Original Article

Journal of Evidence-Based Complementary & Alternative Medicine
2014, Vol. 19(3) 195-199

DOI: 10.1177/2156587214531019
cam.sagepub.com
SAGE

The Effect of Extract/Fractions of *Caralluma tuberculata* on Blood Glucose Levels and Body Weight in Alloxan-Induced Diabetic Rabbits

Khushbakht Sultan, MSc, MPhil[1], Muhammad Zakir, MSc, MPhil[1], Haroon Khan, BPharm, MPhil, PhD[2], Ihsaan Ullah Khan, MSc, MPhil[1], Ali Rehman, MSc, MPhil[1], Noor Ul Akber, MSc, MPhil[1], Naveed Muhammad, BPharm, PhD[2], and Murad Ali Khan, MSc, MPhil, PhD[1]

Abstract

Caralluma tuberculata is a cooked food item in Pakistan especially for diabetics. The current study was designed to explore the antidiabetic potential of extract/fractions of *Caralluma tuberculata* in alloxan-induced diabetic rabbits and its effect on body weight. The crude extract of the plant provoked 24% and 44% antidiabetic action at 25 and 50 mg/kg OP, respectively, after the 24th day of treatment, which was strongly supported by a positive effect on the body weight of animals. On fractionation, pretreatment of the ethyl acetate fraction demonstrated most dominant (25.17% and 34.83%) antidiabetic activity followed by *n*-hexane (19.33% and 32.76%) and aqueous fractions (16.44% and 22.36%) at 25 and 50 mg/kg OP, respectively, after the 24th day of treatment. The corresponding effect on blood glucose was also observed on body weight of diabetic rabbits. In sum, extract/fractions of the plant showed marked antidiabetic action and thus a provided scientific foundation to the use of the plant as an antidiabetic.

Keywords

Caralluma tuberculata, extract/fractions, antihyperglycemic effect

Received February 17, 2014. Accepted for publication March 17, 2014.

The genus *Caralluma* belongs to family Asclepiadaceae consist of approximately 2500 species that are extensively observed in Spain, India, Africa, Saudi Arabia, the Middle East, and Pakistan. *Caralluma tuberculata* and *Caralluma edulis* are the 2 species of the genus that are grown in Pakistan.[1] *Caralluma tuberculata* is a leafless, succulent, and angular plant that grows in the wild. In Pakistan, it is used it as food (cooked) as well as used in the treatment of different disorders such as leprosy, rheumatoid arthritis, irritation and swelling, diabetes, snake and scorpion bites, preventing fever (antipyretic), and for other antinociceptive activities.[2,3] A traditional healer in Saudi Arabia uses this plant in the treatment of ulcers and diabetes, and the juice of the plant is used as ear drops for inflammation and pain.[4] The antinociceptive, sedative, muscle relaxant, phytotoxic, and antioxidant potential of the plant has also been documented.[5,6] Phytochemically, various glycosides, mostly flavone[7] and pregnane, are isolated from the plant.[8,9]

The current study was designed to explore the antidiabetic activity of crude extract and subsequent solvent fractions of *Caralluma tuberculata* in alloxan-induced diabetic rabbits followed by a study of its effect on the body weight of experimental animals.

Methods

Plant Materials

Fresh plants of *Caralluma tuberculata* were purchased from local market in 2013. After collection, a plant taxonomist at the Department of Plant Sciences, KUST, Pakistan, determined the taxonomic identities of the desired plants.

Extract Preparation

Air-dried and coarsely powdered plants were extracted 3 times with methanol. The methanol extracts were evaporated under reduced pressure to give a dark-greenish residue (extract), which was further suspended in water and partitioned successively with *n*-hexane, chloroform, and ethyl acetate to obtain *n*-hexane-soluble,

[1] Kohat University of Science & Technology, Kohat, Pakistan
[2] Abdul Wali Khan University, Mardan, Pakistan

Corresponding Author:
Haroon Khan, PhD, MPhil, BPharm, Department of Pharmacy, Abdul Wali Khan University, Mardan 23200, Pakistan.
Email: hkdr2006@gmail.com

Table 1. Effect of Orally Administrated Extracts of *Caralluma tuberculata* on Blood Glucose Level of Alloxan-Induced Diabetic Rabbits.

			Drug Trial					
Extracts	Drug Dose (mg/kg)	Normal Glucose Level	0th Day	2nd Day	5th Day	10th Day	17th Day	24th Day
Crude + alloxan	25	124 ± 3.93	162 ± 4.07	130 ± 3.36	128 ± 3.27	126 ± 3.77*	125 ± 3.55*	124 ± 3.55*
	50	121 ± 2.77	168 ± 3.66	134 ± 3.44	128 ± 3.74	116 ± 3.48*	112 ± 3.87*	111 ± 3.27*
n-Hexane + alloxan	25	123 ± 3.86	144 ± 3.90	139 ± 3.86	131 ± 3.06	127 ± 3.28	120 ± 3.45	117 ± 3.54
	50	106 ± 4.01	146 ± 3.75	140 ± 2.87	129 ± 3.52	118 ± 3.69	112 ± 3.67	102 ± 3.29*
Chloroform + alloxan	25	115 ± 3.26	160 ± 3.86	150 ± 3.70	152 ± 3.74	152 ± 3.79	159 ± 3.96	155 ± 3.79
	50	117 ± 3.29	154 ± 3.29	141 ± 3.29	144 ± 3.88	145 ± 3.43	151 ± 2.85	151 ± 3.48
Ethyl acetate + alloxan	25	125 ± 3.57	147 ± 3.60	139 ± 3.88	135 ± 3.92	131 ± 3.58	127 ± 3.99*	110 ± 3.45*
	50	130 ± 3.79	155 ± 3.71	130 ± 3.91	128 ± 3.48	125 ± 2.97*	119 ± 2.93*	101 ± 3.90*
Aqueous + alloxan	25	126 ± 299	152 ± 3.99	142 ± 3.53	139 ± 4.06	133 ± 3.88	130 ± 4.02*	127 ± 3.69*
	50	130 ± 3.94	153 ± 3.83	138 ± 4.02	133 ± 3.65	130 ± 4.11*	124 ± 3.87*	118 ± 3.80*
Normal saline	10 mL/kg	127 ± 2.77	154 ± 4.12	162 ± 3.66	175 ± 4.29	182 ± 4.02	193 ± 4.02	203 ± 4.09
Glibenclamide + alloxan	5	110 ± 2.35	167 ± 3.55	140 ± 4.10*	132 ± 2.80*	126 ± 4.10*	120 ± 4.10**	109 ± 3.65**

[a] Values are mean ± standard error of the mean of 4 different rabbits.
*$p < 0.5$ was considered as significant from control.

chloroform-soluble, ethyl acetate-soluble, and aqueous fractions. The crude plant extracts and subsequent solvent-soluble fractions were then dissolved in distilled water individually and stored in a refrigerator at 4°C for future use.

Experimental Animals

Sixty-eight healthy rabbits 1 to 2 years age (1.2-1.5 kg weight) purchased from local market were divided into 17 groups; each group contained 4 rabbits and was assigned to 2 treatments in a completely randomized design at the start of the study. This study was conducted at the Department of Zoology, Kohat University of Science and Technology, Kohat, Pakistan. Management and hygiene measures were properly maintained. Fresh green fodder and tap water were provided to them daily and were placed at room temperature (22-24°C) during the experiment.

Experimental Design

Each group contained 4 rabbits and was assigned to 2 different treatments, 25 and 50 mg/kg OP. The rabbits of negative control were treated with normal saline (10 mL/kg), whereas the positive control was glibenclamide (5 mg/kg). The blood sugar level of each rabbit was thoroughly recorded with the help of glucometer before the start of the experiment. The rabbits were placed for a week under standard laboratory conditions and also to ensure they did not have any type of abnormality with regard to their health. The rabbits were kept in at room temperature (25-30°C) and were given tap water and fresh green fodder daily. These animals were approved for experimental purposes and studies by the Ethical Committee of Kohat Institute of Medical Sciences. This study was performed in the animal shade of the Zoology Department, KUST, Kohat, Pakistan.

Induction of Diabetes

These rabbits were made diabetic by means of alloxan monohydrate. It was administered orally in ice-cold aqueous medium at the dose rate of 150 mg/kg body weight daily for 1 week. This drug inhibits insulin production, due to which rabbits become artificially diabetic.[10] The rabbits shown symptoms of diabetes including acidosis, increased thirst, increased urination, and lack of energy. The rabbits with a blood glucose level of 140 mg/dL and above were considered as diabetic. After 6 days, the blood glucose level of each group of animals was recorded and assumed as zero days during launching the treatment trial. Alloxan monohydrate was given at the same rate of 150 mg/kg body weight. After 2 and 5 days, glucose level determination was started and continued up to the end of the experiment. After giving the extract, their blood was collected from the vein of ear and blood sugar was measured after an interval of 3 hours. At days 10, 17, and 24 their blood glucose levels were compared with the negative control.

Statistical Analysis

All values are presented as the mean ± standard error of mean and analyzed for ANOVA and post hoc Dunnet's *t*-test. Differences between groups were considered significant at $P < .05$. Statistical analysis was carried out on GraphPad PRISM 6 (San Diego, CA).

Results

Effect of Crude Extract of Caralluma tuberculata *on Blood Glucose and Body Weight*

The effect of crude extract of *Caralluma tuberculata* on blood glucose in alloxan-induced diabetic rabbit is presented in Table 1. It caused significant ($P < .05$) reduction in blood glucose, 24% and 44% at 25 and 50 mg/kg OP, respectively (Figure 1A). The effect of crude extract on diabetic rabbit is shown in Figure 2A. It had a marked increase in body weight and thus significant weight recovery after the 24th day of drug administration.

Effect of n*-Hexane Fraction of* Caralluma tuberculata *on Blood Glucose and Body Weight*

The effect of the *n*-hexane fraction of *Caralluma tuberculata* on blood glucose in alloxan-induced diabetic rabbit is

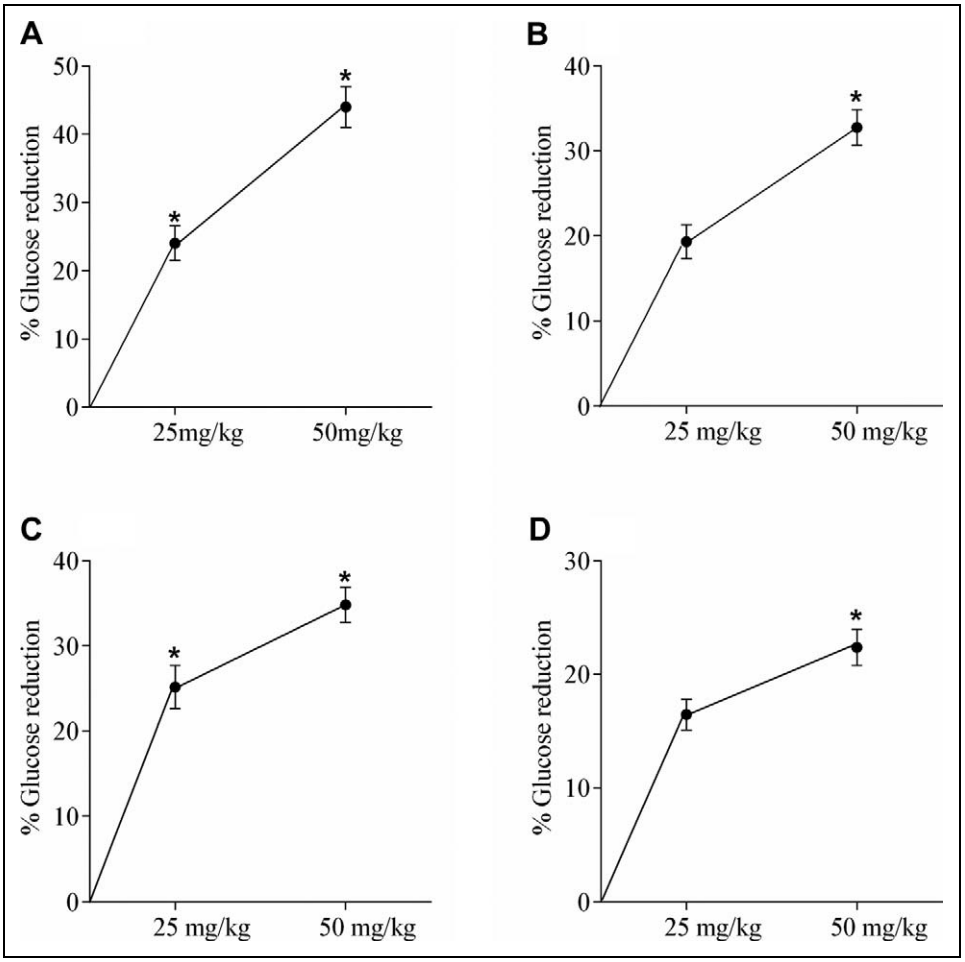

Figure 1. Percent effect of *Caralluma tuberculata* (A) crude extract, (B) *n*-hexane, (C) ethyl acetate, and (D) aqueous fractions on blood glucose in alloxan-induced diabetic rabbit. Values are mean ± standard error of the mean of 4 different rabbits.
*$p < 0.5$ was considered as significant from control.

presented in Table 1. It showed a significant ($P < .05$) effect only after the 24th day of treatment at the 50 mg/kg OP dose. The percent effect was 19.33% and 32.76% 25 and 50 mg/kg OP, respectively (Figure 2B). Overall positive effect on weight recovery was observed on both test doses (Figure 2B) after the 24th day of drug administration.

Effect of Chloroform Fraction of Caralluma tuberculata *on Blood Glucose and Body Weight*

The chloroform fraction of *Caralluma tuberculata* showed insignificant effect on blood glucose in alloxan-induced diabetic rabbits (Table 1). However, marked reduction in body weight of the test animal was observed (Figure 2C) after the 24th day of drug administration.

Effect of Ethyl Acetate Fraction of Caralluma tuberculata *on Blood Glucose and Body Weight*

The result of ethyl acetate fraction of *Caralluma tuberculata* on test doses (25 and 50 mg/kg OP) during various assessment times is illustrated in Table 1. Pretreatment of the fraction evoked significant ($P < .05$) amelioration of blood glucose in alloxan-induced diabetic rabbit. The percent effect was 25.21% and 34.83% at 25 and 50 mg/kg OP, respectively (Figure 1C). The diabetic control was also supported by improvement in overall weight recovery (Figure 2D) after the 24th day of drug administration.

Effect of Aqueous Fraction of Caralluma tuberculata *on Blood Glucose and Body Weight*

The effect of aqueous fraction of *Caralluma tuberculata* on blood glucose in alloxan-induced diabetic rabbit is presented in Table 1. Overall significant effect was observed with 16.44% and 22.36% at 25 and 50 mg/kg OP, respectively (Figure 1D). There was significant recovery in the body weight of test animals (Figure 2E).

Discussion

Hyperglycemia or diabetes mellitus is caused by an inherited or acquired deficiency in production of insulin by the

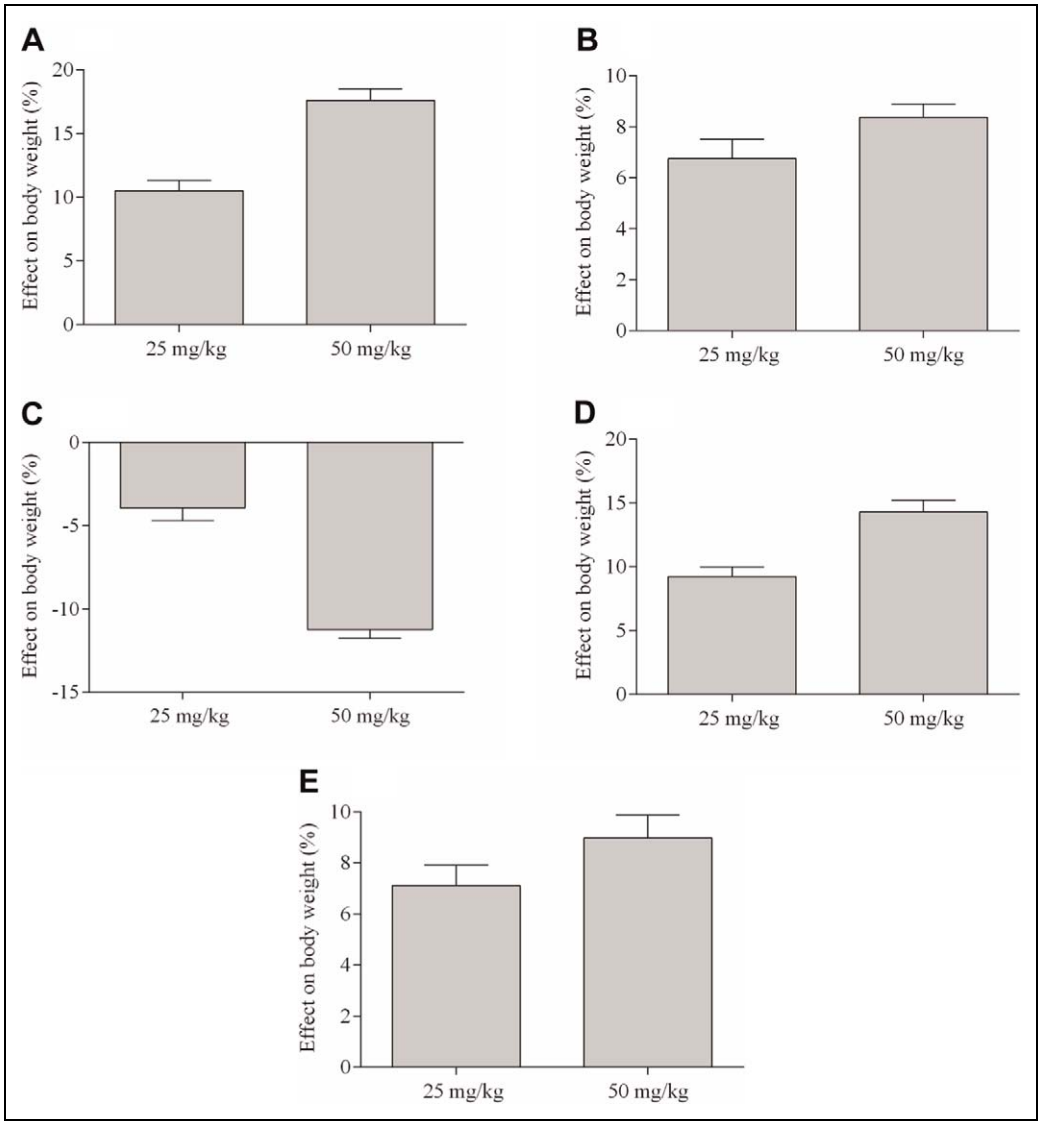

Figure 2. Effect of *Caralluma tuberculata* (A) crude extract, (B) *n*-hexane, (C) chloroform, (D) ethyl acetate, and (E) aqueous fractions on body weight in alloxan-induced diabetic rabbit. Values are mean ± standard error of the mean of 4 different rabbits.

pancreas or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentration of glucose in the blood, which in turn damages many of the body systems in particular the blood vessels and nerves.[11,12] Chronic hyperglycemia during diabetes causes glycation of body proteins, which in turn lead to secondary complications effecting eyes, kidneys, nerves, and arteries. Apart from currently available therapeutic options many herbal medicines have been recommended for the treatment of diabetes. Medicinal plants have the advantage of having no side effects.[13,14]

Different therapies are used in clinical practice for the management of diabetes including insulin and various oral antidiabetic agents such as sulfonylureas, thiazolidinediones, α-glucosidase inhibitors, and so on. These drugs are used as monotherapy or in combination to achieve better glycemic control.[15,16] However, they suffer from multiple problems in terms of patient compliance; the synthetic drugs are not effective in controlling hyperglycemia in the majority of patients.[10,15] Hence, antidiabetic drug discovery has shifted its focus to natural plant sources having minimal side effects.

Therapeutic history has recorded several examples from the plant kingdom primarily due to safety reasons. The World Health Organization is also in favor of alternate therapies for the effective management of diabetes, especially in those countries where results are coming from conventional therapies.[17] The results of our study shows the outstanding antidiabetic potential in crude extract and subsequent solvent fractions of *Caralluma tuberculata* except the chloroform fraction. The most promising blood glucose control was exhibited by the ethyl acetate fraction followed by crude extract in alloxan-induced diabetic rabbits.

In conclusion, the crude extract and subsequent solvent fraction of *Caralluma tuberculata* illustrated marked antidiabetic activity in alloxan-induced diabetic rabbits. The results

on blood glucose control were supported by the corresponding effect on body weight and thus provided a scientific rationale to the folk uses of the plant. In this regard, isolation of bioactive molecules are required to test and confirm the chemical background of such activities.

Author Contributions

KS, MZ, IUK, and AR were involved in plant collection, processing, and carrying out the experimental work. NUA and MAK supervised the overall study. HK and NM drafted the article.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: We are thankful to the Higher Education Commission (HEC) of Pakistan for providing funding for the study.

Ethical Approval

The study was approved by the Ethical Committee of the Kohat University of Science and Technology (KUST), Pakistan.

References

1. Ali S. Flora of Pakistan. *Asclepiadaceae*. 1983;150:1-65.
2. Abdel-Sattar E, Ahmed A, Mohamed-Elamir F, Mohamed A, Al-Yaha M. Acylated pregnane glycosides from *Caralluma russeliana*. *Phytochemistry*. 2007;68:1459-1463.
3. Khan S, Khatoon S. Ethnobotanical studies on some useful herbs of Haramosh and Bugrote valleys in Gilgit, northern areas of Pakistan. *Pak J Bot*. 2008;40:43-58.
4. Gushash A. *Plants in the Mountains of Sarat and Hejaz*. Al Madinah, Saudi Arabia: Sarawat; 2006.
5. Rauf A, Muhammad N, Barkatullah KH, et al. Antinociceptive, sedative and muscle relaxants activity of *Caralluma tuberculata* NE Brown. *Orthop Muscul Syst*. 2013;2(131). doi:10.4172/2161-0533.1000131.
6. Rauf A, Wajid Ur Rehman, Muhammad RJ, Muhammad N. Phytochemical, phytotoxic and antioxidant profile of *Caralluma tuberculata* NE Brown. *Wudpecker J Pharm Pharmacol*. 2013;2(2):21-25.
7. Rizwani G, Usmanghani K, Ahmad M, Ahmad V. Flavone glycosides of *Caralluma tuberculata*. *Pak J Pharm Sci*. 1990;3:27-32.
8. Abdel-Sattar E, Harraz F, Al-Ansari S, et al. Acylated pregnane glycosides from *Caralluma tuberculata* and their antiparasitic activity. *J Nat Med*. 2008;69:2180-2186.
9. Naik J, Jadge D. Anti-bacterial and anti-fungal activity of *Actiniopteris radiata* and *Caralluma adscendens*. *Int J Pharm Tech*. 2010;2:1751-1753.
10. Ahmed M, Kazim S, Ghori S, et al. Antidiabetic activity of *Vinca rosea* extracts in alloxan-induced diabetic rats. *Int J Endocrinol*. 2010;2010:841090.
11. Gao C, Aqie K, Zhu J, et al. MG132 ameliorates kidney lesions by inhibiting the degradation of Smad7 in streptozotocin-Induced diabetic nephropathy. *J Diabetes Res*. 2014;2014:918396.
12. Ukwe CV, Ubaka CM. Hypoglycemic activity of leaves of *Acanthus montanus* T. Anderson (Acanthaceae) in rats. *Int J Diabetes Dev Ctries*. 2011;31(1):32-36.
13. Ahmad M, Qureshi R, Arshad M, Khan MA, Zafar M. Traditional herbal remedies used for the treatment of diabetes from district Attock (Pakistan). *Pak J Bot*. 2009;41:2777-2782.
14. Patel D, Prasad S, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac J Trop Biomed*. 2012;2:320-330.
15. Nabi S, Kasetti R, Sirasanagandla S, Krishna T, Kumar M, Rao C. Antidiabetic and antihyperlipidemic activity of Piperlongum root aqueous extract in STZ induced diabetic rats. *BMC Complement Altern Med*. 2013;13:37-46.
16. Marles R, Farnsworth N. Antidiabetic plants and their active constituents. *Phytomedicine*. 1995;2:137-189.
17. Santhakumari P, Prakasam A, Pugalendi KV. Antihyperglycemic activity of Piper betle leaf on streptozotocin-induced diabetic rats. *J Med Food*. 2006;9:108-112.