ORIGINAL ARTICLE

HYPOTENSIVE AND ANTIHYPERTENSIVE PROPERTIES OF AQUEOUS EXTRACT OF *Cassia occidentalis* (Caesalpiniaceae) ROOTS ON ANESTHETIZED RABBITS

| Tohandrainy, Mbola Adrien [1] | Rakotoarison, Olivier [2]* | Lahadson, Stanislas Fulgence [3] | Rabemandroso, Faramalala Louise [3] | Ravelomanantsoa, Germier [4] | and | Rajemiarimiraho, Manitriniaina [3,5] |

[1.] University of Antananarivo | Mention Chimie I Parcours Arôme Cosmétique Phytomédicament |Faculté des Sciences | Madagascar |
[2.] University of Antananarivo | Faculty of Sciences | Antananarivo | Madagascar | Laboratoire de Pharmacodynamie |
[3.] University of Antananarivo | Faculty of Sciences | Laboratoire de Chimie des Substances Naturelles et Chimie Organique Biologique (LCSN/COB)| Antananarivo | Madagascar |
[4.] University of Antananarivo | Faculty of Sciences | Laboratoire de Physique nucléaire et physique de l'environnement | Antananarivo | Madagascar |
[5.] University of Antananarivo | Mention Génie Rural | Ecole Supérieure Polytechnique | Madagascar |

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ABSTRACT

Background: *Cassia occidentalis* (Caesalpiniaceae) is used in traditional medicine to treat many diseases including hypertension. **Objectives:** This study evaluated the potential hypotensive and antihypertensive effects of aqueous extract of its roots. **Methods:** Roots extract (EABCO) was made by decoction. The activity of EABCO was tested in vivo on normotensive rabbits under anesthesia for arterial pressure lowering effect. The responses were measured using Ludwig manometer. **Results:** With dose going from 100 to 500 mg/kg of body weight (b.w.) EABCO, there is an induction of hypotension comparable to that seen with acetylcholine at the doses from 1 to 20ɣ/kg. The study of Adrenalin-EABCO antagonism has shown an inhibit hypertensive property of EABCO at a dose of 25, 50 or 100 mg/kg of body weight. EABCO act as a noncompetitive inhibitor of adrenoceptor, justified by a parallel shift to the right from the cumulative dose-response curve and no attained bend. **Conclusions:** Results indicate that aqueous extract of roots of *Cassia occidentalis* possess hypotensive and antihypertensive properties. Cholinomimetic and/or noncompetitive adrenoceptor blocking actions may explain in part the antihypertensive potential of this plant and provides a potential pharmacological base to its medicinal use in the management of hypertension.

Keywords: *Cassia occidentalis, hypotension, antihypertension.*

1. INTRODUCTION

Hypertension is one of the high-prevalence diseases in the world. Mortality risks were significantly increased among hypertension patient especially in untreated or uncontrolled blood pressure [1]. Due to the high cost of antihypertensive drugs in developing countries, a plausible approach to reducing hypertension-related fatalities is the use of local traditional remedies. People have customarily used the plant(s) or plant(s)-derived preparations to combat many disorders. Detailed laboratory and clinical evaluations are needed to justify their use as medicines. However, only few of them have been controlled clinically, or studied chemically and biologically to identify their active constituents or mechanism of action [2]. *Cassia occidentalis* is a plant of the family Caesalpiniaceae, which is locally known as Tsotsoronangatra, bemaimbo, voanembanalika, Tsatsinangatra, famônoakoho or Sarongazany. Its roots are widely used by residents of Salobe in the Betioky-Sud district (South-west of Madagascar) to treat various human ailments including hypertension and paludism. Previous study carried by August (2010) shown that this plant possess an anti-hyperglicemic activity [3]. Since no pharmacological data concerning their antihypertensive properties are available, to the best of our knowledge, the present study was performed to verify the hypotensive and antihypertensive effects of its roots. The main aim was to justify the empirical use of this plant in the treatment of hypertension.

2. MATERIALS AND METHODS

2.1.1. Plant material

C. occidentalis were collected at Salobe in the Betioky-Sud district of Toliara Province (southwest of Madagascar) on January 2021. The plant name has been checked with http://www.theplantlist.org and specimen was identified by the botanist at the CNARP (Centre National d'Applications de Recherches Pharmaceutiques) where a voucher was deposited for future references. The roots (119.296 g) of *C. occidentalis* were air-dried at 36°C till dryness. Dried roots (80.344 g) were reduced to a powder using electrical crusher. The dried and powdered roots were extracted using distilled water (2 L) for 30 min at 100°C. The samples were cooled to room temperature and filtered with joseph's paper to obtain the first filtrate. The residue was re-extracted with distilled water (2 L) under the same conditions and then filtered. Both filtrates were pooled, concentrated in a rotary evaporator (BÜCHI type R-114, Switzeland) under reduced pressure at a temperature below 60°C. The concentrated extract thus obtained was called EABCO with a dry weight of 9,506 g, yield of 11.83 %.

2.1. Chemicals

Heparin, thiopental, acetylcholine (ACh) and adrenaline (ADr) were purchased from local pharmacy. All other chemicals and drugs used were of analytical grade. All drugs and the extract were dissolved in saline and then the solution was

*Corresponding author Author | Rakotoarison, Olivier |

vortexed (HEIDOLPH type REAX1, Germany) in order to mix the mixture thoroughly.

2.2. In-vivo experiments

2.2.1. Animals

Normotensive rabbits of either sex (*Oryctolagus cuniculus,* Leporidés), weighing between 1.7-2.5 kg were obtained from the Animal House of the department of Animal Physiology and Pharmacology, University of Antananarivo. Experiments performed were approved by the Ethical Committee of CNARP (Centre National d'Applications de Recherches Pharmaceutiques), Antananarivo.

2.2.2. Experimental protocol

A method described by Léandre K., (2008) with slight modification was used [4]. Animals were anesthetized with an intra-peritoneal injection of thiopental (40 mg/kg b.w), fixed on a dissecting table; a small mid tracheal incision was made to expose trachea, right jugular vein, and carotid artery. The trachea was cannulated to maintain the spontaneous respiration. The right jugular vein was cannulated to facilitate the intravenous injections of the standard drugs and test materials. The carotid artery was cannulated for arterial pressure recording. Heparinized saline were used to prevent blood clotting. The body temperature of the animal was maintained by using an overhead lamp. After the surgical procedure, the arterial pressure was allowed to stabilize before injection of the test substances. Acetylcholine (ACh; from 1 to 20 ɣ/kg b.w.) and adrenaline (ADR: 10, 20 and 30 ɣ/kg b.w.) were chosen as the drug standard curve of hypotensive and hypertensive mechanisms, respectively.

2.2.3. Statistics

All experiments were repeated twice and the results were expressed as mean ± standard error of mean (SEM). Statistical analysis was carried out by Students *t*-test using Microsoft Excel 2013. All data for statistical comparisons have to be tested for homogeneity of variances. Differences were considered significant at $p < 0.05$.

3. RESULTS

3.1. Comparative study of hypotensive activity of EABCO and ACh in normotensive rabbits

As shown by the tracing from typical experiments (figure 1), EABCO at higher dose (500 mg/kg) induced a sustained lower level of arterial pressure when injected intravenously. At a lower doses (100 and 250 mg/kg) it produced a temporary decrease in arterial pressure, returning to baseline values within less than 10 minutes. Moreover, administration of acetylcholine at the doses of 1 or 10 ɣ/kg produced a similar temporary reduction in the blood pressure (Figure 1). Variation observed was more rapid than that induced by the EABCO. At a higher dose (20 ɣ/kg) it induced a marked fall in arterial pressure ($p<0.05$). The logarithms of the dose were used for in depth analysis. The average of hypotensive effects (in %, n=2) calculated from the maximum responses can be noted from the dose response curve presented in figure 2. It shows that the doses of EABCO and acetylcholine (ACh) necessary to elicit 50% of its maximum responses (ED50) were 251 mg/kg and 5ɣ/kg, respectively (Figure 2).

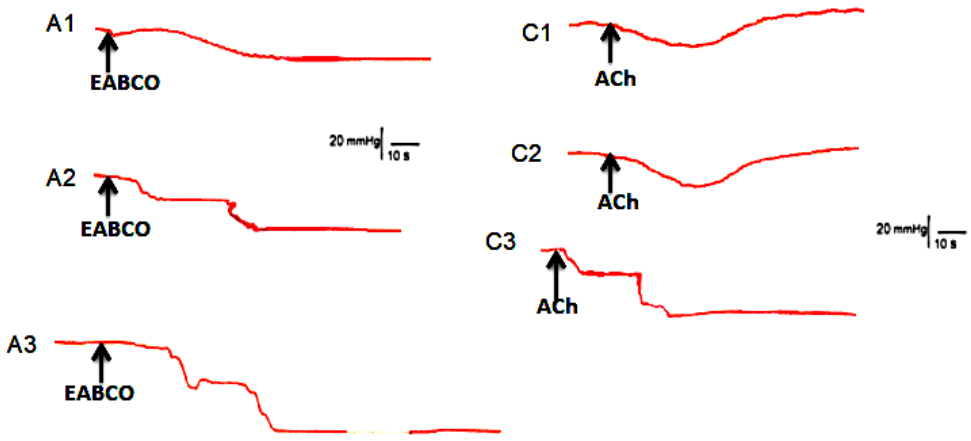

Figure 1: Tracing from typical experiments performed on anesthetized normotensive rabbits: effect of graded dose of EABCO (A1: 100 mg/kg, A2: 250 mg/kg and A3: 500 mg/kg) and Acetylcholine (C1: 1 ɣ/kg, C2: 10 ɣ/kg, C3: 20 ɣ/kg) on arterial pressure of normotensive anesthetized rabbits. 1 ɣ = 1 µg = 10^{-3} mg = 10^{-6} g.

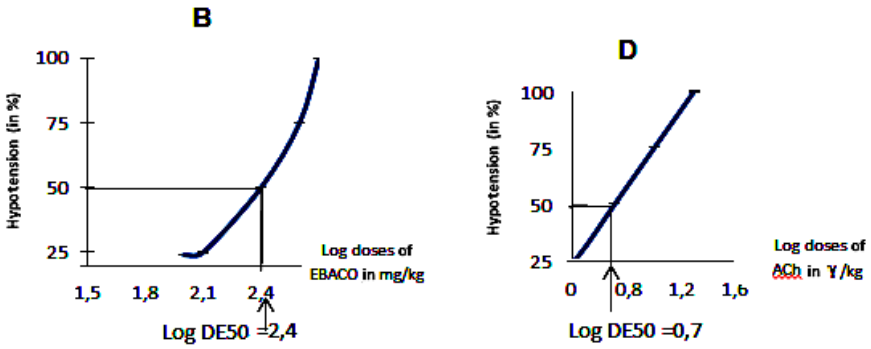

Figure 2: Effects of EABCO (B) and acetylcholine (D) on arterial pressure of normotensive anesthetized rabbits.
Log DE50: The logarithms of the doses of EABCO or acetylcholine (ACh) necessary to elicit 50% of its maximum responses.

3.2. Antihypertensive activity of EABCO

Baseline arterial pressure was 90 ± 6.70 mmHg in normotensive anesthetized rabbits (n = 2). The tracing from typical experiments are presented in Figure 3 and 4.

Figure 3 shows that the intravenous injection of adrenaline (20 ɣ/kg) caused a temporary increase in arterial pressure with maximum value of 126.00 ± 6.27 mmHg (approximately 36 mmHg of hypertension). The three (3) injections of EABCO (25, 50 and 100 mg/kg) produced a marked decreases on the hypertension induced by adrenaline (Figure 3). The arterial pressure rapidly exceed its nadir (<90 mmHg) after the second injection with EABCO at the doses of 50 mg/kg. These data suggest that EABCO have an antihypertensive activity on adrenaline-induced hypertension.

Figure 4 shows the effect of intravenous injections of adrenaline with and without EABCO pretreatment on the arterial pressure of anesthetized rabbits. The mean of arterial pressures were, respectively, 169±1.4, 180±2 and 181±1.5 mmHg in the group of rabbits treated with only adrenaline at the doses of 10, 20 and 30 ɣ/kg. Also, pretreatment with EABCO at the doses of 25, 50 or 100 mg/kg affect the responses to adrenaline. Thus, EABCO at 25 mg/kg, (i.v.) reduced the effects of adrenaline (10, 20 and 30 ɣ/kg) on arterial pressure to 165±0.6, 174±1 and 174±0.6 mmHg (n = 2), respectively. The EABCO at the doses of 50 or 100 mg/kg, reduced the responses to adrenaline to 163±1, 171±0.6 and 171±1.3 mmHg (50 mg/kg, n = 2) or to 160±0.5, 169±1.5, 169±2 mmHg (100 mg/kg, n = 2), respectively. Differences observed were significant (p<0.05) when compared to the group receiving only adrenaline (table 1).

For in depth analysis, data values were transformed by converting all doses of adrenaline to their logarithms and by using the maximal response as 100% of hypertension ($Emax$). The resulting dose-response curves are shown in figure 5; parallel shift to the right observed with EABCO pretreatment indicates antagonism. Indeed, the dose of adrenaline necessary to elicit 50% of its maximum response (EC50) was modified. The maximum effect (Emax) of adrenaline was also reduced from 100% to less than 96% by pretreatment with EABCO. The EABCO induced modifications of Emax and ED50 of adrenaline were dose related, as demonstrated in this study. These finding indicates that EABCO act as a noncompetitive inhibitors of adrenoceptor.

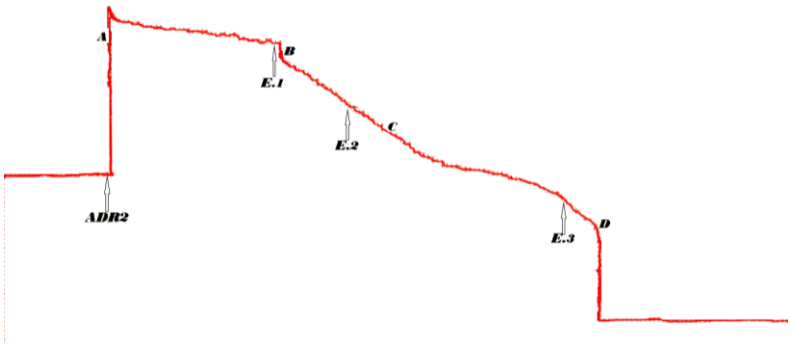

Figure 3: Tracing from typical experiments performed on anesthetized rabbits: antihypertensive effect of EABCO on the hypertension induced by intravenous injection of adrenaline. ADR2: adrenaline at the dose of 20 ɣ/kg, E.1: EABCO at the dose of 25 mg/kg, E.2: EABCO at 50 mg/kg, E.3: EABCO at the dose of 100 mg/kg.

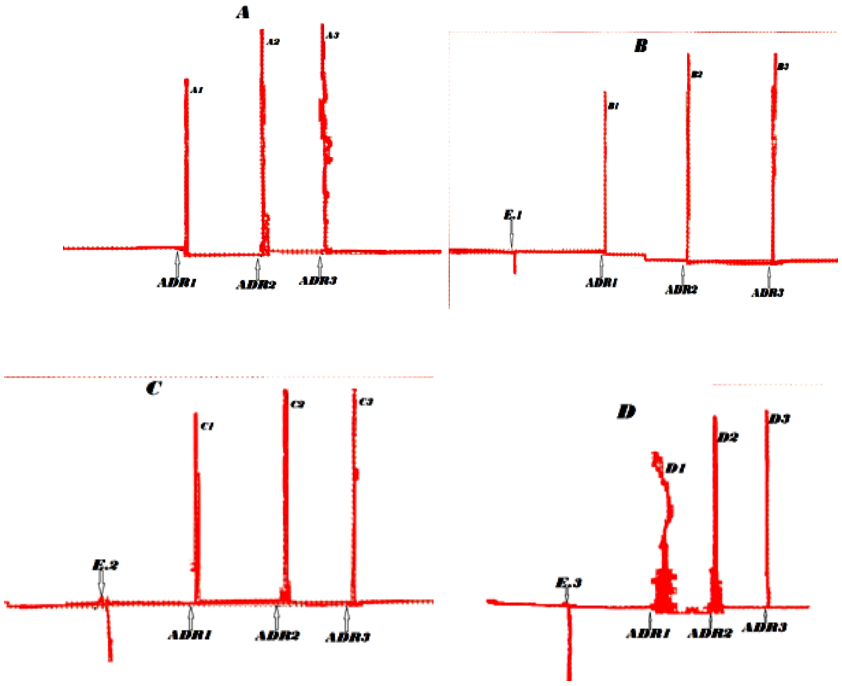

Figure 4: Tracing from typical experiments performed on anesthetized rabbits: effect of intravenous injections of adrenaline at the doses of 10, 20 and 30 ɣ/kg with and without EABCO pretreatment on the arterial pressure of anesthetized rabbits. A: Without EABCO pretreatment and B, C, D: with EABCO at the doses of 25 (B), 50 (C) and 100 mg/kg (D).

Table 1: Statistical analysis: tests for equality of variances and means:

Adrenaline (in ɣ/kg)	10	20	30
matrice2 compared to matrice1			
F TEST (p>0.05)	0.516	0.590	0.484
T TEST (p<0.05)	0.036	0.034	0.018
matrice3 compared to matrice1			
F TEST (p>0.05)	0.790	0.371	0.909
T TEST (p<0.05)	0.021	0.014	0.011
matrice4 compared to matrice1			
F TEST (p>0.05)	0.437	0.819	0.819
T TEST (p<0.05)	0.007	0.009	0.008

Matrice1: effects of ADR without EABCO pretreatment, **matrice2 - 4**: with EABCO at the doses of 25 (matrice2), 50 (matrice3) and 100 mg/kg (matrice4).

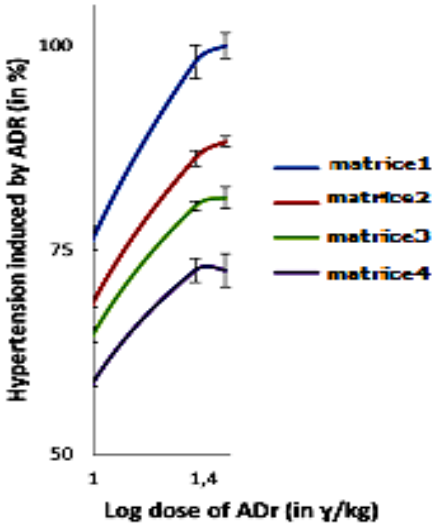

Figure 5: Effect of intravenous injections of adrenaline (10, 20 and 30 ɣ/kg) with and without EABCO pretreatment on the arterial pressure of anesthetized rabbits. matrice1: without EABCO pretreatment, matrice2 - 4: with EABCO at the doses of 25 (matrice2), 50 (matrice3) and 100 mg/kg (matrice4). Vertical bars indicate the mean ± SEM (n = 2).

4. DISCUSSION

The present study was performed to verify the hypotensive and antihypertensive effects of *C. occidentalis* which was selected based on its traditional use in ethnomedicine. The main aim was to justify the empirical use of its roots in the treatment of hypertension. The results of the present study show that *Cassia occidentalis* decreases the arterial pressure of anesthetized normotensive rabbits. Its effects seemed to depend on the dosage used. Thus, following the injection of EABCO at higher doses, the duration of the hypotensive effect became longer as evidenced by the sustained lower level of arterial pressure whereas at lower doses it produced only a temporary hypotension. These types of hypotension promoted by EABCO seemed to be comparable to that seen with acetylcholine.

Moreover, antihypertensive activity of EABCO was assessed in vivo using models of hypertension. Adrenaline was used as an agonist in the production of the hypertensive effect. The hypertension promoted by adrenaline was reduced by EABCO, as observed from the results. It suggests that adrenoceptors may play a role in the hypotensive mechanism of this extract. However, the study of adrenalin-EABCO antagonism revealed that EABCO act as a noncompetitive adrenoceptor blocking agent. Since various mechanisms for antihypertensive effect have been reported in literature, many studies have shown that plant extracts exert antihypertensive effects through the combination of the activities of its bioactive components [5, 6].

These findings suggest that the curative effect of *C. occidentalis* extract on hypertension is probably related to the presence of antihypertensive and hypotensive methabolites, which justifies its empirical use in the treatment of hypertension.

5. CONCLUSION

Aqueous extract of roots of *Cassia occidentalis* exhibited a hypotensive and antihypertensive actions in normotensive and in adrenaline-induced hypertensive models, respectively. Its actions seemed to be similar to that seen with acetylcholine. Cholinomimetic and/or other noncompetitive adrenoceptor blocking effects may explain the antihypertensive mechanism of the roots extract of this plant. These properties justify the empirical use of *Cassia occidentalis* in the treatment of hypertension. Further studies are required to identify the active compounds in the extract.

6. REFERENCES

1. Yeni F., and Kharimah F. T. Adverse Drug Reactions Study of Antihypertensive Drugs in Primary Care Settings. JMPF. 2020;10(4): 224-228. Available: DOI:10.22146/jmpf.56975
2. Lahadson S. F., Randrianjatovo M., Rabemandroso F. L., Toghueo K. R. M., Rakotoarison O., Ravelomanantsoa G., Randrianasolo R., Nkengfack E. A., and Rasoanaivo H. L. Biological activity of leaves extracts and active ent-kaurane from *Erythroxylum ferrugineum* cav. (Erythroxylaceae). *American Journal of Innovative Research and Applied Sciences*. 2020;11(3):131-136. Available: https://american-jiras.com/Lahadson-Ref1-ajira290720.pdf
3. Verma L., Khatri A., Kaushik B., and Patil U. Antidiabetic activity of *Cassia occidentalis* (Linn) in normal and alloxan-induced diabetic rats. Indian J Pharmacol. 2010 ; 42(4): 224-228. doi: 10.4103/0253-7613.68422
4. Léandre K. K., Kouakou J-C. ABO, Traore F. and Ehouan E. E. Effet antihypertensif de BpF2, une fraction d'extrait aqueux de feuilles de *Bidens pilosa* L. (Asteraceae) chez le lapin. *Sci. Nat.* 2008;5(1): 29-37. Available: https://www.readcube.com/articles/10.4314%2Fscinat.v5i1.42149
5. Curin Y. and Andriantsitohaina R. Polyphenols as potential therapeutical agents against cardiovascular diseases. *Pharmacological Reports.* 2010; 57: 97-107. Available: https://pubmed.ncbi.nlm.nih.gov/16415490/
6. Oh K. S., Ryu S. Y., Oh B. K., Seo H. W., Kim Y. S., and Lee B. H. Antihypertensive, vasorelaxant, and antioxidant effect of root bark of *Ulmus macrocarpa*. *Biological and Pharmaceutical Bulletin*. 2008; 31(11): 2090–2096. Available: DOI:10.1248/bpb.31.2090

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