

Antifertility Activity Of Aqueous Extract Of Phyllanthus Niruri In Male Albino Rats

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Abstract

The effects of aqueous extract of P.niruri on epididymal sperm characteristics, fructose and testosterone levels in male albino rats were investigated. The treatment of the extract for 14days resulted in appreciable decrease in the fructose level of the seminal fluid, sperm motility, sperm count, and sperm viability of the treated groups ($p<.05$) when compared with the control. These decreases were dose dependent. Testosterone levels were lower in the treated animals as compared to the control but not significantly different. These findings suggest that the aqueous crude extract of phyllanthus niruri has antifertility activity. However, the observation of progressive weakness and reduction in agility across the group may be as a result of a parameter that did not form part of this research.

INTRODUCTION

The use of herbs in the treatment of ailments in Africa has been an age long practice. Man's continuous reliance on herbs for therapeutic and nutritional benefits cannot be overemphasized. Phyllanthus niruri (stonebreaker) commonly called 'enyikwonwa' in the Ibo dialect, a wide spread tropical plant commonly found in coastal areas that grows 40-70cm tall is one of such herbs. All parts of this herb have been proved to have a wide range of therapeutic effects. P.niruri has been reported to reduce significantly blood sugar level (Nwanjo H.U, 2007). Adedapo et al (2005) reported the immune stimulating effect of this herb. P.niruri plays Hepatoprotective and antioxidant role as reported by Lee (2006) Chatterjee et al (2006) and Nwanjo (2006). Phyllanthus niruri acts as a hypolipemic agent (Khanna et al, 2002), anti-lithic agent (Barros, 2003) (Freitas et al, 2002) anti-viral (Naik and Juvekar, 2003) (Iam, 2006) and anti-malarial properties (Subeki 2005) (Cimanga 2004) (Simons et al 1995).

Many anti-malarial agents have been shown to have anti-fertility effects. The anti-fertility actions of quinine and chloroquine (Meisel, 1993) (Adeeko and Daada, 1998) and Dihydroartemisinin (Nwanjo et al 2006) have been reported. Similar reports have been documented in herbs that have anti-malarial activity, Oze et al (2007) and Raji et al (2003) reported antifertility effects of Alstonia boonei and Azadirachta indica respectively; plants of high anti-malarial

activity. These are a few of the much documentation on the anti-fertility actions of anti-malarial agents. The evaluation of anti-malarial agents for possible toxicity and anti-fertility actions becomes imperative due the global concern of malaria and infertility and thus relationships has to be established to guide the common man.

In the absence of any information on the effect of P.niruri on the fertility of male rats, this research is undertaken to determine if there are such negative effects on certain parameters such as sperm count, sperm viability, sperm motility, fructose and serum testosterone levels.

MATERIALS AND METHODS

ANIMALS

Twenty-five albino rats (150-200g) obtained from the animal house of the department of Biochemistry, University of Nigeria, Nsukka were used for this study. They were housed in metal cages and kept in the animal house of the department of biochemistry, Anambra State University. They were allowed to acclimatize to the new environment for 7days before the commencement of treatment. Throughout the period of the experiment the animals were fed normal feed.

PREPARATION OF THE EXTRACT

The entire plant was sun dried for 12days before powdering using manual grinder. 20g of P.niruri was soaked in 500ml of distilled water in a beaker. The mixture was shaken and

allowed to stand for 24hours before filtering with a cheese cloth. The filtrate was evaporated using an oven at 50-60°C. Appropriate weights of the residue were prepared in distilled water to obtain the various concentrations used for the experiment.

EXPERIMENTAL DESIGN

Five experimental groups of five albino rats were used in the experiment. Each group was treated and fed as follows for two weeks

Group A: served as the control and received nothing but normal feed

Group B: 100mg/kg body weight of the extract

Group C: 200mg/kg body weight of the extract

Group D: 250mg/kg body weight of the extract

Group E: 300mg/kg body weight of the extract

BODY AND ORGAN WEIGHTS

Initial and final body weights of the animals were recorded. At the end of the treatment period, the animals were sacrificed 18hours after the last dosage. The testis was removed and weighed, the mean value was recorded.

HORMONAL ASSAY

18hours after the last doses were administered, the animals were sacrificed and whole blood was collected by cardiac puncture. The blood sample was spun at 2500rpm for 10min using a centrifuge. Serum sample were assayed for testosterone using enzyme linked immunoassay technique.

SPERM CHARACTERISTICS AND FRUCTOSE TEST

The seminal fluid was collected by macerating the reweighed and dissected testis in normal saline; after centrifuging at 12000rpm for 5minutes, the supernatant were assayed for sperm qualities and characteristics were assayed as described by Cheesbrough (1984) and the fructose level of the seminal fluid assayed by spectrometric technique.

RESULTS

BODY AND ORGAN WEIGHT CHANGES

Table 1 shows the effect of P.niruri on the body and organ weight of the rats. The entire group showed no significant weight gain or loss throughout the experiment though the level of agility of the animals decreased remarkably across the group. There was significant increase in the weight of the

testis in all the treated groups when compared with the control.

Figure 1

Table 1: The Body weight and organ weight of the animals.

	Mean initial body weight (g)	mean final body weight (g)	Mean body weight change (g)	Mean testis weight (g)
GROUP A(control)	167.29±11.58	167.57±13.42	0.28±0.04	0.78±0.08
GROUP B	167.76±8.46	168.79±8.29	1.03±0.11	0.84±0.21*
GROUP C	174.85±15.73	175.80±15.06	0.95±1.42	0.97±0.09*
GROUP D	172.50±13.71	171.94±13.37	0.56±0.66	1.13±0.04*
GROUP E	171.54±14.54	170.44±14.36	1.10±0.22	1.21±0.15*

* (p<0.05) significantly different from the control)

SPERM MOTILITY

The sperm mobility was significantly reduced (p<0.05) in rats treated with the 250mg/kg and 300mg/kg of the extract when compared to that of the control. (Table 2).

SPERM COUNTS

There was a visible reduction (p<0.05) in the sperm counts of the treated groups D and E when compared with that of the control (Table 2).

SPERM VIABILITY

The sperm viability of the treated groups shows a significant deviation (p<0.05) from what was obtained in the control (Table 2).

SPERM FRUCTOSE LEVEL

The fructose concentration of the seminal fluid of the animals treated with the extract showed significant decrease (p<0.05) when compared with that of the control (Table 2)

SERUM TESTOSTERONE LEVEL

Table 2 shows as well the serum testosterone level of both the treated rats and that of the control. There was progressive mild decrease (p<0.05) in the testosterone level of the animals and this decrease was dose dependent.

Figure 2

Table 2: sperm motility, sperm count, sperm viability, testosterone level and fructose level.

	SPERM MOTILITY (%)	SPERM COUNT (%)	SPERM VIABILITY (%)	TESTOSTERONE LEVEL (mg/ml)	FRUCTOSE LEVEL (µg/ml)
GROUP A (CONTROL)	74±8.09	71±6.04	93±8.46	1.63±0.17	3.32±0.25
GROUP B	66±8.57	68±14.48	81±10.77	1.59±0.26	2.01±0.64*
GROUP C	69±18.56	61±5.98	78±7.61*	1.50±0.61	1.28±0.75*
GROUP D	56±3.81*	51±6.31*	61±13.01*	1.61±0.53	0.99±0.32*
GROUP E	53±9.35*	55±7.60*	49±6.27*	1.35±0.11*	0.46±0.57*

*($p < 0.05$) significantly different from the control

DISCUSSION

The decrease in fertility potentials reported after the treatment of male rats with dihydroartemisinin has been attributed to impairment in sperm motility and viability (Nwanjo et al 2007). treatment of animals with antimalarial drugs usually result in reducing the sperm counts, motility, viability and visible alteration alters the morphology of the sperms; such impairment of male fertility has been reported with chloroquine and halofantrine treated rats (Adeeko and Daada 1998; Orisakwe et al 2003). Similar reports have been reported in herbs that have anti-malarial activity, Oze et al (2007) and Raji et al (2003) reported antifertility effects of *Alstonia boonei* and *Azadirachta indica* respectively; plants of high anti-malarial activity.

The findings of the present study showed that the aqueous extract of *P. niruri* could significantly alter the fertility potential of male rats. The mere fact that there was lack of effect on the body weight on treated animals does not rule out the possibility of a systemic toxicity at the doses treated due to behavioral alterations observed within the treated group. The treated groups showed progressive decrease in agility. Furthermore the significant increase in the weight of the organs ($p < 0.01$) of the treated groups indicates that the extract may have toxic effect on this organ. Simons et al (1995) noted that increase or decrease in weight of an organ after the administration of a chemical agent is an indicator of a toxic effect of such agent.

The significant depletion of the fructose level of the seminal fluid and reduction of sperm viability, sperm motility and sperm counts shows that the extract has the potential to penetrate the blood-testis barriers. Baddesarini (1980) reported that effect of chemical agents on sperm composition is attributed to their ability to penetrate this barrier. This depletion of seminal fructose across all the treated groups ($p < 0.05$) invariably affects the sperm motility and viability since fructose serves as the driving energy of the sperm and since fructose is androgen-dependent may indicate reduction in circulating androgen levels. Similarly, the decrease in sperm qualities points to reduction in the circulating androgen level. I would take caution in suggesting that the near absence of fructose is an indication of an obstruction either in vas deferens or the epididymis (Zhu et al 2006; Gonzales 1997) due to the technique used in collection of the sample.

Any chemical agent that can affect reproductive activity will

as well affect the quality and quantity of the sperm. The sperm counts, motility and viability of the treated samples were significantly decreased in groups D and E ($p < 0.05$) when compared to the control, such decrease can be attributed anti-androgenic property of the extract. Animals fed with 100mg/kg showed no significant change in all the parameters tested except the fructose level which indicates that at this concentration the extract may not be toxic.

Summarily, these observations show that the aqueous extract of *phyllanthus niruri* may have antifertility effects in albino rats at doses above 200mg/kg. But since there has been no such documentation of the plant with respect to humans research should be directed towards this area to assess the effect of the extract on man.

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