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Biological Effects of an Aqueous Extract of *Phthirusa pyrifolia* (Kunth) Eichler Leaves

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Research Article

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ABSTRACT

Phthirusa pyrifolia (Kunth) Eichler is claimed in medicinal practice in Brazil, to be useful in the treatment of respiratory diseases and liver injury, aphrodisiac effect, and also for to its antimicrobial properties, and is also used in Peru to treat fractures and sprains. The aim of this research was to evaluate the effects of an aqueous extract from *P. pyrifolia* leaves in male rats submitted to oral administration. Animals in experimental protocol were submitted to natural oral ingestion of *P. pyrifolia* leaves aqueous extract over to 12 days. Total blood aliquots were collected for hormonal and biochemical-hematological analysis. After the treatment period, the rats were subcutaneously anesthetized, euthanized and afterwards orchidectomized. The biochemical parameters revealed a significant decrease in aspartate-aminotransferase, alanine-aminotransferase and alkaline-phosphatase enzyme levels by about 40%, 27% and 52%, respectively. However, the extract does not cause liver injury and no impairment of renal function as well no affect any hematological parameters, but the histological analysis revealed a somatic action on the testes. The testosterone hormone levels of treated rats were drastically affected and showed a higher decrease ($p<0.05$) of about 82.31% than compared with the control, 46.0 (± 8.1) ng/dL and 260.0 (±4.1) ng/dL, respectively. We believe that the aqueous extract may be responsible to promote a decrease in the libido and reproduction in male rats, and induces hepatic-protective effects. Our results contribute towards validation of the traditional use of the plant.

INTRODUCTION

Ethnopharmacological studies have suggested that a large number of medicinal plants have provided a source for novel biological assays which may affect even reproduction in rats and various mammals through the presence of secondary metabolites [1]. Plant extracts have been shown to have biological activity among the following: antihypertensive, anti-inflammatory, hepatoprotective, anti-tumor, antioxidant, anti-diarrheic, anti-spermatogenic, stimulate spermatogenesis, and increase or decrease serum testosterone levels [2]. Several animal studies with rats showed that plant extracts may have contraceptive or anti-fertility effects following long-term treatment at low or high doses. Generally, these researchers have observed changes in morphological-morphometric testicular parameters and levels of testosterone hormone [3].

Phthirusa pyrifolia (Kunth) Eichler, hemi-parasitic plant that belongs to the Loranthaceae family (mistletoe), is popularly

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known in Brazil as "*Erva de Passarinho*". The leaves have been used, in Brazil and Peru, as a popular medicine against respiratory diseases, liver damage and also for their antimicrobial properties. Alarcón described the use of this plant to treat fractures and sprains. Recently, our research group isolated and purified a lectin from the leaves of *P. pyrifolia* aqueous extract that showed effective antimicrobial activities [4,5].

With regard to the reported properties of plant extracts on fertility and libido, the current investigation proposed to evaluate the effects of *P. pyrifolia* aqueous extract on the testosterone hormonal levels of male Wistar rats given an oral administration.

MATERIALS AND METHODS

Aqueous Extract (AE)

P. pyrifolia leaves were collected at the Federal University of Pernambuco campus, Brazil, from the top of a *Bauhinia monandra* host plant, and the Botanical specimen was identified at the DÁRDANO DE ANDRADE LIMA Herbarium of the *Instituto Agronômico de Pernambuco* (IPA), where a Voucher was deposited [Botanical identification N°18/2006; Identification code IPA-80.066; *P. pyrifolia* (Kunth) Eichler]. Dried leaves were powdered and submitted to extraction with distilled water [10% (w/v)] under orbital agitation at 4°C for 2 h. Afterwards, the mixture was filtered through gauze and centrifuged at 11,180 g at 25°C for 15 min and the supernatant was identified as aqueous extract (AE). For animals' implications, the standardization of the AE was realized with protein content according to Lowry et al. [6].

Experimental Treatment

All experimental procedures involving the animals were approved by the Committee for Ethics in Animal Experimentation of the Federal University of Pernambuco (No. 23076.012671/2006-09). Male Wistar rats (n=30), aged 23 weeks and weighing 378.5 ± 5 g, were housed under controlled environmental conditions (photoperiod of 12 h light/dark, temperature of 23 ± 1°C). The animals were divided into two groups: control (n = 8) and test (n = 22) and the groups were fed standard food *ad libitum*. The control group had free access to water while the test group had free access to the AE (67 mg of protein/kg body weight) by natural ingestion over 12-days. Each three days of intervals, whole blood aliquots (500 µL) were collected for analysis.

Histomorphologic Study

After the treatment period, the rats were subcutaneously anesthetized and euthanized through left ventricular perfusion with a fixing solution of 4% glutaraldehyde in 0.05 M phosphate buffer, pH 7.4, and afterwards orchidectomized. The testes were clearly dissected out, weighed, fixed in buffered formalin. Fixed tissue was dehydrated, cleared, infiltrated, and paraffin embedded by an automatic tissue processor and subsequently cut into thin slices (4–6 µm in thickness) with a microtome (Leica, model RM-2245). For the histomorphologic study the sections were stained with hematoxylin–eosin and examined under a light microscope. Morphological analysis included observation of diameters of seminiferous tubule (DST), height of seminiferous epithelium (HSE) and Gonadosomatic indices (GSI). These data were obtained from the 30 cross-sections measurement of seminiferous tubules for each animal [Cross-sectional area (A_c) of the seminiferous tubules was determined from the formula:

$A_c = \pi D^2/4$ (where π is equivalent to 3.14 and D is the mean diameter of the seminiferous tubules]. Image capture system examined them by the image processing program Scion Image at a magnification of 360×.

Biochemical, Hematological and Hormonal Levels

Blood samples were obtained from each three days interval by caudal puncture and placed into coagulant and anticoagulant micro-blood tubes. The whole blood was used to evaluate hematological parameters, and the clotted blood was centrifuged at 3,000 xg, for 15 min, at 25°C. The serum was used to evaluate biochemical parameters by an automated random-access clinical chemistry analyzer (Abbott-Aerosets). The hormonal assay kits for testosterone was analyzed by the chemiluminescent method (Immulite-2000) using the Access imunoassay system testosterone calibrator calibrated for the animal blood.

Statistical Analysis

Parameters were analyzed using the Mann-Whitney test with the SIGMASTAT program for repeated measures with parametric Student's t-test. The level of statistical significance was set at $p<0.05$.

RESULTS

Experimental

Throughout the experimental period, the daily intake of AE dose in the test group was 67.0 mg of protein/kg body weight through natural ingestion (30 mL/rat/day), representing about 603.0 mg of protein/rat/day. After the twelve days of this course of feeding, the average body weight of the test group fed with AE (369.0 ± 4 g), was significantly less than the control group (409.0 ± 6 g).

Histopathological Studies

The effects of the AE on the body weight, testicular weight and gonadosomatic indices (GSI) of the rats are shown in **(Figure 1A.)**

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The height of the seminiferous epithelium (HSE) was thick and usually comprised five to six layers of closely packed cells in the test group (HSE = 66.0 ± 1 µm) and showed clear signs of spermatogenesis when compared with the control group (HSE = 71.0 ± 1 µm) **(Figure 1B)**. The diameter (DST) and the cross-sectional area (A_C) of the seminiferous tubules were significantly lower ($p<0.05$) in the test group (DST = 254.0 ± 2 µm; 50.0 ± 0.6 x 10^3 µm^2), **Figure 1C**, than in the control group (DST = 265.0 ± 2 µm; 56.0 ± 0.7 x 10^3 µm^2), **(Figure 1D).**

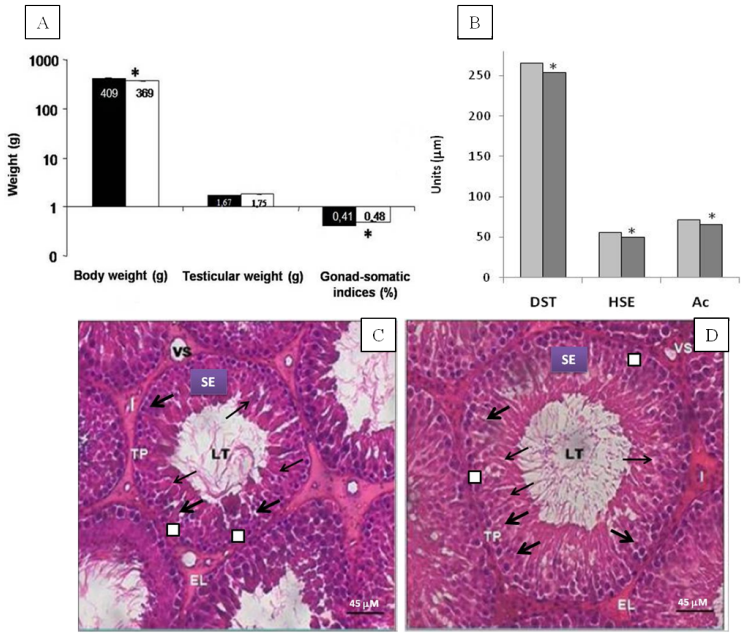

Figure 1.Histomorphological analysis. (A) Effect of aqueous extract on body and testis weights of rats, and on gonadosomatic indices (GSI). (B) Histogram illustrating the diameter of seminiferous tubules, heights of the epithelium and cross-sectional area of the seminiferous tubules. (DST) diameter of seminiferous tubule; (HSE) height of the seminiferous epithelium and (AC) cross-sectional area of the seminiferous tubules. *Statistical significance (Student t-test) $p< 0.05$. Histochemical analyses of rat testicular compartments in Test group (C) and Control group (D). I- interstice; EL- lymph capsule; VS- blood vessel; TP- tunica; SE- seminiferous epithelium and LT- lumen. The LT contains sperm tails. Large arrows, primary spermatocytes; double small arrows, spermatids. The seminiferous epithelium (SE) comprises many layers of sex cells. The lumen is distinct. Numerous spermatogonia with small dark nuclei (squares) are clearly visible at the periphery of each tubule H and E. Bar ___45 µm. Control group (■) and Test group (□). *Statistical significance analyzed by Mann-Whitney test, $p< 0.05$.

Biochemical, Hematological and Hormonal Serum Analysis

The biochemical, hematological and testosterone hormone levels were presented in **Table 1**. We observed that AST, ALT and ALP enzyme levels were reduced by about 40%, 27% and 52%, respectively. The analysis of serum testosterone in test group showed a significant decrease ($p<0.05$) of about 82.31% when compared with the control group, 46.0 ± 4.0 ng/dL and 254.0 ± 8.0 ng/dL, respectively. All the results for hematological parameters did not show any significantly differences.

Table 1. Effect of the Aqueous Extract of P. pyrifolia Leaves on Biochemical, Hematological and Hormonal Constituents.

Biochemical Parameters	Control[a]	AE[a,b]
Aspartate aminotransferase (IU L^{-1})	122.6 ± 7.70	77.3 ± 10.10[b]
Alanine aminotransferase (IU L^{-1})	38.8 ± 6.10	28.3 ± 2.10[b]
Alkaline phosphatase (IU L^{-1})	228.0 ± 4.28	108.0 ± 9.20[b]
Total bilirubin (mg dL^{-1})	0.2 ± 0.01	0.2 ± 0.01
Direct bilirubin (mg dL^{-1})	0.1 ± 0.01	0.1 ± 0.01
Urea (mg mL^{-1})	36.9 ± 2.00	34.3 ± 1.80
Creatinine (mg mL^{-1})	0.6 ± 0.08	0.6 ± 0.03
Sodium (mEq L^{-1})	144.1 ± 2.60	145.1 ± 1.90
Potassium (mEq L^{-1})	6.75 ± 0.22	5.86 ± 0.90
Chloride (mEq L^{-1})	103.7 ± 1.10	104.5 ± 1.10
Hematological parameters	**Control[a]**	**AE[a,b]**
Red blood corpuscles (RBC) 10^6 uL^{-1}	7.37 ± 0.1	7.64 ± 0.3
Hemoglobin (HGB) g dL^{-1}	14.20 ± 0.2	14.40 ± 0.5
Hematocrit (HCT)%	42.60 ± 0.5	43.36 ± 2.8
Mean cell volume (MCV) fL	57.17 ± 0.8	56.63 ± 1.6
Mean corpuscular hemoglobin (MCH) pg	19.04 ± 0.1	18.86 ± 0.3

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Mean corpuscular hemoglobin concentration (MCHC) g dL^{-1}	33.33 ± 0.3	33.25 ± 1.1
Red cell distribution width (RDW)%	13.92 ± 0.1	14.20 ± 0.1
Count of Platelet (PLT) 10^3 μL^{-1}	906.50 ± 1.2	910.00 ± 0.1
Mean platelet volume (MPV) fL	5.50 ± 0.1	5.50 ± 0.1
White blood corpuscles (WBC) 10^3 μL^{-1}	5.22 ± 0.5	4.44 ± 0.6
Neutrophils (NE) %	17 ± 2.0	19 ± 3.0
Lymphocytes (LY) %	71 ± 2.0	70 ± 2.0
Monocytes (MO) %	7 ± 1.0	5 ± 1.0
Eosinophils (EO) %	1 ± 0.1	1 ± 0.1
Basophils (BA) %	8 ± 2.0	6 ± 1.0
Period of oral administration	**Hormonal Parameters Testosterone levels ng/dL**	
	Control[a]	**AE[a,b]**
Initial experiment	256.0 ± 3.10	257.0 ± 2.10
Third day	256.0 ± 1.10	254.0 ± 2.10
Sixth day	254.0 ± 1.10	128.0 ± 6.10[b]
Ninth day	256.0 ± 2.10	59.0 ± 5.10[b]
Twelfth day	260.0 ± 4.10	46.0 ± 8.10[b]

[a] Values are represented as mean ± SD (n=6).[b] $p<0.05$ compared with control group; Student t-test was used to assess the statistical significance.

DISCUSSION

P. pyrifolia leaf AE did not cause the death of any animal during the experimental treatment of the present work, indicating that the extract has no lethal effect. In addition, behavioral changes, rejection of extract ingestion and decreases of thirst and hunger mechanisms (data not shown), were also not detected.

The study of the serum biochemical components from hepatic metabolism provides valuable parameters for the evaluation of hepatic function as far as it is concerned with diseases, directly or indirectly, related to the liver. The AE reduced the levels of all serum enzymes tested. When high plasmatic levels of urea and creatinine are found, they provide evidence of renal overload, acute renal failure, or increase in protein catabolism [7].

Concerning the hormone testosterone, we observed a steady decline of serum testosterone in the test group compared to the controls after the fourth day. The decrease in serum testosterone may have resulted either from the direct effect of the extract on Leydig cells or indirectly as a consequence of decreased LH levels thereby affecting steroidogenesis. Testosterone is synthesized in the Leydig cells via several important enzymes, carrier proteins or receptors from cholesterol synthesized de novo [8]. According to Mishra and Singh treatment with *Curcuma longa* causes suppression of spermatogenesis in mice testes, although the mechanism of this suppression remains poorly understood. Besides, in immature male rats, *Curcuma comosa*, another species of *Curcuma*, has been suggested to act directly on the testes or indirectly to inhibit gonadotropin secretion, which consequently reduces testosterone production, or to act by both means [9]. In contrast to our results, Yakubu and Afolayan revealed that the AE of *Bulbine natalensis* increased testosterone levels and this may be adduced to the induction of hormone synthesis by the Leydig cells, as these cells are the main source of testosterone [10]. The data found in our study about the hormone testosterone may be relevant to the treatment of one of the diseases that most affect man, nowadays, prostate cancer. Hormones, especially androgens, are believed to play a key role in the etiology of prostate cancer [11].

The results of histopathological testicular studies showed significant differences between the control and test groups. GSI were significantly higher in the control group than in the test group. However, our results disagree with another study carried out by Yakabu et al., which revealed an increase of GSI in rats treated with *Fadogia agrestis*, a plant used to treat sexual disorders. These parameters can also be used to evaluate the normal functioning capacity of the testes [12]. According to Wankeu-Nya et al., implications in gonad somatic indices may occur due to inflammatory or cell constriction processes [13]. Secondary metabolites of plants have been studied for their androgenic effects in mammals. Subsequently, the studies carried out by Yakubu and Jimoh showed decreased levels in the weight of reproductive organs in male rats submitted to treatment with extract of seeds from *Vitex negundo*, and suggested that the flavonoids present in high concentrations in the extract may be related to the results [14].

CONCLUSION

In conclusion, the results of the current study revealed that the oral administration of an AE of *P. pyrifolia* was not lethal, but promoted a severe decrease in testosterone hormone levels, consequently, the fertility in male rats may be affected, proposing the extract as possible use as in an adjuvant therapy of prostate cancer.

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