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Research Article

Diuretic Activities of Root Bark Aqueous and Ethanolic Extracts of *Parquetina nigrescens*: I-Effects on Urinary Excretion in Wistar Rat

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Abstract



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Background and objective: The root bark of *Parquetina nigrescens* is used in traditional medicine in the treatment of hypertension and edema for its diuretic properties. This study was conducted to examine the effects of this medicinal plant on the excreted urinary volume and contribute to scientific knowledge on its diuretic action. **Methods:** Water-overloaded rats were treated separately with increasing doses (5-70 mg/kg) of *Parquetina nigrescens* root bark aqueous and ethanolic 70 % extracts of and furosemide (FURO) at 5 mg/kg. Excreted urine was collected and measured. **Results:** Plant extracts caused increased urine excretion in rats. PNea 15 mg/kg and PNee 25 mg/kg were the most active doses. They caused an increase of volume of urine comparable to that induced by FURO 5 mg/kg. A very significant elimination of water overload at $p < 0.001$ was observed. And the recorded values were $42.73 \pm 0.26\%$ (PNea 15 mg/kg), $45.39 \pm 0.190\%$ (PNee 25 mg/kg) and $73.6 \pm 0.24\%$ (FURO 5 mg/kg) against $29.73 \pm 0.24\%$ (Saline solution NaCl 0.9%). **Conclusion and perspectives:** Increase of urine volume excreted induced by the extracts partly confirms the diuretic virtue of *Parquetina nigrescens* justifying its empirical use to treat hypertension and edema. However, these results, on their own, do not make it possible to elucidate the mechanism underlying diuretic activity of this plant. Additional studies should be carried out for this purpose.

Keywords: Diuretic activity, urinary excretion volume, *Parquetina nigrescens*, Furosemide

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INTRODUCTION

The hypotensive and antihypertensive effects of substances used in the treatment of arterial hypertension are based on their actions, combined or not, on the cardiovascular system (heart and vessels) and on blood volume ¹. Generally in modern therapy, diuretics are, alongside ACE inhibitors and β -blockers, used as first-line therapy in the management of hypertension ^{2, 3}. The use of diuretics in the treatment of hypertension aims to normalize blood pressure by promoting reduction of blood volume. This decrease of blood volume is the consequence of a significant elimination of water and electrolytes caused by diuretic substances administered to hypertensive patients ¹.

However, because of the high cost of diuretics and other pharmaceutical products used for the treatment of hypertension, populations have more recourse to medicinal plants ⁴⁻⁶. Indeed, many plants deemed diuretic by traditional medicine are commonly used to treat hypertension and edema ⁷. *Parquetina nigrescens* is one of these plants ⁸. *Parquetina nigrescens* (Periplocaceae) is an herbaceous, perennial plant commonly found in West African countries, especially Nigeria, Ghana, and around villages in Senegal ⁹. Previous works on the

chemical composition and the toxicity as well as *in vitro* and *in vivo* tests have shown that this plant has various pharmacological properties justifying its traditional use in the treatment of various diseases ¹⁰⁻¹³. Unfortunately, despite this fairly extensive literature on *Parquetina nigrescens*, it is noted practically the inexistence of works on the diuretic activity of the root bark of this medicinal plant.

The diuretic activity of a substance can be evaluated through its ability to induce an elimination of water overload and electrolytes in the animal. Also, falling within a global context of verification of the diuretic virtue that traditional medicine grants to *Parquetina nigrescens*, the present study was carried out with the aim of evaluating the effects of two extracts of root bark of this plant on urinary excretion volume in rat.

MATERIALS AND METHODS

Ethical considerations

The experimental procedures and protocols used in this study were performed according to the recommendations of the Ethics Committee of Biological Sciences, Félix Houphouët-Boigny University. These guidelines were in accordance with

the internationally accepted principles for laboratory animal use and care ^{14,15}.

Animals

72 Male Wistar rats weighing 175 and 279 g were used. They were obtained from the *Vivarium* (animal house), Ecole Nationale Supérieure (ENS), Abidjan, Côte d'Ivoire. The animals were grouped and housed in metabolic cages and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet and water *ad libitum*. Animals are fasted 18 hours before the experiment.

Plant material and extractions

Fresh leaves and root bark of *Paquetina nigrescens* were collected in July 2005 in Grand-Bassam forest (Region des Lagunes, Southern region of Côte d'Ivoire). These leaves and root bark of *Paquetina nigrescens* were certified to be an identical sample at the specimen herbarium of Centre National Floristique (CNF), Felix Houphouët-Boigny University (Abidjan, Côte d'Ivoire). This medicinal plant was authenticated by a Botany expert, Prof. Aké-Assi Emma of the CNF.

Root barks of *Paquetina nigrescens* were dried under shade and powdered with a machine (Mark RETSCH, type SM 100, Germany). The extraction process was implemented according to the method described by some authors ^{16,17}. One hundred grams (100 g) of the root barks powder were macerated separately during 24 hours in 1 l water and 2 l ethanol-water (70:30 v/v) for 3 times until complete exhaustion. The mixtures were filtered (Whatman n°1) and concentrated under reduce pressure using a rotary evaporator (Büchi R110, type MKE 6540/2) at a temperature of 45 °C. The concentrated extracts obtained (PNae: *Paquetina nigrescens* aqueous extract and PNee: *Paquetina nigrescens* ethanolic 70 % extract) were stored at 4 °C until experiments.

Drug and chemicals

The following reference drugs were used: Ethanol (alcohol-Nadal, France) and Furosemide (Lasilix®, Sanofi-Aventis, France). The concentrations of drugs (PNae, PNee and Furosemide) to be tested were prepared extemporaneously by dilution in saline solution (NaCl 0.9 %).

Measurement of urinary excretion

The method described by Amonkan et al. was employed, with modification, for the assessment of diuretic activity ^{18,19}. The day of the experiment, the animals received fluid overload. Fluid overload was carried out with distilled water in an amount of 50 ml/kg. Animals divided into 12 groups of 6 rats and treated by intraperitoneal administration. Groupe 1 served as normal and the others groups served as test animal. Group 1 received a saline solution (NaCl 0.9 %), while Group 2 received furosémide (FURO) at 5 mg/kg. Groups 3, 4, 5, 6 and 7 were treated with PNae (5; 15; 25; 50; 70 mg/kg respectively). Groups 8, 9, 10, 11 and 12 received PNee (5; 15; 25; 50; 70 mg/kg respectively). Animals were placed individually in metabolic cages where urine is collected every 2 hours for 8 hours. The urinary volumes thus collected made it possible to evaluate the diuretic power of the substances studied by the determination of the urinary excretion volume (UEV). The UEV was determined from the ratio of the total volume of urine and the volume of fluid overload ^{18,19}.

Statistical Analysis

The values were expressed as mean with standard error of the mean ($m \pm \text{sem}$). The data were evaluated by analysis of variance followed by Tukey-Kramer with GraphPad Instat software (Microsoft, San Diego, California, USA) method. The graphical representations of data were performed by the GraphPad Prism 5 software (Microsoft, San Diego, California, USA). The difference between the averages is considered statistically significant when $p < 0.05$.

RESULTS

Effect of *Parquetina nigrescens* aqueous extract on urinary excretions volume

The urinary excretions volume (UEV) induced by the aqueous extract, saline solution (NaCl 0.9 %) and furosemide (FURO) administered to Wistar rats are reported in Table 1. The largest PNae-induced UEV were recorded at 15 mg/kg and 25 mg/kg. Six hours after the treatment, they were estimated with PNae 25 mg/kg at 31.14 ± 0.21 %. Higher UEV values of 39.31 ± 0.32 % and 42.73 ± 0.26 % were observed respectively 6 and 8 hours after PNae 15 mg/kg administration. UEV was evaluated at 29.73 ± 0.244 % in animals treated with saline solution (0.9 % NaCl) 8 hours after treatment whereas with FURO 5 mg/kg, a value of 73.6 ± 0.24 % was recorded 2 hours after its administration to rats.

Table 1: Effect of *Parquetina nigrescens* aqueous extract on urinary excretion volume in Wistar rat

Time of treatment (h)	Urinary excretion volume (%)						
	Control	FURO 5 mg/kg	PNae 5 mg/kg	PNae 15 mg/kg	PNae 25 mg/kg	PNae 50 mg/kg	PNae 70 mg/kg
2	11.33±0.19	28.7±0.26***	10.7±0.24 ^{ns}	17.5±0.23***	17.21±0.23***	7.3±0.30***	1.61±0.36***
4	16.97±0.49	48.27±0.22***	18.08±0.35 ^{ns}	30.92±0.27***	25.31±0.16***	12.82±0.27**	2.34±0.24***
6	20.93±0.26	60.87±0.28***	20.12±0.32 ^{ns}	39.31±0.32***	30.28±0.30***	15.05±0.36**	2.64±0.37***
8	29.73±0.24	73.6±0.24***	22.27±0.32***	42.73±0.26***	31.14±0.21 ^{ns}	17.84±0.20***	3.08±0.45***

$m \pm \text{sem}$; $n = 6$; ^{ns} $p > 0.05$: no significant; ^{**} $p < 0.01$: significant; ^{***} $p < 0.001$: very significant; Control: Saline solution NaCl 0.9 %; PNae: *Parquetina nigrescens* aqueous extract; FURO: Furosemide.

Analysis of these results indicated that all doses of PNae and FURO failed to induce total elimination of water overload throughout the experiment. However, it was observed that the comparison of the UEV values of the substances studied with

those of the controls at the end of the experiments indicated that FURO 5 mg/kg and PNae 15 mg/kg favored a very significant elimination of the water overload ($p < 0.001$). Examination of PNae-induced UEV values showed that 15

mg/kg was the dose that resulted in the greatest elimination of water overload. This dose of the aqueous extract provoked the strongest UEV. Compared to the control, this dose very significantly increased UEV in a time interval of two hours ($p < 0.001$).

Effect of *Parquetina nigrescens* ethanolic 70 % extract on urinary excretions volume

Regarding the ethanolic extract (PNee), the two highest UEV values were observed with the doses of 25 mg/kg and 15 mg/kg (Table 2). The UEV values of 38.95 ± 0.29 %; $42.93 \pm$

0.17 % and 45.39 ± 0.19 % were measured respectively 4, 6 and 8 hours after treatment of the animals with PNee 25 mg/kg. Administration of PNee 15 mg/kg caused an UEV of 37.35 ± 0.26 % after 8 h. As the other doses of PNee (5, 50 and 70 mg/kg), these two doses did not lead to a total elimination of the water overload. Compared to that of the controls (29.73 ± 0.24 %), the value of 45.39 ± 0.19 % recorded with PNee 25 mg/kg reflects a very significant increase of UEV ($p < 0.001$). The EUV caused by this dose of PNee (25 mg/kg) is however very low at $p < 0.001$ compared to that of FURO 5 mg/kg measured at 73.6 ± 0.24 %.

Table 2: Effect of *Parquetina nigrescens* ethanolic extract on urinary excretion volume in Wistar rat

Time of treatment (h)	Urinary excretion volume (%)						
	Control	FURO 5 mg/kg	PNee 5 mg/kg	PNee 15 mg/kg	PNee 25 mg/kg	PNee 50 mg/kg	PNee 70 mg/kg
2	11.33 ± 0.19	$28.7 \pm 0.26^{***}$	11.85 ± 0.23^{ns}	13.91 ± 0.33^{ns}	$22.54 \pm 0.28^{***}$	12.71 ± 0.26^{ns}	$7.69 \pm 0.40^{***}$
4	16.97 ± 0.49	$48.27 \pm 0.22^{***}$	17.75 ± 0.35^{ns}	$30.02 \pm 0.27^{***}$	$38.95 \pm 0.29^{***}$	$24.69 \pm 0.21^{***}$	15.81 ± 0.24^{ns}
6	20.93 ± 0.26	$60.87 \pm 0.28^{***}$	21.14 ± 0.23^{ns}	$35.59 \pm 0.32^{***}$	$42.93 \pm 0.17^{***}$	$27.26 \pm 0.39^{***}$	23.51 ± 0.10^{ns}
8	29.73 ± 0.24	$73.6 \pm 0.24^{***}$	33.37 ± 0.24^{ns}	$37.35 \pm 0.26^{***}$	$45.39 \pm 0.19^{***}$	30.72 ± 0.11^{ns}	30.20 ± 0.26^{ns}

m $\pm$ sem ; n = 6 ; $^{ns}p > 0.05$: no significant ; $^{**}p < 0.01$: significant ; $^{***}p < 0.001$: very significant ; Control : Saline solution NaCl 0.9 % ; PNee : *Parquetina nigrescens* ethanolic extract 70 % ; FURO : Furosemide.

Compared effects of two active doses of *Parquetina nigrescens* extracts on urinary excretion volume

As indicated in Figure 1, FURO 5 mg/kg showed the strongest power to eliminate water overload even though it failed to completely eliminate it after 8 hours of experimentation. The UEV recorded with the most active doses of PNae and PNee were significantly higher at $p < 0.001$ than those of control (solution saline NaCl 0.9 %). When the UEV of the plant extracts are compared with each other, the results did not show any significant difference ($p > 0.05$) at the beginning and at the end of the experiment. This is well illustrated by the

most active doses of *Parquetina nigrescens* extracts (PNae 15 mg/kg and PNee 25 mg/kg). Indeed, after two hours of observation, the PNae 15 mg/kg dose induced an UEV of 17.5 ± 0.23 % when that of PNae 25 mg/kg was estimated at 22.54 ± 0.28 %. At the end of the observation time, the EUVs had increased to 42.73 ± 0.26 % (PNae 15 mg/kg) and 45.39 ± 0.19 % (PNee 25 mg/kg). The extracts of *Parquetina nigrescens* exhibited an action with similar kinetics to that of the reference substance (furosemide) even though the UEV levels achieved by the medicinal plant extracts were much lower than those of FURO 5 mg/kg.

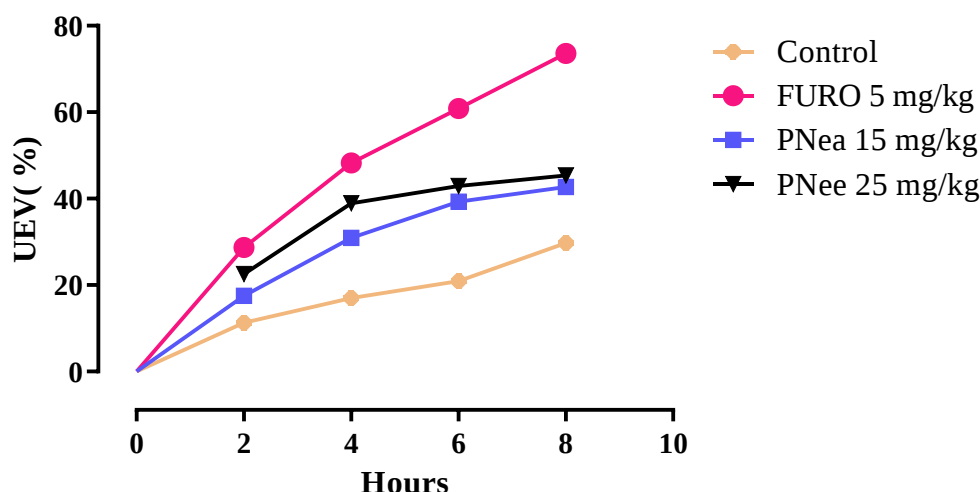


Figure 1: Evolution of urinary excretion volume in the presence of the most active doses of *Parquetina nigrescens* extracts. The substances studied were administered intraperitoneally. Furosemide caused the strongest UEV. PNee 25 mg/kg and PNae 15 mg/kg respectively followed with kinetics of action similar to that of FURO. Control: Saline solution NaCl 0.9 % ; PNae : *Parquetina nigrescens* aqueous extract ; PNee : *Parquetina nigrescens* ethanolic 70 % extract ; FURO : Furosémide ; UEV : Urinary excretion volume.

DISCUSSION

The administration of aqueous (PNae) and 70 % ethanolic (PNee) extracts of *Parquetina nigrescens* caused an increase of urinary excretion volume in water overloaded rats. This increase depends on both the dose of the drug and duration of treatment. These results are similar to those of previous works on the diuretic activities of medicinal plants in mammals. Patell et al. (2009) showed the dose-dependent effects of aqueous and alcoholic extracts of *Lepidium sativum* (Crucifereae) on diuresis in rats ²⁰. Hailu and Engidawork (2014) also made this observation in mice treated separately with aqueous and alcoholic (80%) extracts of *Ajuga remota* (Lamiaceae) ²¹. Several other studies including that of Ntchapda et al. (2010) with the aqueous extract of *Ficus glumosa* (Moraceae) ²² and that of Amonkan et al. (2013) with the aqueous extract of *Ficus exasperata* (Moraceae) showed similar results in rats ¹⁹.

According to the results recorded, the aqueous (PNae) and 70% ethanolic (PNee) extracts have the same potential for eliminating water overload in rats. The most active dose of the aqueous extract (PNae 15 mg/kg), although lower than the most active dose of the ethanolic extract (PNee 25 mg/kg), promoted a higher UEV of 42.73 ± 0.26 %. PNee 25 mg/kg caused a UEV of 45.39 ± 0.19 %. These two UEV values are statistically identical. These results are different from those of Patel et al. ²⁰. Working on the diuretic effect of *Lepidium sativum* (Crucifereae), these authors showed a significant increase in the baseline diuresis induced by the aqueous extract (49.89 %) compared to that of the alcoholic extract (41.05 %). A higher elimination value of the water overload (52.67 %) was obtained by Diallo (1983) with an aqueous extract of *Boerhaavia diffusa* (Nyctaginaceae) ²³.

Analysis of the measured UEV revealed a faster and shorter diuretic action of PNee (70 % ethanolic extract) compared to that of PNae (aqueous extract). The effect of the alcoholic extract of *Parquetina nigrescens* (PNee) appeared two hours after application and had greatly diminished by the third hour. These results are in agreement with those of several previous works. These have shown that the speed of action of a plant on diuresis depends on the type of extract. Aziz et al. (2014) came to the same conclusion with *Mentha viridis* (Labiatae) ²⁴. Das et al. (2014) showed that the methanolic extract of *Ludwigia hyssopifolia* promoted greater, faster and shorter diuretic activity compared to the other two extracts (hexane and ethyl acetate) ²⁵.

The diuretic effects of *Parquetina nigrescens* extracts could be attributed to the secondary metabolites contained in the extracts and favorable to diuresis such as flavonoids, flavonoids and steroids highlighted by previous works ²⁰. The metabolites could act in synergy or not to induce urinary excretion according to one or more mechanisms of action in agreement with Lullman et al. ²⁶. These authors point out that the use of a plant extract for the treatment of a disease involves the administration of various molecules with different mechanisms of action. Also at this stage of the study of the diuretic properties of *Parquetina nigrescens*, several axes can therefore be evoked to explain the diuretic effects observed. Saponins, steroids and flavonoids may stimulate renal artery vasodilatation. This action would increase glomerular filtration to induce greater diuresis. The secondary metabolites could also inhibit tubular reabsorption. The similarity of the UEV kinetics of FURO 5 mg/kg, PNae 15 mg/kg and PNee 25 mg/kg could suggest the same mechanism of action. In other words, the extracts of *Parquetina nigrescens* could follow the furosemide pathways. Furosemide is a loop of Henlé diuretic. It inhibits the reabsorption of water and

sodium at the level of the loop of Henlé ¹⁹. Therefore, extracts of *Parquetina nigrescens* could promote strong diuresis. Finally, the plant extracts (PNae and PNee) could behave as angiotensin receptor antagonists. In this case, they would prevent the binding of angiotensin II to its receptor in the adrenal glands. This action would reduce the plasma levels of vasopressin and aldosterone to cause greater diuresis ²⁷.

CONCLUSION

The aqueous (PNae) and ethanolic 70 % (PNee) extracts of the root bark of *Parquetina nigrescens* (Periplocaceae) caused an increase in urine excretion in rats. This would militate in favor of the use of this plant in traditional medicine as a diuretic to treat various diseases. However, comparative studies with various reference substances (Spironolactone, Hydrochlorothiazide, Furosemide, etc.) in the presence of specific blockers (β -blockers, ACE inhibitors, etc.) must be carried out in animal models (Normotensive, DOCA-salt hypertensive, etc.). These works will make it possible to evaluate the effects of *Parquetina nigrescens* extracts not only on urinary excretion but also on the electrolytes and on some biochemical parameters (creatinine, urea, etc.) in blood and urine in order to better understand the mechanisms of action for a rational and efficient use of this medicinal plant.

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AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

DECLARATIONS

We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere

AUTHORS' CONTRIBUTION

BAK proposed the research idea. BAK and KBK collected the data. BAK and ZFK organized the data in computer, did the analysis, interpretation and wrote the manuscript. KBK and YPA revised the manuscript for scientific content and did the language check. All authors read and approved the final manuscript.

CONSENT FOR PUBLICATION

This manuscript does not contain any individual person's data, and further consent for publication is not required.

COMPETING INTERESTS

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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