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Mostua hirsuta (Geselmiaceae) extract stimulates the reproductive and cardiovascular systems in male Wistar rats

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Abstract
A species' ability to reproduce itself by transferring life and creating new living beings is a fundamental process that ensures its survival. Unfortunately, many couples experience infertility, among other reproductive health difficulties. The primary goal of this study was to see how extracts from *Mostuea hirsuta* affected the reproductive system of wistar rats.
The various aqueous extract doses were determined based on traditional healers' guidance. The mice received different doses of Mustuea hirsuta aqueous extract (6, 12, and 18 mg/kg) every day for 30 days. The rats received the medicine for thirty days and were weighed every two days. At the end of the thirty days, the rats were slain, and their organs and blood were collected for analysis in accordance with normal protocols.
The study found that providing *Mostuea hirsuta* extract at a dose of 12 mg/dL significantly enhances blood testosterone levels and sperm density in seminal fluid. Asparatate aminotransferase and alanine aminotransferase, two indicators for liver and kidney function, as well as blood cell counts, showed no detrimental effects from varied doses of the extract. Furthermore, the varied extract doses increased both total and HDL cholesterol levels. A phytochemical test found that *Mostuea hirsuta* extract has naturally high levels of tannins, flavonoids, and polyphenols.
The current study's findings back up the traditional use of *Mostuea hirsuta* extract in the treatment of male infertility.

Keywords: Infertility, *Mostuea hirsuta*, spermatogenesis, adverse effects

Introduction
The transfer of life and the birth of a new living creature through reproduction ensures a species' continuing survival. The genital tract, which produces and mixes gametes during fertilization, is associated with human sexual reproduction. As a result, both males and females can create reproductive cells known as spermatozoa in men and ovarian cells in women [1]. Couples face a variety of reproductive health challenges, including infertility.
Couple infertility is defined by the World Health Organization (WHO) as a couple's failure to conceive after a year of marriage and regular sexual activity without the use of contraception [2]. Its affects 10% to 15% of married couples worldwide, with 40% of instances attributed to a male component [3, 4]. Approximately 10% of couples worldwide experience primary or secondary infertility [5]. 25% to 63% of women and 10% to 52% of men suffer from this serious medical illness, which disrupts social and biological relationships [6]. Male reproductive health is declining worldwide [7]. Male infertility can be caused by a variety of factors, including erectile dysfunction, genetic disorders, varicocele, genital duct obstruction, low sperm production, and poor semen quality [8]. According to Ngalle [9], the prevalence of infertility in Cameroon is 11.9%. Surgical methods, medications, and natural therapies are utilized to treat infertility. There are numerous phony pill goods on the global market that promise fast therapy. However, the majority of them are associated with negative consequences, which are commonly linked to addiction and symptom suppression [10]. However, the interest in natural substances in general, and phytomedicine in particular, is justified by the wide range of applications for plant products, their efficacy in treating chronic diseases, and the need to develop new medications.

Because over 25% of contemporary drugs are derived from plants, the use of medicinal plants to treat diseases and dysfunctions has a long history and has had a substantial impact on the development of pharmaceutical products [5]. According to the WHO, more than 60% of the world's population has used plant-based products for medical purposes for over 20 years.

Several plants have been demonstrated to improve both male and female reproductive function in Cameroon in earlier studies [11-16]. Despite the fact that the inhabitants of Adamaoua use medicinal herbs to treat infertility, and despite the region's abundance of plants, there has been little scientific investigation in this part of Cameroon. *Mostuea hirsuta* is used in traditional medicine to improve respiratory function, regulate heart rate, and alleviate discomfort. Furthermore, Neuwinger [17] reports that it is used to treat aphrodisiacs, sleep disturbances, infantile umbilical hernias, and colds. This plant has long been used to treat male infertility issues in the village of Roblin, in the Meiganga district of Cameroon's Adamaoua region; however, according to our bibliographic research, no research has been conducted on the biological activity of this plant on the male reproductive system. In light of this, we conducted this study, with the primary purpose of determining the effect of *Mostuea hirsuta* extracts on wistar rat male reproductive function. The study aimed to assess the effects of aqueous Mostua hirsuta extract on reproductive hormones, oxidative stress, and biochemical parameters in rats.

Materials and Methods

Materials

This investigation was conducted at the Sunshine biomedical analysis and research laboratory and at the biology laboratory at the University of Ngaoundéré. This occurred over five months, from March 8, 2023 to July 20, 2023.

The plants were collected in January 2023 at Roblin village, Meiganga district, Mbéré department, Adamaoua region of Cameroon. It was identified at the Cameroonian national herbarium by Dr. Tchiengue Barthélemy who used existing records and samples from plants in the same genus to make the identification (Flora of West Cameroon vol.2 P44).

The trial included mature male Wistar albino rats aged seven to eight weeks, with an average weight of 152g ± 35g. These animals were obtained from the Department of Biological Sciences' animal facility at the University of Ngaoundéré's Faculty of Science.

Methods

Preparation of the extract

In a flask containing 2,000 ml of distilled water, 249 g of fine plant powder was added. The mixture was then brought to a boil for one hour. After cooling, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated by evaporating the water in a preheated oven at 40 °C. The extracted material was stored at 4°C until ready for use.

Animal allocation and treatment

The animals underwent a five-day acclimatization period prior to the commencement of the actual treatment. Amidst the acclimatisation process, the animals were accommodated in suitable enclosures that maintained a light-dark cycle of 12 hours and a temperature of 22±2°C. Throughout the 29-day treatment period, the animals were categorized into six groups of five rats each (N=5) and subjected to the following endo-gastric tube treatments:

- The first normal control group of animals received 1 mL per 100g body weight of the extract;
- Groups 2, 3, and 4 of rats were given the extract at the therapeutic and multiples of the therapeutic dosage;
- Animals in groups 5 and 6 (which served as the positive control) were administered Provirion and Viagra, respectively.

Treatments were performed daily between 8 am and 10 am for 29 days. Throughout the 29-day treatment period, the animals were provided with sufficient feed and water and weighed every two day.

Sacrifice and sampling

All animals were slaughtered under chloroform anaesthesia on the 29th day of treatment, following a 12-hour fast. Blood was taken by cardiac puncture in both dry and EDTA tubes. After standing for 30 minutes at laboratory temperature, the blood obtained in the dry tubes was centrifuged at 3,000 rpm for 15 minutes, and the serum was collected in creos tubes for various assays. The blood cell count (CBC) was conducted using blood taken under anticoagulant conditions.

Organs such as the liver, testicles, kidneys, prostate, lungs, and epididymis were carefully removed and cleaned with 0.9% NaCl before being weighed on a sensitive BIOBASE balance. The homogenate was prepared whit 0.56 g of liver and kidney. These samples were ground with 2 mL of phosphate buffer (1.5 mol, PH= 7.4), centrifuged at 3000rpm for 15 minutes, and the supernatant was recovered and frozen for the determination of biochemical parameters indicating oxidative stress (MDA, NO), as well as total proteins. The serum was tested for toxicity markers such as ASAT, ALAT, urea, creatinine, lipid profile some spermatogenesis involved hormones. spermatogenesis involved hormones and antioxidant markers titration.

Testosterone, FSH, and LH assays these were performed using the ELISA technique

A micropipette was employed to transfer 50 µl of both the standard and sample onto the microplates. One hundred microliters of conjugated enzyme and fifty microliters of biotin reagent were added. Following 30 seconds of homogenization at room temperature, the plate was covered and incubated for an additional 60 minutes. Following this, the contents of the wells were empty and they were rinsed with the wash solution. Following the addition of 100 µl of TMB substrate to the plates, incubate at room temperature for 30 minutes. LabTech ELISA readers should be used to measure absorbance at 450 nm after adding 50 µl of stop solution to each well.

Evaluation of antioxidant makers

250 ml of homogenate/50 mM tris-HCL buffer, 150 mm KCL (blank), 200 µl of 20% trichloroacetic acid, and 500 µl of 0.67% thiobarbituric acid should were introduced in a test tubes. After capping the tubes with glass beads, they were heated to 90°C for ten minutes in a water bath. After being cooled with tap water, they were centrifuged for 15 minutes at room temperature at 3000 rpm. The optical density of the supernatants were measured at 530 nm.

Using the Beer-Lambert equation, the amount of malondialdehyde, expressed in mol/L/g of organ, was calculated using the following formula: $C = \frac{DOXVt}{\varepsilon X Vi X M}$

Avec:

$$\varepsilon = 1{,}56 \times 10 \exp 5 \frac{M}{cm}.$$

Vt=total volume of homogenate
M=mass of organ used for homogenate
Vi=volume used for assay

Nitric oxide assay

A tw fold serial dilution of the NaNO2 solution was performed in 13 test tubes. The maximum concentration was 1mM. In the first tube, 200µl of NaNO2 was introduced, while the next 12 tubes received 200µl of distilled water. After adding 200µl of NaNO2 to the second tube, it was homogenized by vortexing. Then, 200µl was removed and added to tube No. 3, and so on until tube No. 13. In the final tube (N 13), 200 µl were removed and discarded.

To tubes 1-13, add 200µl of Griess reagent were added and the absorbance of each tube was read at 570 nm after 10 minutes. This initial series of tubes was used to create the calibration curve. To measure NO levels in different samples, add 200 µl of GRIESS reagent to 200 µl of organ homogenate supernatant. After homogenization, the optical density of each tube was measured at 570 nm with a spectrophotometer (PD-303S). After 10 minutes of incubation at room temperature.

Identification of toxicity biomarkers titration

ASAT and ALT assay

A micropipette was used to mix 1000µl of working reagent with 100µl of patient serum. The mixture was homogenized and incubated at 37°C for 1 minute, with the sample's initial absorbance measured every minute for 4 minutes. The ASAT/ALAT activity in the sample was obtained by multiplying the main A/min by 1750.

Creatinine measurement

After allowing the kit to warm up for 25 minutes at room temperature, 1000 µl of working reagent were added to 50 µl of standard or sample and the absorbance (A1) was immediately read and (A2) after 1 minute. The creatinine concentration in the various samples was calculated using the following formula:

$$\text{Creatinine Conc. mg/dl} = \frac{\Delta AT}{\Delta AS} \times 2$$

Determination of urea

To 10 µl of the standard or sample were added 1000 µl of the working reagent. Afther 5 min incubated at 37 ºC the absorbance (A) of the standard and sample was read at 580nm.

Determination of lipid profil

Using a micropipette, 1000 µl of the working reagent was added to 10 µl of the standard or sample. Afther 5 minutes incubation at 37 ºC, the absorbance (A) of the standard and sample at 580nm triglycerides and 505nm for total and HDL cholesterol. The concentration of triglyceride and total cholesterol in a given sample was calculated using the following formula:

$$CTr = \frac{\text{Standard A}}{\text{Sample A}} \text{ X } 200$$

The concentration of LDL cholesterol was calculated using the using the Friedewald formula:

$$LDL = \text{Total cholesterol} - \text{HDLc} - \text{TG}/5$$

Statistical analyzes

The results obtained in this study are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) with post hoc Waller-Duncan multiple range tests using SPSS 26.0 for Windows. $p<0.05$ was considered significant.

Results and Discussion

Results

The effect of an extract of *Mostuea hirsuta* on particular reproductive hormones

Figure 8 below depicts the distribution of testosterone in respect to the various treatment doses. The results show that administering *Mostuea hirsuta* extract at doses of 12 and 18 mg/kg caused a significant increase in testosterone levels when compared to the negative control, proviron, and the positive control, viagra.

The distribution of serum LH levels in test rats based on various treatments is depicted in the figure 9. When compared to the negative control, there was a drop in LH levels at 12 and 18 mg/kg, while there was a modest increase in the proviron positive control group.

Figure 10, which shows the distribution of FSH levels according to treatment, shows that Mostuea hisurta extract caused no change in serum FSH levels compared with the controls.

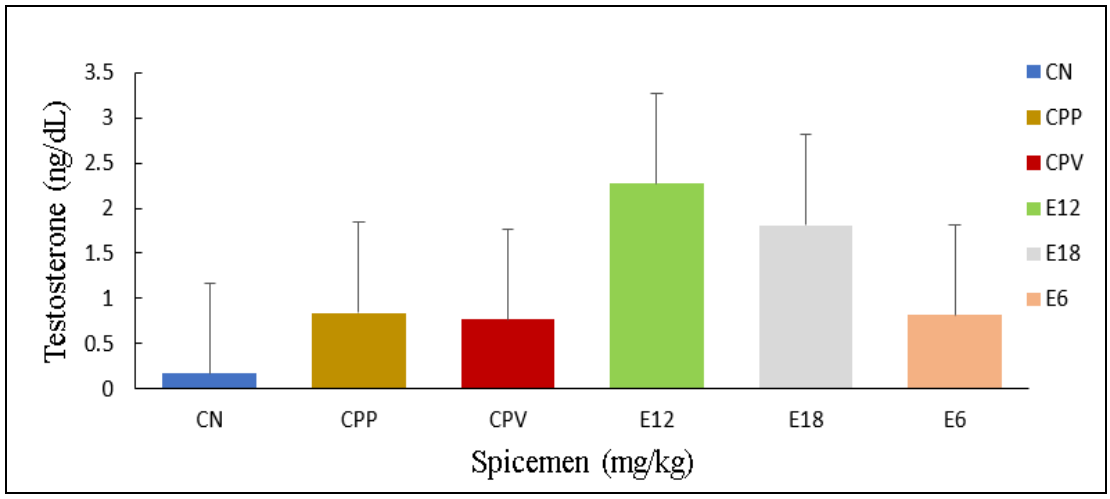

Fig 1: Testosterone levels according to different treatment

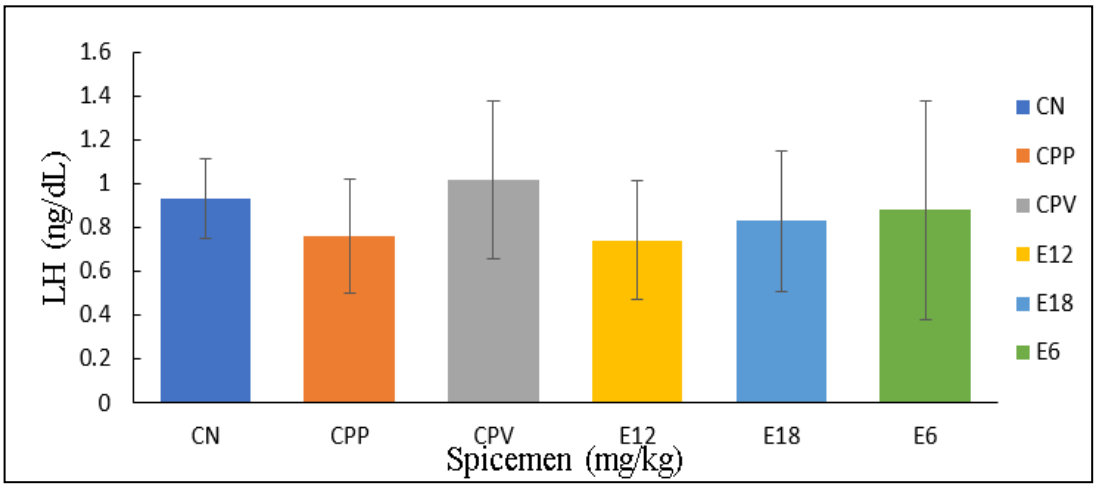

Fig 2: LH levels in different groups

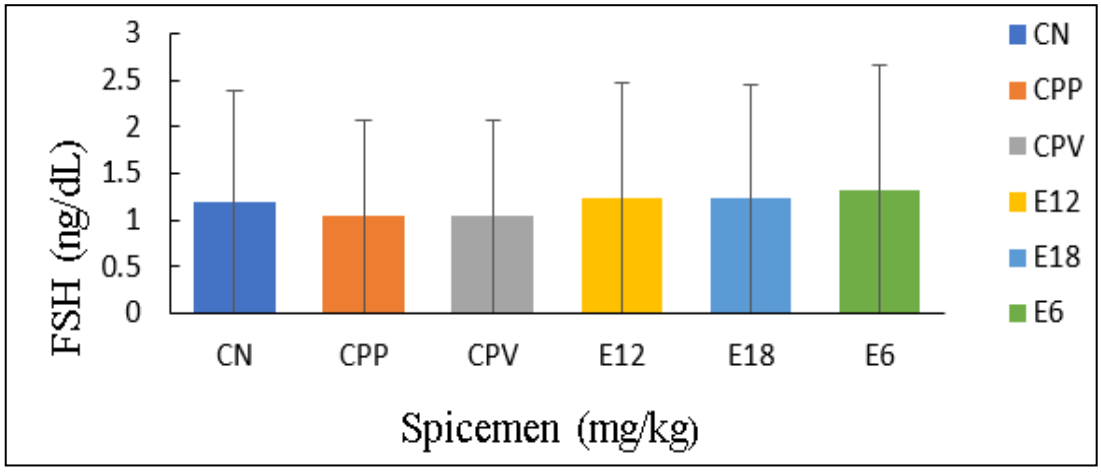

Fig 3: FSH levels of different animal groups

Side effects related to the administration of this extract in rats

Effect of administration of *Mostuea hirsuta* extract on animal and organ weight

The animal weight alterations as a result of the various treatments are illustrated in Figure 11. The animals' masses in each group exhibited a corresponding increase in variation as the days progressed. Nevertheless, the CPP group exhibited a modest increase in animal mass in comparison to the other groups.

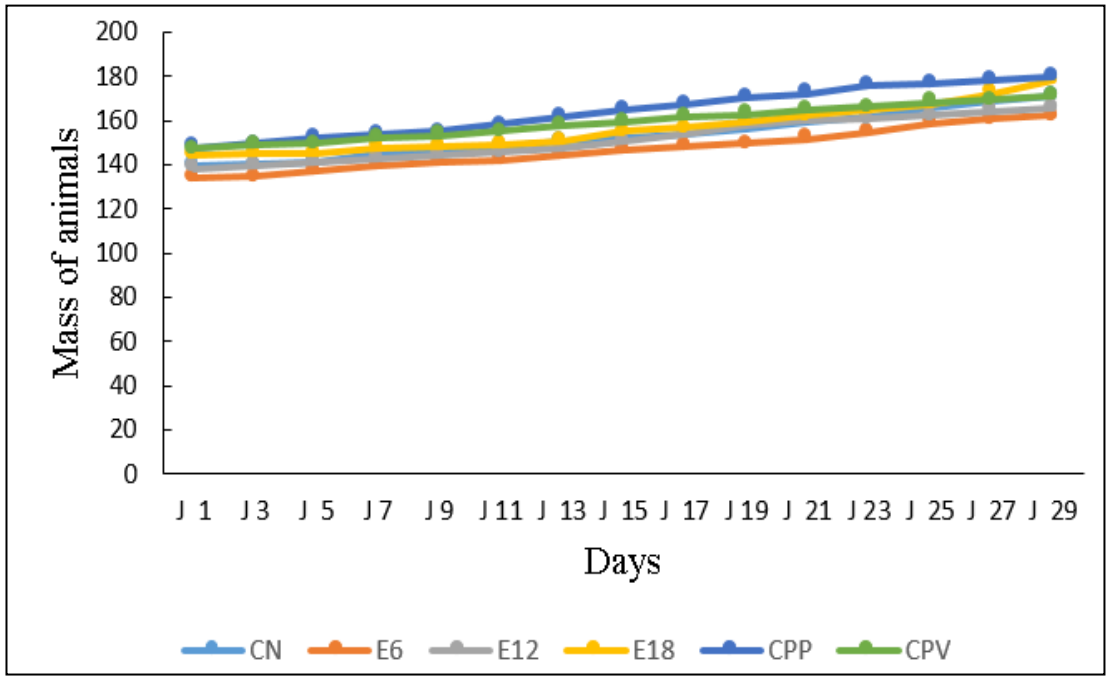

Fig 4: Mass distribution of animals according to samples

The table below illustrates the distribution of relative organ mass in relation to treatment. It is evident that, with the exception of the CPV control group's increased prostate mass, no other substantial alterations in relative organ weights were observed.

Table 1: Relative organ weights according to treatments

Extraits	Liver	Testicules	Prostate	Epididymis	Kidney	Lungs	Spleen	Heart
CN	5,41±1,41	2,36±0,42	0,15±0,15	0,61±0,13	1,04±0,18	1,03±0,16	0,82±0,13	0,55±0,09
CPP	5,46±0,76	2,36±0,31	0,13±0,05	0,81±0,22	1,05±0,13	1,07±0,10	0,68±0,20	0,55±0,06
CPV	5,24±1,01	2,15±0,26	0,21±0,11	0,83±0,21	1,01±0,18	1,10±0,17	0,63±0,17	0,58±0,11
E12	5,26±0,93	2,27±0,32	0,11±0,05	0,70±0,14	1,00±0,21	0,91±0,11	0,67±0,07	0,57±0,09
E18	6,26±1,07	2,48±0,43	0,10±0,03	0,77±0,18	1,12±0,18	1,16±0,11	0,95±0,39	0,55±0,08
E6	5,45±0,99	2,15±0,40	0,12±0,07	0,71±0,13	0,96±0,15	0,93±0,14	0,67±0,13	0,52±0,08
k	3,03103	3,17291	4,32033	5,24113	1,90788	9,36355	6,53276	1,54505
P	0,695201	0,673349	0,504276	0,387166	0,861739	0,0954129	0,257772	0,907813

K: Kriskal-walis Test; P: P-Value

Effect of administration of *Mostuea hirsuta* extract on serum protein levels

Figure 5 illustrates the protein distribution based on different dosages of *Mostuea hirsuta* extract. This demonstrates that, when compared to the controls, the administration of *Mostuea hirsuta* extract at various doses had no influence on serum protein levels.

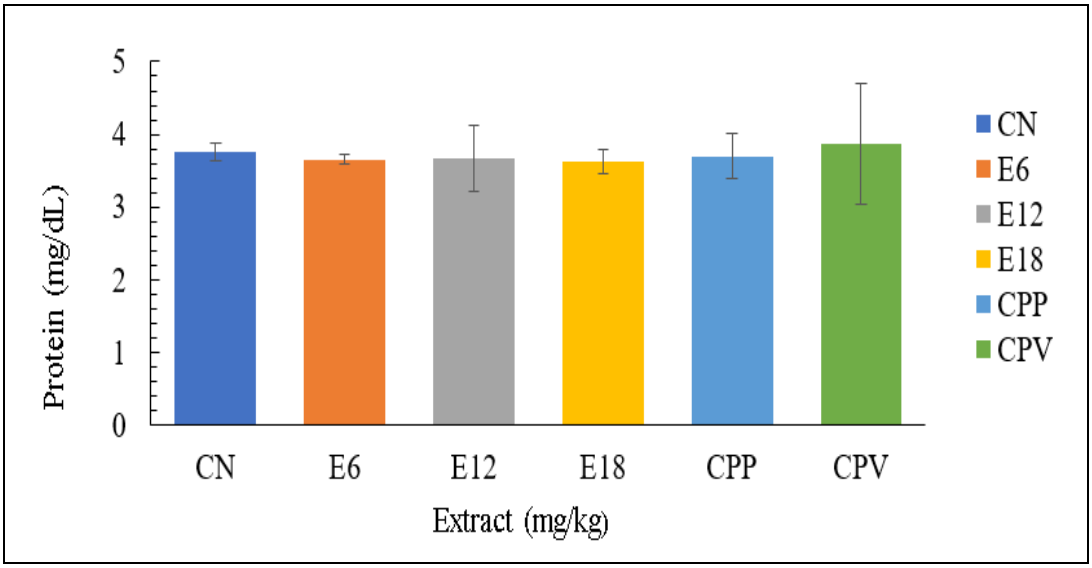

Fig 5: Protein levels of the different samples

Effect of different doses of *Mostuea hirsuta* extract on creatinine and urea levels

The figure 14 below shows creatinine and uraemia levels according to the different doses of extract. It can be seen that, compared with the controls, administration of *Mostuea hirsuta* extract at different doses did not significantly affect urea and creatinine levels.

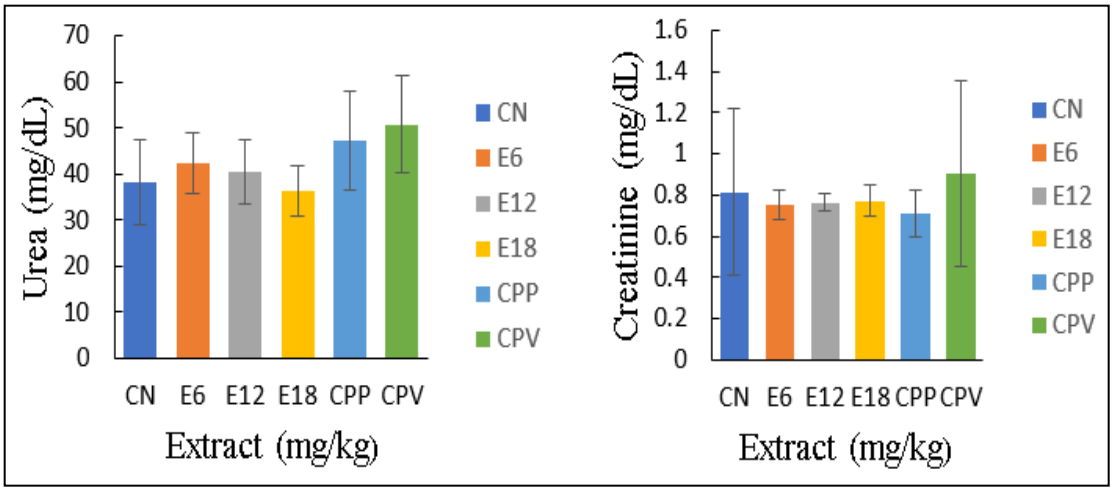

Fig 6: Creatinine and serum urea levels in fifferent animal groups

Effect of administration of *Mostuea hirsuta* extract on lipid profil: Figures 7, 8, and 9 illustrate the distribution of triglyceride, total cholesterol, and HDL cholesterol values among the different treatments. With the exception of the CPV control, which had slightly higher triglyceride levels than the other groups, no other changes were found (Fig. 15). Although not statistically significant, the doses of *Mostuea hirsuta* extract and the Viagra positive control (CPV) increased total cholesterol levels somewhat (Fig. 16). Figure 17 shows that extract doses of 6, 12, and 18 mg/kg raised HDL cholesterol levels when compared to positive controls.

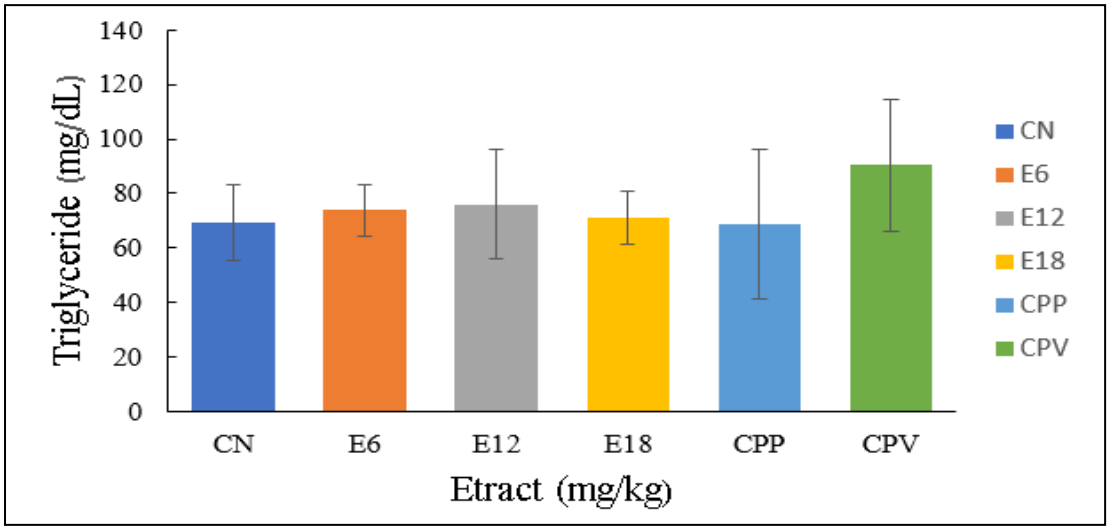

Fig 7: Serum triglyceride levels as a function of treatment

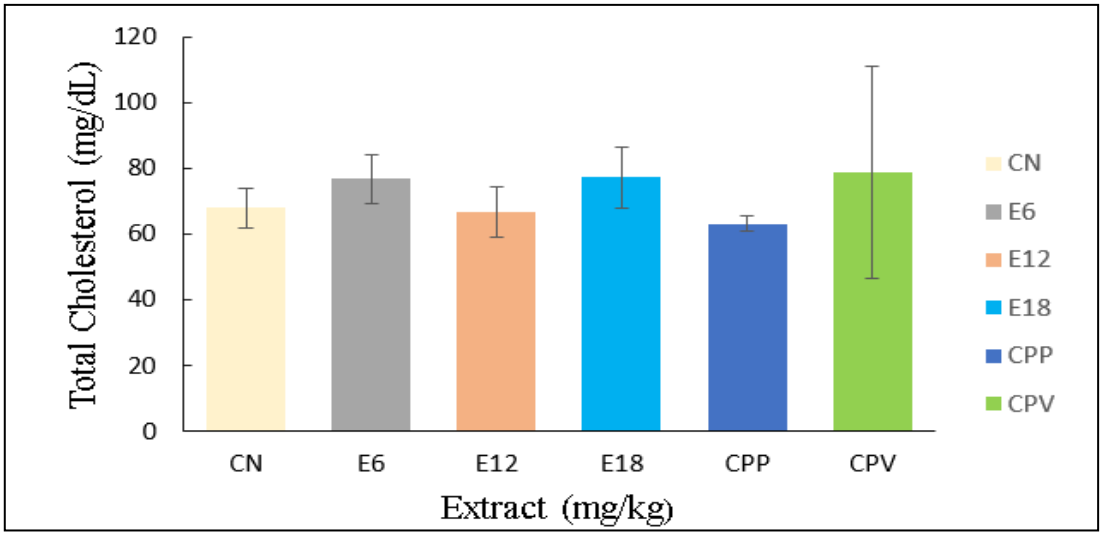

Fig 8: Total cholesterol in relation to treatments

Effect of treatments on HDL cholesterol levels

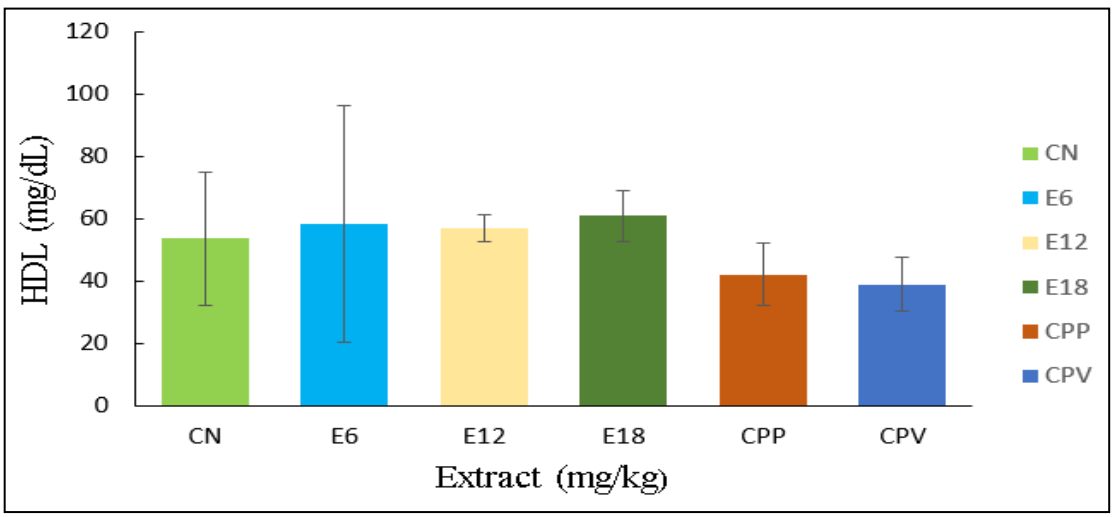

Fig 9: HDL cholesterol levels as a function of treatments

III-1.2.5. Impact of *Mostuea hirsuta* extract administration on ALAT and ASAT activity

The analysis of figures 16, which depict the distribution of AST and ALT levels in relation to the various treatments, indicates that, despite the absence of statistical significance, the varying doses of extract resulted in an increase in AST activity in comparison to the negative control and CPV. On the contrary, ALT levels experienced a modest decrease.

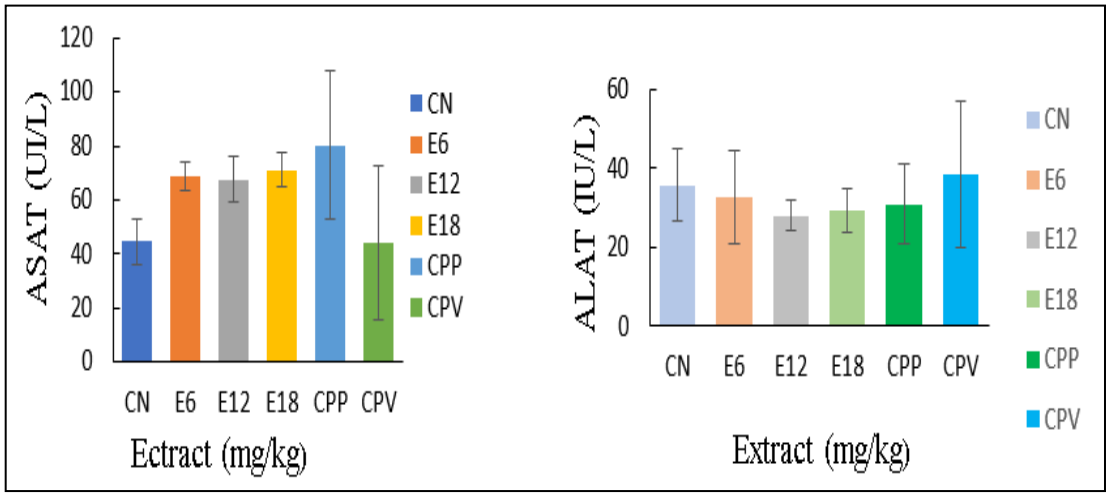

Fig 10: ASAT and ALAT activity as a function of treatments

III-1.2.6. Effect of administration of *Mostuea hirsuta* extract on blood count parameters.

It can be seen from table 5 that there was a significant difference between the granulocyte counts of the groups receiving *Mostuea hirsuta* extract with the proviron positive control and the viagra positive control.

Table 5: Blood count parameters according to samples

Extraits	*WBC*	GRAN	RBC	HGB	HCT	MCV	TCMH	CCMH	PLT	MPV
CN	9,02±2,02	26,96±5,98 *#	7,48±0,58	13,26±1,11	38,08±2,00	51,56±1,65	17,84±0,97	34,68±1,50	763,20±92,90	5,68±0,16
CPP	8,95±1,47	0,63±0,13	7,92±0,40	15,15±0,50	40,93±0,35	51,88±2,68	19,13±0,39	36,98±1,19	692,00±72,14	5,93±0,39
CPV	6,02±1,97	8,22±10,98	7,46±0,78	14,02±1,73	38,54±4,76	51,84±3,48	18,76±1,27	36,34±1,00	591,60±229,07	5,82±0,24
E12	8,26±1,15	22,98±6,82 *	7,36±0,59	13,14±1,03	36,72±2,82	49,82±1,91	17,80±0,60	35,72±0,26	682,80±126,35	6,12±0,42
E18	6,58±3,91	20,48±10,89 *	7,36±0,41	13,70±1,00	37,33±3,44	50,75±1,86	18,53±0,36	36,70±0,70	696,00±163,00	6,28±0,17
E6	8,44±2,83	25,32±8,49 *#	7,52±0,48	13,50±0,81	38,34±3,58	50,98±1,83	17,90±0,37	35,26±1,43	670,40±101,75	6,06±0,43
K	5,76132	14,3894	3,30296	7,95507	5,91842	3,0553	10,1126	10,0454	2,67856	9,23619
P	0,33014	0,0133165	0,653389	0,158729	0,314238	0,69146	0,0721082	0,0739598	0,749396	0,100006

# = comparaison avec CPV, *=Comparaison avec CPP
CPP=Contrôle Positif Proviron CPV= Contrôle positif viagra, CN=Contrôle Négatif
E12=Extrait à la dose 12, E6=Extrait à la dose 6, E18=Extrait à la dose 18
# = comparison with CPV *=comparison with CPP
CPP=Proviron Positive Control, CPV=Viagra Positive Control, CN=Negative Control
E12=Extract at dose, 12 E6=Extract at dose 6, E18=Extract at dose 18

Discussion

The use of medicinal plants in the treatment of male infertility has been of great interest in biomedical research. As a result, several studies have been carried out on medicinal plants. The present study was therefore devoted to investigating the possible effects of *Mostuea hirsuta* extracts on the male reproductive system, in order to justify their use in traditional medicine.

The results of this work show that, compared with controls, Mostuea hisurta extract has no adverse effect on relative organ weights. The increase in prostate size in proviron-treated animals may be due to the fact that proviron leads to a general increase in muscle mass (Notice).

In male physiology, testosterone is one of the essential hormones. It is responsible for the development, growth and normal functioning of the testicles and male accessory reproductive glands, and is also involved in stimulating and maintaining spermatogenesis, muscle development, etc.

Aqueous extracts of *Mostuea hirsuta* administered at a concentration of 12 mg/dL lead to an increase in testosterone levels, as well as in total and HDL cholesterol, resulting in an increase in androgenic activity. This result is thought to be due to the presence in this extract of phytochemical compounds such as saponins, flavonoids and alkaloids and bioactive antioxidant compounds in extracts of this plant [18], which stimulate the synthesis of cholesterol, a precursor metabolite of androgen synthesis, or spermatogenesis. An increase in testicular cholesterol levels stimulates the synthesis of luteinising hormone, which strengthens molecular defence in the testes, increasing blood flow to the testes and thus improving testosterone production by Leydig cells and, in turn, spermatogenesis [19]. On the other hand, although not significant, the drop in LDL-cholesterol levels in animals treated with doses of 12 and 18 mg/kg means that consumption of this extract does not lead to metabolic syndrome (arteriosclerosis, for example) or cardiovascular disorders. In fact, it is known that an increase in LDL levels is a definite risk factor for developing metabolic syndrome or cardiovascular disorders (unstable angina, myocardial infarction) [20, 21].

The anteropituitary gonadotropic cells secrete the gonadotropins LH and FSH, glycoproteins involved in regulating androgen production. Schematically, FSH promotes spermatogenesis, while LH stimulates the secretion

of androgens by Leydig cells. In the present study, a decrease in LH levels was recorded at doses of 12 and 18 mg/kg of *Mostuea hirsuta* extract compared with controls. This may be explained by the fact that *Mostuea hirsuta* extract increased testosterone levels at doses of 12 and 18 mg/kg. Testosterone decreases the expression of the LH gene subunit [22], causing negative feedback to the hypothalamus and slowing down the hypothalamic pulse generator and therefore the release of LH [23].

Creatinine and urea are the main parameters for investigating renal function [24] in general, and glomerular filtration in particular. Any dysfunction of the glomerulus leads to an increase in serum creatinine followed by a decrease in urine output. Administration of *Mostuea hirsuta* extract at different doses had no significant effect on serum creatinine levels.

ALAT is an intracellular enzyme found mainly in liver cells. It is therefore an important marker of liver function. Administration of *Mostuea hirsuta* extract did not result in any change in AST and ALT activity compared with the negative control and control. This allows us to confirm that this extract does not cause hepatic cytolysis at the doses tested.

The haematopoietic system is also one of the most sensitive targets for toxic compounds. It is an important indicator of the physiological and pathological state of humans and animals [25]. In this respect, the state of bone marrow activity and intravascular effects are often monitored by haematological examinations. Administration of the various doses of *Mostuea hirsuta* extract had no effect on the blood count. This means that it did not interfere with erythropoiesis, haemoglobin formation or the immune system of the test rats.

Conclusion

Ultimately, the aim was to assess the effect of *Mostuea hirsuta* extracts on reproductive function in wistar rats. More specifically, to evaluate the effects of administration of the aqueous extract of *Mostuea hirsuta* on changes in body mass and the mass of certain organs, to evaluate the effects of administration of the aqueous extract of *Mostuea hirsuta* on the level of certain reproductive hormones, to evaluate the effect of administration of this extract on oxidative stress and to evaluate the secondary effects associated with administration of this extract in rats by measuring certain biochemical parameters that are markers of toxicity. The results showed that administration of the aqueous extract of *Mostuea hirsuta* had no adverse effect on the weight growth of the test animals. Determination of biochemical toxicity markers showed that the extract had no negative effect on blood count, urea, creatinine, lipid profile or liver enzyme activity. On the contrary, administration of this extract leads to an increase in total and HDL cholesterol levels. The effect on the reproductive system showed that this extract, at a dose of 12 mg/kg, led to an increase in testosterone levels, an increase in sperm density and a reduction in prostate mass. At the doses tested, this extract also showed no pro-oxidant effects.

Contributions

The study was designed by BPT and DMY. RGN, SFSN, RDN and DMY performed experiments and analyzed data. The manuscript was written by DMY and ZIE. Funding to carry out the work reported in the manuscript was provided by DMY. Both the authors reviewed and approved the final manuscript.

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