

Antihypertensive Activity of Residue From "Gebto Arekei", Locally Distilled Medicinal Spirit From a Brew Containing *Lupinus albus* Seeds in Renovascular Hypertensive Guinea-Pigs

Cherinet Ambaye¹, B. Pharm, Tesfaye Tolessa², MSc, Abebe Abera², MD, MSc, Hassen Taha Sherief^{2*}, MD, PHD, Dawit Abebe¹, PHD, Kelbessa Urga¹, MSc

ABSTRACT

BACKGROUND: "Gebto Arekei", the traditional medicinal spirit prepared from a fermented brew containing *Lupinus albus* seeds is assumed to represent one of the forms of use of this plant in the traditional treatment of hypertension. The present work was undertaken to see the effect of "Gebto Arekei" on blood pressure of renovascular hypertensive guinea-pigs and evaluate its traditional medicinal use.

METHODS: The study was conducted between Oct 2000 and January 2001 in Addis Ababa. In this experiment, guinea-pigs were used. Hypertension was induced by using surgical procedures involving clamping of the left renal artery. Blood pressure was recorded invasively by direct cannulation method using surgical procedures involving the right common carotid artery. Thereafter, the effect of "Gebto Arekei" on blood pressure was investigated.

RESULTS: "Gebto Arekei" residue, when infused at dose of 2 mg/kg, 20 mg/kg and 200 mg/kg body weight, caused a dose-dependent reduction of blood pressure in anaesthetized renovascular hypertensive guinea-pigs (n=5). The fall in blood pressure had an acute and slowly recovering sustained phases. During the sustained phase, the residue at doses of 2 mg/kg, 20 mg/kg and 200 mg/kg body weight decreased the systolic pressure by 3.02%, 23.37% (p<0.005) and 43.11% (p<0.0005), respectively; whereas, diastolic pressure was decreased by 3.49%, 26.89% (p<0.005) and 49.75% (p<0.0005), respectively. Similarly, the mean blood pressure was reduced by 13.58%, 25.54% (p<0.005) and 47.02% (p<0.0005), respectively. The calculated ED₅₀ for the mean blood pressure was 19.69 mg/kg body weight. The duration of action for each of the dose infused was 3.4±0.50 min, 12±1.70 min and 24.5±1.66 min, respectively.

¹Department of Drug Research, Ethiopian Health and Nutrition Research Institute, Addis Ababa, P.O. Box, 1242, Addis Ababa, Ethiopia.

²Department of Physiology, Faculty of Medicine, Addis Ababa University, Addis Ababa, P. O. Box 9086, Addis Ababa, Ethiopia.

*Corresponding author

The pulse pressure was significantly reduced only during infusion with a dose of 200mg/kg body weight.

CONCLUSION: In this study, the result of the experiment using a residue of "Gebto Arekei", seems to suggest an antihypertensive effect in anesthetized renovascular hypertensive guinea-pigs.

KEY WORDS: Antihypertensive effect, blood pressure, "Gebto Arekei", "Gebto", guinea-pigs, *Lupinus albus*.

INTRODUCTION

Traditional treatment of different kinds of diseases is a common practice in many countries, especially in developing countries where access to modern medications is less. In our country as elsewhere in the African populations, more than 80% of our people rely on traditional treatment of diseases (1). Hypertension as one of the common health problems treated traditionally has a prevalence rates of 1.8 to 7.5% for rural and up to 11.8% for urban adult population in some parts of Ethiopia, and is indicated as one of 13 leading death causing diseases (2,3,4).

Drugs of different plant sources are used traditionally to ease cardiovascular problems (5). "Gebto Arekei" is a vernacular name of one of locally made antihypertensive medicinal preparation. It is medicinal spirit obtained by distilling a fermented brew prepared in the same way for other cereal-based alcoholic beverages, except that in this case seeds of *Lupinus albus* are used as one of the substrates (6).

The plant *Lupinus albus* L. (family Leguminosae) which is one of the traditionally known medicinal plant and the source of the seed component of the brew is a short hairy annual herb indigenous to the Mediterranean region and India. "Gebto" as this plant is known locally in Ethiopia grows in the regions of Gojam, Gonder, Shew and Harrar (7).

The medicinal properties attributed to the seeds of *Lupinus albus* are: diuretic, anthelmintic, and emmenagogue. Furthermore, the seeds bruised and soaked in water are applied to ulcer (8). Powder of cooked seeds is eaten daily mixed with butter and milk to rejuvenate or to make the skin look fresh (5).

Some results of pharmacological studies indicate the presence of parasympathetic and hypoglycemic properties (10,11,12). Effects on cardiac arrhythmia and atrial fibrillation of the alkaloid sparteine and its derivatives from the seeds of the plant have been reported (10).

The traditional system of medicine in Ethiopia claims the antihypertensive medicinal properties of the plant, and prescribes one of the two forms of use of the plant material. This could be either eating whole seeds or the use of "Gebto Arekei", which are said to have the same medicinal effect. In contrast to the antihypertensive activity of *Lupinus albus* seeds and "Gebto arekei" claimed by the traditional system, no pharmacological investigations concerning their effect on blood pressure has been reported on either mode of preparation.

Pharmacodynamic study to assess the potential efficacy of traditional medicines is the basis to realize the rationality of traditional practices in the society. The present work, therefore, was undertaken to see the effect of "Gebto Arekei" which is

one of the mode of preparation of the medicinal plant on blood pressure of renovascular hypertensive guinea-pigs and evaluate its traditional medicinal use.

MATERIALS AND METHODS

"Gebto Arekei" and the residue

"Gebto Arekei" was collected from distillers in the town of Debre Markos, Gojam, Ethiopia. "Gebto Arekei" was evaporated under reduced pressure using a rotary evaporator and the residue lyophilized resulting in a yellowish-brown powder. The powder was then dissolved in normal saline (0.9% NaCl) for further pharmacological investigation.

Preparation of animals for anti-hypertension studies:

Five male guinea-pigs (Addis Ababa, Ethiopia) weighing 400-700 gm were used in this study. The experimental protocol was approved by the Ethiopian Science and Technology Commission, (ESTC), for animal experimentation.

The animals were housed in the faculty of medicine under appropriate condition with free access to food and water. Before surgery, animals were injected phenobarbital to induce anaesthesia and analgesia, therefore experiments were conducted free of pain. At the end of the experiment animals were sacrificed using air embolus injection.

The experiments were performed at the Department of Physiology (Faculty of Medicine, Addis Ababa University) on guinea-pigs (n=5) that were anaesthetized with sodium phenobarbital (Apoteksbolaget, Umea, Sweden) 50 mg kg⁻¹ body weight intraperitoneally. At the stage of light anesthesia indicated by lack of pain sensation and spontaneous movements, tracheal cannulation was made

and the animals were artificially ventilated (Bioscience, 815-51190-1, Sheerness, Kent, UK). Then the right carotid artery was cannulated with heparinized saline-filled catheter connected to a pressure transducer (BBC, Goetz Metrawatt, Model SE 120) for continuous recording of blood pressure. Similarly, the right jugular vein was cannulated with similar tubing for intravenous infusion of saline and the "Gebto Arekei" residue. After allowing blood pressure to stabilize for a period of one hour, the animals were subjected to a second surgical procedure to ligate renal artery. In the procedure, a midline incision was made through the abdominal wall. The intestine was carefully extirpated and then the left renal artery was clamped.

Design of pharmacodynamic studies in hypertensive model:

All experiment started with a control recording of blood pressure in induced renovascular hypertensive guinea-pigs with its measurable systolic and diastolic levels during a period of 30 minutes. The residue, dissolved in 0.5 ml of saline, was given through jugular vein as a bolus injection over a duration of 15 sec. Change in blood pressure during treatment with the residue was recognized as the difference between the steady state value before and the changed state of blood pressure seen after infusion. The recovery was recorded when the increased blood pressure came to a steady state as seen during the control period.

Data processing and statistical analysis:

The values for the systolic, diastolic pressure and duration of action for the residue was calculated from the calibration scale made at the beginning of each

experiment. Pulse pressure was taken as the difference between the systolic and diastolic pressure. Mean blood pressure was calculated as diastolic pressure plus one-third pulse pressure.

Data was expressed as mean and standard error of the mean of n experiments. The concentration-response curve with ED_{50} values representing the negative logarithm for the residue concentration causing half maximum response were calculated using the Least-Square method. Data were statistically compared as repeated measures using ANOVA followed by paired observations using Student's t -test. $P < 0.05$ in two tailed tests was considered significant.

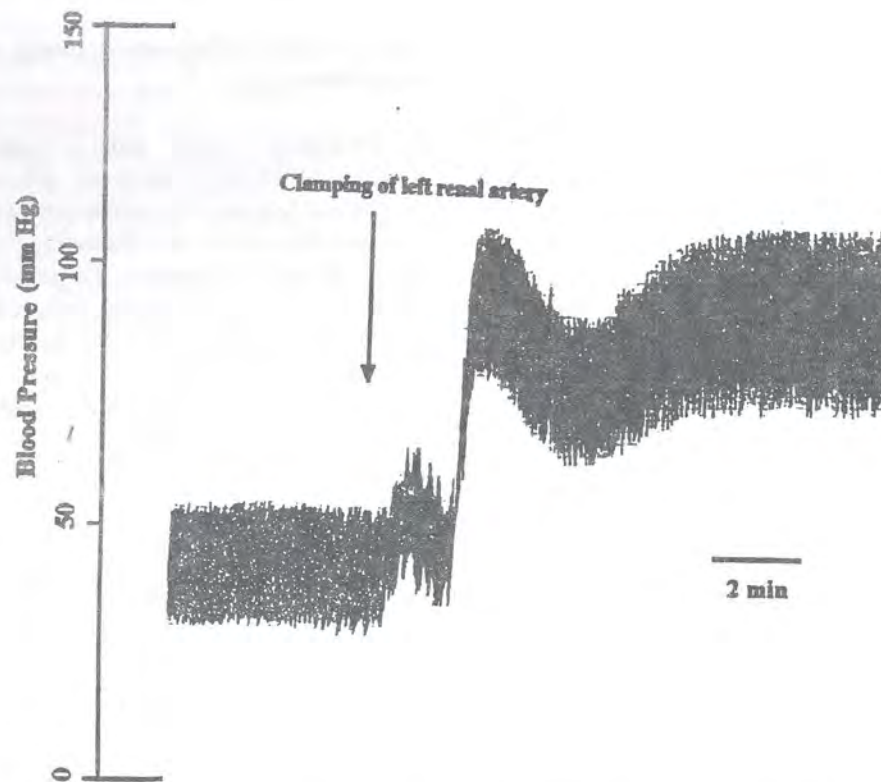


Figure 1. Recording of blood pressure by carotid artery cannulation from a guinea-pig before and after clamping of the left renal artery (Goldblatt or renovascular hypertension).

RESULTS

In anaesthetized guinea-pigs, clamping of the left renal artery raised the systolic pressure from 50.00 to 109.09, diastolic pressure from 27.27 to 75.45 and mean blood pressure from 34.84 to 86.66 (Fig. 1). Residue of "Gebto Arekei" caused a fall in systolic, diastolic and mean blood pressure in a dose-dependent manner as shown by the tracing from a typical experiment (Fig. 2).

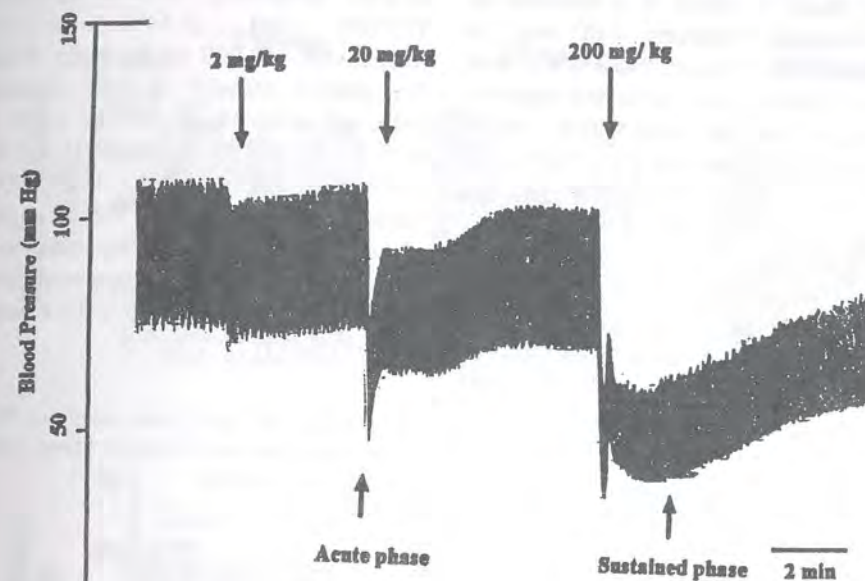


Figure 2. Recording of blood pressure by cannulation of the right carotid artery showing the effect of increasing intravenous doses (2mg/kg, 20mg/kg and 200mg/kg body weight) of "Gebto Arekei" residue in renovascular hypertensive guinea-pigs.

The antihypertensive effect had two phases. Phase 1 which is a transient or acute response returning half way to normal with in a minute. Phase 2 which is relatively prolonged or sustained response returning to base line level within 3 to 30 minutes depending upon the dose given. At a dose of 2 mg/kg body weight, the residue reduced systolic, diastolic, pulse and mean blood pressure during the sustained phase by 3.02% 3.49%, 1.01% and 13.58%, respectively. At a dose of 20 mg/kg body weight, the residue reduced systolic, diastolic, pulse and mean blood

pressure during the sustained phase by 23.37% ($p < 0.005$), 26.89% ($p < 0.005$), 17.774% and 25.54% ($p < 0.005$), respectively. At 200 mg/kg body weight, the residue reduced systolic, diastolic, pulse and mean blood pressure by 43.1% ($p < 0.0005$), 49.75% ($p < 0.0005$), 28.82% ($p < 0.05$) and 47.59% ($p < 0.0005$), respectively (Table 1). The percentage change in blood pressure parameters in response to the residue during acute phase parallels that measured during the sustained phase (Table 1).

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Table 1. Effect of i.v infusion of doses 2 mg/kg, 20mg/kg and 200 mg/kg body weight of "Gebto Arekei" residue on systolic pressure, diastolic pressure; pulse pressure and mean blood pressure expressed as percentage response in reno-vascular hypertensive guinea-pigs

Dose (mg/kg) vs Response (%)		SP	DP	PP	MBP
2	Acute	7.20	7.19	6.80	12.22
	Sust	3.02	3.49	1.01	13.58
20	Acute	29.33***	35.03**	17.74	32.28**
	Sust	23.37**	26.89**	17.33	25.54**
200	Acute	43.38***	53.84***	45.43**	52.27***
	Sust	43.11***	49.75***	28.82*	47.02***

The responses are shown as acute and sustained (sust) phases expressed as a percentage changes from the steady state base-line value. SP= systolic pressure; DP= diastolic pressure; PP= pulse pressure; MBP= mean blood pressure. Mean values and standard error of the mean (n=5). * $p<0.05$; ** $p<0.005$; *** $p<0.0005$.

Table 2. Duration (min) of action of "Gebto Arekei" residue on mean arterial blood pressure following i.v. infusion of doses of 2 mg/kg; 20mg/kg, and 200 mg/kg body weight in renovascular hypertensive guinea-pigs

Doses in mg/kg body weight	Duration in minutes
2	3.40±0.50
20	12.00±1.70
200	24.50±1.66

Mean values and standard error of the mean (n=5)

There was a significant reduction in systolic, diastolic and mean blood pressure at doses of 20 mg/kg and 200 mg/kg (Fig. 3&4). The pulse pressure was significantly reduced at doses of 200 mg/kg (Fig.2). The duration of action for each of the three doses given as measured during the sustained response was 3.40 ± 0.50 min, 12± 1.70 min and 24.50± 1.66 min, respectively (Table 2). The maximum reduction in mean blood pressure observed after infusion of a dose of 200 mg/kg was 47.02% (Table 1). The ED₅₀, which is a concentration of the residue producing half maximum response, was 19.69 mg/kg body weight (Fig. 5).

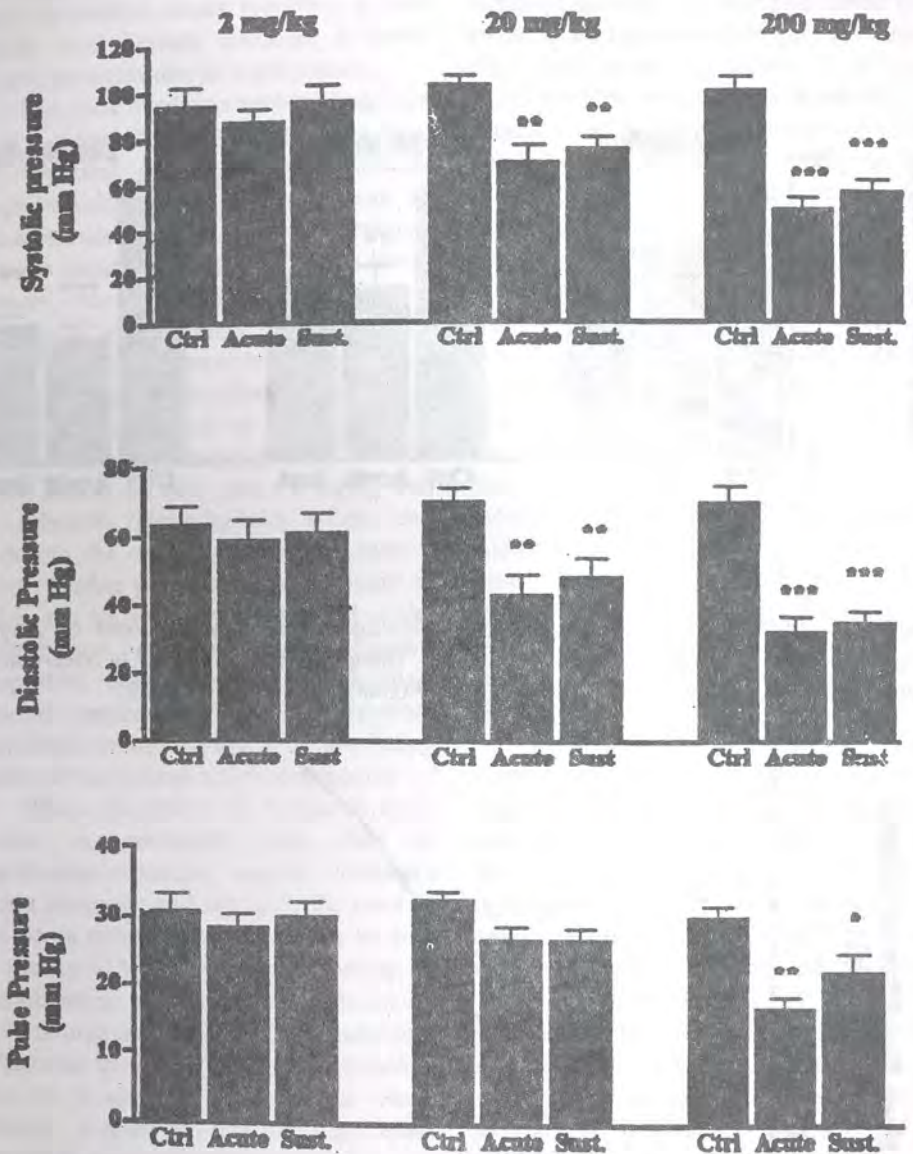


Figure 3. Composite diagram of the effect of "Gebto Arekei" residue on systolic pressure, diastolic pressure and pulse pressure in renovascular hypertensive guinea-pigs (n=5) during acute and sustained response (sust) as compared to the control (ctrl) values. Mean values and standard error of the mean. * $p<0.05$; ** $p<0.05$, and *** $p<0.0005$.

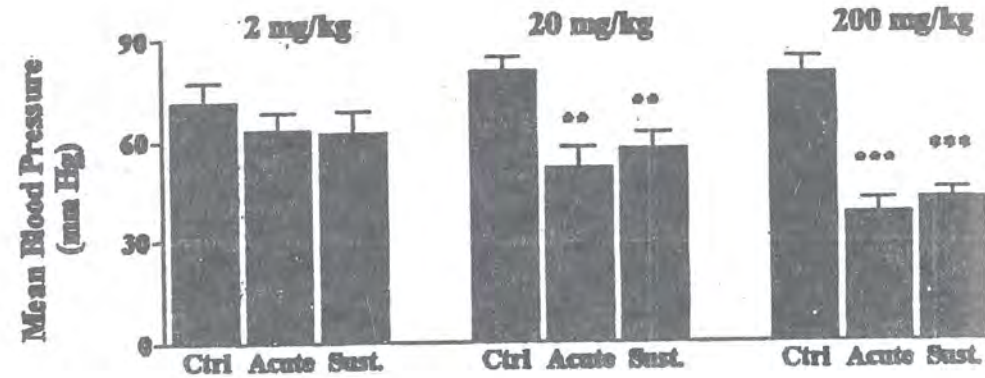


Figure 4. Mean blood pressure changes following i.v infusion of doses of 2mg/kg; 20mg/kg and 200 mg/kg body weight of "Gebto Arekei" residue in renovascular hypertensive guinea-pigs (n=5). *p<0.05; ** p<0.0005, and *** p<0.0005.

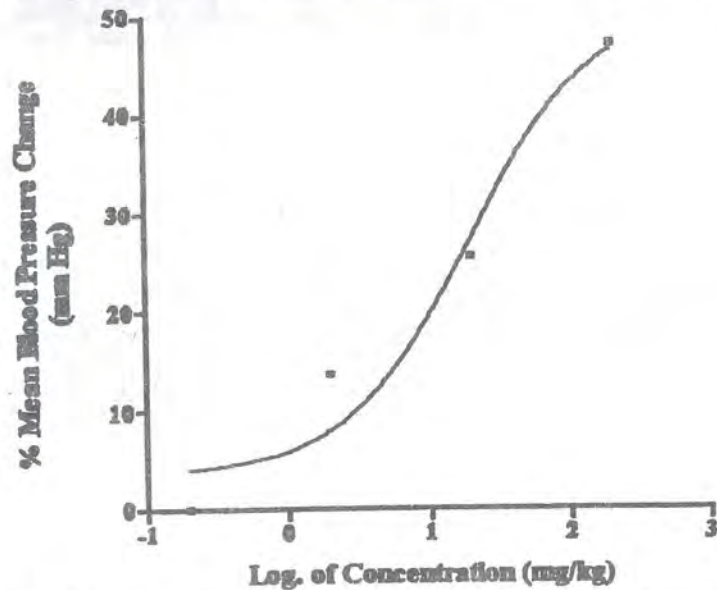


Figure 5. Dose-response curve showing the effect of increasing doses of "Gebto Arekei" residue on mean blood pressure in renovascular hypertensive guinea-pigs (n=5).

systolic pressure, while coronary blood flow depends on the diastolic pressure. Since the residue causes reduction in both systolic and diastolic pressure, it might prevent accidents due to hypertension.

The best antihypertensive drugs are those that are effective over 24 hours (13). The circadian pattern of cardiovascular events, are affected by several factors. In post-awakening period there is increased vascular resistance, higher systemic blood pressure, heart rate, blood viscosity, and a reduction in coronary blood flow. However, further research is required on the residue to know its effects on the 24 hrs circadian rhythm and its haemodynamic changes (20). Its relatively short duration of the action, 12 min with 20 mg/kg and 24.5 min with 200mg/kg body weight, may be due to the crude nature of the extract. Hence, further purification is necessary to isolate the active component found in the crude extract. Following isolation of active component, elucidation of its chemical structure assists in structural alteration. This helps in identifying a 24 hrs acting analog of the residue active components

When the effect of "Gebto Arekei" residue is compared with that of angiotensin-converting enzyme inhibitors such as Enalapril and Lisinopril, the latter in oral doses reduced blood pressure by 6 to 15 mmHg (14, 15) in humans, while i.v administration of the residue reduced by 19.74 mmHg in guinea-pigs. In future trials in humans, the crude residue is assumed at least to produce a reduction in blood pressure similar to that of angiotensin converting enzyme inhibitors.

In this particular experiment, the renovascular model of hypertension was used. It is known that in rats with experimentally induced kidney disease, long-term administration of calcium antagonists protected against deterioration

of renal functional, histological damage, nephrocalcinosis & myocardial calcification (16). It was postulated that calcium antagonists may provide some renal and cardiac protection in patients with impaired renal function. It can also be assumed that "Gebto Arekei" residue might give similar protection on renal impaired patients since the result obtained in this experiment was based on the model of renovascular hypertensive guinea-pigs.

Angiotensin-converting enzyme inhibitors such as Lisinopril when given to two kidney, one-clip Goldblatt hypertensive rats, significantly increased carotid compliance of both normotensive and hypertensive rats (17), thus showing that modification of arterial wall were independent of blood pressure changes. Although arterial wall compliance was not tested, the present drug tested in a two kidney, one-clip Goldblatt hypertensive guinea-pigs might bring a change in arterial compliance during the blood pressure reduction.

In conclusion, the residue from "Gebto Arekei" in this model has shown a significant reduction in blood pressure in induced renovascular hypertension. Therefore, it seems that the residue is not only useful in hypertension associated with renal impairment cases but also in those cases with essential hypertension at least in guinea-pigs. Whether this can be inferred to human subjects requires clinical trials. Furthermore, additional research is warranted to improve its duration of action, to know its mechanism of action and to establish doses in oral administration.

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