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Original Research Article

Peperomia pellucida (L.) Kunth Piperaceae has the Expected Reproductive Toxicity of Antimalarial Drugs?

Gideon O. Alade[1*], Blessing O. Ariri[1], Tolulope O. Alade[1], Kola' K. Ajibesin[1]

[1]Department of Pharmacognosy & Herbal Medicine, Niger Delta University, Wilberforce Island, Nigeria

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Abstract: In folklore medicine, *Peperomia pellucida* (L.) Kunth (Piperaceae), has demonstrated a good activity in malaria treatment and this has also been proven pharmacologically. Most antimalarial agents have demonstrated negative effects on spermatogenesis. For effective development and formulation of this plant resource into a dosage form that will be well tolerated by humans, the leaf extract of the plant was therefore evaluated for reproductive toxicity associated with antimalarial drugs in male Wistar rats at 250, 500 and 1000mgkg^{-1} doses for it effect on the sperm characteristics as well as the histology of the testes. Rats, divided into three groups (n=6) administered with 250, 500 and 1000mgkg^{-1} doses of the extract and a fourth one which was the control (administered with distilled water), were used for a period of twenty days following standard procedure. The result indicated, although the normal sperm morphology was not significantly affected, a significant reduction of sperm count and active motility by 93 and 71 ($p<0.05$) observed at 1000 mg/kg and interstitial necrosis of the testes at 500 which became severe at 1000 mg/kg. The extract was however safe at 250 mg/kg. The usual spermatotoxicty of antimalarials was therefore indicated at 500 and 1000mg/kg. The study has proven that *P. pellucida* leaf educed the usual antimalarial spermatotoxicity.

Keywords: fever, sperm, toxicity, antimalarials, testes.

INTRODUCTION

Peperomia pellucida (L.) Kunth is a member of the family, Piperaceae and very useful in the treating fever in folkloric medicine among several uses [1, 2]. Laboratory investigation on the antipyretic activity of the plant has been successfully performed to justify its folkloric use in fever [3]. One of the leading causes of mortality and morbidity in underdeveloped world is malaria fever. It poses a very serious problem in public health [4, 5]. Issue of resistance to orthodox treatment is a cause of treatment failure [6] and this has necessitated the development of alternative from medicinal plants [7-9]. One of the serious concerns of antimalarial drugs has been linked to reproductive dysfunction in male. For instance, chloroquine has been shown to elicit reduction of sperm motility [10]. Halofantrine has been shown to exhibit adverse sperm morphology [11], pyrimethamine caused was reported to cause arrest of spermatogenesis [12] while a study showed that artemether adversely altered sperm motility, viability, and count [13-14]. The current study was therefore aimed to deduce the value/effect of *P. pellucida* leaf on sperm characteristics.

Plant collection and extraction

Peperomia pellucida. sample was harvested from plants growing in the wild on the campus of Niger Delta University, Bayelsa State, Nigeria at N 039 57' 09.2'' E 0056 06' 50.8'' on the 10th of February, 2022. Proper plant identification and authentication were carried out at the Herbarium that is domiciled in the Department of Pharmacognosy & Herbal Medicine of the same University. An herbarium voucher specimen (NDUP237) was deposited followed by bulk plant collection for the purpose of extraction. Coarsely powdered dried leaves were prepared and 1050g of this was subjected to cold extraction using ethanol (50%) for 3 days. The filtered extract was subjected to concentration *in vacuo* at a temperature of 30°C to afford 8.7% yield. The extract for oral administration was prepared in distilled water.

Animals

Procurement of the thirty male animals employed for the current study which were 140-160 kg Wistar rats was made at the Animal house of the Department of Pharmacology, Faculty of Pharmacy,

***Corresponding Author:** Gideon O. Alade
Department of Pharmacognosy & Herbal Medicine, Niger Delta University, Wilberforce Island, Nigeria

Niger Delta University, Bayelsa State, Nigeria. The animals were kept under standard environmental conditions of equal daily hours of light and darkness (1:1) and were freely permitted to have access to water and standard rat diet. Approval to initiate and proceed with the work was secured from the Ethical committee in the same department while adhering to the guideline of the National Institute of Health on animal care [15].

Experimental Design

The animals were picked at random and grouped into four comprising six each. The first; Group A (Control) received distilled water only while Groups B - D (Test) received the leaf extract of *P. pellucida* at graded doses (250, 500 and 1000 mg/kg *p.o*) for a span of twenty days. Following an all-night long fasting, on the 20th day of the oral administration of the extract, animals were sacrificed by using ketamine i.p (20 mg/kg). The epididymis and testicles of each rat were carefully excised and used to evaluate sperm count histology, respectively.

Assessment of sperm parameters

Using the epididymis, sperm concentration (x 10^6/cells), sperm motility (%) and morphology (%) were evaluated using standard method with the aid of microscope slide and improved Neubauer Haemocytometer [16] by visualising under microscope (x 40 objective).

Histological examination of testes

A 10%$^{v}/_{v}$ solution of Formalin was used for fixing the obtained testes and routine processing was performed by embedding them in paraffin wax. A 5 µm-thick section was torn and stained (haematoxylin and eosin) and thereafter viewed under a microscope at x 100 and 400. This was photographed with a digital camera borne on the microscope [17].

Data analysis

The values that were obtained were subjected to statistics by using GraphPad Prism version 6.0.0 for Windows (GraphPad Software, www.graphpad.com). Analysis was performed with one-way analysis of variance and Tukey's multiple comparison test. They were reported as mean ± standard error of the mean, and a value of $P < 0.05$ was taken to be significant.

RESULTS AND DISCUSSION

Sperm concentration was adversely affected in a dose dependent manner; a reduction of 11.9, 79.7 and 93.2% was observed at 250, 500 and 1000 mgkg^{-1}, respectively. Also, the percentage of active motile sperms showed 3.6, 50.7 and 70.7% at 250, 500 and 1000 mg/kg. These were all significant at 250 and 500 mg/kg ($p<0.05$). Normal morphology was also adversely with approximately 23, 19 and 19% reduction at 250 and 500 mg/kg (Table 1).

Table 1: Outcome of *P. pellucida* leaf extract on sperm characteristics

Parameters	**Distilled water**	**250 mgkg^{-1}**	**500 mgkg^{-1}**	**1000 mgkg^{-1}**
%				
Active motile	14.0 ± 1.10	13.5 ± 1.58 (3.6)	7.0 ± 0.92 (50.0*)	4.10 ± 1.21 (70.7*)
Sluggish motile)	65.9 ± 2..66	69.0 ± 3.96 (-4.4)	83.0 ± 3.50 (-20.6*)	86.1 ± 1.12 (-23.4*)
Non motile	20.1 ± 1.32	17.5 ±1.10 (12.9)	10.60 ± 1.10 (47.3*)	9.8 ± 0.10 (51.2*)
Normal morphology	75.0 ± 2.26	58.0 ± 3.86 (22.6*)	46.2 ± 2.70 (18.8)	38.0 ± 3.21 (18.8)
(x 10^6 cells/mL)				
Sperm count	5.9 ± 0.05	5.2 ± 0.33 (11.9)	1.20 ± 0.05 (79.7*)	0.4 ±0.01 (93.2*)

Values = mean ± standard error of the mean, where N = 6, *$P < 0.05$

Key: ( ) – percentage reduction, $p< 0.05$

An estimate of about 1 in every 50 male with infertility problem will exhibit 'suboptimal' sperm characteristics. A combination of two or more of these parameters (reduced sperm concentration, sluggish motility and abnormal morphology) is a major cause [18]. In essence; semen deficiencies are an important factor in male infertility [19]. Factors such as reduced sperm count referred to as 'oligospermia', negatively altered motility referred to as 'asthenospermia' as well as abnormal morphology known as 'teratospermia' are significant among others [20]. About nine-tenth of male with cases of infertility challenges arises from low sperm count and as well as negatively altered motility and morphology [21].

The results of the histology of the testes reveal that the control group and 250mg/kg body weight group showed rich epithelium with seminiferous tubules, spermatogonia, Sertoli and Leydig cells congruous with the histology of the testis. This is evidenced in the insignificant differences exhibited by the sperm parameters. However, those administered with the extract at 500mg/kg body weight showed slight areas of necrosis while those with 1000mg/kg showed severe areas of interstitial necrosis and exudation consonant with atypia (Plate 1). Necrosis of the testes can sometimes be a complication of 'Epididymo-orchitis' [22, 23]. This shows that oral administration of the extract higher concentrations may be injurious to the testis. This has actually shown that apart from orthodox antimalarial drugs, plant drugs having antimalarial property should also be taken with caution.

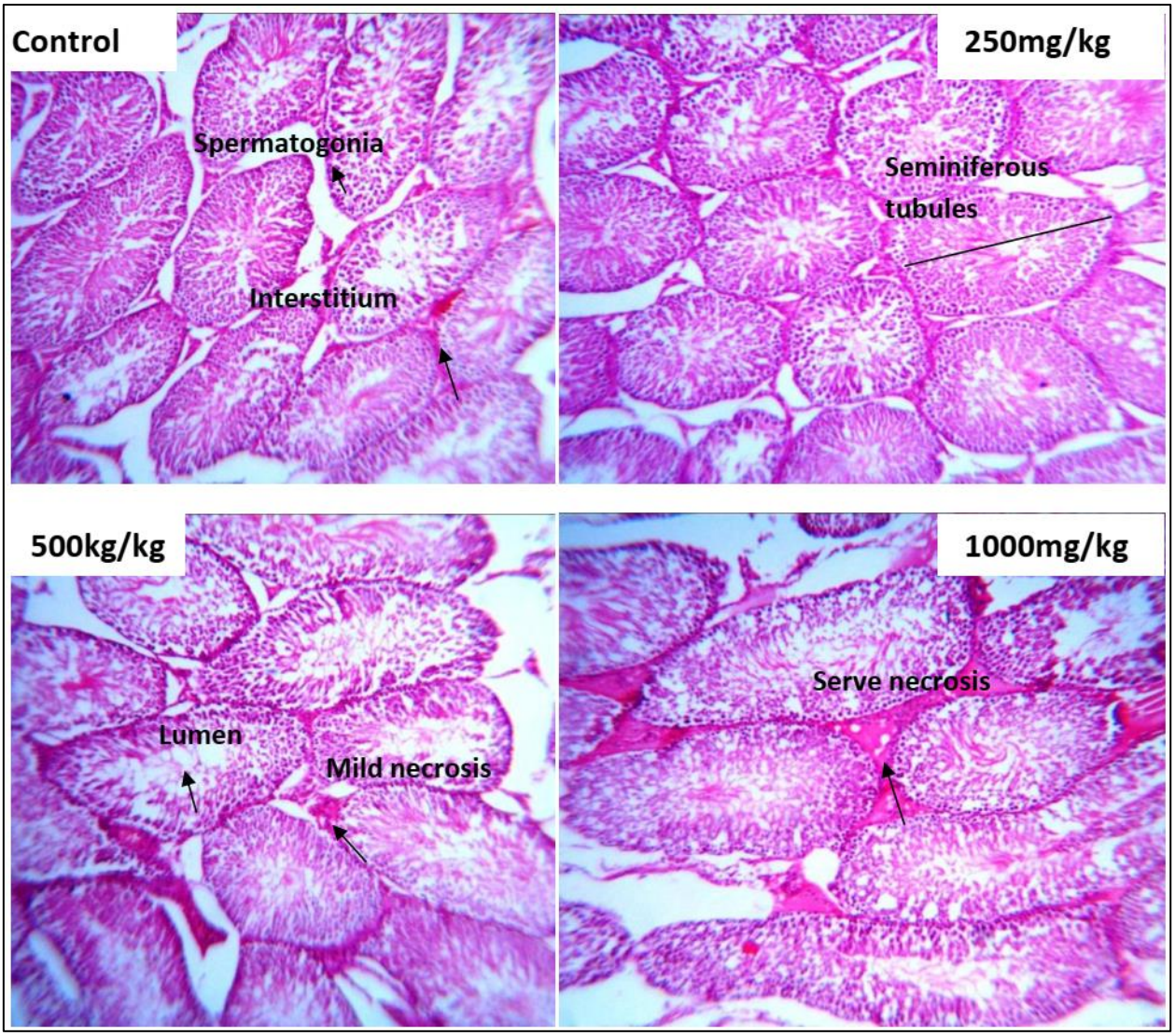

Plate 1: Photomicrograph of transverse section of the testis tissue stained with Haematoxylin and Eosin X400 Magnification of rats administered with *P. pellucida* leaf extract

CONCLUSION

The leaf of *P. pellucida* is not shorn of the reproductive toxicity expected of antimalarial drug. It elicited about 93% reduction of sperm concentration and approximately 70% reduction in motility at 1000mg/kg dose. Hence, this drug should therefore be taken with caution as higher doses of the extract may be injurious to the testis.

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