Research Paper

Effect of the aqueous extract of dry fruits of *Piper guineense* on the reproductive function of adult male rats

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Received: 19.9.2003
Revised: 28.10.2003
Accepted: 8.9.2004

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ABSTRACT

Objective: To study the effect of the aqueous extract of *Piper guineense* (Piperaceae) on male reproductive function in Wistar rats.

Materials and Methods: Male rats, 3 months old, weighing 180-210 g were administered (by gastric intubation) the aqueous extract of dry fruits of *Piper guineense* at two doses, 122.5 and 245 mg/kg for 8 days and 122.5 mg/kg for 55 days. The control group received distilled water for the same duration. Animals were sacrificed and the blood, testes, epididymis, seminal vesicles and prostate were collected for biochemical analyses.

Results: There was a significant increase in the level of testosterone in the serum and testes, cholesterol in the testes, α-glucosidase in the epididymis and fructose in the seminal vesicles after 8 days of treatment, while with 55 days of treatment, the levels of cholesterol in the testes increased by 75%, while the levels of α-glucosidase in the epididymis and the seminal vesicle fructose decreased by 24 and 21% respectively. On the other hand, there was a 20% reduction of fertility in the *P. guineense*-treated rats after 55 days of treatment.

Conclusion: The aqueous extract of *P. guineense* at both doses (122.5 and 245 mg/kg) had a positive impact on the male reproductive function since it stimulated the secretions of the testes, epididymis and seminal vesicles. The use of *P. guineense* could negatively influence male fertility.

KEY WORDS: α-glucosidase, cholesterol, guinea pepper, male fertility, testosterone

Introduction

In many developing countries, traditional medicines are widely utilized in the treatment of various ailments on an empirical basis. A variety of plants have been used for the treatment of ulcer, hypertension, diabetes and male reproductive function. *Litsea chinensis* and *Ochis maculata* are used for their aphrodisiac activity.[1] The plant of *Striga orobanchioides* has antiandrogenic and antispermatogenic effects.[2] Leaves *of Hibiscus macranthus* and *Basella alba* have androgenic activity.[3] Thus medicinal plants are used for the treatment of disorders linked to male infertility. Some of the factors responsible for this infertility are linked with hormonal secretion, erectile impotence, disorders of ejaculation, and toxic effects on the testes and accessory sex organs. Leaves of *Piper guineense* are used for respiratory infections and for female infertility while its fruits are used as an aphrodisiac.[4] However, previous studies in our laboratory have shown that the aqueous extract of *Piper guineense* fruits at 122.5 mg/kg have been shown to stimulate sexual behavior of mature male rats[5] by decreasing mount and intromission latencies and by increasing mounting, anogenital sniffing and penile erection index. Since *Piper guineense* has an impact on penile erection and copulatory behavior which are controlled by androgens, the present study was undertaken to evaluate the effects of the dry fruits of *Piper guineense* on some male reproductive parameters such as the secretory activities of the testis and some accessory sexual organs which are also controlled by androgens.

Materials and Methods

Animals

Male Wistar rats, 3 months old, weighing between 180-210 g were used. The rats were kept at a room temperature of 22 ± 3° C and 12-h natural light-dark cycle, in the animal house of the Faculty of Science, University of Yaounde I. Rats were fed on standard laboratory chew and water *ad libitum*. Female rats (5 months old; weight 170-215 g), of proven fer-

tility were used for the fertility test. The experiments were performed following approval by the University Ethics Committee of the University of Yaounde I.

Preparation of the plant extract

Fruits of *P. guineense* Schum and Thonn (Piperaceae) were collected during the first week of November 2000 in the peripheral region of Yaounde in the Central Province of Cameroon and identified in the National Herbarium (IRA), Yaounde, where a voucher specimen (43129-HNC) was deposited. The fruits were dried at 34° C using an oven type P-SELECTA and crushed. The dried powder (100 g) was macerated in 200 ml of distilled water for 12 h at room temperature and filtered to obtain a final extract concentration of 28 mg/ml.

Experimental protocols

Short-term treatment (8 days)

Fifteen rats were divided into three groups of five animals each and treated for 8 days. Through a gastric tube, the control group received 2 ml of distilled water and the two test groups, the aqueous extract of *P. guineense* at doses of 122.5 and 245 mg/kg respectively, as recommended by traditional practitioners. Two doses were used because the traditional practitioners used one or two teaspoon(s) of *P. guineense* fruits to treat the reproductive problem of their patients.

Sub-acute treatment (55 days)

Two groups of five male rats each received by gavage, either the plant extract (122.5 mg/kg) or distilled water as recommended by traditional practitioners. The dose of 122.5 mg/kg was recommended by traditional practitioners. For the mating test, each male of 55 days of treatment was introduced in a Plexiglas cage with 2 females of proved fertility for 8 days. This occurred 3 times before the end of the treatment by the 8th, 24th and 47th day. After each mating test, each female was observed for delivery (21-24 days following the mating test) as a criterion of successful insemination.

After 55 days of the sub-acute feeding, male rats were sacrificed for biochemical analyses.

Biochemical analyses

At the end of each treatment period (8 and 55 days), the rats were sacrificed by cervical dislocation and blood collected in dry tubes. The blood samples were centrifuged and the serum was aliquoted and frozen for biochemical analyses. The prostate, seminal vesicle, left testis and epididymis were dissected out and frozen for biochemical analyses. Levels of testosterone in the serum and testes were determined using the radioimmunoassay (RIA) method.[6] Total protein levels were determined in the serum and sexual organs (testes and epididymis) using colorimetric methods described by Gornal et al[7] and Bradford8 respectively. Fructose and α–glucosidase levels were determined in the seminal vesicles and epididymis using protocols described in a WHO manual.[9] The cholesterol levels in the testes were determined using the colorimetric method described by Forbes.[10]

Statistical analysis

The data are expressed as mean±SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) and the comparison between the control and experimental groups was done using the Dunnett's test. $P<0.05$ was regarded as significant.

Results

Body weight and weight of organs

There was a significant increase in body weight both in control and *P. guineense* treated rats, but the increase was much higher in *P. guineense* treated rats. The daily administration of *P. guineense* extract after 8 days (245 mg/kg) and 55 days (115 mg/kg) did not increase the weight of the testes, seminal vesicles, epididymis and prostate (Table 1).

The fertility of 122.5 mg/kg *P. guineense*-treated rats was not affected after 16 and 32 days of treatment but there was a 20% reduction after 55 days of treatment. The mean number of litters/animal in the control and treated groups was 8.2 ± 0.66 and 5.4 ±1.4 respectively.

Biochemical changes in the serum and sexual organs

Changes after 8 and 55 days of treatment are outlined in Table 2. Testosterone levels in the serum and testes were significantly higher in *P. guineense*-treated rats after 8 days of treatment compared to the control group. The testicular cholesterol level increased significantly in both 8 days and 55 days *P. guineense* treated groups. The epididymis alpha-glucosidase increased significantly in 8 day *P. guineense* treated rats.

Discussion

The present study shows a significant increase in the body weight of *P. guineense*-treated rats after 55 days of treatment, besides a significant increase of total serum proteins. The increase in the body weight of *P. guineense*-treated rats could be due to the androgenic properties of this plant since androgens possess anabolic activity.[11] The present study revealed that an acute treatment of male rats with *P. guineense* extract at doses of 122.5 mg/kg and 245 mg/kg stimulated testosterone biosynthesis as shown by the increase of serum and testicular levels of this hormone. Our results revealed that *P. guineense* at both doses displays a steroidogenic activity after 8 days of treatment like *Hibiscus macranthus* and *Basella alba*.[3] The leaves of these plants stimulated testosterone production *in vivo*.[3] The significant increase of testicular cholesterol after 8 and 55 days of treatment is important since cholesterol is the starting material for androgen biosynthesis.[11] The increased levels of α–glucosidase and fructose in the epididymis and seminal vesicles, respectively, after 8 days, could be justified by the increase of testosterone in the serum, since those accessory sex organs are controlled by androgens.[13] Since the low dose of *P. guineense* extract (122.5 mg/kg) was as active as the high dose (245 mg/kg), the study of fertility was carried out with the low dose. Our data showed a 20% decrease in the fertility of *P. guineense*-treated rats after 55 days of treatment. The decrease of fertility noticed here could be justified by the significant decrease of α–glucosidase and fructose levels. In fact, α–glucosidase plays an important role in the

Table 1

Changes in the body weight and male reproductive organs of rats after *Piper guineense* treatment.

Group n=5	*Treatment (days)*	*Body weight (g)*			*Weight of male reproductive organs (g/100g body weight, mean ± SEM)*			
		Initial	*Final*	*% increase*	*Testis*	*Epididymis*	*Seminal vesicle*	*Prostate*
Control	8	200.6 ± 3.4	216.8 ± 5.3*	7.9	0.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.1
P. guineense (122.5 mg/kg)	8	186.0 ± 3.3	213.6 ± 6.2*	14.8	0.6 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
P. guineense (245 mg/kg)	8	188.2 ± 4.0	218.6 ± 8.1*	16.2	0.6 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
Control	55	209.8 ± 5.1	274.0 ± 11.3*	30.6	0.6 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
P. guineense (122.5 mg/kg)	55	196.8 ± 3.7	286.5 ± 7.7*	45.6	0.6 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0

Data expressed as mean±SEM. *P<0.05 *Vs* initial body weight (Paired 't' test)

Table 2

Biochemical changes in the serum, testis, epididymis and seminal vesicle of *P. guineense*-treated rats

Group (mg/kg) n=5	*Treatment (days)*	*Total proteins*			*Testosterone*		*Cholesterol*	*α-glucosidase*	*Fructose*
		Serum (g/l)	*Testis (mg/g)*	*Epididymis (mg/g)*	*Serum (ng/ml)*	*Testis (ng/g)*	*Testis (mg/g)*	*Epididymis (mU/g)*	*Seminal vesicle (µmol/g)*
Control	8	85.7 ± 3.7	0.4 ± 0.0	0.2 ± 0.0	0.5 ± 0.2	4.0 ± 0.7	3.2 ± 0.1	4.4 ± 0.1	4.7 ± 0.6
P. guineense (122.5)	8	87.9 ± 4.0	0.5 ± 0.0	0.3 ± 0.0	3.0 ± 0.1*	9.1 ± 0.7*	6.6 ± 0.2*	7.0 ± 0.7*	8.2 ± 0.6
P. guineense (245)	8	88.1 ± 3.9	0.6 ± 0.1	0.3 ± 0.0	2.5 ± 0.1*	7.9 ± 0.9*	7.6 ± 0.4*	6.3 ± 0.5$^{\#}$	8.6 ± 1.7
One-way ANOVA				F	87.5	11.92	76.0	7.24	
				df	2,12	2,12	2,12	2,12	
				P	<0.0001	0.0014	<0.0001	0.0087	
Control	55	91.8 ± 8.0	0.5 ± 0.0	0.3 ± 0.0	ND	ND	4.4 ± 0.5	5.3 ± 0.3	5.0 ± 0.7
P. guineense (122.5)	55	98.5 ± 5.8	0.8 ± 0.1	0.7 ± 0.1	ND	ND	7.7 ± 0.2^{+}	4.0 ± 1.3	3.9 ± 0.6

Data expressed as mean ± SEM. ND: Not determined. *P<0.01 *Vs* respective control; $^{\#}$P<0.05 *Vs* respective control (Dunnett's test) and $^{+}$P=0.0003 *Vs* respective control (Unpaired 't' test)

motility of spermatozoa which is an important factor for fecundation.[11] Fructose is also important for fecundation since it is the energy source for spermatozoa motility. This was in opposition to the root bark extract of *Mondia whitei* (400 mg/kg) which reduced by 50% the fertility of rats after 55 days of treatment as shown by previous study in our laboratory and this reduction was due to testicular lesions.[14]

In conclusion, the significant increase of the testosterone levels in the serum and testes, cholesterol in the testes, α–glucosidase in the epididymis and fructose in seminal vesicles show that *P. guineense* stimulated the male reproductive function after 8 days of treatment since it increased the secretion of the sex organs. These findings could explain the traditional use of this plant for male reproductive problems, especially those linked to secretion by sex glands. *Piper guineense* is consumed in an addictive manner by some individuals and well directed studies in such individuals may reveal whether there are any such adverse effects as seen in the present animal studies.

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