thereby reversing male infertility (Ahmad et al., 2010).

Aspalathus linearis (rooibos) a well-known tea in South Africa for its antioxidative properties when treated on sperm *in vitro* using the fermented form, attenuated the production of reactive oxygen species, DNA fragmentation and improved sperm motility and vitality (Takalani et al., 2021).

Mono herbal preparations of aphrodisiac plants and plant products have been explored over the years. *Chlorophytum borivilianum*- an aphrodisiac plant is said to have positive effects on semen profiles and improve plasma biochemical profiles in infertile men. *C. borivilianum* is referred to as nature's wonder drug mainly because of its aphrodisiac properties. At doses of 125 and 250 of *Chlorophytum borivilianum* using Wistar albino rats, the lower dose of 125 demonstrated aphrodisiac properties during the 3-hour observation. Furthermore, sperm count continued to increase for the next 3 weeks (Ray et al., 2014). However, basic science research on this plant for its aphrodisiac and fertility potential is very minimal. Similarly, *Eulophia campestris* though reported to have similar effects on male infertility has little or no scientific data available on its fertility and aphrodisiac potential as a monoherbal plant.

The art of combining monoherbal therapeutic plants to produce a poly herbal product is an ancient art in Ayurvedic medicine. Such combinations have been seen for disease such as diabetes (Madić et al., 2021) and hypertension (Tian Shen et al., 2019).

Chlorophytum borivilianum, Eulophia campestris and Mucuna pruriens was investigated as a combined product for the improvement of semen quality and motility in infertile males involving administration of the poly herbal product to male albino rats for 40 days and oligospermic males for 90 days. Results from the former (albino rats) resulted in significant sperm motility and density increase as well as reproductive organs and accessories (testes and epididymes).

Similar results in sperm motility and density were observed in the oligospermic cohort. Furthermore, these significant changes in the human study were attributed to serum testosterone increase.

As the search for newer and safer plant products continues, *Mucuna pruriens* seed is one of such with insufficient empirical data albeit purported to improve libido and testosterone levels (Choowong-In et al., 2021). Furthermore, controversies surround the appropriate safe dose of *M. pruriens* due to dopamine levels contained in its seeds. Again, animal studies demonstrating its fertility outcomes amongst normal subjects do not exist. The aim of the present study was therefore to determine the safety, fertility potential and appropriate dose of *M. pruriens* using animal models. It was hypothesized that *Mucuna pruriens* if indeed aphrodisiac, could improve male fertility hormones and subsequently affect semen production, motility and viability, at a dose that would not be toxic.

Materials and method

Plant extract preparation

M. pruriens seed were donated by Prof. George Awuku Asare. Seeds were received from Catholic Missionaries visiting Battor Catholic Hospital (Sogakofe Volta Region), and identified as such, by the National Herbarium, University of Ghana. Seeds were sun-dried for 3 days and roasted lightly to break the hard coat followed by grounding with an electronic high-speed multifunctional grinder (Zhejiang, China) into fine powder. The powder was used to prepare the crude extract of concentrations 500 mg/kg b.wt, 1000 mg/kg b. wt and 2000 mg/kg b. wt. The selected dose was based on a previous study that determined the LD_{50} to be above 2000 mg/kg b.wt (Jayakumar et al., al.,2016).

Experimental animals

The Ethics and Protocol Review Committee of the School of Biomedical and Allied Health Sciences, University of Ghana approved the study and issued ethics number SBAHS/AA/MLAB/10,607,885/2020–2021. A total of 28 male Sprague-Dawley (SD) rats were obtained from the Noguchi Memorial Institute of Medical Research (NMIMR), University of Ghana and housed at the Department of Animal Experimentation. Rats were housed in stainless steel cages with soft wood shavings and fed rodent feed pellet (AGRIFEEDS, Kumasi) with access to drinking water *ad libitum*. Four (4) groups were established (7 rats per group) after being randomized. Animals were allowed to acclimatize for seven days under 12-hour light and 12-hour darkness. Temperature and humidity were 22±3 °C and 4–45%, respectively. Groups comprised the control group (distilled water), low dose group (500 mg/kg b.wt *M. pruriens*), medium dose group (1000 mg/kg b.wt *M. pruriens*) and high dose group (2000 mg/kg b.wt *M. pruriens*) administered by gavage over a period of 90 days. Rats were weighed weekly, and the dose adjusted to the weight. On the 91st day, animals were sacrificed, and blood samples taken by cardiac puncture for various haematobiochemical analyses. Organs such as testes, prostate and seminal vesicle were harvested alongside systemic organs. Epididymal sperms samples were taken for laboratory analysis.

Physical weights of rats and organ

Weights of animals were determined initially. Subsequent weights were taken on weekly basis throughout the period of study.

Blood collection

The rats were sacrificed on the 91st day. Rats were first anaesthetised using ethyl ether and seven millilitres (7 ml) of the blood samples were

drawn by cardiac puncture with 25 G needles and discharged into gel separator and EDTA tubes for various assays.

Organ harvesting and tissue processing

Organs harvested included prostate, testes, seminal vesicle and other systemic organs such as liver, kidney, and heart. Organs were weighed, rinsed in normal saline solution, and were preserved in 10% buffered formalin except for testes which were preserved in Bouin's solution for histological analysis. The histological examination of organs was according to the study of (Owagboriaye et al., 2017). Tissues were processed using Leica TP 1020 tissue processor (Wetzlar, Germany) which employed the routine paraffin embedding procedure. The embedded tissues were subjected to serial sectioning of 4 μm thickness using a rotary microtome and were subsequently processed in alcohol-xylene series which were stained with hematoxylin and eosin (H & E). Slides prepared were examined at x4, x10 and x40 objective lens using Olympus CX23 light microscope (Tokyo, Japan).

Semen collection

Caudal epididymis from each side of the testis of the rats were removed and placed into 2 mls of 0.85% warmed physiological saline. Sterile needle was used to puncture the caudal epididymis several times to allow sperms to seep into the saline to produce a saline sperm suspension. A volume of 10 μl was dispensed onto a pre-warmed microscope slides and were covered with cover slips. Slides were observed immediately under Olympus CX23 light microscope (Tokyo, Japan) for the motility of the sperms. Sperm count estimation, was carried out by thoroughly mixing saline semen suspension and semen diluting fluid (sodium bicarbonate 5 g, formalin 1 ml, and distilled water 99.0 ml). A drop of sperm suspension was loaded into a haemocytometer and was allowed to settle in a humid place for 5 to 10 min afterwards, spermatozoa were counted under the light microscope [Olympus CX23 (Tokyo, Japan)]. Sperm count was estimated by using the formula (Sperm count = number of sperm counted x dilution factor / volume x 1000).

Haemato-biochemical analyses

Hormonal analyses

Hormones were analysed using the MAGLUMI 600 chemistry autoanalyser (Shenzhen, China) with Testosterone, FSH, LH and Oestrogen hormonal test kits (Shenzhen, China). The testosterone test was based on a competitive chemiluminescence immunoassay. Oestrogen estimation was a competitive immunoluminometric assay employed. LH and FSH assays were based on a sandwich chemiluminescence immunoassay.

Determination of haematological and biochemical parameters

Full blood count (FBC) was performed on whole blood samples using Sysmex haematology autoanalyzer (Kobe, Japan). Liver function tests (Total and conjugated bilirubin, liver enzymes, total protein, and albumin) as well as renal function tests (sodium, potassium, urea and serum creatinine) were determined using SELECTRA JUNIOR Version 04 autoanalyzer (Vital Scientific, Spankeren, The Netherlands).

Statistical analysis

Repeated measures design was used to control factors that caused variability between subjects. Continuous variables were expressed as mean ± SEM and statistical significance determined by ANOVA. Bonferroni post hoc was used to determine specific groups where significant differences were found. Pearson correlation was used to determine the association between hormonal levels and sperm count. The significant level was set at $p < 0.05$.

Results

From Table 1, all groups gained significant weight after 90 days. The greatest change was observed in the high dose group.

For relative organ weights Post hoc analysis on the liver and spleen showed significant difference ($p < 0.05$) between the control and or other dose groups. For the kidney, differences were between the control group and low dose group. For the prostate, differences occurred between the control and low dose, low and medium dose and medium and high dose ($p < 0.05$, in all cases).

Hormonal analyses

Testosterone showed a gradual increase across the groups. However, this change was not significant. Oestrogen declined but not in a dose dependant manner with the greatest decline occurring in the medium dose. FSH on the other hand increased only with the high dose group but was not statistically significant (Table 3).

Semen analyses

From Table 4, motility increased significantly for all the experimental groups with the highest seen in the medium dose group. Conversely, immotility decreased across the experimental groups with the lowest in the medium dose groups. Analysis of variance (ANOVA) for motility and immotility was statistically significant with p-values 0.013 and 0.013, respectively. A decrease in sperm count was observed in the low and high dose groups with the highest observed in the medium dose group. Again, the difference was statistically significant ($p < 0.01$) (Table 4). However, no correlation existed between sperm count and hormonal levels.

Post hoc analysis for motility (Table 5), demonstrated that significant difference existed between the control and the medium dose group ($p = 0.022$) as well as control and high dose groups ($p = 0.029$). Post hoc analysis of immotility, showed that significant changes occurred between the control and the medium dose group ($p = 0.03$) as well as control and the high dose group ($p = 0.004$).

For sperm count, post hoc analysis revealed significant differences between the control group and the medium dose group as well as the low dose group and medium dose groups ($p < 0.05$ respectively). Furthermore, significant difference was observed between the medium and high dose groups ($p = 0.000$).

Liver function test

Liver function test (TBIL, DBIL, IBIL, ALT, AST, ALP, GGT, AST/ALT, TP, ALB, Glo, Alb/Glo) did not show any significant difference for the various analytes except for ALT ($p = 0.042$), (Table 6).

Renal function test

For renal function test (Na, k, Creatinine, Urea), only creatinine was

Table 1
Body weight profile of rats within groups after 90 days administration of the crude aqueous extract of *Mucuna pruriens*.

Groups	Initial body weight (g)	Weight after 90 days of administration	P – value
Control	147.7 ± 13.9	273.53 ± 18.2	<0.001
Low dose	196.3 ± 7.9	291.7 ± 35.3	<0.001
Medium dose	190.9 ± 14.28	298.0 ± 27.9	<0.001
High dose	185.0 ± 27.9	317.0 ± 16.3	<0.001

Values are expressed as mean±SEM; $n = 7$ per group; statistically significant at $p < 0.05$. Data were analysed by a paired T-test.

significant ($p = 0.043$) for ANOVA (Table 7).

Post hoc analysis of biochemical parameters

Post hoc analysis of ALT (showed significant difference ($p = 0.033$) between the medium dose and high dose group with a reduction in the high dose group. However, post hoc analysis did not reveal any significant difference in creatinine (Table 8).

Effect of extract on haematological parameters

Red cell indices in Table 9 (RBC, HGB, HCT, MCV, MCH, MCHC, RDWCV, RDWSD) showed no statistically significant difference with the exception of RDWCV and RDWSD and ($p = 0.007$; 0.002) respectively. From Table 4.12, WBC and platelet parameters [WBC (tov tal), Neu, Lym, Mon, Eos, Bas, Bas%, PLT, PDW, PCT, PLCC, PLCR] did not show any significant change with the exception of basophils% which showed significant change ($p = 0.036$).

Post hoc analysis for haematological parameters

Post hoc analysis in Table 11 revealed differences existed between the control and the medium dose group as well as conrol and low dose group for RDW-SD ($p = 0.013$; $p = 0.012$, respectively). Significant differences in RDW-CV existed between low dose and medium dose groups, as well as medium dose and high dose groups ($p = 0.039$; $p = 0.022$) respectively. For BAS% differences existed between the Control group and the High dose group (0.046).

Histological analysis

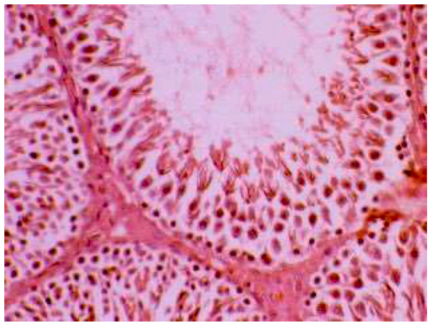
Histology so far does not confirm any adverse effects of the aqueous crude seed extract of *Mucuna pruriens* on systemic organs. Furthermore, reproductive organs herein presented also showed normal architecture (Figs. 1-2).

Discussion

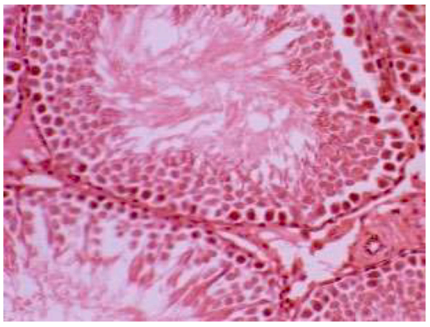
To the best of our knowledge, this present study is the only experimental investigation involving *M. pruriens* seed powder that has been conducted over a 90-day period regarding its efficacy and safety in the remedy of male infertility. All previous studies created pathological conditions of some disease, mainly diabetes. This present study however demonstrates findings on non-pathological conditions, thereby resolving the incline of what happens when normal people use *M. pruriens* to boost fertility.

Rats exhibited normal growth weight over the 90-day period (Table 1). *M. pruriens* on its own does not appear to cause weight increase. The weight increase is the normal growth pattern as even seen in the control group. Indeed, *M. pruriens* improves body composition and subsequently prevents obesity (Tavares et al., 2021). Brain size increased non-significantly in a non-dose dependant manner but not in the relative organ weight (Table 2). *M. pruriens* therefore does not have detrimental effects on the brain. In one study, remarkable protection was observed in the brains of experimental rats treated with *M. pruriens* as opposed to those not treated who suffered from cerebral ischaemia (Nayak et al., 2017).

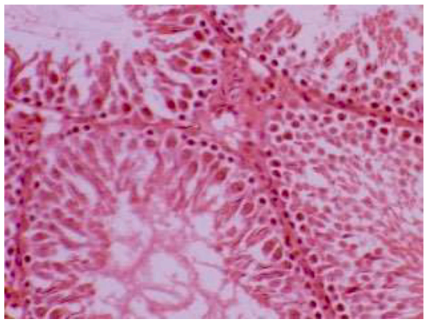
Spleen organ weight demonstrated a reduction in all groups with the lowest weight in the medium dose group. For relative organ weigh the change was still significant ($p < 0.05$). The spleen is responsible for WBC production. This did not reflect in the total WBC; indeed, all parameters of WBC were normal except for basophils which showed a significant reduction comparing the high dose group to the control group (Table 10). A reduction in basophils is often associated with some allergy or infection. However, all animal were produced pathogen-free. This change may not be pathological. Other haematological parameters that showed differences included the red cell distribution width coefficient of variation and Red Cell Distribution Width SD (Table 9). Slight decreases were observed from medium dose and high dose, compared to the control. These parameters are associated with anaemia. A decrease will suggest an improvement in the haemoglobin level. Indeed, haemoglobulin levels improved slightly in all groups receiving *M. pruriens* in a dose dependant manner, although not significant



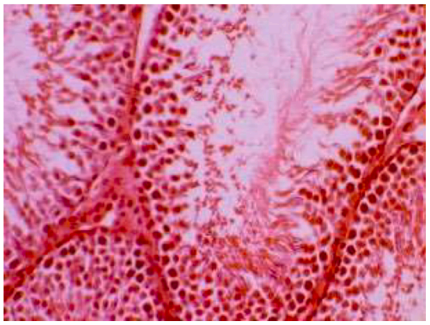
Control group



Low dose group (500 mg/kg)



Medium dose group (1000 mg/kg)



High dose group (2000 mg/kg)

Fig. 1. . Photomicrograph of cross section of the testis (400X). This figure shows the cross section of the testis in S-D rats following treatment with low dose (500 mg/kg), medium dose (1000 mg/kg) and high dose (2000 mg/kg) of M. pruriens for 90 days. In the control group, the testicle appeared covered by a capsule of connective tissue (the tunica albuginea). Testicular parenchyma consisted of seminiferous tubules which appeared rounded or oval with regular contour. The interstitial spaces in-between the tubules contain a delicate loose connective tissue and Leydig cells. H and E-stained testicular samples of the low dose group, medium dose group, and high dose group revealed the same histological features of the testis. (Hematoxylin and Eosin stain, 400X).

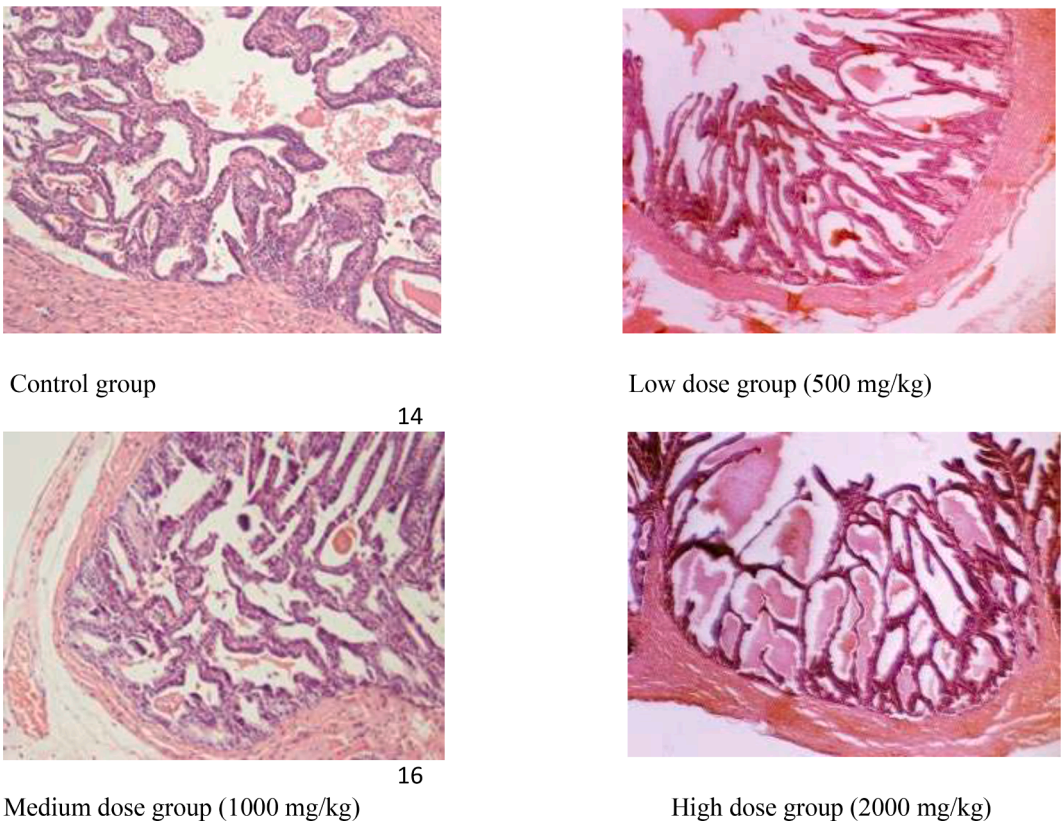


Fig. 2. Photomicrograph of the seminal vesicles (100X). This figure shows the effect of the crude extract of M. pruriens on the histology of the seminal vesicles (Hematoxylin and eosin). All groups showed normal tissue histology. All groups showed mucosal folds/ridges extending into the lumen. The lining epithelium of seminal vesicle was pseudo-stratified columnar epithelium, composed mainly of a single layer of tall columnar principal cells and triangular shaped basal cells. The lumen of seminal vesicle was filled with eosinophilic secretion. The epithelium lies on a thin layer of connective tissue lamina propria. The lamina propria was lined by inner circular and outer longitudinal smooth muscle fibres. Mucosal alveoli or crypts showed small amount of secretion.

Table 2
Effect of the crude aqueous extract of *Mucuna pruriens* on relative organ weight (g) of vital organs of rats after 90 days administration by gavage.

Organs (g)	Control	Low dose	Medium dose	High dose	p-value
Heart	0.350	0.388	0.388	0.362	NS
Liver	3.400^{β}	$3.100^{\S}$	$2.900^{\S}$	$3.000^{\S}$	<0.05
M. Kidney	0.315^{θ}	0.290^{α}	0.270	3.000	<0.05
Lungs	0.735	0.750	0.750	0.670	NS
Spleen	0.330^{ϕ}	0.230*	0.200*	0.220*	<0.05
Brain	0.550	0.577	0.925	0.880	NS
M. Testes	0.880	0.935	0.935	0.853	NS
Seminal vesicle	0.850	0.750	0.725	0.800	NS
Prostate	0.270^{Ψ}	0.375^{Ω}	$0.263^{\ddagger}$	0.385^{χ}	<0.05

NS = Statistically not significant. Statistically significant at $p < 0.05$. M. Kidney= Mean of kidneys. M. Testes= Mean of testes. Post hoc revealed: ϕ compared to *<0.05; θ compared to $\alpha < 0.05$; β compared to $\S$ <0.05; Ψ compared to Ω <0.05; Ω compared to $\ddagger$ <0.05; $\ddagger$ compared to $\chi < 0.05$.

(Table 9). One study also alludes to the fact that *M. pruriens* could improve anaemia. (Abidemi and Fadeyibi 2011). The only other study that examined the effect of *M. pruriens* on haematological parameters in a 28-day study showed that PCV, Hb, RBC, MCV, HCHC, MCHC and total WBC were normal as similarly observed in this study. However, regarding red blood cell indices, platelet count decreased at a dose of 400 mg/kg b.wt. Furthermore at doses of 50 and 200 mg/kg b.wt neutrophils decreased (Abidemi and Fadeyibi 2011). Contrarily, neuatrophils increased in this study although not significantly.

Biochemically, the increase in ALT was in the medium dose group, with a sharp decrease in the high dose group (Table 6). Post hoc analysis revealed that differences were between the high dose and the medium dose groups (Table 8). However, histologically there were no liver lesions. In one study, when liver tissues were damaged by cobra venom in rats, the pre-treatment of rats with *M. pruriens* extract protected vessels in these organs from damage as seen histopathologically (Jung and Schuppe, 2007), thereby suggesting hepatoprotective properties of *M. pruriens*.

Although creatinine was significantly elevated in the medium dose groups (Table 7 & 8), this did not lead to any concomitant increase in urea nor were histological lesions observed. On renal function, when rats were fed with high fructose to induce renal damage by oxidative stress mechanisms (increased renal MDA), 200 mg/kg daily *M. pruriens* modulated the kidney nuclear transcription factors thereby restoring kidney function after 8 weeks of deterioration (Ulu et al., 2018). Additionally, the protective effect of *M.pruriens* against arsenic-induced liver and kidney dysfunction has been recorded (Concessao et al., 2020). Contrary findings have however been presented by (Gbotolorun et al., al.,2018) in which the authors observed that medium dose was associated with mesengial expansion, hypercellularity of the glomeruli as well as tubular necrosis. These adverse effects differences may be due to the use of *M. pruriens* methanolic extract, if the methanol was not properly cleaned. Furthermore, the apparent nephrotoxicity was not accompanied by increase in creatinine and was observed only in the medium dose group (100 mg/kg. b.wt) for 2 weeks.

In this study, hormonal assays demonstrated a dose-dependant increase in testosterone, though not statistically significant. However, other hormones such as oestrogen and FSH did not show any dose-dependant changes (Table 3). Perhaps the positive effects of the testosterone increase can be supported by data from semen analysis.

Table 3
Effect of *Mucuna pruriens* crude aqueous seed extract on hormonal parameters (Testosterone, Oestrogen and Follicle Stimulating Hormone (FSH).

Parameter	Control	Low dose (500 mg/kg)	Medium dose (1000 mg/kg)	High dose (2000 mg/kg)	p-Value
Testosterone	5.59 ± 0.4	6.58 ± 0.7	6.65 ± 0.4	7.58 ± 0.6	NS
Oestrogen	210.6 ± 22.4	180.8 ± 26.0	135.7 ± 16.6	152.6 ± 28.0	NS
FSH	2.9 ± 0.2	2.9 ± 0.2	2.9 ± 0.2	3.0 ± 0.3	NS

Values are expressed as mean± SEM. $n = 7$; NS = Statistically not significant. Data were analysed.
by one-way ANOVA.

Similar positivity of semen analysis and improvement in sexual behaviour was also reported by (Suresh and Prakash 2012) where the levels of FSH and LH in diabetic rat models administered *M. pruriens* improved significantly. This was coupled with improvement in daily sperm production. The authors also reported improved spermogram parameters. In this present study, sperm count was highest in the medium dose group (1000 mg/kg b.wt) and statistically significant. Motility was also significantly high in this group (Tables 4 & 5). In mice models, *M. pruriens* seeds at a dose of 600 mg/kg positively affected degenerative seminiferous epithelium. Testosterone levels and sexual behaviour also improved (Choowong-In et al., 2021).

In humans 5 g of *M. Pruriens* administered over 3 months in infertile men increased testosterone levels to the levels of controls (Gupta et al., 2011). At this same dose, sperm count, and motility increased (Shukla et al., 2009). These results are similar to our results where normal rats had testosterone increase dose-dependently (but not significantly) over 3 months. It is proposed that levodopa in *M. Pruriens* antagonizes prolactin. Increase prolactin level supresses testosterone and libido (Shukla et al., 2009). Similarly in this study, sperm count and motility increased significantly thereby mimicking the study of (Gupta et al., 2011) in humans.

Although behavioural studies were not undertaken in this study, the increasing testosterone and sperm count would suggest improved sexual behaviour. Improved sexual behaviour was observed as increase in mounting frequency, intromission frequency, and ejaculatory frequency amongst others. Sperm quality and daily sperm production improved. *M. pruriens* at 200 mg/kg of *M. pruriens* seed extract is said to have restored mitochondria dysfunction and DNA damage of sperm in diabetic rats over a period of 60 days (Suresh et al., 2013). The authors of that study indicated that activities of sexual behaviour were however maximized and effective at their medium dose of 200 mg /kg. in rats

Table 4
Effect of *Mucuna pruriens* on semen analysis (motility, immotility and sperm count) of rats after 90 days oral administration with low dose (500 mg/kg), medium dose (1000 mg/kg) and high dose (2000 mg/kg) of crude aqueous extract of *Mucuna pruriens*.

Parameter	Control	Low dose (500 mg/kg)	Medium dose (1000 mg/kg)	High dose (2000 mg/kg)	p – Value
Motility (X 100%)	0.36 ± 0.04	0.50 ± 0.01	0.57 ± 0.07	0.56 ± 0.04	0.013
Immotility (X 100%)	0.64 ± 0.03	0.50 ± 0.01	0.43 ± 0.05	0.44 ± 0.03	0.013
Sperm Count (n x 10^6)	273.0 ± 12.1	260.3 ± 10.7	339.3 ± 14.5	257.8 ± 6.4	<0.001

Values are expressed as mean ± SEM (n = 7); statistically significant at $p < 0.05$. Data were analysed by one-way ANOVA

Table 5
Post hoc analysis for motility (%), Immotility (%),sperm count of rats after 90 days oral administration with low dose (500 mg/kg), medium dose (1000 mg/kg) and high dose (2000 mg/kg) of crude aqueous seed extract of *Mucuna pruriens*.

Parameter	Low dose (500 mg/kg)	Medium dose (1000 mg/kg)	High dose (2000 mg/kg)
Motility (%)			
Control	0.208	0.022*	0.029
Low dose		1.000	1.000
Medium dose			1.000
Immotility (%)			
Control	0.061	0.003*	0.004*
Low dose		1.000	1.000
Medium dose			1.000
Sperm count			
Control	1.000	0.003*	1.000
Low dose		<0.001**	1.000
Medium dose			

*statistically significant at $p < 0.05$; **statistically significant at $p < 0.001$.

Table 6
The effect of the crude aqueous seed extract of *Mucuna pruriens* on liver function after 90 days administration by gavage.

	Control	Low dose (500 mg/kg)	Medium dose (1000 mg/kg)	High dose (2000 mg/kg)	P-Value
TBIL	0.746 ± 0.05	0.865 ± 0.04	0.872 ± 0.08	0.773 ± 0.11	NS
DBIL	0.104 ± 0.05	0.232 ± 0.07	0.163 ± 0.04	0.122 ± 0.01	NS
IBIL	0.638 ± 0.07	0.605 ± 0.08	0.713 ± 0.09	0.618 ± 0.11	NS
ALT	104.6 ± 10.2	106.6 ± 13.2	151.7 ± 27.3	74.6 ± 12.8	0.042*
AST	400.9 ± 110.6	286.1 ± 38.6	420.02 ± 122.9	273.4 ± 66.8	NS
ALP	314.7 ± 20.0	305.5 ± 40.4	333.1 ± 49.4	289.7 ± 28.4	NS
GGT	5.2 ± 0.25	4.03 ± 0.80	5.28± 0.9	5.32 ± 0.6	NS
AST/ALT	3.58 ± 0.69	2.76± 0.32	2.63 ± 0.36	3.53 ± 0.41	NS
TP	65.8 ± 2.24	73.1 ± 2.65	76.03 ± 2.97	70.43 ± 4.32	NS
ALB	34.16 ± 0.87	37.12 ± 1.30	36.75 ± 1.14	40.0 ± 1.7	NS
Glo	31.7 ± 1.41	36.07 ± 1.76	39.28 ± 2.42	34.5 ± 2.7	NS
Alb/Glo	1.08 ± 0.02	1.033 ± 0.05	0.967 ± 0.06	1.05 ± 0.03	NS

Values are expressed as mean ± SEM. $n = 7$. NS = Statistically not significant; *statistically significant at $p < 0.05$. Data were analysed by one-way ANOVA. TBIL: Total bilirubin (μmol/L); DBIL: Direct bilirubin (μmol/L); IBIL: Indirect bilirubin (μmol/L); ALT = Alanine aminotransferase (U/L); GGT = Gamma-glutamyl transferase (U/L); ALP = Alkaline phosphatase (U/L); AST: Aspartate transaminase (U/L); Glo: Globulin (g/L); TP: Total protein (g/L); Alb: Albumin (g/L).

(Suresh et al., 2009).

Erectile dysfunction in aged rats observed by a reduction in the number of myelinated fibres, indentation of the myelin sheath with degenerative changes of the dorsal nerve of the penis with its implication on erectile function, were all recovered following *M. pruriens* supplementation (Seppan et al., 2020).

Histologically, no abnormality was found in the testicles of rats receiving *M. pruriens* in this study (Fig. 1). A study on testicular damages

Table 7
Effect of the crude aqueous extract of *Mucuna pruriens* on renal function test (Na, K, Urea, Crea.) after 90 days administration by gavage.

	Control	Low dose (500 mg/kg)	Medium dose (1000 mg/kg)	High dose (2000 mg/kg)	P-Value
Na	142.6 ± 0.9	142.8 ± 1.6	141.7 ± 0.56	143.8 ± 1.08	NS
K	3.56 ± 0.11	3.62 ± 0.10	3.55 ± 0.04	3.57 ± 0.09	NS
Urea	6.64 ± 0.43	6.23 ± 0.37	6.96 ± 0.27	6.72 ± 0.38	NS
Crea	102.8 ± 3.8	106.5 ± 6.2	120.5 ± 4.7	103.7 ± 2.5	0.043*

Values are expressed as mean ± SEM ($n = 7$); NS = Statistically not significant; * statistically.
significant at $p < 0.05$. Data were analysed by one-way ANOVA. Na: Sodium (mmol/L); K:.
Potassium (mmol/L); Crea: Creatinine (mmol/L); Urea (mmol/L).

Table 8
Post hoc analysis of ALT and creatinine of rats after 90 days oral administration with low dose (500 mg/kg), medium dose (1000 mg/kg) and high dose (2000 mg/kg) of crude aqueous extract of *Mucuna pruriens*.

Parameter		Low dose (500 mg/kg)	Medium dose (1000 mg/kg)	High dose (2000 mg/kg)
ALT				
	Control	1.000	0.508	1.000
	Low dose		0.500	1.000
	Medium dose			0.033*
Creatinine				
	Control	1.000	0.092	1.000
	Low dose		0.240	1.000
	Medium dose			0.095

*Statistically significant at $p < 0.05$.

Table 9
. Effect of the crude aqueous extract of *Mucuna pruriens* on red cell indices after 90 days of oral administration by gavage.

	Control	Low dose (500 mg/kg)	Medium dose (1000 mg/kg)	High dose (2000 mg/kg)	P-Value
RBC	7.6 ± 0.3	8.2 ± 0.4	8.47± 0.1	8.3 ± 0.3	NS
HGB	14.6 ± 0.5	15.3 ± 0.4	15.4 ± 0.3	15.4 ± 0.5	NS
HCT	42.5 ± 1.5	44.7 ± 0.8	44.2 ± 0.8	44.6 ± 1.3	NS
MCV	56.1 ± 0.6	54.6 ± 1.4	52.2 ± 0.8	54.0 ± 1.1	NS
MCH	19.6 ± 0.2	18.7 ± 0.4	18.2 ± 0.3	18.6 ± 0.3	NS
MCHC	34.5 ± 0.2	34.3 ± 0.2	34.8 ± 0.2	34.5 ± 0.2	NS
RDWCV	15.2 ± 0.2	15.5 ± 0.4	14.2 ± 0.3	14.0 ± 0.2	0.007
RDWSD	32.5 ± 0.6	32.3 ± 1.0	28.3 ± 0.8	29.0 ± 0.8	0.002

Values are expressed as mean ± SEM, $n = 7$; NS = Statistically not significant; Statistically significant at $p < 0.05$. Data were analysed by one-way ANOVA. RBC: red blood cell count ($x10^6$); HGB: haemoglobin concentration (g/dL); HCT: hematocrits (%); MCV: mean corpuscular volume (fL); MCH: mean corpuscular haemoglobin (pg); MCHC: mean corpuscular haemoglobin concentration (g/dL); RDW-CV= red cell distribution width coefficient of variation (%); RDW-SD (fL): red cell distribution width-standard deviation.

Table 10
The Effect of the crude aqueous seed extract of *Mucuna pruriens* on White blood cells and platelets after 90 days of oral administration by gavage.

	Control	Low dose (500 mg/kg)	Medium dose (1000 mg/kg)	High dose (2000 mg/kg)	p-Value
WBC	3.7 ± 1.0	7.1 ± 1.8	7.0 ± 1.1	7.4 ± 1.9	NS
Neu	0.6 ± 0.2	1.3 ± 0.3	1.4 ± 0.1	1.3 ± 0.3	NS
Lym	2.9 ± 0.8	5.6 ± 1.6	5.4 ± 0.9	5.6 ± 1.5	NS
Mon	0.17 ± 0.06	0.17 ± 0.09	0.14 ± 0.03	0.50 ± 0.20	NS
Eos	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.0	0.02 ± 0.01	NS
Bas	0.01 ± 0.002	0.004 ± 0.002	0.01 ± 0.004	0.0 ± 0.0	NS
Bas %	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.02 ± 0.01	0.036
PLT	409.0 ± 172.1	571.0 ± 73.0	401.9 ± 101.5	677.4 ± 35.6	NS
PDW	15.7 ± 0.3	15.4 ± 0.1	15.5 ± 0.1	15.4 ± 0.1	NS
PCT	0.28 ± 0.11	0.42 ± 0.05	0.29 ± 0.07	0.50 ± 0.04	NS
PLCC	41.7 ± 12.9	65.6 ± 9.02	48.6 ± 11.7	85.2 ± 11.4	NS

Values are expressed as mean ± SEM ($n = 7$). NS = statistically not significant; *statistically significant at $p < 0.05$. Data were analysed by one-way ANOVA. WBC: white blood cell count ($x10^3$); Eos: Eosinophils (U/L) count; Mon: Monocyte (U/L); Bas: Basophils count (U/L); Neu: Neutrophils count (U/L); Lym: lymphocytes (%); PLT: platelet count (%); LYM: lymphocyte (%); MPV: mean platelet volume (fL); PDW: platelet distribution width (fL); PCT: Platelet ($x10^3$); PLCR: Platelet large cell ratio; PLCC: Platelet Large cell count (fL).

Table 11
Post hoc analysis for haematological parameters (RDW-SD, RDW-CV, basophils % and PLT) of rats after 90 days oral administration with low dose (500 mg/kg), medium dose (1000 mg/kg) and high dose (2000 mg/kg) of crude aqueous extract of *Mucuna pruriens*.

		Low dose (500 mg/kg)	Medium dose (1000 mg/kg)	High dose (2000 mg/kg)
RDW-SD				
	Control	1.000	0.013*	0.070
	Low dose		0.012*	0.074
	Medium dose			1.000
RDW-CV				
	Control	1.000	0.249	0.132
	Low dose		0.039*	0.022*
	Medium dose			1.000
BAS%				
	Control	0.413	1.000	0.046*
	Low dose		1.000	1.000
	Medium dose			0.168

*Statistically significant at $p < 0.05$.

induced by ethanol ingestion through MDA accumulation in rats was subsequently reversed by *M. pruriens* seed extract administration. This is in agreement with our testicular histology. Indeed, testicular damage will not support the several findings that suggested that *M. pruriens* could improve spermatogenesis. Histologically, the seminal vesicles did not show any abnormality.

The concept of polyherbalism has been in existence since the era of Ayurvedic medicine as exemplified by triglize usage (Parasuraman et al., 2010). In Ayurvedic medicine it is believed that combining plants boosts the levels of certain phytocompounds which otherwise would exist in minimum quantities. The phenomenon of synergy suggestively led to combinations such as black pepper, long pepper and ginger (mucous--reducing as well as "heating" effect) or ginger and neem to take care of

"warmth" and "cold" extreme conditions (Pole et al., 2013). Scientifically, the concept of pharmacodynamics and pharmacokinetics synergy in most cases support polyherbalism (Spinella et al., 2002). The efficacy of *Mucuna pruriens* (Linn), *Chlorophytum borivillianum* (Sant and Fernand), and *Eulophia campestris* (Wall) as a polyherbal drug for the management of male infertility has been demonstrated and fully discussed elsewhere (Mahajan et al., 2012).

Conclusion

This study primarily examined the effect of *M. pruriens* using rat models without pathological conditions. Systemic organs did not show any toxicity histologically in tandem with blood biochemistry results. The area that has been well researched is the effect of *M. pruriens* on fertility. In this study and the only human study available on *M. pruriens* administration to normal humans or animals, increased sperm count and motility were observed. Hormonal changes are still a matter of conjecture.

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Contribution of authors

PD undertook experimentation/investigations, contributed to writing; SA drafting and reviewing manuscript; BYA supervision and reviewing; GNAH histology slides preparations/reading; EZ statistics; RO-K experimentation/investigation; ICO writing; GAA conceptualization and writing manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper.

Supplementary materials

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