

ORIGINAL ARTICLE

**Hypotensive Activity of Residue From "Gebto Arekei",
Locally Distilled Medicinal Spirit From a Brew Containing
Lupinus Albus Seeds in Normotensive Guinea pigs.**

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ABSTRACT

BACKGROUND: *The plant Lupinus albus, the source of the seed component of the brew, is a short hairy legume known for its traditional medicinal value in the treatment of hypertension. The aim of the study was to see the blood pressure reducing effect of the residue from "Gibto Arekei" in the alternative normotensive laboratory animal model and thereby consolidate the finding of the study.*

METHODS: *The study was conducted between Oct. 2000 and January 2001. In this experiment, male guinea-pigs were used. 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: *Residue from "Gebto Arekei" when administered at doses of 2 - 200 mg/kg body weight caused a dose-dependent decrease of blood pressure in anesthetized normotensive guinea-pigs (n=6). The fall in blood pressure had an acute and sustained phases. During the sustained phase which had slow recovery period, the residue at doses of 2 mg/kg, 20 mg/kg and 200 mg/kg body weight decreased the systolic pressure by 12.69%, 20.42% (p<0.005) and 39.45% (p<0.0005) whereas, diastolic pressure was reduced by 15.14%, 29.34% (p<0.005) and 47.16% (p<0.005), respectively. The mean blood pressure was reduced by 11.72%, 26.22% (p<0.005) and 43.59% (p<0.0005), respectively. The calculated ED₅₀ for the mean blood pressure was 14.86 mg/kg. The duration of action for each of the dose infused was 3.1±1.28 min, 13.16±2.03 min and 21.33±1.89 min, respectively. The pulse pressure was not significantly affected even after infusion with 200 mg/kg.*

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CONCLUSION: *In this study, the result of the experiment using a residue from "Gebto Arekei" seems to suggest a hypotensive effect in anesthetized normotensive guinea-pigs.*

KEY WORDS: *Blood pressure, "Gebto Arekei", hypotensive effect, Lupinus albus, normotensive guinea-pigs.*

INTRODUCTION

Lupinus albus (L. family Leguminosea) is a herbaceous annual plant, native to the Mediterranean regions. In Ethiopia it is cultivated particularly in Gondar, Gojam, Arsi, Harrargeh and Shewa regions (1).

The plant is reported to have hypoglycemic properties (2-4). Pharmacological activities of the alkaloids from *Lupinus albus* include: affinity at nicotinic and muscarinic receptors and effects on cardiac activity (5). Its use as a vermifuge is also indicated (6).

Lupinus albus, "Gebto" as it is called in Ethiopia is well known traditionally in the treatment of hypertension. Its seeds are used in the traditional practice in the forms of maceration, infusion, powder, tincture and fluid extract (6). "Gebto Arekei", the traditional medicinal spirit which is prepared from a fermented brew containing *Lupinus albus* seeds is assumed to represent one of the forms of use of this medicinal plant in the traditional therapeutic application of treating hypertension.

Investigation of the antihypertensive effect of "Gebto Arekei" using the residue obtained from it has shown a dose-dependent decrease of blood pressure in renovascular hypertensive guinea-pigs (7)

Normotensive animals have been used as one of experimental models to show blood pressure reducing effects of plant material extracts (8-11). Since both the hypertensive and the normotensive animal models are equally recommended to be employed in antihypertensive drug

screening studies (12), the relevance of the study aims at observing the blood pressure reducing effect of the residue from "Gebto Arekei" in the alternative normotensive laboratory animal model and thereby consolidate the finding of the study.

MATERIALS AND METHODS

The study was conducted between Oct 2000 and January 2001 in Addis Ababa.

"Gebto Arekei" and the residue:

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

Preparation of animals for blood pressure studies:

A total of 6 normotensive male guinea-pigs (Addis Ababa University, Faculty of Medicine, Ethiopia) weighing 400-650 gm were used in this study. The experimental protocol was approved by the Ethiopian Science and Technology Commission, (ESTC), for animal experimentation. All the experiments were conducted under phenobarbital induced anesthesia and analgesia and therefore free of pain.

Normotensive male guinea-pigs were anesthetized with sodium phenobarbital (50 mg/kg, i.p.). At the stage of light

anesthesia, a midline incision was made through the anterior of the neck to expose the trachea, the right common carotid artery and the left jugular vein. The trachea was halfway cut along its circular axis, then endotracheal cannula was inserted and the animal was artificially ventilated (Bioscience 815-51190-1, Sheemess, Kent, UK). Then the right carotid artery was then cannulated with heparinized saline-filled catheter connected to a pressure transducer (BBC, Goetz Metrawatt, Model SE 120) for recording of blood pressure. The right jugular vein was cannulated with similar tubing for intravenous infusion of saline and the residue. The exposed surface was covered with cotton wool moistened in warm saline.

Design of the experiment for pharmacodynamic studies:

After surgery, the blood pressure was allowed to stabilize during a period of 60 min. Then all the experiments started with a control recording of basal blood pressure with stable systolic and diastolic phases. Then the drug dissolved in normal saline was slowly administered as bolus injection in 0.5 ml syringe during 15 sec. The change in blood pressure during treatment with the residue was recognized as the difference between the steady state value before and the changed blood pressure state after infusion of the residue.

Data collection and statistical analysis

The main characteristic features of blood pressure recorded by a pen writer connected to a cannula inserted in carotid artery through a transducer are the measurable systolic and diastolic phases. Their values and duration are calculated from the calibration scale made at the beginning of each experiment. Pulse pressure was taken as the algebraic difference between the systolic and diastolic pressure. Mean blood pressure was calculated as the diastolic pressure plus one-third pulse pressure.

Data are expressed as mean and standard error of the mean of n experiments. The concentration response curve with ED_{50} value representing the logarithm of the concentration of residue producing 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 test was considered statistically significant.

RESULTS

In anaesthetized guinea-pigs, "Gebto Arekei" residue caused a fall in systolic, diastolic and mean blood pressure in a dose-dependent manner as shown by tracing from a typical experiment (Fig. 1).

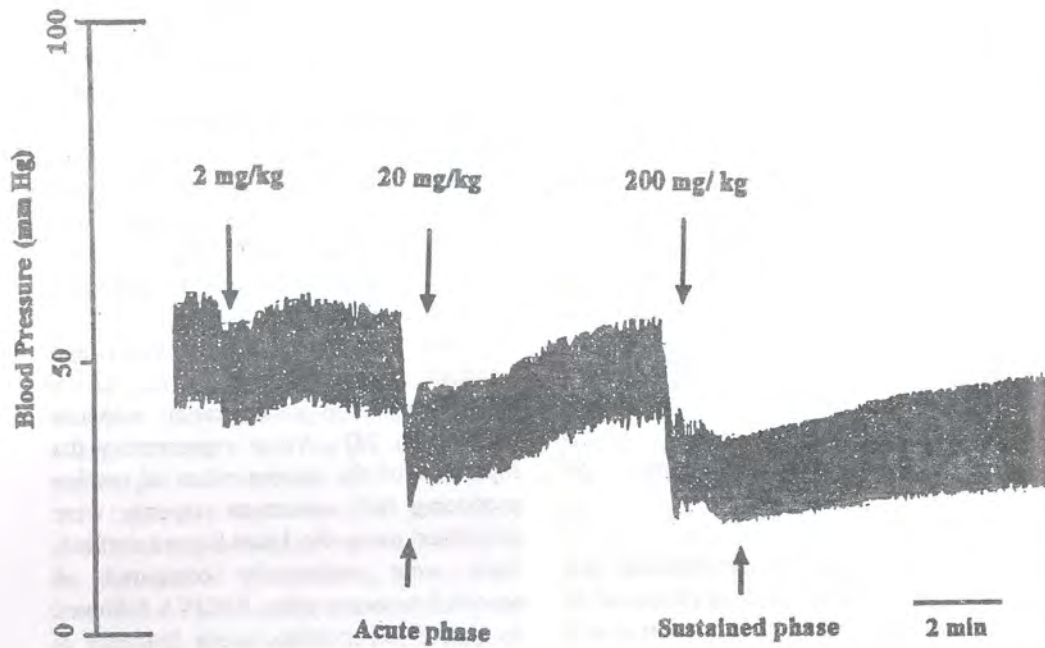


Figure 1. Recording of blood pressure by cannulation of the right carotid artery showing the effect of increasing intravenous doses of 2mg/kg, 20mg/kg and 200 mg/kg body weight of "Gebto Arekie" residue in normotensive guinea-pigs.

The hypotensive effect had two phases; Phase 1, which is a transient or acute response returning half way to normal within a minute. Phase 2, which is a relatively prolonged or sustained response returning to base line level with in 3 to 28 minutes depending up on the dose given. At doses of 2 mg/kg body weight, the residue reduced systolic, diastolic, pulse and mean blood pressure during the sustained phase by 12.69%, 15.14%, 9.27% and 11.72%, respectively. At a dose of 20 mg/kg body weight, the residue reduced systolic, diastolic, pulse and mean blood pressure by 20.42% ($p<0.005$), (29.34%

($p<0.005$), 8.11% and 26.22% ($p<0.0005$), respectively. 200 mg/kg body weight of the residue reduced systolic, diastolic, pulse and mean blood pressure by 39.45% ($p<0.0005$), 47.16% ($p<0.0005$), 25.89% ($p<0.05$) and 43.59% ($p<0.0005$), respectively (Table1). The percentage change in blood pressure variables measured during acute phase is similar to that of sustained phase (Table 1). There was a significant reduction in systolic, diastolic and mean blood pressure following infusion with 20 mg/kg and 200 mg/kg body weight of the residue (Fig. 2 & 3).

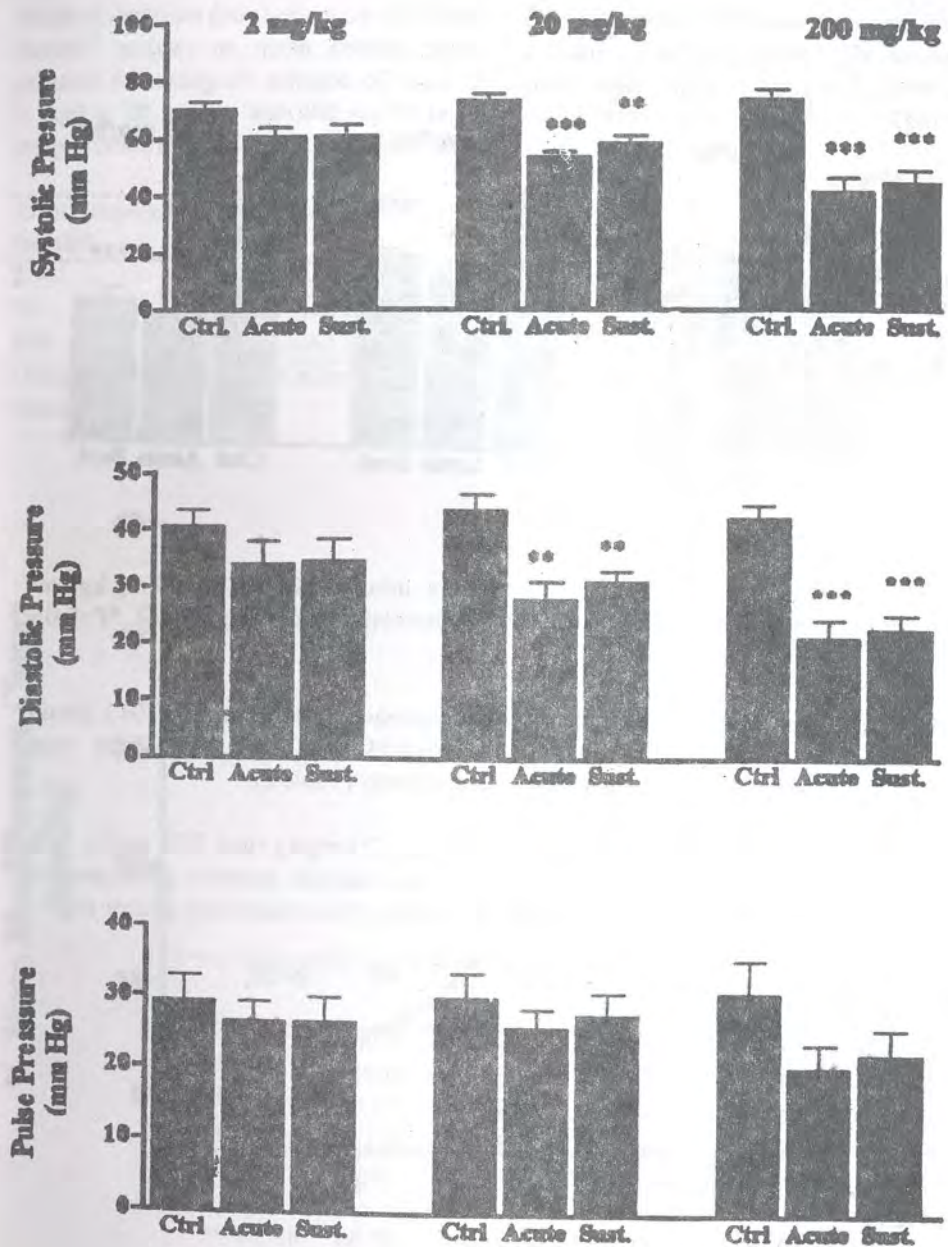


Figure 2. Composite diagram of the effect of "Gebto Arekei" residue on systolic pressure, diastolic pressure, pulse pressure and mean blood pressure in guinea pigs (n=6) during acute and sustained response (sust.) as compared to the control (ctrl) values. Mean values and standard error of the mean. * $p<0.05$; ** $p<0.005$, and *** $p<0.0005$.

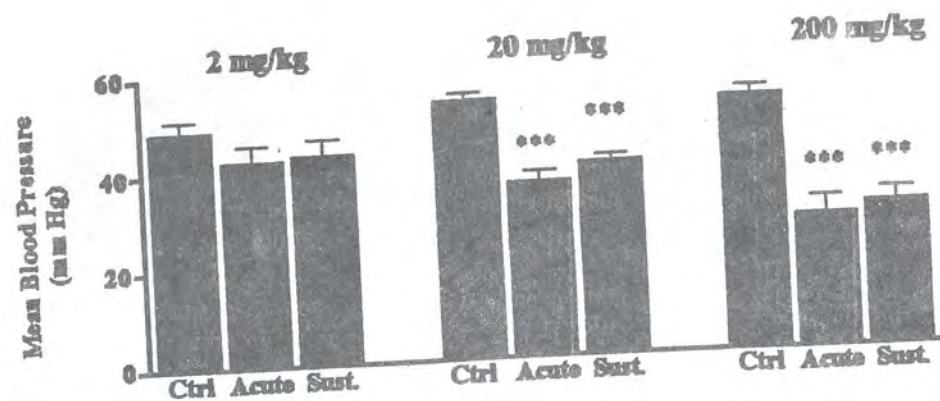


Figure 3. Mean blood pressure change following i.v. infusion of 2 mg/kg, 20 mg/kg, and 200 mg/kg body weight of "Gebto Arekei" in normotensive guinea-pigs (n=6). *P<0.05; **p<0.005, and ***p<0.0005.

The pulse pressure was not significantly affected. The duration of action for each of the three dose given as measured during the sustained response were 3.10±1.28min, 13.16±2.03 min and 21.33±1.89 min, respectively (Table 2).

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

Dose (mg/kg)		SP	DP	PP	MBP
Response (%)					
2mg/kg	Acute	12.48	16.52	9.26	12.84
	Sust.	12.69	15.14	9.27	11.72
20 mg/kg	Acute	27.06***	36.48**	13.76	32.17**
	Sust.	20.42**	29.34**	8.11	26.22**
200 mg/kg	Acute	44.00***	50.75***	34.43	47.74***
	Sust	39.45***	47.16**	25.89	43.59***

Mean values and standard error of the mean (n=6). *p<0.05;***p<0.0005. SP= systolic pressure; DP= diastolic pressure; PP= pulse pressure; MBP= mean blood pressure. ^aComparisons were made between steady state and base line blood pressure and changes in blood pressure after each dose of the residue was infused.

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, 20 mg/kg, and 200 mg/kg body weight in normotensive Guinea-pigs.

Dose (mg/kg body weight)	Duration (minutes)
2	3.10±1.28
20	13.16±2.03
200	21.33±1.89

Mean values and standard error of the mean (n=6)

The maximum reduction in mean blood pressure observed during the sustained phase after infusion with a dose of 200 mg/kg body weight was 43.59% (Table 1). The calculated ED₅₀, which is a concentration of the residue producing half the maximum response was 14.86 mg/kg body weight (Fig. 4).

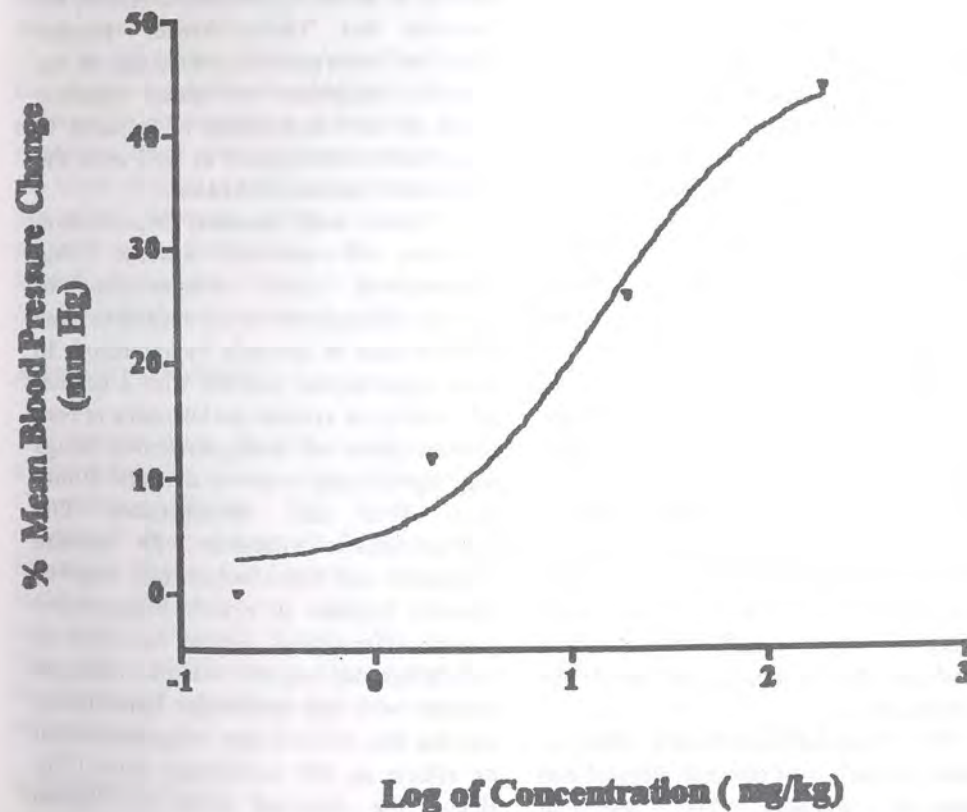


Figure 4. Dose-response curve showing the effect of increasing doses of "Gebto Arekei" residue on mean blood pressure in normotensive guinea pigs (n=6).

DISCUSSION

The traditional system of medicine in Ethiopia claims the antihypertensive medicinal property of *Lupinus albus*, and prescribes one of the two forms of use of the plant material. That is either eating whole seeds of the plant or use of "Gebto Arekei", which are said to have the same medicinal effect.

The result of this study demonstrates that infusion of a residue from "Gebto Arekei" at doses of 2 mg/kg, 20 mg/kg and 200 mg/kg had a dose-dependent hypotensive effect on the normal blood pressure. At a dose of 20 mg/kg, the systolic and diastolic pressures were reduced by 20.42% and 29.34% respectively. Previous studies made on "Gebto Arekei" residue showed that systolic and diastolic pressure dropped by 23.37% and 26.89%, respectively at dose of 20 mg/kg body weight, in renovascular hypertensive guinea-pigs (7). This demonstrates the effectiveness of "Gebto Arekei" residue in reducing blood pressure both in normotensive and hypertensive guinea-pigs.

Other pharmacological studies have also demonstrated the characteristic hypotensive activity of other related medicinal plants, such as *Othostegia integrifolia* and *Urtica cernis* (10,11). These clues of pharmacological evidence, in addition to their use in search for new drugs and the improvement of the already existing ones, are of paramount importance in realizing the rationality of indigenous medicinal plants.

The observed hypotensive effect of "Gebto Arekei", is of pharmacological and therapeutic interest. The use of antihypertensive therapy is increasing because a number of intervention trials have demonstrated that lowering blood

pressure reduces the incidence of cardiovascular morbidity and mortality (13). Trials in patients with non-malignant hypertension revealed the antihypertensive treatment reduced the incidence of fatal and nonfatal stroke by 42%, and the incidence of coronary heart diseases by 14% (14).

"Gebto Arekei" residue had more pronounced effect on diastolic component of blood pressure. It also reduced the mean blood pressure without marked change of the pulse pressure. The diastolic pressure is normally an important index in terms of hypertension with 85 mm Hg defined as borderline, 95 mm Hg as hypertensive, 100 mm Hg as severe hypertension. Hence, we speculate that "Gebto Arekei" residue which had more pronounced change on the diastolic component of blood pressure seems to have a potential to be used in hypertensive emergencies as well as in the treatment of mild hypertension.

Patients with essential hypertension also have left ventricular diastolic filling abnormalities (14). Abnormality of diastolic filling is one of the earliest cardiac manifestations of systemic hypertension. In those hypertensive patients with a normal left ventricular systolic performance at rest, administration of antihypertensive drugs may significantly improve diastolic filling (15). This also demonstrated that antihypertensive treatment with calcium antagonist and beta-blockers can improve diastolic function in elderly hypertensive patients. Teratolol, one of the series of such drugs, can improve diastolic filling in patients with left ventricular hypertrophy and that this effect occurs independently of its effects as left ventricular mass (16). Hence, the observed effect of "Gebto Arekei" residue in the reduction of diastolic pressure might also contribute to

the improvement of left ventricular diastolic filling.

The effective concentration of "Gebto Arekei" residue is found to be 14.89 mg/kg in normotensive and 19.69 mg/kg in hypertensive guinea-pigs (7). The result demonstrated that there was a correlation in the effect from treatment with the residue, on normotensive and hypertensive arterial pressure. Therefore, the observed effect indicates that there was no major difference in the models used in terms of efficacy and therapeutic index of the drug. However, since the drug administration was i.v., in our study, further research is needed to determine the reduction of blood pressure during oral administration.

In conclusion, "Gibto Arekei" residue has shown a significant reduction in blood pressure in normotensive guinea-pigs. The relatively small effective concentration of the crude residue (14.89 mg/kg) producing the effect observed seems to make this plant more attractive for further investigation in the aspects of purification, mechanism of action and clinical trials.

ACKNOWLEDGMENTS

The Authors thank the financial assistance of the Ethiopian Science And Technology Commission (ESTC). The study was conducted in collaboration between the Ethiopian Health and Nutrition Research Institute (EHNRI) and Addis Ababa University, Faculty of Medicine, Department of Physiology. We appreciate the sound and exemplary research activity undertaken.

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