

## Antihypertensive and antiatherogenic effects of *Tanopati* a traditional recipe used for the treatment of high blood pressure

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### ABSTRACT

**Background:** The objective of the present study was to investigate the possible effect of *Tanopati* on induced hypercholesterolemia in rats and hypertension in rabbits.

**Methods:** Twenty wistar rats were divided into 4 groups each and then subjected to different treatments. Hypercholesterolemia was induced by induced dietary cholesterol; the rats are then treated with *Tanopati* and Questran®. Sixteen rabbits males, divided in four lots with four rabbits each, were used in this study. Hypertension was induced by adrenalin (1 mg/ml for 2 weeks intramuscularly) in the lots 2 to 4. After induction of hypertension in animals, they were treated with the extract of *Tanopati*. The cardiovascular parameters of rabbits (systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) was taken with an electric manometer. These three cardiovascular parameters were then compared to the witness group.

**Results:** The treatment with the *Tanopati* and with Questran® significantly improved ( $p < 0.05$ ) these parameters by decreasing the concentrations of total cholesterol, triglyceride and LDL-cholesterol against an elevation of HDL cholesterol levels. After 8 days of treatments on rabbits induced hypertension with *Tanopati* or with Tenordate®, cardiovascular parameters decreased significantly up to their normalization values.

**Conclusions:** The results obtained confirm the antihypertensive effect of *Tanopati* and justify its traditional use in treatment of high blood pressure.

**Keywords:** *Tanopati*, Antihypercholesterolemia, Cardiovascular index, Adrenaline

### INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality worldwide and hypertension remains the most common cardiovascular disease and a major public health issue in both developed and developing countries.<sup>1</sup>

Hypertension, according to the National High Blood Pressure Education Program (NHBPEP), is defined as systolic blood pressure (SBP) equal or greater than 140 mmHg and diastolic blood pressure (DBP) as equal or more than 90 mmHg, taking antihypertensive medication,

or being told twice by a physician or other professional that one has hypertension. It is also defined as a condition in which the arterial blood pressure is chronically elevated. Hypertension is considered an independent, useful and powerful prognostic indicator for cardiovascular and renal disease, whereas it is significantly associated with the increased morbidity and mortality from cerebrovascular disease, myocardial infarction, congestive heart failure and renal insufficiency.<sup>1</sup> Various studies indicate that high levels of serum cholesterol are closely linked to atherosclerosis and increased risk of various cardiovascular diseases.

Cardiovascular diseases, including coronary heart disease, stroke (stroke) and hypertension have some major health threats worldwide today. High plasma lipids (hyperlipidemia and other abnormal blood lipid profile) are among the risk factors commonly involved in most cardiovascular problems.

Under these conditions, lipids and other related substances accumulate on the arterial wall, forming a plate which occludes the lumen and obstructs blood flow to vital organs such as the heart, brain, liver, or kidneys. Lipids with significant elevations are involved in these diseases are cholesterol and triglycerides. They are generally transported in the form of lipid-protein complexes called lipoproteins, which are classified according to their density and their loads. Cholesterol and high density lipoprotein (HDL-C) transports lipid blood cells in the liver, while the low-density lipoprotein cholesterol (LDL-C) mobilizes lipids in the cells and blood vessels. The increased levels of triglycerides, total cholesterol and LDL-C and HDL-C reduction promote the development of atherosclerosis and related cerebrovascular disorders.

The treatment management is usually a lifelong treatment and the cost of treatment is not affordable to the majority of the developing countries population that has recourse to a recipe from medicinal plants.

In the last three decades, a lot of concerted efforts have been channeled into researching into local plants with hypotensive and antihypertensive therapeutic values, with the therapeutic potentials of some of these medicinal plants either validated or outrightly disproved. However, attempts by the low-income group, particularly the rural dwellers in the developing countries, to control hypertension and its attendant complications in the face of the scarce socioeconomic resources, have led more people opting for herbal remedy.<sup>2</sup>

Identification of antihypertensive foods and herbal remedies is currently interested because they are expected to prevent hypertension with lower side-effects than antihypertensive drugs.<sup>3</sup> But, these drugs have various side effects that could lead to the malfunctioning of the body function e.g. formation of gall stones, gall bladder diseases, itching and diarrhea. Therefore, much attention has been shifted to the use of natural products from plants with very few side effects in the treatment of atherosclerosis.<sup>3</sup> The mechanisms of these products are elicited to counteract the effect of hypertension and associated risk factors such as hypercholesterolemia, hypertriglyceridemia, and oxidative stress on blood vessel walls. They include direct vasodilation of the blood vessel, blocking of calcium channels, inhibition of  $\alpha$ -adrenoreceptor response, induction of negative inotropic response of smooth muscle, inhibition of platelet aggregation, reduction of vascular resistance, and improvement of pulmonary oxygen utilization.<sup>4</sup>

Enhanced activity of nitric oxide and improved handling of intracellular calcium has also been found to play a critical role in the reduction of vascular resistance and blood pressure that are elevated in hypertensive rats and humans.<sup>5</sup>

Many researchers are actively looking for antihypertensive compounds derived from various natural products for the use in functional foods. Several studies have shown that food ingredients rich in flavonoids and other polyphenols can lower blood pressure.<sup>6</sup> Ours earlier studies have demonstrated that the extract of *Tanopati*, a polyherbal formulation used in treatment of high blood pressure is rich in phenolic compounds such as tannins, flavonoids, leucoanthocyanins, anthocyanins.<sup>7</sup>

The aim of the present study was to investigate the possible effect of *Tanopati* on induced hypercholesterolemia in rats and hypertension in rabbits.

## METHODS

### Chemicals

Quesran<sup>®</sup>, was obtained from a pharmaceutical company in Abidjan (Cote d'Ivoire). HDL -Cholesterol, Total Cholesterol and Triglyceride Kits, Potassium Phosphate buffer and Cholesterol, were obtained from Roche Diagnostics (France).

### Plant material

The plant material is a recipe obtained from the decoction of roots, leaves and bark of ivoirian traditional medicine plants. These are: *Ageratum conyzoides*, *Newbouldia laevis*, *Phyllanthus muellerianus*, *Aloe vera* and *Cassia occidentalis*. This recipe was appointed by the health *Tanopati* traditional healer.

### Animals

Male's wistar rats weighing 150-200 g procured from the animal house of the faculty of pharmaceutical and biological sciences, Félix Houphouët-Boigny University of Abidjan. Rabbits males' of the species *Oryctolagus cuniculus*, aged 10 weeks weighting 1800 to 2200 g were used. These animals were brought from a poultry farm in Abidjan, were acclimated for two weeks at the Animal unit of the faculty of pharmaceutical and biological sciences, Félix Houphouët-Boigny University of Abidjan. All these animals were housed in plastic cages where they had free access to water and food, and kept at a temperature of  $22\pm 3^\circ$  C with a relative humidity of 50.15%. The cycle of light and darkness was 12 h/12 h. All the experimental procedures were approved by the Ethical Committee of Health Sciences, Félix Houphouët-Boigny University of Abidjan. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals.

### **Preparation of lyophilized extract *Tanopati***

This recipe was provided by M. Adou Tano Albert, an Ivorian traditional practitioner. The decoction was then lyophilized and stored in the freezer for the study.

### **Evaluation of antiatherogenic activity of lyophilized extract *Tanopati***

This study was to induce hypercholesterolemia with dietary cholesterol and to evaluate the antihypercholesterolemic effect of *Tanopati* using Questran® as reference product.

#### *Experimental Protocol*

The experiment was conducted according to the method of Erukainure et al.<sup>8</sup>

Twenty (20) male albino rats were divided into 4 groups of 5 animals each and then subjected to different treatments.

- Group 1 (normal control) has received from corn oil (0.3 ml / rat) throughout the experiment.
- Group 2 (experimental control) RCU has only cholesterol (40 mg/kg bw) for the duration of the experiment.
- Group 3 (*Tanopati*) has received cholesterol (40 mg/kg bw) then *Tanopati*
- Group 4 (Questran®) has received cholesterol (40 mg / kg BW) followed Questran® (260 mg/kg bw).

The administration of Questran®, *Tanopati*, and cholesterol were given orally 5 times a week for 6 weeks using an intragastric tube to the volume of 5 mL/kg bw. Corn oil was used as a vehicle to *Tanopati*, Questran® and cholesterol. The dose of the extract of *Tanopati* was calculated from the dosage given by the health triadpractitioner.

At the end of 6 weeks of treatment, animals were sacrificed under light anesthesia with ether vapor. The blood collected in EDTA tubes was centrifuged at 3000 revs/min for 10 min and the supernatant (plasma) was used for the determination of total cholesterol, HDL cholesterol and triglycerides.

#### *Estimation of biochemical parameters of interest*

Total cholesterol was assayed by the method of cholesterol oxidase (CHOD)/peroxidase (POD).<sup>9</sup> HDL cholesterol was measured by the method precipitation.<sup>10</sup> The concentration of serum LDL is determined by the difference method of the equation by Friedewald et al.<sup>11</sup> Triglycerides were assayed by the method of the oxidase glycerol 3-phosphate (GPO)/peroxidase (POD).<sup>12</sup>

The atherogenic index was calculated by the following formula:

$$\text{Atherogenic index} = \frac{\text{Cholesterol total}}{\text{Cholesterol HDL}}$$

### **Effect of *Tanopati* on blood pressure and heart rate**

#### *Chemicals and drugs*

Adrenalin and Ténordate® were obtained from local office, Abidjan, Cote d'Ivoire, *Tanopati* was gifted by an Ivorian traditional practitioner, all reagents, solvents and chemical compounds used for analysis met the quality criteria in accordance with international standards

#### *Experimental protocol*

The experiment was performed by the method described by Tiekpa et al.<sup>13</sup> Sixteen (16) male rabbits were divided into 4 lots of 4 rabbits (Lot1to 4). Lot 1 or witness lot received throughout the duration of the experiment distilled water; Lot 2-6 or experimental lots were intramuscularly injected with an insulin syringe, adrenaline dosed at 1 mg/ml to cause elevated blood pressure or hypertension, which was later stabilized after 14 days of treatment. Blood pressure of rabbits was taken with an electric manometer which cuff was adapted to the leg of rabbit. The cuff was wrapped around the left hide leg of the animal and its inflation gauge enabled with the on button. The cuff inflates to the maximum to tighten up the leg of the animal and deflates immediately. It appears on the manometer screen, systolic pressure, diastolic pressure and the heart rate of rabbit. These three cardiovascular parameters of rabbits were then compared to the witness group. After induction of hypertension in animals, they were treated with the *Tanopati* and Ténordate® a reference antihypertensive sold in the market. Each lot was treated as follows:

LOT 1: Witness + distilled water

LOT 2: Witness treated with adrenaline (Adr)

LOT 3: Treated with Adrenalin + Ténordate® (Atenolol + Nifedipine) (10 mg/kg bw)

LOT 4: Treated with Adrenalin + *Tanopati* (10 mg/kg bw)

#### *Statistical analysis*

The results are expressed as mean±SEM. The results were analyzed using one-way ANOVA followed by Turkey's multiple comparison tests. Data was computed for statistical analysis by using graph pad prism 5 Software. P values <0.05 were considered as significant.

## **RESULTS**

### **Effect of the extract of *Tanopati* on induced hypercholesterolemia in rats**

The results show that plasma concentrations of total cholesterol, triglyceride and LDL cholesterol were significantly higher (p <0.05) in the group addicted to

dietary cholesterol, while HDL cholesterol rate is lower than the normal group control. The treatment with the Questran® and *Tanopati* significantly improved ( $p < 0.05$ ) these parameters by decreasing the concentrations of total cholesterol, triglyceride and LDL-cholesterol against an elevation of HDL-cholesterol levels compared to rats of hypercholesterolemia group. Furthermore we see that there's a significant difference ( $p > 0.05$ ) results between the group treated with *Tanopati* and the other treated with Questran® regarding the concentrations of total cholesterol and LDL cholesterol, but no significant difference ( $p > 0.05$ ) results in terms of triglyceride and HDL for both groups.

The link between dyslipidemia cardiovascular diseases is particularly well established. The elevation of Total Cholesterol and LDL Cholesterol is associated with an increased cardiovascular risk. A low concentration of HDL can be regarded as an additional risk factor, then a high concentration of the HDL is a protective factor. Because of this inverse relationship between cardiovascular risk and LDL one hand, the other hand HDL, it has been proposed that the ratio [total cholesterol/HDL] or atherogenic index as precher cardiovascular risk. Particularly cardiovascular risk increases when this ratio exceeds 5. Figure 36 shows the estimated cardiovascular risk of different groups. The results indicate that cardiovascular risk is very High significantly ( $p < 0.05$ ) in the group of rats addicted to cholesterol compared to that of animals of normal control groups. Treatment with *Tanopati* and Qestran® extract significantly decreased ( $p < 0.05$ ) this risk with values below normal for *Tanopati*.

**Effect of *Tanopati* on blood pressure and heart rate in rabbits (BP and HR)**

Table 1 shows the values of cardiovascular parameters systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) of animals of the control lots, sick not treated and treated with *Tanopati* and Tenordate® (atenolol+nifedipine).

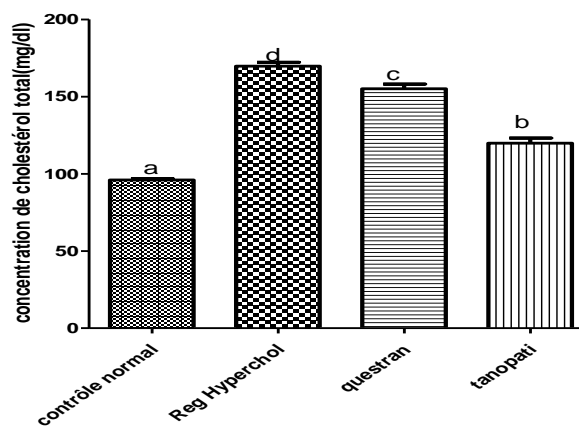
Cardiovascular parameters values for animals in witness lot, SBP, DBP and HR were respectively 123.4±0.2 mmHg for SBP; 81.1±3 mmHg for DBP and 230.8±5.7 beat/min for HR. These values increased significantly ( $p < 0.05$ ) after the induction of hypertension in rabbits respectively to 178.5±0.5 mmHg for SBP, 117.43±1.33 mmHg for DBP and 329; 97±2.3 beat/min for HR that is a percentages increase of 44, 65.43%, 44.79%, 42,96% respectively. These parameters (SBP, DBP and HR) decreased significantly ( $p < 0.05$ ) up to their normalization with the treatment of Tenordate® (10 mg/kg bw) and *Tanopati*, at doses of 10 mg/kg bw. The SBP values change from 178.5±0.5 mmHg to 120.7±1.6 mmHg for SBP, 117.43±1.33 mmHg to 83±1.3 mmHg for DBP and 329; 97±2.3 mmHg to 231±1; 21 mmHg for after 14 days of treatment with *Tanopati*. With Tenordate® (10 mg/kg.bw), the values obtained are

117.84±0.7 mmHg, 79.61±2.1 mmHg and 223±4.5 mmHg for SBP, DBP and HR respectively.

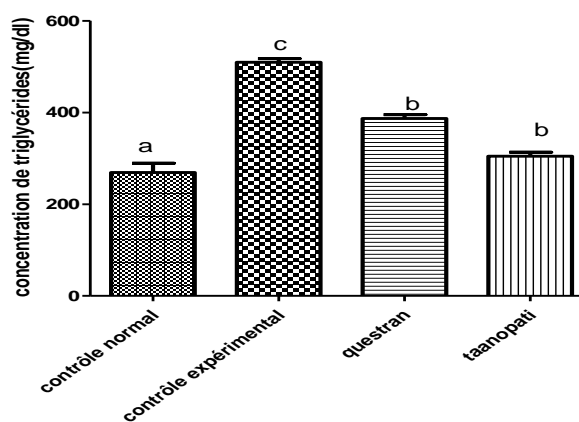
**Table 1: Values of SBP, DBP, and HR in rabbits treated and not treated with adrenaline, the Tenordate and *Tanopati* after 14 days of treatment.**

Treatments	Cardiovascular parameters		
	SBP	DBP	HR
Distilled water	123.4± 0.2 <sup>a</sup>	81.1± 3.0 <sup>a</sup>	230.8± 5.7 <sup>a</sup>
Adrénaline	178.5± 0.5 <sup>b</sup>	117.43± 1.33 <sup>b</sup>	329.97± 2.3 <sup>b</sup>
Adrénaline+ <i>Tanopati</i>	120.7± 1.6 <sup>a</sup>	83±1.3 <sup>a</sup>	231± 1.21 <sup>a</sup>
Adrénaline+ Ténordate®	117.8± 0.7 <sup>a</sup>	79.61± 2.1 <sup>a</sup>	223± 4.5 <sup>a</sup>

Values are means±SEM for 4 rabbits. a and b are row values with different superscripts are significantly different ( $p < 0.05$ ). SBP: systolic blood pressure, DBP: diastolic blood pressure, HR heart rate.



**Figure 1: Effect of the extract of *Tanopati* on total cholesterol.**



**Figure 2: Effect of the extract of *Tanopati* on triglycerides.**



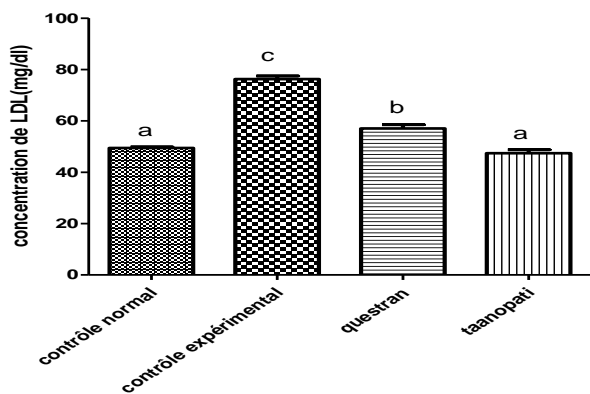


Figure 3: Effect of the extract of *Taanopati* on LDL.

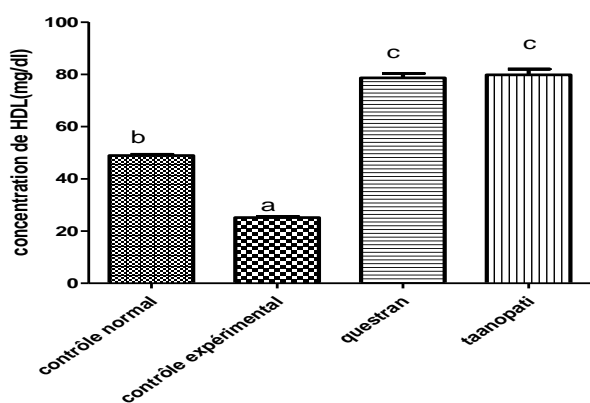


Figure 4: Effect of the extract of *Taanopati* on HDL.

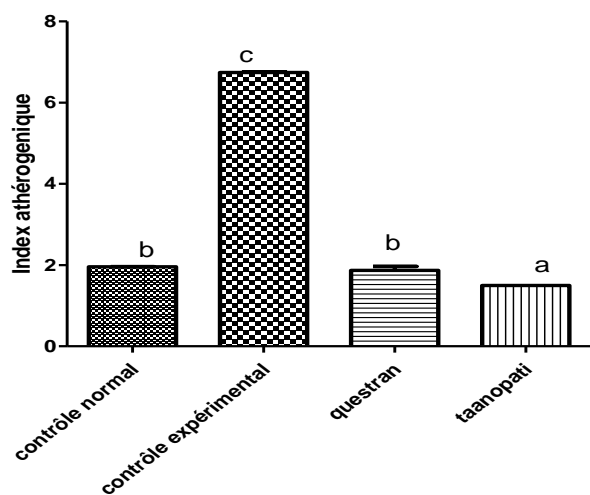


Figure 5: Effect of the extract of *Taanopati* on atherogenic index.

## DISCUSSION

The results show that dietary cholesterol intake five times a week for six weeks in rats causes hypercholesterolemia resulted in an increase in total cholesterol, triglycerides

and LDL cholesterol and decrease HDL cholesterol. In those circumstances, the lipids accumulate on the arterial wall, thereby forming a plate which occludes the lumen and reduces blood flow to vital organs such as the heart, brain, liver, or kidney. This narrowing of the vascular lumen would result in the elevation of blood pressure. The increase in the level of triglycerides, total cholesterol and LDL cholesterol and reduced HDL cholesterol thus promote the development of atherosclerosis and hypertension.<sup>14</sup>

Our results indicate that the extract of *Taanopati*, administered at the doses of 10 mg/kg bw causes a decrease of the concentrations of triglycerides, total cholesterol and rendered LDL serum cholesterol in rats hypercholesterolemic by consumption of dietary cholesterol. The underlying mechanism by which the extract *Taanopati* exercised its hypocholesterolemic effect may be a decrease in intestinal absorption of cholesterol by binding with the bile acids in the intestine and increased biliary excretion.<sup>15</sup>

*Taanopati* could also act by reducing cholesterol biosynthesis specifically by decreasing the activity of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase.<sup>16</sup> In addition, the *Taanopati* could lower the concentration of serum cholesterol by modifying the lipoprotein metabolism, enhancing the absorption of LDL by the LDL receptors increase and/or increasing the activity of lecithin acyl transferase-cholesterol (LCAT).<sup>17</sup>

The link between dyslipidemia and cardiovascular diseases is particularly well established. The elevation of Total Cholesterol and LDL Cholesterol is associated with an increased cardiovascular risk. A low concentration of HDL-C can be regarded as an additional risk factor, and then a high concentration of HDL-C is a protective factor. Because of this inverse relationship between cardiovascular risk and LDL one hand, the other hand HDL, it has been proposed to use the ratio [total cholesterol/HDL-C or atherogenic index as cardiovascular risk predictor. The cardiovascular risk increases particularly when this ratio exceeds 5 according to the Framingham study. Our results indicate a ratio of 6.7 in rats made hypercholestérmiques, meaning exposure to high risk of cardiovascular disease. However, the treatment with the *Taanopati* significantly reduced this index in hypercholesterolemic animals by passing 6.7±40.2 to 1.5.

This could still justify antihypercholestérolémique effect of the extract of *Taanopati*. According to Nwankpa et al, *Phyllanthus* sp has a protective role against cardiovascular disease by significantly reducing the serum total cholesterol, LDL, triacylglycerides and atherogenic index which is a predictor of risk factor, and by the significant increase in HDL.<sup>18</sup> The presence of *Phyllanthus* sp, *Taanopati* in the recipe so to justify the low atherogenic index and antihypercholestérolémique effect observed.

Furthermore, the phytochemical screening extract *Tanopati* indicated that it contains flavonoids, tannins and saponin glycosides. Flavonoids and tannins play an important role in lipid metabolism. Flavonoids block specific enzymes. For example, flavonoids block converting enzyme (ACE), which increases blood pressure, cyclooxygenase (COX) and prostaglandin-endoperoxide synthase (PTGS), catalyze the formation of prostaglandins from arachidonic acid.<sup>19</sup> Flavonoids also protect the vascular system and strengthen capillaries that carry oxygen and vital nutrients to all cells.<sup>19</sup> As tannins, they form complexes with proteins and make them unavailable for the cells.<sup>20</sup> The significant increase in serum concentration of triglycerides observed may be due to the ability of tannins extract *Tanopati* to form complexes with the HMG CoA reductase inhibitor and cyclooxygenase. These tannins and prevent normal metabolism enzymes leading to lipid accumulation in the serum. Extract *Tanopati* thus improves the lipid profile in rats and reduces atherogenic risk.

Hypertension was induced in rabbits after 14 days of daily treatment with adrenalin. This hypertension would settle according to various molecular mechanisms<sup>7</sup>. I) At the heart, the link coupled with the G protein (Gs) to  $\beta 1$  receptors leads to activate adenylyl cyclase (AC), which converts ATP into cAMP. The cAMP activate protein kinase A (PK-A) which in the turn allows phosphorylation of the calcic channels thus involving the increase the flow of the calcium from endoplasmic reticulum. It follows from there an increase in the force (positive inotropic effect) and the frequency (positive chronotropic effect) of the heart. This increase in force and frequency of the heart then cause elevation of blood pressure.<sup>21</sup> (ii) The adrenaline in the blood vessels directly activates natriuretic peptide and vasopressin which activate the calcium channels in the endothelium causing the release of calcium and increased calcium flow as a result of vasoconstriction leads to the elevation of blood pressure.<sup>22</sup> (iii) The adrenaline binds to the  $\beta$ -adrenergic receptor present on the juxtaglomerular kidney unit to activate adenylyl cyclase (AC), which converts ATP into cAMP. The cMPA activates protein kinase A which involves the release of renin. Renin converts angiotensin I to angiotensin II, leading to vasoconstriction and thus high blood pressure.<sup>23</sup>

In this study, the results show that the extract of *Tanopati* normalizes in eight days cardiovascular parameters namely systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) of hypertensive animals. The phytochemical screening showed that the extract *Tanopati* is rich in polyphenol, while according to Lorenz et al.<sup>24</sup> polyphenols act by rapid dose-dependent and sustained activation of the PI3K/PKB (phosphatidylinositol 3-kinase/protein kinase B) pathway which phosphorylates the endothelial nitric oxide synthase (eNOS). This phosphorylation occurs on serine 1197 independently of the intracellular  $Ca^{2+}$  and activation of eNOS in response to the increase in

intracellular  $Ca^{2+}$  via the  $Ca^{2+}$  complex-CAM (calcium-calmodulin).<sup>24</sup> The production of NOS would thus involve a vasodilation and consequently a normalization of cardiovascular parameters.

The vasodilator and vasculoprotective effect of polyphenols is potentiated by amplification of the effectiveness of endogenous antioxidants, like catalase, inhibition of the pro-oxidant enzymes (NADPH oxidase) and the dismutation of the surrounding ROS. We used Ténordate® as an antihypertensor of reference because Ténordate® is an association of atenolol and nifedipine. Our results show that the extract of *Tanopati* has the same effects as Ténordate®, it could thus act by its mechanism.

Indeed, Nifedipine is a calcium antagonist and atenolol, a  $\beta$  blocker. Ténordate® acts by inhibiting the calcium channel, preventing the entry of calcium into the contractile structures, thus causing the decrease in cardiovascular parameters or by binding to adrenergic  $\beta$  receptors, consequently preventing the release of calcium into the intracellular environment. This results in vasodilation and a decrease in systolic blood pressure, diastolic blood pressure and heart rate.<sup>25</sup> The extract of *Tanopati* could therefore contain antagonist compounds of calcium and/or  $\beta$  blockers.

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