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Assessment of Acute Antihypertensive Activity of Tanopati, a Concentrate of Plant Extracts During Adrenaline Induced Experimental Hypertension in the Rabbit

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Original Research Article

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Abstract: The aim of this study was to evaluate the antihypertensive effect of *Tanopati* in hypertensive rabbits. *Tanopati* is a traditional recipe made from the parts of five plants, namely *Ageratum conyzoides, Newbouldia laevis Phyllanthus muellerianus, Cassia occidentalis* and *Aloe vera*. The extract of *Tanopati* was obtained after drying of its decoction at 40 °C for 5 days. The experimental hypertension was induced by intramuscular injection with 0.75 mL of adrenalin at a dose of 1 mg/ml in normotensive rabbits. Once installed hypertension, these rabbits of test group received by gavage 1 ml of *Tanopati* at a dose of 11 mg/kg bw and those of control group 1 mL of furosemide® at dose of 20 mg/kg bw by gavage. Results showed that *Tanopati* at dose 11 mg/kg bw increased significantly urinary excretion of sodium compared to the control ($p < 0.001$). *Tanopati* while reducing the plasma level of creatinine increased significantly ($p < 0.001$) its urinary rate as well as its clearance. So, this elimination of sodium would be the consequence of an increase in the glomerular filtration rate, like diuretics of loop such as furosemide®. The antihypertensive effect of *Tanopati* would thus be exerted by a natriuretic effect, ie the urinary elimination of sodium.
Keywords: *Tanopati*, hypertension, antihypertensive, natriuretic, furosemide.

INTRODUCTION

According to World Health Organization [1], more than one billion people suffered from hypertension worldwide with a prevalence of nearly 40% in adults aged 25 or older. Africa with a prevalence of more than 46%, is one of the most affected continents [2].

According to [3] for the treatment of this disease, nearly 80% of the world population and more than 90% of the population of the developing countries, resort to medicinal plants. This popularity, although linked to the high price of antihypertensives sold in pharmacies, explains mainly the relatively low costs and the accessibility of the decoctions of plants proposed by traditional healers. In Côte d'Ivoire, *Tanopati* appears on the list of decoctions of plant extracts proposed to hypertensive patients. It is a concentrate of plant extracts prepared from five (5) plants including *Ageratum conyzoides, Newbouldia laevis, Phyllanthus muellerianus, Cassia occidentalis* and *Aloe vera* [4]. Pharmacological studies carried out by [4] on this decoction demonstrated antiradical, antioxidant, antihypercholesterolemic and cardioprotective properties. The toxicological study carried out by these same authors showed that this decoction is non-toxic orally.

The purpose of the present study was to evaluate the antihypertensive activity of *Tanopati* in hypertensive rabbits through sodium urinary excretion.

MATERIALS AND METHODS

Animal

Male rabbits of *Oryctolagus cuniculus* species, about 10 weeks old and weighing between 1500 g and 1900 g were used. They came from farms in Bingerville (suburbs of Abidjan). They were acclimatized for two weeks in the laboratory before the beginning of the experiment.

Experienced product

Tanopati was supplied in the form of a decoction by a traditional healer who produced and marketed it. It is a fluid solution of brown color and bitter taste prepared from different fragments of five plants including *Ageratum conyzoides, Newbouldia laevis, Phyllanthus muellerianus, Cassia occidentalis* and *Aloe vera*. .

Chemical substances

The chemicals were composed of furosemide® (Sanofi, France) and adrenalin (Sanofi, France) respectively used as diuretic and hypertensive. NaCl 0.9%, a physiological solution of was also used.

Experimental devices

A handheld tensiometer (Berheur® WHO, Germany) was used for the measurement of hypertension and biochemical analyzer type CYANStart were used for the determination of electrolytes and creatinine.

METHODS

Drying the *Tanopati*

Tanopati was evaporated in an oven at 40 °C for 5 days. The dry brown color extract obtained was used for the preparation of the dose used during this study.

Induction of experimental hypertension

The induction of hypertension was performed according to the method described by [5]. So, rabbits in the experimental lots received 0.75 mL of adrenalin solution at a dose of 1 mg/ml per day intramuscularly injection for 7 days. An automatic sphygmomanometer (Berheur® WHO, Germany) made it possible to determine daily the cardiovascular parameters (systolic blood pressure and diastolic blood pressure) to realize the installation of the hypertension. Once the hypertension was installed, these rabbits were divided into three lots of 6 rabbits each. The three test lots were:

- Lot HNT: lot of untreated hypertensive rabbits,
- Lot HF lot: lot of hypertensive rabbits treated with furosemide®,
- lot HT: lot of hypertensive rabbits treated with *Tanopati*

Experimental protocol

After induction of hypertension, the three hypertensive lot and the control lot (normotensive) were deprived of water and food for 18 hours. After that, each of these rabbits received orally 2.5 mL/100 g of NaCl 0.9 % in order to impose a uniform charge of water [6]. Forty-five (45) minutes later, rabbits from the control lot (normotensive) and those from the HNT lot received each 1 mL of distilled water. Those of the HF lot received 1 mL of furosemide® at a dose of 20 mg/kg bw and authors of the lot HT 1 mL of *Tanopati* at dose of 11 mg/kg bw. After administration of the substances, the rabbits were placed individually in metabolic cages.

Determination of urinary levels of electrolytes

Six (6) hours after treatment, the cumulative urine volume excreted by each rabbit was measured and samples were taken to determine the urinary concentrations of sodium (Na^+), Potassium (K^+) and chloride (Cl^-). The urinary concentrations of the electrolytes were determined using the CYAN® Start controller according to the manufacturer's instruction manual with appropriate reagents. The urinary levels of the electrolytes were used to calculate the saliuretic index of Na^+, K^+ and Cl^-; the aldosterone secretion index (Na^+/K^+); the thiazide diuretic index (Na^+/Cl^-) and carbonic anhydrase inhibition index [$Cl^-/(Na^+ + Cl^-)$].

Determination of diuretic index

The diuretic index was determined using computational formulas proposed by [7]: Diuretic index = Urinary volume of the experimental lot/Urinary volume of the experimental lot

Determination of creatinine clearance

$$\text{Clearance (mL/min)} = \frac{U \times V}{P \times T}$$

Where,

U: urinary creatinine
P: plasma creatinine
T: Time of urine collection (Time equal to 24 h)
V: volume of urine collected at 24 h

Twenty-four (24) hours later, urine and arteriovenous blood samples were taken for the determination of plasma and urinary creatinine levels. These rates were determined using the CYAN® Start biochemical analyzer. Then, the creatinine clearance was determined by the formula proposed by [8]:

Statistical analysis

Analyzes and graphical representations of the data were carried out using the Graph Pad Prism 5.0. The values expressed are the means of three experiments with standard error of the mean (Mean ± SEM). The significance of the difference when compared to control was achieved using ANOVA-one way followed by the Dunnett multiple comparison test. Differences were considered significant at p-value less than 0.05.

RESULTS

Effect of *Tanopati* and furosemide® on the urinary concentration of electrolytes in rabbits after 24 h of treatment

Table-1 showed the urinary level of Na^+, K^+ and Cl^- of the 24 hours urine and, on the other hand, the aldosterone secretion index (Na^+/K^+), the thiazide diuretic index Na^+/Cl^-) and the inhibition index of carbonic anhydrase [$Cl^-/ (Na^+ + Cl^-)$].

Urinary levels of Na^+, K^+ and Cl^- in the lots treated with *Tanopati* and furosemide® were significantly increased ($p < 0.05$) compared to controls and HNT. Concerning, HT (127.3 ± 3.2 mmol/L) and the HF (158.00 ± 4.72 mmol/L) Na^+ were significantly

higher ($p < 0.001$) than those of the control (95.6 ± 1.45 mmol/L). The urinary K^+ level of animals in the HT lote (70.67 ± 3.48 mmol/L) and HF (62 ± 1.15 mmol/L) showed a significant increase ($p < 0.05$) compared to control lots (52.00 ± 0.58 mmol/L) and HNT (51.18 ± 0.58 mmol/L). Cl^- levels of HT (69.33 ± 0.33 mmol/L) and HF (67.67 ± 0.88 mmol/L) were significantly ($p < 0.001$) higher than control (62.00 ± 0.00 mmol / L) and HNT (51.18 ± 0.58).

Tanopati and furosemide® increased higher only sodium saliuretic index of Na^+ than those of the control and HNT lot.

Tanopati did not increase significantly aldosterone secretion index and thiazide diuretic index but decreased slightly carbonic anhydrase inhibition index

Table-1: Effect of *Tanopati* and furosemide® on the urinary concentration of electrolytes in rabbits after 24 h treatment.

Lots	Urinary concentration of electrolytes (mmol/L)			Saliuretic Index			Na^+/K^+	Na^+/Cl^-	$Cl^-/(Na^+ + K^+)$
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-			
Control	93.67 ± 0.33	52.00 ± 0.58	62.00 ± 0.00	1.00	1.00	1.00	1.8	1.51	0.42
HNT	95.3 ± 1.45^{ns}	51.18 ± 0.58^{ns}	63.67 ± 0.88^{ns}	1.13	0.98	1.02	1.86	1.49	0.43
HF	158.00 ± 4.72***	62.00 ± 1.15*	67.67 ± 0.88***	1.68	1.19	1.02	2.55	2.33	0.3
HT	127.3 ± 3.2***	70.67 ± 3.48**	69.33 ± 0.33***	1.35	1.36	1.12	1.8	1.83	0.35

Values are expressed as mean ± SEM with n=6 in each group. *** $p < 0.001$: significant difference compared the control lot,

ns: there was no significant difference compared to the control lot at $p > 0.05$. Distilled water, HNT: untreated hypertensive lot,

HF: lot Treated with furosemide at the dose of 20 mg / kg bw, HT: lot treated with *Tanopati* at a dose of 11 mg/kg bw.

Saliuretic index = Concentration of excreted solute of the lot/Concentration of solute excreted of the control lot

Na^+/K^+: aldosterone secretion index

Na^+/Cl^-: thiazide diuretic index

$Cl^-/(Na^+ + K^+)$: carbonic anhydrase inhibition index

Effect of *Tanopati* and furosemide® on serum creatinine concentration in hypertensive rabbits after 24h treatment

Figure-1 showed the variation of serum creatinine in hypertensive animals treated with *Tanopati* and furosemide®. This figure showed that in the animals treated with *Tanopati* (11 ± 0.45 mg/L) and furosemide® (10.66 ± 0.33 mg/L), serum creatinine levels were significantly lower than of the untreated hypertensive animals (13.1 ± 0.5 mg/L), with the respective decrease percentage of -16.03 % and -18.62 %)

Effect of *Tanopati* and furosemide® on urinary creatinine concentration in hypertensive rabbits after 24 h of treatment

Figure-2 showed the variation in urinary creatinine in hypertensive rabbits treated with *Tanopati* and furosemide®. This figure showed that *Tanopati* and furosemide® increased significantly urinary creatinine level ($p < 0.001$) compared to the HNT lot. Indeed, the creatinine urinary level of the HNT lot is (4.42 ± 0.2 mg/L). The percentages of increase were 93.66 % for the lot treated with *Tanopati* and 130.32 % for the lot treated with furosemide®.

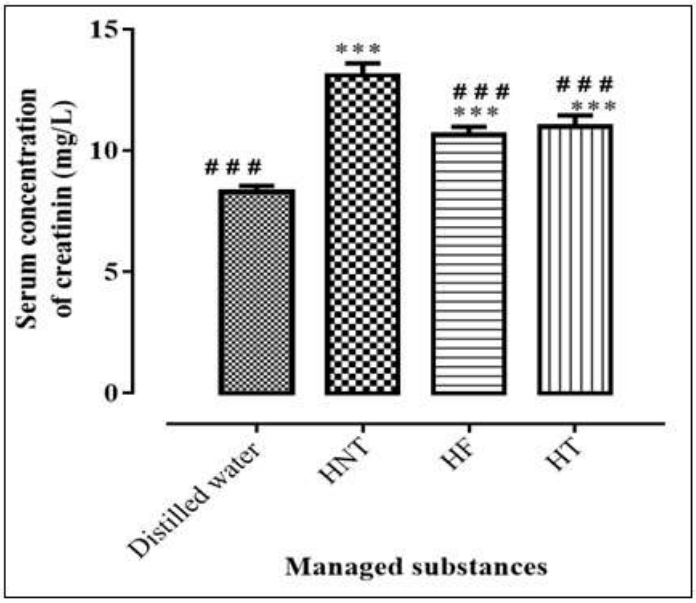

Fig-1: Effect of *Tanopati* and furosemide® on serum creatinine in hypertensive rabbits after 24 h of treatment.

Values are expressed as mean ± ESM, with n=6 in each group. *** p <0.001: significant difference of creatinine urine concentration compared to the control lot, ### p <0.001: significant difference of urinary creatinine concentration compared to lot HNT. ns: no significant difference compared to control lot at p> 0.05. HNT: untreated hypertensive lot, HF: lot treated with furosemide® at a dose of 20 mg / kg bw, HT: Lot treated with *Tanopati* at the dose of 11mg/kg bw.

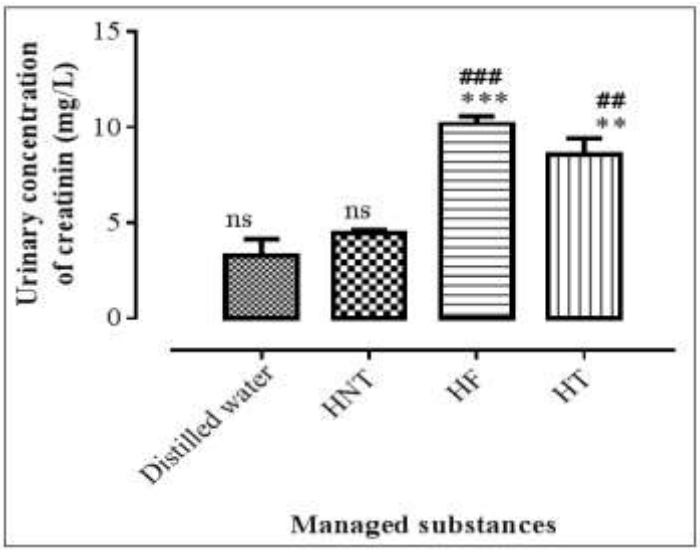

Fig-2: Effect of *Tanopati* and furosemide® on the urinary concentration of creatinine in hypertensive rabbits after 24 h of treatment

Values are expressed as mean ± ESM, with n=6 in each group. *** p <0.001: significant difference of creatinine urine concentration compared to the control lot, ### p <0.001: significant difference of urinary creatinine concentration compared to lot HNT. ns: no significant difference compared to control lot at p> 0.05. HNT: untreated hypertensive lot, HF: lot treated with furosemide® at a dose of 20 mg / kg bw, HT: Lot treated with *Tanopati* at the dose of 11mg / kg bw.

Effect of *Tanopati* and furosemide® on creatinine clearance in hypertensive rabbits after 24 h of treatment

Figure-3 showed the evolution of the creatinine clearance of the animals in the control lot, the lot of untreated hypertensives animals, lot of animals treated with *Tanopati* and furosemide®. This figure showed that the creatinine clearance of the animals in the HNT lot is (8.03 ± 0.89 mL/min). In lot treated with *Tanopati* (30.91 ± 1.56 mL/min) and furosemide (40.81 ± 2.3 mL/min), creatinine clearance increased

significantly ($p < 0.001$) compared to control. The percentages of increase induced by *Tanopati* and furosemide® were respectively of 216.92 % and 315.54 %.

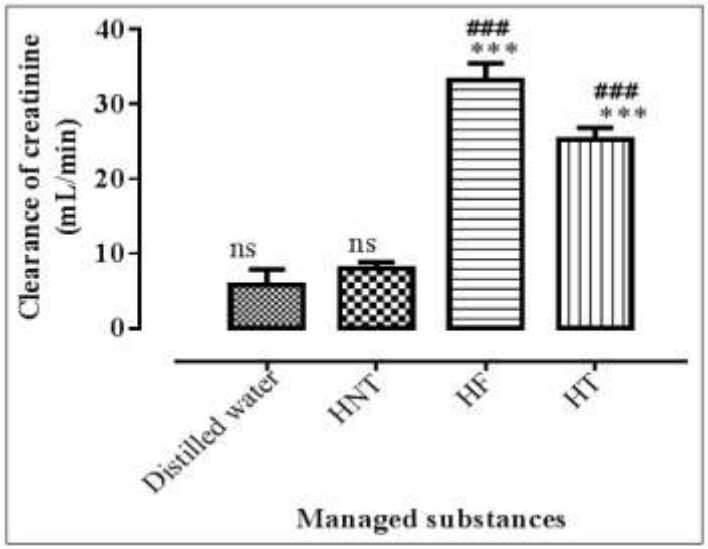

Fig-3: Effect of furosemide® Tanopati et on creatinine clearance in hypertensive rabbits after 24 h of treatment.

Values are expressed as mean ± ESM, with n=6 in each group. *** p <0.001: Significant difference from creatinine clearance compared to the control lot, ### p <0.001: Significant difference compared to creatinine clearance in the HNT lot.
ns: there was no significant difference from the creatinine clearance of the control lot at $p > 0.05$. HNT: untreated hypertensive lot, HF: lot treated with furosemide® at the dose of 20 mg / kg bw, HT: lot treated with *Tanopati* at the dose of 11 mg/kg bw.

DISCUSSION

The antihypertensive effect of *Tanopati* was evaluated through its potential urinary excretion of electrolytes. The results showed that after 6 h of experimentation, the urine was hypernatremic, hyperkalaemic, hyperchloraemic compared to the control. *Tanopati* did not increase the aldosterone secretory index (Na^+/K^+) urine, suggesting that the increase in urine output is different from potassium-sparing diuretics. These diuretics act on the distal tube of the loop of Henle by antagonizing the aldosterone and increasing the ratio (Na^+/K^+) [9]. The aldosterone opens the sodium and potassium channels, and stimulates the Na^+/K^+-ATPase pump. It reabsorbed sodium and urinated potassium. When the action of this hormone is blocked, the epithelial sodium channel and the potassium channel remain closed. The Na^+/K^+-ATPase pump becomes less active, the Na^+ is not reabsorbed and the potassium can no longer exit into the tubular light (potassium sparing). This leads to a decrease in kaliuresis and a risk of increasing the concentration of K^+ in the extracellular medium.

Moreover, the non-significant variation of the thiazide diuretic index of the lot treated with *Tanopati* compared to the control lot suggests that the increase in urinary excretion observed is not of the thiazide type. Indeed, thiazide diuretics increase the urinary levels of Na^+ and K^+ as well as the thiazide diuretic index. They also inhibit the Na^+/Cl^- cotransporter in the distal circumferential tubule of the nephron by competition for Cl^- binding sites and increase Na^+ excretion by inhibiting its reabsorption [10, 11].

In addition, the reduction the carbonic anhydrase inhibition index excludes an inhibition of the enzymatic activity of carbonic anhydrase in the distal circumferential tubule. These findings might suggest that *Tanopati* would not act as an inhibitor of carbonic anhydrase. Indeed, the action of the inhibitors of this enzyme is mainly reflected by a decrease in the proximal reabsorption of sodium and bicarbonates, causing hypovolemia [12].

On the contrary, *Tanopati* caused an increase in the saliuretic index of Na^+, K^+ and Cl^-. These observations suggest that *Tanopati* could act as a loop diuretic. In fact, these diuretics caused an increase in the urinary excretion of the electrolytes, in particular Na^+, K^+ and Cl^-, by inhibition of the $Na^+/K^+/2Cl^-$ cotransporter in the thick region of the ascending branch of the Henle loop [13].

In order to understand the mechanism that *Tanopati* used to induce its diuretic effect, an evaluation of the glomerular filtration rate was made by determining the clearance of creatinine, the indirect marker of choice for the evaluation of the glomerular filtration rate [14]. After one day of treatment, *Tanopati* as well as furosemide® significantly increased (p <0.001) the urinary rate and creatinine clearance. These

results suggest that both *Tanopati* and furosemide increased glomerular filtration in hypertensive rabbits. These results are similar to those obtained with the aqueous extracts of *Retama raetam* [15]. In view of all the above, the antihypertensive activity of *Tanopati* would be exerted through the urinary excretion of sodium. This property may be due to the phytomolecules contained in *Tanopati*, particularly flavonoids and tannins. This hypothesis was developed by [16-17]. These compounds would act either individually or by synergy. The exact mechanism of this activity is not clearly established. It may be due to the stimulation of blood flow or to initial vasodilation, or by the inhibition of tubular reabsorption of water and electrolytes [15-18]. Antihypertensive drugs of *Tanopati* would be similar to those of furosemide®, a loop diuretic.

CONCLUSION

Tanopati is a concentrate of plant extracts. This nontoxic oral preparation revealed an antihypertensive effect which would be exerted by a urinary elimination of the electrolytes, in particular sodium. *Tanopati* therefore has a saliuretic effect as does furosemide®. For this purpose, this traditional recipe would be an alternative therapeutic solution in the treatment of hypertensive pathology.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests regarding the publication of this paper.

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