Cardiovascular effect of the fermented seeds of *Parkia biglobosa* on rabbits *Oryctolagus cuniculus*

ABSTRACT

Background and objectives: Fermented seeds of *Parkia biglobosa* or "African mustard" are widely consumed in Togo. These fermented seeds are known to have a protective effect against the occurrence of cardiovascular diseases. The objective of the present study is to evaluate the effect of the decoction of fermented seeds on cardiovascular system. **Methods:** The fermented seeds have been subjected to physicochemical tests. A decoction was prepared and underwent qualitative and quantitative phytochemical tests. The decoction was also administered intravenously to rabbits at cumulative doses (17.5 mg / kg; 35 mg / kg; 70 mg / kg and 140 mg / kg) to assess the effect of *Parkia biglobosa* fermented seeds on the cardiovascular system. Parameters such as blood pressure, heart rate, and electrocardiogram (ECG) were then recorded. **Results:** the fermented seeds had a water content of 8.1 ± 0.2%, the total ash content was 21 ± 0.4% and the content of ash insoluble in hydrochloric acid of 2.3 ± 0.1%. The extract contained alkaloids, cardiac glycosides (with a content of 13.9 ± 0.2 µg equivalent of Digoxin / mg of extract) and saponins. The decoction causes a dose-dependent hypotension and bradycardia at 140mg / kg. The ECG shows a decrease of the amplitude of QRS complex at doses of 70 mg / kg and 140 mg / kg. **Conclusion:** the decoction has a dose-dependent hypotensive effect probably linked to its cardiotonic glycosides.

Keywords: *Parkia biglobosa*; fermented seeds, decoction, cardiac glycosides; hypotension; ectrocardiogram.

1. INTRODUCTION

Cardiovascular disease is the prevalent cause of morbidity and mortality in the world, affecting many millions of individuals every year [1]. According to a report by the World Health Organization in 2017, over 17.7 million deaths worldwide are related to cardiovascular disease and three-quarters of these deaths occur in low-income countries and middle [2]. Difficult access to adequate health services, the high cost of drugs, the harm that these drugs can cause and the low purchasing power of populations ultimately leads to non-compliance with treatments. [3,4].This leads most people to turn to herbal medicine as prime alternative related to its availability, its accessibility and the existence of plant species that can help prevent or treat cardiovascular disease [5,6,7]. *Parkia biglobosa* (Mimosaceae) more known as "néré", in Togo is one of the plants which can be helpful in cardiovascular disorders management. Studies have reported beneficial effects associated with the consumption of the plant on the cardiovascular system including antihypertensive and cardioprotective

effects [8,9].The upstream prevention against occurrence of risk factors of these diseases is the best way to fight and there is a correlation between our diet and the occurrence of certain risk factors, including hypertension, dyslipidaemia and diabetes; be cured by feeding becomes important [8]. The fermented seeds of *Parkia biglobosa* gives a condiment commonly known as "afitin" or "dzotu" which is used by several people [8] mainly to flavor sauces. The aim of this work is to evaluate the effect of the outcome of this condiment decoction on the cardiovascular parameters of rabbits.

2. MATERIALS AND METHODS

2.1. Plant material

Fermented seeds of *Parkia biglobosa* were purchased at Tchaoudjo'smarket in Sokode, a town in the northern part of Togo.

2.2. Preparation of the decoction

In a flask 2 g of fermented seeds were added to 100 mL of distilled water and the whole was heated under reflux at 100°C for 15min. The decoction obtained was filtered with filter paper and kept refrigerated until use.

2.3. Physicochemical tests

The physicochemical tests were made to assess the quality of the fermented seeds. Methods recommended in the European Pharmacopoeia were followed for determining the water content, total ash value and acid insoluble ash content in fermented seeds of *Parkia biglobosa* [10,11].

2.4. Determination of cardiac glycosides

For the determination of the cardiac glycosides 1 mL of the extract was mixed with 10 mL of Baljet's reagent. The mixture was incubated for one hour and diluted with 20 mL of distilled water. The mixture's absorbance was then read on a spectrophotometer at 495 nm. The content of cardiotonic glycosides was determined from a calibration range with Digoxin (10-100 µg / mL) and results were expressed in mg of Digoxin equivalent per g of dry extract (mg DEQ / g). Three tests were performed for the sample [12].

2.5. Animals

The rabbits (*Oryctolagus cuniculus)* were used for the experiment, they weighed between 1.2 and 2 kg and were purchased from a breeder at Adidogomé neighborhood in Lomé. These animals were acclimated at the animal unit of the Faculty of Science (FDS) of the University of Lomé for a week before handling. They had free access to water and food and subjected to a regular 12 hours of light and 12hours of dark cycle.

2.6. Experimental design

Six rabbits were used for this experiment. The rabbits were anesthetized with ethyl carbamate 80% at 1.2 g / kg body weight according to the method of Van Vliet [13]. Anesthetized rabbit was placed supine and his jugular vein and carotid artery were intubated after dissection. The jugular vein was used for administration of different doses of decoction in the general blood flow and changes in blood pressure were recorded at the carotid artery. The value of 80 mmHg was set as the base value on the pressure sensor before the introduction of the catheter into the carotid artery. Any pressure change after the introduction of the catheter is the normal blood pressure of the animal. The animals were prepared as described above and received single doses of extract (17.5;35;70; 140 mg / kg) at 1 mL / kg; the reference solution used was that of Marc Ewen glucose at pH 7.4.At the same time, the electrocardiogram electrodes were inserted under the skin in the armpits and in the folds of the groin. The parameters such as blood pressure (BP), heart rate (HR) and electrocardiogram (ECG) were recorded after each administration.

2.7. Heart rate and blood pressure recording

The measurement of blood pressure was performed through a sensor provided with a pressure-sensitive membrane. This sensor consists of a transducer (Reusable Bridge Transducer Pod) is used to transmit pressure variations at the carotid artery to a module (ADInstruments Bridge Pod, PowerLab 26T) which receives the pulses with this transducer. This module transmits the pulse to a recording system provided with a computer through software (LabChart 8).

2.8. Statistical analysis

The results were expressed as mean ± standard error of the mean. They were treated with the software GraphPad Prism 8.0 (GraphPad Software Inc., California) and Excel 2019. The analysis of variance ANOVA followed by Tukey's test was used to compare means. Significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

The results of the physicochemical tests allowed us to assess the quality of the fermented seeds. The water content in the sample was 8.1 ± 0.2%; this content is less than 10% set by the European Pharmacopoeia [9].This shows that the fermented seeds of *Parkia biglobosa* were well dried without risk of damage[14]. The total ash content in the sample was 21 ± 0.4%, this content is well above the 14%recommended by the European Pharmacopoeia. This high content can be explained by a significant proportion of minerals in sample [14]; it is also related the sample clean condition [15].The content of insoluble acid ash is 2.3 ± 0.1%. This content exceeds 2% set by the European Pharmacopoeia; this can be explained by the presence of traces of sand adhered to the drug[14]. The contents of total ash and ash insoluble in hydrochloric acid give information on the hygienic preparation of our sample (Table 1).

Table 1: Table of physicochemical tests on fermented seeds of Parkia biglobosa.

Parameters	***Parkia biglobosa***
Water content (%)	21 ± 0.4
Ash value (%)	8.1 ± 0.2
Acid insoluble ash content (%)	2.3 ± 0.1

Parameters are expressed as mean ± SEM: Standard Error of the Mean (n = 3).

The phytochemical screening of the decoction showed the presence of saponins, alkaloids and cardiac glycosides; on the other hand, there is an absence of tannins, flavonoids and anthracene heterosides (Table 2). These results are similar to those described by Biobakuand *al*.(2017) [16,17].They differ from those obtained by Ouolouhoand *al*. (2017), in particular on the presence of flavonoids, tannins and the absence of alkaloids in the aqueous and ethanolic extracts of fermented seeds [18]. These differences may be related to intrinsic factors, the solvent used and the extraction procedure that can influence the composition [19,20].

Table 2: Phytochemical screening of the decoction of fermented seeds of Parkia biglobosa.

Chemical groups	**Decoction of *Parkia biglobosa***
Alkaloids	+
Flavonoids	-
Anthracene glycosides	-
Cardiac glycosides	+
Total phenols and tannins	-
Saponins	+

+ : presence ; - : absence.

The content of cardiac glycosides of the decoction of fermented seeds of *Parkia biglobosa* was determined from a linear regression curve of Digoxin (OD = 0.1011C-0.2562; R^2 = 0.9809). The content obtained is 13.9 ± 0.2 mg DEQ / g of extract. The value of cardiac glycosides is expressed as mean ± SEM (n = 3) (Figure 1). Note that cardiac glycosides are highly toxic compounds even at low doses due to their very narrow therapeutic range [21].

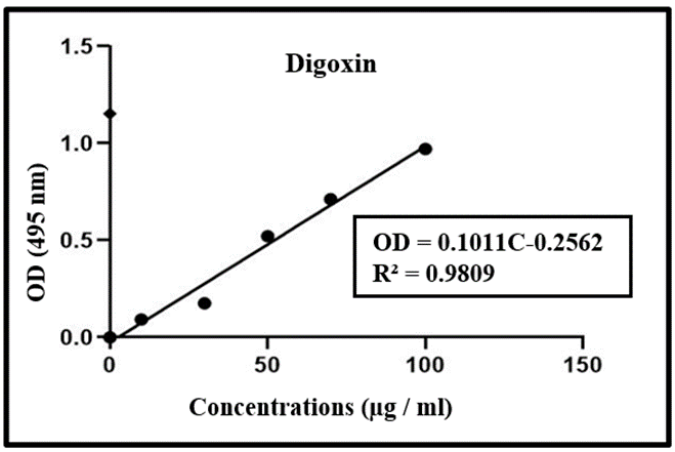

Figure 1: Linear regression curve of Digoxin.

OD: optic density; R^2: correlation coefficient; C: concentration

The decoction induced a dose-dependent reduction in blood pressure (Table 3 and Figure 2); this reduction is significant at the dose of 140 mg / kg (32.5 ± 2.9 mm Hg; $p<0.05$) compared to the control. There was also a significant decrease in heart rate at the dose of 140 mg / kg (217 ± 4.2 beats / min; $p<0.01$) compared with the control (Table 4). These results show that the decoction acts on the cardiovascular system (heart and blood vessels) by inducing hypotension and bradycardia. These effects were likely related to the presence of cardiac glycosides which are cardiac tropism compounds. Indeed, cardiac glycosides are compounds that enhance, regulate and slow down the heart [21].The slowing of the heart rate or negative chronotropic could explain the decrease in heart rate. Blood pressure is dependent on cardiac activity, one can also hypothesize hypotension by negative chronotropy.

Table 3: Effects of different doses of the decoction of fermented P. biglobosa seeds on blood pressure.

		Doses (mg/kg) n=6			
	Control	**17.5 mg / kg**	**35 mg / kg**	**70 mg / kg**	**140 mg / kg**
BP (mmHg)	59.6 ± 2.8	58.4 ± 3	57.7 ± 3.7	40.6 ± 3.9	32.5 ± 2.9*

BP: blood pressure; results are expressed as means ± ESM. Significantly different compared to Control * $p<0.05$.

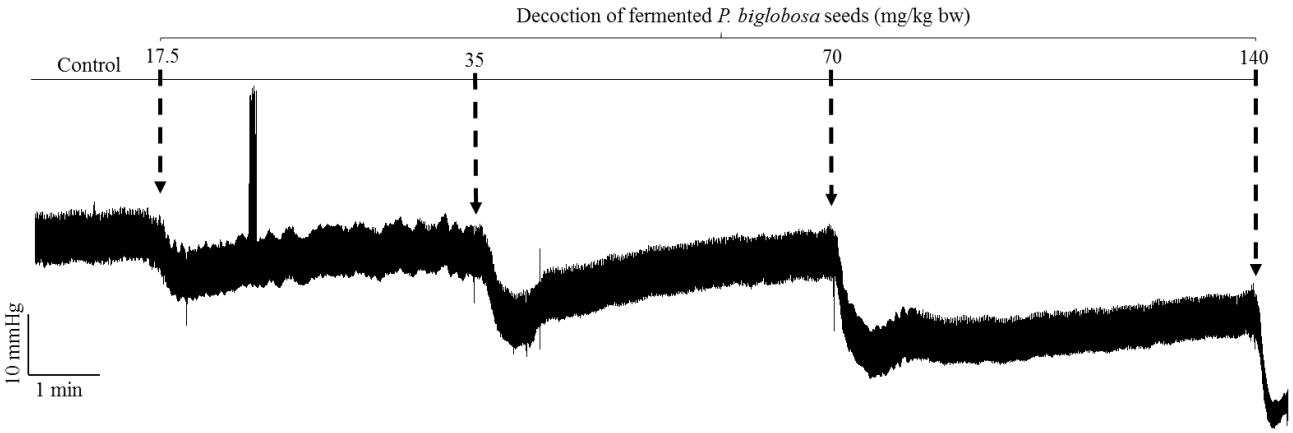

Figure 2: Appearance of the blood pressure tracing after administration of each dose.

Table 4: Effects of different doses of the decoction of fermented Parkia seeds biglobosa on heart rate.

		Doses (mg / kg) n=6			
	Control	**17.5 mg / kg**	**35 mg / kg**	**70 mg / kg**	**140 mg / kg**
HR (beats / min)	245 ± 4.2	241.7 ± 7.8	246 ± 8.3	232.2 ± 10.4	217 ± 4.2**

The results are expressed means ± ESM. Significantly different compared to Control ** $p<0.01$.

The rabbits'ECG before administration of our extract shows a characteristic appearance with the negative wave P (Figure 3); one is in the presence of an ECG rhythm with the coronary sinus. Coronary sinus rhythm is an accessory pacemaker that is particularly active in some subjects and has no pathological value [22].In our study, administration of the decoction showed changes in the ECG compared to the control. A very significant decrease of the amplitude QRS is noted at the doses of 70 mg / kg and 140 mg / kg compared with the control; this decrease is more visible at the dose of 140 mg / kg with a QRS complex in appearance almost iso-biphasic (Figure 3). There is also a very significant decrease in the duration of the ST interval at a dose of 140 mg / kg compared with the control. The significant decrease in the amplitude of the QRS complex may be related to inhibition of ventricular depolarization that would decrease the force of contraction of the myocardium, which is reminiscent of a negative inotropic effect [23].This is contrary to the inotropic effect (+) that is to say, the increase in strength and the contraction of the heart rate that normally induce cardiac glycosides. This suggests the likely toxicity of the extract at the dose of 140 mg / kg. The decrease in the ST segment may be related to early ventricular repolarization or at least in part to delayed depolarization. It could also be linked to the bradycardic effect induced by the extract [24].

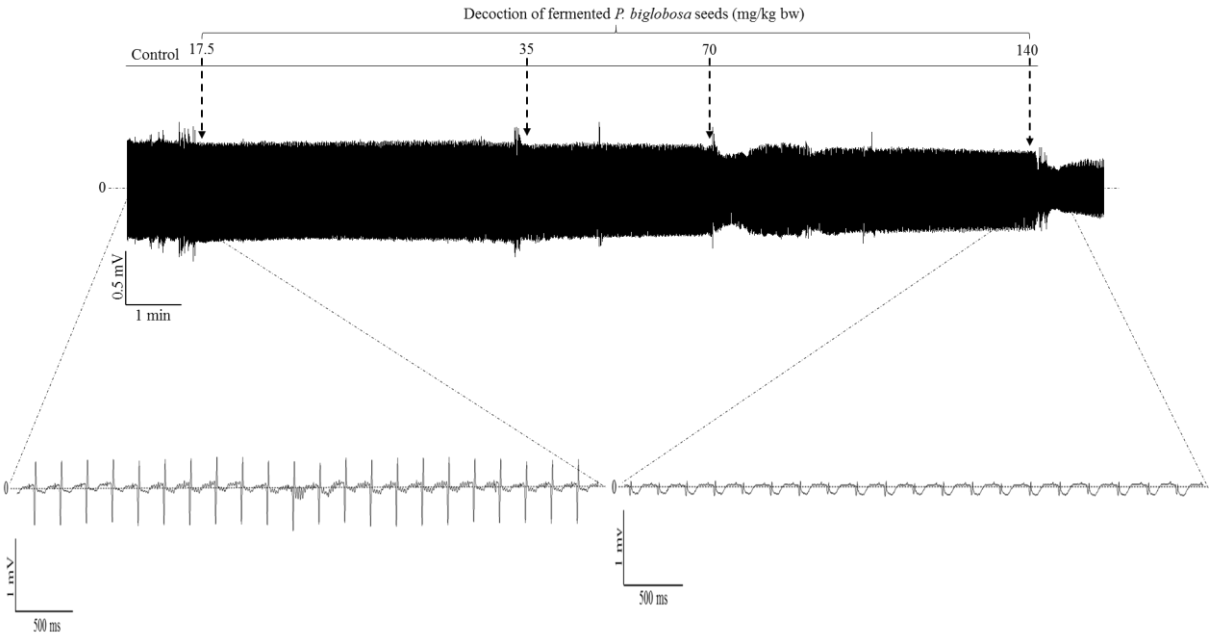

Figure 3: Appearance of the ECG before and after administration of decoction to rabbits.

4. CONCLUSION

These fermented seeds of *Parkia biglobosa* are known to have a protective effect against the occurrence of cardiovascular diseases in traditional medicine in Togo. the results of this study show that this fermented seeds has blood pressure lowering effect, a bradycardic effect and induces a decrease of the QRS complex. These results may justify the use of this plant in the management of

hypertension. Further studies need to be conducted to confirm these cardiovascular effects and identify active molecules.

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AUTHOR CONTRIBUTIONS

Benjamin Apedjinou, Mindede Assih, Sabrina Sanvee, Komlan M. Dossou-Yovo, Aboudoulatif Diallo and Batomayena Bakoma conceived and realized the present study.

Sabrina Sanvee and Benjamin Apedjinou wrote the article and runned the chemical assays.

Benjamin Apedjinou, Mindede Assih, and Komlan M. Dossou-Yovo runned the biological tests.

Each author read and approved the manuscript.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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