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Seminal Fluid Analyses of Wistar Rats Exposed to *Hippocratea africana* Root Bark Extract

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Abstract: ***Malaria chemotherapy and antimalarial herbs have been linked with infertility. This study was carried to examine the effect of Hippocratea africana root bark extract used traditionally in the South Eastern region of Nigeria for the treatment of malaria on seminal fluid. Twenty-four (24) mature male albino Wistar rats weighing between 110-150g used for the study were randomly divided into four (4) groups with six (6) rats in each group. Group 1 served as the control and was administered 1ml of distilled water, while Groups II, III and IV were the test groups and were orally administered 100, 200 and 300mg/kg body weight of H. africana root bark extract respectively for fourteen (14) days using syringe attached to a cannula. The result showed a significant (P < 0.05) increase in groups III and IV for Total Cell Concentration when compared with the control. Motile sperm showed a dose dependent increase in all the test groups when compared with the control. Beating Cilia Frequency showed increase in all test groups, however the increase was only significant (P <0.05) in group III and IV compared with the control. Percentage progressivity showed a dose dependent increase when compared with the control. Indices of sperm cell abnormalities; head anomality, body anomality and total anomality showed significant (P < 0.05) decreases in all the test groups compared with the control. Red Blood Cell and White Blood Cell recorded decreases in test groups when compared with control group animals. The overall result showed an improvement in seminal fluid parameters which may be associated with the phytochemicals present in the herb. The result suggest that the herb is safe for use for its antiplasmodial property***

Keywords: *Hippocratea africana,* Seminal Fluid, Chemotherapy, Infertility, Malaria

1. Introduction

Malaria ranks among the major health and development challenges of the world and despite great international efforts, malaria still inflicts an enormous toll on human lives, especially in Africa. It is regarded as the single most destructive and dangerous infectious agents in the developing countries of the world [1]. High cost of antimalarial drugs especially the WHO recommended artemisinin combination therapies (ACTs), unavailability, development of resistance and ignorance of rural inhabitants have militated against the use of chemotherapies in malaria treatment [2][3]. This has led to increasing research into medicinal plants and their utilization in the treatment of malaria. Medicinal plants have been used in the treatment of ailments for centuries and have played significant roles in the general provision of good health globally [4].

Malaria chemotherapies and anti-malarial herbal preparations have been linked with several toxicities including reproductive dysfunction and infertility. [5] reported hepatotoxicity of artesunate, while anti-fertility effects of dihydroartemisinin-piperaquin [6], artemether-lumefantrine [7], quinine, artemether and fansidar [8] have been reported. Furthermore, reduced concentrations of sex hormones have been reported following the administration of antimalarial herbs; *Cylicodisus gabunensis, Nauclea latifolia* and *Araliopsis soyauxil* to male albino Wistar rats [9].

Though current trends in malaria treatment is towards the development of more effective chemotherapies [10] and vaccines [11]. The present interest should be on the discovery and safety of antimalarial herbs due to their readily availability and cost advantage in rural communities.

The anti-malarial property of *Hippocratea africana* has been reported in literature [12]. Medicinal and biochemical effects of the plant in experimental animals have been documented; antidiarrheal and antiulcer activities [13], hepatoprotective effect [14], analgesic and anti-inflammatory effects [15], effects on some clinical indices [3], renal status [16] and effects on lipid profile [17].

Despite its promising antimalarial potential, there is paucity of information on the reproductive effect of *H. africana*. This led to the present study which attempts to evaluate the effect of root bark extract of *H. africana* on seminal fluid in male albino Wistar rats.

2. Materials and Methods

2.1 Plant Sample

Fresh root bark of *Hippocratea africana* was obtained from Afaha Etok forest in Ibesikpo-Asutan Local Government Area of Akwa Ibom State. The plant was identified and authenticated by a taxonomist in the Department of Botany, University of Uyo, Uyo.

The fresh roots of *Hippocratea africana* were washed gently with tap water to remove sand, scrapped to remove the bark, cut into pieces and air-dried for two weeks. The air-dried sample was pulverized using manual grinder. 2kg of the pulverized sample was macerated in 80% ethanol (sigma Aldrich) and allowed to stand for 72 hours for the solvent to solubilize the active ingredients. The clear orange filtrate obtained was carefully siphoned off the residue using a tube and concentrated in a water bath at 45^{o}C to obtain a crude extract.

2.2 Experimental Animal

Twenty-four (24) matured male albino Wistar rats weighing between 110 – 150 g were obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo. The animals were selected randomly into four groups of six rats per group. They were housed in a ventilated room in standard cages under standard laboratory conditions. The animals were fed grower rat chow and allowed water *ad libitum*.

Group I animals served as the control group and were administered 1ml of distilled water. Group II, III, and IV were the test groups and received 100, 200 and 300 mg/kg body weight of *H. africana* root bark extract respectively orally using a cannula attached to syringe for 14 days.

At the end of the treatment period, the rats were fasted overnight but still allowed access to water *ad libitum*. They were anaesthetized using chloroform and dissected to harvest the testes. The cauda epididymis from each side of the testes was cut into pieces and suspended in 1 ml buffered saline to allow the spermatozoa swim up.

2.3 Seminal Fluid Analysis

Semen analysis was carried out using Computer-Assisted Semen Analysis (CASA) in accordance with the Breanna Tilley (2007) and WHO (1999) criteria. Freshly collected semen samples were diluted appropriately in mixed agglutination reaction (MAR) test buffer (9 mmol/L KH_2PO_4, 28 mmol/L Na_2HPO_4, 11 mmol/L NaCl), and the diluted sample were pipette into a Makler Chamber, which was placed on a heated microscope stage (37°C). Video recordings were made from four different fields of the chamber using a 20x magnification objective on the microscope. The CASA analysis was based on capturing sequences of 64frames per field and counting a minimum of 100 spermatozoa. The following measurements were obtained; Total cells detected (10^6/ml), Total cell Concentration (TCC) (10^6/ml). Motile sperm (MS) (%), Beating Cilia Frequency (BCF) in Hz, Head Anomality Rate (%), Body Anomality Rate (%), Total Anomality Rate (%), motile sperm rate (%), Red Blood Cell count ($x10^6$/µL), White Blood Cell count ($x10^3$/µL) and Progressivity (%).

2.4 Statistical Analysis

Analysis of variance (ANOVA) and Least Significance Difference post hoc multiple comparisons of the data were evaluated using Windows SPSS Version 20.0. The results are expressed as mean ± standard deviation. Values at $P < 0.05$ were considered statistically significant.

3. Results

The results of the study on the effect of the *Hippocratea africana* ethanolic root bark extract on seminal fluid analysis on male albino Wistar rats is presented in Table 1. The concentration of total cell per ml of the seminal fluid was observed to increase significantly in Groups III and IV. Percentage of motile sperm and beating cilia frequency were increased and particularly significant in Group IV when compared with the control group. Statistically significant decrease in the test groups when compared with the control group was observed for the following indices; beating cilia frequency, head, body and total anomality rate, red blood cell as well as white blood cells.

Table 1: Seminal Fluid Analyses of male rats exposed to *Hippocratea africana* root bark extract

Parameters	Group 1	Group 2	Group 3	Group 4
Total Cell Concentration (TCC) (10^6/ml)	59.00 ± 6.44	58.67 ± 9.77	68.33 ± 2.87[a]	75.17 ± 3.60[a]
Motile Sperm (MS) (%)	59.67 ± 7.22	62.83 ± 3.97	63.67 ± 3.20	72.83 ± 3.43[a]
Beating Cilia Frequency (HZ)	4.67 ± 1.03	6.33 ± 1.63	8.00 ± 1.89[a]	11.17 ± 1.32[a]
Head Anomality Rate (%)	22.40 ± 2.57	10.00 ± 1.41[a]	9.17 ± 1.16[a]	6.00 ± 1.89[a]
Body Anomality Rate (%)	26.00 ± 3.08	12.00 ± 1.41[a]	9.50 ± 1.64[a]	6.50 ± 1.37[a]
Total Anomality Rate (%)	46.73 ± 3.98	24.00 ± 2.89[a]	18.67 ± 1.75[a]	12.50 ± 2.50[a]
Red Blood Cell ($x10^6$/µL)	8.20 ± 2.56	5.40 ± 1.95[a]	3.33 ± 0.51[a]	2.17 ± 0.41[a]
White Blood Cell ($x10^3$/µL)	3.40 ± 1.14	2.20 ± 0.45[a]	2.00 ± 0.63[a]	1.00 ± 0.00[a]
Progressivity (%)	52.67 ± 4.27	60.83 ± 2.31[a]	65.00 ± 3.34[a]	68.83 ± 2.04[a]

Data presented as Mean ± Standard Deviation (SD)
a = significantly different when compared to Group 1 (control) at $p < 0.05$.

4. Discussion

Malaria remains the scourge of the subtropical region of the world even with the increasing efforts in the development of more potent antimalarial agents as a result of the challenge emanating from the resistant strains of malaria parasite. These drugs are unavailable and unaffordable to the low income earners and peasants who are mostly affected by the disease. This has necessitated a fall back to herbal remedy which is a global trend, not only in malaria therapy. The evaluation of anti-malarial herbal agents for possible anti-fertility actions becomes important.

Semen analysis remains the corner stone of male infertility investigation [18]. The assay is not a direct measure of fertility although the results may correlate with fertility [19]. This study reports the effect of administration of *Hippocratea africana* on seminal fluid of mature male rats. The result showed an increase percentage sperm motality in a dose dependent manner. This suggests that the extract did not permeate the blood-testis barrier and the inner part of the seminiferous tubules thus did not create a different micro-environment in the walls different from its outer part. It may also suggest that the bioactive components of the extract did not affect the sperm quality. However, [9] [20] and [21] reported decrease in sperm motility following administration of *A. soyauxii*, arthemether and dihydroartemisinin respectively.

There was increase in Total Cell Concentration of the test groups compared with the control group in a dose dependent manner too, suggesting an improvement in the epididymal sperm reserve by the herb, which may have led to larger spermatocyte production in a dose relate manner. This corroborates with a study on animal subjects that sesame can improve epididymal sperm reserve and increase Spermatocyte size [22]. A contrary report indicates that exposure to arthemether causes impairment to reproductive activity exhibited by reduction in sperm count [20]. It is reported that sperm production, development and maturation are processes that are vulnerable to interferences in the internal environment of the reproductive organs [23].

The sperm cells are propelled by beating of cilia and flagella. In males, immobility of sperm can lead to infertility, although conception remains possible through the use of in-vitro fertilization as they have been reported cases where sperms were able to move [24]. In this study, the rate of beating cilia frequency was observed to increase as the dose increases. This suggests that the bioactive components inherent in this herb implicated a positive effect on the seminal fluid of the experimental animals and thus improves the movement of the sperm cell.

There was decreased percentage of abnormal sperm (Head anomality rate, Total anomality rate and Body anomality rate) in a dose dependent manner. This suggests that the herb will not have a negative effect on conception. In contrast, treatment with antimalarial drugs such as chloroquine and halofantrine increased the abnormal sperms [25]. The decrease in both WBC and RBC when compared with the control recorded in this study further adds credence to the herb. The concomitant reduction in RBC, WBC, Head Anomality, Total Anomality and Body Anomality may be due to the quantity of flavonoids, alkaloids and tannins present in the herb. This compouds have been reported to possses strong ant-oxidant capacity [26] and therefore could inhibit haemolysis of red blood cells [27].

Progressivity shows how sperm swim from one place to another, not just twitching or going around in circles. There was a dose dependent increase in percentage progressivity. A decreased percentage progressivity following both short and long term administration of artesunate is however reported by [28]. The significant reduction observed in the progressive sperm motility was suggested to be due to free radical generating capacity of the drug. Free radicals have been implicated in male infertility by decreasing sperm motility [29]. *H. africana* have been reported to be rich in flavonoids and cardiac glycosides which are good indices of free radical scavenging [17].

The improvement of sperm parameters may be due to the anti-oxidant properties of *Hippocratea africana.* Experimental investigation is also needed on its effect on the female reproductive indices to further confirm its safety as an antiplasmodial herb.

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