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Original Research Article

Effects of the Hydromethanol Leaf Extract of *Craterispermum schweinfurthi* on Sperm Characteristics and Haematological Indices in Male Wistar Rats

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Abstract: Sexual and haematological abnormalities are on the increase, hence the need to identify possible ameliorative agents. The present study evaluates the effects of the hydromethanol leaf extract of C*raterispermum schweinfurthi* on sperm characteristics and haematological indices in male wistar rats. A total of 20 male wistar rats weighing between 100-250g were randomly divided into 4 groups of 5 rats each and treated as follows for 28days: Group A: Negative control: Group B, C and D received 250mg/kgbw, 500mg/kg bw and 750mg/kg bw of extract. On day 29, the rats were placed under chloroform anaesthesia and blood samples collected for the estimation of serum total white blood cell, red blood cell and platelet counts. Also, rats caudal epididymides were isolated for sperm characteristics estimation. Compared to Group A rats, significantly higher values of sperm count, active sperm, and population of normal sperm were observed amongst Groups B-D rats in a dose dependent manner ($p<0.05$). For sperm volume and viability, significant increases were observed amongst Group D rats only compared to Group A rats ($p<0.05$): Suggesting a possible greater potency of the extract at 750mg/kg body weight. Population of abnormal sperm, sluggish sperm and dead sperm were significantly decreased amongst Groups B-D rats compared to Group A rats ($p<0.05$). Similarly, a significant and dose dependent increase in total WBC, RBC and PLT counts following graded extract doses administration were observed amongst rats in B, C and D Groups compared to Group A rats: Demonstrating a possible blood boosting potentials of the extract in male wistar rats. The extract apparently exhibits potential beneficial effects on sperm characteristics and haematopoiesis in male wistar rats.
Keywords: Sexual dysfunction, haematological abnormalities, C*raterispermum schweinfurthi*.

1.0 INTRODUCTION

Infertility is characterized by the inability to conceive after about a year of unprotected sexual exposure [1]. Many factors are involved in conception which affects both men and women, however, approximately 40 to 50% of infertility cases are attributed to male infertility [2]. The initial steps in the assessment of male infertility involves the determination of semen quality: sperm concentration, morphology, and motility [3]. The ability of sperm to move towards an oocyte is called sperm motility [4]. Sperm motility is a crucial factor in the evaluation of semen quality, lack of adequate sperm motility is observed as one of the prominent causes of male subfertility or infertility [4]. The capacity of sperm to fertilize an ovum is not only dependent on motility but also on other functions like viability,-and sperm DNA fragmentation [5, 6].

Blood is a connective tissue that consists of plasma in which are suspended a number of formed elements. Blood cells exist at fairly constant levels, suggesting the existence of feedback regulatory mechanisms [7, 8]. Blood delivers necessary substances such as nutrients and oxygen to the cells, and transports metabolic waste products away from same cells [9]. The blood cells are mainly red blood cells (also called RBCs or erythrocytes), white blood cells (also called WBCs or leukocytes), and in mammals platelets (also called thrombocytes) [10]. The most abundant cells in vertebrate blood are red blood cells [11]. These contain hemoglobin, an iron-containing protein, which facilitates oxygen transport by reversibly binding to this respiratory gas thereby increasing its solubility in blood, White blood cells help to resist infections and parasites, platelets are important in the clotting of blood [11].

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There is an increasing dependence on native African (medicinal) plants for the prevention and treatment of various illnesses around the world [12, 13]. It has been estimated that, a large proportion of the population in developing countries depend mainly on medicinal plants of therapeutic value and the services of traditional medicine practitioners [12]. The scientific usefulness and importance of the use of medicinal plants is derived from its accessibility, affordability and minimal side effects amongst other peculiar benefits compared to orthodox medications [14]. Specific important compounds identified in most medicinal plants are effective in the treatment, management, and prevention of disease conditions [13]. These bio-active compounds are frequently characterised, extracted and used as raw materials in the production of many drugs [12, 13]. Widely distributed in tropical Africa, Madagascar, and the Seychelles [15, 16]. *Craterispermum schweinfurthi* species are shrubs or small trees with axillary or supra-axillary inflorescences, paired at the nodes and often condensed. The anecdotal applications of C*raterispermum schweinfurthi* in traditional medicine are numerous. For instance, in traditional folklore medicine the seed, leaves, and inner bark have been described to have beneficial effects in cases of stomach afflictions, ulcer, infertility, anemia, diabetes and fever [17]. Though, scientific studies on the anecdotal benefits of the leaves of *Craterispermum schweinfurthii* are relatively scanty in our environment.

Therefore, based on its many described anecdotal applications, the present study attempts an evaluation of the potential beneficial effects of hydromethanol leaf extract of C*raterispermum schweinfurthi* using male Wistar rats as models.

2.0 MATERIALS AND METHODS

2.1 Collection, Identification and Extraction of Plant Materials

Fresh leaves of *Craterispermum schweinfurthii* were obtained from the University of Port Harcourt Botanical Garden. Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port-Harcourt, Nigeria identified and authenticated the specimen and assigned a reference code; UPH/V/296. Voucher specimen was subsequently deposited in the University Herbarium for future reference. The plant leaves were gathered, and all extraneous materials carefully removed. The leaves were air dried at room temperature for a minimum of 7 days after which it was pulverized into powder and the weighed quantity of 670.6g dissolved using Soxhlet device in 390ml of water-methanol mixture (25:75% v/v BDH) for three days in a jar. It was filtered and concentrated using a rotary evaporator at 40°C and the yield was 73%. Obtained extract was preserved in airtight containers and stocked at room temperature prior administration.

2.2 Procurement and Handling of Experimental Animals

Wistar rats weighing between 100-250g were used for the study. Animals were acquired from the Department of Physiology Animal House, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria. Rats were placed in different compartments, one for each experimental group and cared for under standard laboratory conditions. Wood shavings and beddings were changed on a daily basis to prevent any infection due to unkept beddings. The animals were acclimatized for two weeks and subsequently grouped for the study.

2.3 Ethical Approval and Acute Toxicity Studies

Ethical approval was sought and obtained from the University of Port Harcourt Ethical Committee vide a communication referenced: UPH/CEREMAD/REC/MM82/024 and dated 23rd November 2021. The acute toxicity of the hydromethanol extract of C*raterispermum schweinfurthi* leaves was determined using Karber's method as modified by Aliu and Nwude, 1982 [18]. Lethal dose (LD_{50}) of the extract was found to be 3968mg/kg body weight. The study was conducted in accordance with the guidelines for the care and use of laboratory animals [19].

2.4 Research Design

A total of 20 male wistar rats weighing between 100-250g were used for the study. After two weeks of aclimatisation, the rats were randomly divided into 4 groups of 5 rats each designated Groups A to D and treated as follows for 28days:

Group A: Negative control; received extract vehicle only.

Group B: Low dose extract; received 250mg/kgbw of the leaf extract of C*raterispermum schweinfurthi.*

Group C: Medium dose extract; received 500mg/kg bw of the leaf extract of C*raterispermum schweinfurthi.*

Group D: High dose extract; received 750mg/kg bw of the leaf extract of C*raterispermum schweinfurthi.*

By the end of 28 days, the rats were placed under chloroform anaesthesia (cotton wool soaked in 3.5% chloroform) and blood samples collected via direct cardiac puncture and immediately transferred into plain sample tubes for the estimation of serum concentration of total white blood cell, red blood cell and platelet counts. Also, rats caudal epididymides containing sperm were excised for the determination of sperm characteristics. The samples were immediately used for the estimation of the different variables.

2.5 Determination of Sperm Characteristics and Haematological Indices

The method described by Nwafor *et al*., 2021 [20], was used to obtain sperm cells from the caudal epididymis. The testis was excised and caudal epididymis carefully isolated and placed in a petri dish containing 3 ml of sodium bicarbonate (NaHCO3) buffered Tyrodes' solution. Many (1 mm) incisions were made on it and sperm carefully drawn into a plastic pipette and transferred into 5 ml test tubes and energetically shaken for homogeneity and dispersal of sperm cells. Sperm cells were then evaluated to determine sperm motility, sperm count, sperm viability, active sperm, sluggish sperm, dead sperm amongst others following standard procedures [21].

Blood samples were procedurally analysed using an automated cell counter (Coulter Electronics, Bedfordshire, UK) with established standard calibrations, as specified in the available manufacturer's manual for analysis of human blood specifically total white blood cell (WBC) count, red blood cell (RBC) count and platelet count.

2.6 Statistical Analysis

Results are as presented in Tables 1 and 2 as Mean ± Standard Error of Means (SEM). Significant differences were determined using one-way ANOVA and LSD Post Hoc test. A p value of less than 0.05 was considered statistically significant.

3.0 RESULTS

Table 1: Values of sperm characteristics following leaf extract of C*raterispermum schweinfurthi* administration

	A	B	C	D
	Control	250mg/kg Extract	500mg/kg Extract	750mg/kg Extract
Volume (ul)	0.2±0.001	0.2±0.002	0.2±0.00	0.3±0.006[a]
Ph	8,00±0.007	8.00±0.004	8.00±0.003	8.00±0.006
Viability (%)	80.00±0.002	80.00±0.003	80.00±0.005	90.00±0.009[a]
Sperm Count	550.00±0.001	600.00±0.001[a]	700.00±0.002[a]	800.00±0.001[a]
Normal (%)	70.00±0.001	75.00±0.001[a]	80.00±0.001[a]	90.00±0.002[a]
Abnormal (%)	30.00±0.001	25.00±0.002[a]	20.00±0.000[a]	10.00±0.001[a]
Active (%)	70.00±0.007	75.00±0.006[a]	80.00±0.009[a]	90.00±0.006[a]
Sluggish (%)	10.00±0.000	10.00±0.003	5.00±0.002[a]	5.00±0.001[a]
Dead	20.00±0.002	15.00±0.001[a]	10.00±0.001[a]	5.00±0.000[a]
Appearance	Milky	Milky	Milky	Milky
Viscosity	Normal	Normal	Normal	Normal

Values are shown as Mean ± SEM; n=5; [a] Significant at P<0.05 compared to control.

Significantly higher values of sperm count, active sperm, and population of normal sperm were observed amongst Groups B-D rats in a dose dependent manner compared to Group A rats (p<0.05): Suggesting a possible beneficial effects of the extract. For sperm volume and viability, significant increases were observed amongst Group D rats only, administered 750mg/kg of the extract compared to Group A (p<0.05): Suggesting a possible greater potency of the extract at 750mg/kg body weight. Population of abnormal sperm, sluggish sperm and dead sperm were significantly decreased amongst B, C and D Groups compared to Group A (p<0.05). These findings indicate a likely beneficial effects of the extract on sperm parameters.

Table 2: Values of total WBC, RBC and PLT counts following leaf extract of C*raterispermum schweinfurthi* administration

Groups	WBC ($X10^9$/L)	RBC ($X10^9$/L)	PLT ($X10^9$/L)
Control	20.13±0.061	9,28±0.008	282.20±0.610
250mg/kg Extract	21.14±0.032[a]	9.51±0.007[a]	294.51±0.300[a]
500mg/kg Extract	24.00±0.034[a]	10.12±0.008[a]	298.29±0.183[a]
750mg/kg Extract	25.91±0.061[a]	10.37±0.022[a]	309.93±0.198[a]

Values are shown as Mean ± SEM; n=5; [a] Significant at P<0.05 compared with control.

There was a significant and dose dependent increase in total WBC, RBC and PLT counts following leaf extract of C*raterispermum schweinfurthi* administration at 250mg/kg body weight, 500mg/kg body weight and 750mg/kg body weight doses were observed amongst rats in B, C and D Groups compared to Group a rats. Demonstrating a possible blood boosting potentials of the extract in male wistar rats.

4.0 DISCUSSION

There are many ways by which sexual dysfunction and blood abnormalities can be managed amongst male and female subjects [22]. This include the use of mechanical maneuvers, natural products and orthodox medications amongst others. Many extracts of plants are traditionally applied in different cultures of the world to manage and restore sexual inadequacies and blood disorder [23, 24]. The use of herbs and medicinal

plants in the management, prevention and treatment of ailments and disease conditions is gaining prominence and relevance in the scientific community the world-over [17-26].

The marked improvement observed in sperm characteristics in this study suggest that the administration of the leaf extract of C*raterispermum schweinfurthi* in male rats successfully increased the indices of sperm. This positive effect may be due to the inhibition of lipid peroxidation which occurs due to the hydroxyl radical scavenging functions of the extract [27]. Apparently, leaf extract of C*raterispermum schweinfurthi* suppresses the adverse effects associated with reactive oxygen species (ROS) production [28, 27]. ROS functions by signaling series of cascades as essential intermediate messenger molecules in the process of apoptosis [29]. Therefore, inhibition of ROS production by the extract provided a positive effect on sperm characteristics achieved by anti-apoptotic effects of C*raterispermum schweinfurthi* [30]. These findings are suggestive of the presence of antioxidant agents and other phytochemicals like tannins, flavonoids, minerals, phytosterol, neophytadiene and vitamins: These constituents stimulate the proliferation of sperm cells. These findings are in agreement with Zade *et al.,* (2013) [31], and Guohua *et al*., (2009) [32], for *Moringa oleifera* and *Allium tuberosum*.

The effect of C*raterispermum schweinfurthi* on haematological parameters in rats was investigated in this study. Graded doses of the extract caused an increase in the total white blood cell count, red blood cell count and platelet count. The assessment of haematological indices remains valuable indicators to determine the functional capacity of the hematopoietic system of experimental animals [33]. The observed significant increases in assayed haematological parameters following the administration of extract suggest that the extract has a beneficial effect on haematopoiesis and possibly stimulate haemopoietic factors with direct influence on blood synthesis [34-24]. These findings are consistent with the reports of Saronee *et al*., (2023) [24], Mohammad *et al.,* (2019) [35], Anslem *et al.,* (2017) [36], and Switti *et al.,* (2011) [37], in which extracts of plants improved haematological indices in laboratory animals.

CONCLUSION

The hydromethanol leaf extract of C*raterispermum schweinfurthi* apparently exhibits potential beneficial effects on sperm characteristics and haematopoiesis in male wistar rats. These findings therefore, validates the use of the leaves of the plant in folklore medicine in our environment.

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