Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Ethnopharmacology 102 (2005) 465–469
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Ethnopharmacological communication

Effect of the extracts and fractions of *Baccharis trimera* and *Syzygium cumini* on glycaemia of diabetic and non-diabetic mice

Antônio Carlos P. Oliveira, Denise C. Endringer, Luiz Alberto S. Amorim, Maria das Graças L. Brandão, Márcio M. Coelho *

Faculty of Pharmacy, Federal University of Minas Gerais, Avenida Antônio Carlos 6627, 31270-901 Belo Horizonte MG, Brazil

Received 8 October 2004; received in revised form 7 June 2005; accepted 10 June 2005
Available online 1 August 2005

Abstract

In the present study, we investigated the effects of extracts and fractions of *Baccharis trimera* and *Syzygium cumini* on glycaemia of diabetic and non-diabetic mice. Crude ethanolic extracts and aqueous and butanolic fractions of the aerial parts *of Baccharis trimera* and leaves of *Syzygium cumini* were evaluated. None of the extracts or fractions (200 or 2000 mg/kg, per os) induced any effect after acute administration. Seven-day treatment with crude ethanolic and aqueous and butanolic fractions (200–2000 mg/kg, twice daily, per os) of *Syzygium cumini* reduced glycaemia of non-diabetic mice. However, this effect was associated with a reduction of food intake and body weight, indicating that this may not be a genuine hypoglycaemic effect. In diabetic mice, only the aqueous fraction of *Baccharis trimera* (2000 mg/kg, twice daily, per os) reduced the glycaemia after a 7-day treatment. This effect was not associated with a body weight reduction. The results suggest that *Baccharis trimera* presents a potential antidiabetic activity and indicate that food intake and body weight must be determined when evaluating metabolic parameters after prolonged administration of plant extracts.

Keywords: *Baccharis trimera*; *Syzygium cumini*; Tannins; Glycaemia; Diabetes; Antidiabetic

1. Introduction

Plants have been used as sources of drugs for the treatment of diabetes in developing countries where the cost of the conventional medicines represents a burden to the population. Many species have been reported to present antidiabetic activity (Grover et al., 2002). *Baccharis trimera* (Less.) D.C. and *Syzygium cumini* (L.) Skeels (synonym: *Eugenia jambolana* Lam), from the Myrtaceae and Asteraceae families, respectively, are among the most commonly medicinal plants used to treat diabetes in Brazil. Although decoction, infusion and tincture of *Baccharis trimera* have been widely used to treat diabetes in Brazil, there is no report of their antidiabetic activity either in experimental animals or in humans. Furthermore, other species of the genus *Baccharis* may present marked toxicity (Jarvis et al., 1996; Varaschin and Alessi, 2003), raising concerns about the use of plants from this genus. On the other hand, the potential antidiabetic effect of *Syzygium cumini* has been previously evaluated. Both the aqueous and ethanolic extracts from the seeds reduced the glycaemia of diabetic animals (Prince et al., 1998; Grover et al., 2000; Sharma et al., 2003), an effect that may be associated with some inorganic constituents (Ravi et al., 2004). However, the decoction of leaves has neither presented antidiabetic activity in rats (Teixeira et al., 1997; Teixeira et al., 2000; Pepato et al., 2001) nor altered the glucose tolerance test in non-diabetic humans (Teixeira et al., 2000). Although the decoction is widely used by the population, its preparation may inactivate substances with potential pharmacological activity and may have contributed to the reported lack of effect in the last studies.

In the present study, we evaluated the potential antidiabetic effect of the crude ethanolic extract of *Baccharis trimera* and *Syzygium cumini* as well as the aqueous and butanolic fractions of these extracts. In addition, we evaluated if any

* Corresponding author. Tel.: +55 31 3499 6965; fax: +55 31 3499 6730.
E-mail address: mmcoelho@farmacia.ufmg.br (M.M. Coelho).

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.06.025

change in the glycaemia induced by a prolonged treatment with these extracts or fractions could be associated with a change of food intake and body weight.

2. Materials and methods

2.1. Plant material

Aerial parts of *Baccharis trimera* and leaves of *Syzygium cumini* were collected in Lagoa Formosa (MG, Brazil) and identified by J.R. Stehmann, Department of Botany, Federal University of Minas Gerais (BHCB 64921 and BHCB 46216, respectively).

2.2. Preparation of extracts and fractions

The plant material was dried, powdered and defated with hexane and then with ethanol 70%. The extracts were evaporated to dryness at 40 °C. A part of this crude ethanolic extract was used in the experiments. The other part was resuspended in water and successively extracted with dichloromethane and *n*-butanol. The fractions were evaporated to dryness at 40 °C and the aqueous fractions were lyophilised. The crude ethanolic extracts (EE), aqueous (AF) and butanolic fractions (BF) of *Baccharis trimera* and *Syzygium cumini* were used in the experiments.

2.3. Animals

Female Swiss mice with 22–27 g were used. The animals were maintained on a 12:12 h light–dark cycle and food and water were available ad libitum. Experiments were performed in accordance with the guidelines specified by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais. We used five to seven animals in each experimental group throughout the study.

2.4. Administration of extracts, fractions and drugs

Solutions or suspensions of extracts and fractions were prepared in saline immediately before the experiments. Tannic acid (TA), a mixture of gallotannin and tannin (Sigma, USA), was also suspended in saline. Solutions or suspensions were administered per os in a volume of 100 μl/25 g. Streptozotocin (200 mg/kg, Sigma, USA) and human NPH insulin (2 U/kg, Biobrás, Brazil) were injected intravenously and subcutaneously, respectively.

2.5. Determination of glycaemia

Mice were decapitated and blood was collected in tubes containing 50 μl of Na_2EDTA:NaF (60/30 mg in 1 ml of distilled water). Glycaemia was determined by an enzymatic method (glucose oxidase and aminoantipyrine – Labtest, Brazil).

2.6. Determination of body weight and food consumption

Daily food consumption of the whole experimental group and body weight of each animal treated with the extracts, fractions or TA were determined daily for 7 days.

2.7. Experimental protocols

2.7.1. Acute treatment of non-diabetic mice

Two doses of the extracts or fractions (200 and 1000 or 200 and 2000 mg/kg, depending on the protocol) or insulin were administered, food was removed and glycaemia was determined 3 h after.

2.7.2. Seven-day treatment of non-diabetic mice

Two daily doses (08:00 and 18:00 h) of the extracts or fractions (200, 1000, 1500 or 2000 mg/kg, depending on the protocol) or TA (50 or 500 mg/kg) were administered and glycaemia determined 3 h after the last dose. The first dose of the extracts, fractions or TA was administered at 18:00 h on day 1 and the last dose was administered at 08:00 h on day 8. Food was removed immediately after the last dose. One experimental group was treated with insulin in the last day and glycaemia was determined 3 h later.

2.7.3. Seven-day treatment of diabetic mice

Streptozotocin (200 mg/kg) was dissolved in buffer citrate (pH 4.5) and injected i.v. In this protocol, the animals were treated with the extracts or fractions (200, 1500 or 2000 mg/kg, depending on the protocol) for 7 days, two daily doses (08:00 and 18:00 h), with the first dose administered 24 h after the treatment with streptozotocin. The treatment with the extracts, fractions and insulin and also glycaemia analysis were carried out as described for the non-diabetic mice.

2.7.4. Acute treatment of non-diabetic mice submitted to glucose overload

To evaluate the effect of the extracts, fractions or TA on the elevation of glycaemia induced by the per os administration of glucose, non-diabetic mice were fasted for 15 h, before treatment. The extracts, fractions or TA were administered 30 min before glucose (1000 mg/kg) and glycaemia determined 1 h after.

2.8. Chemical characterization of extracts and fractions

The assays were performed using a HPLC Hewlett Packard model 1100, with a DAD detector. Column: RP-18 Chromolith 100 × 4.6 mm (Cat. 1.02129.0001, Merck). The eluent was acetonitrile:water (EM Science, AX0142-1), with gradient of 10–90% of acetonitrile in 30 min. Detection: UV spectra at 254 nm. Injection volume: 20 μl. Each sample (1 ml) was evaporated to dryness at less than 50 °C, dissolved in acetonitrile 20%, filtered (Minisart RC-15, 0.45 μm,

Sartorius) and directly injected in the HPLC. The identification of the compounds was carried out by analysis of the retention time (Rt) versus the characteristic bands in the UV spectra. Quercetin, rutin and chlorogenic acid were used as standards. Tannins concentration was determined according to the British Pharmacopoeia (British Pharmacopoeia, 1993).

2.9. Statistical analysis

Glycaemia and body weight were expressed as means ± S.E.M. Analysis of variance followed by Newman-Keuls post-hoc test was used. A level of significance of 0.05 was used. As food intake was determined for the whole experimental group and not for each mouse, the data obtained were not analysed.

3. Results

As in most of the protocols we did not observe any effect of the extracts and fractions of *Baccharis trimera* and *Syzygium cumini* on glycaemia of non-diabetic and diabetic mice, only the positive results are shown in the form of graphics.

3.1. Effect of acute treatment with the extracts and fractions of Baccharis trimera and Syzygium cumini on the glycaemia of non-diabetic mice

Glycaemia was not changed after acute per os treatment with EE, AF and BF of *Syzygium cumini* and *Baccharis trimera* (200 or 2000 mg/kg). However, insulin, used as a positive control, reduced glycaemia by 20–30%.

3.2. Effect of a 7-day treatment with the extracts and fractions of Baccharis trimera and Syzygium cumini and tannic acid on the glycaemia of non-diabetic mice

Glycaemia was reduced by a 7-day treatment with the AF and BF of *Syzygium cumini* (2000 mg/kg), but not with the EE and AF of *Baccharis trimera* (200 or 2000 mg/kg) and EE of *Syzygium cumini* (200 or 1000 mg/kg). However, this effect was associated with a reduction of food intake and also a significant reduction of body weight (Fig. 1). Glycaemia of the animals treated with the EE of *Syzygium cumini* (2000 mg/kg) were not analysed as they presented marked body weight reduction and sickness behaviour. Insulin reduced glycaemia by 20–30%.

To investigate if glycaemia reduction was due to reduced food intake, we administered the EE of *Syzygium cumini* (1500 mg/kg) to one group of animals that had free access to food, and saline to a second group fed with the same amount of food consumed by the first group the day before. Food restriction induced reduction of glycaemia and body weight that did not differ statistically from that induced by the EE in animals with free access to food (Fig. 1).

Bark and seeds of *Syzygium cumini* present a high concentration of tannins (Bhatia and Bajaj, 1975; Ramirez and Roa, 2003). In the present study, we observed concentrations of tannins ranging from 20 to 35% in the EE, AF and BF of the leaves. As tannins may present hypoglycaemic activity (Thompson et al., 1984), we investigated their effect on glycaemia, food intake and body weight. We used TA to simulate the tannins found in the extracts and fractions. The doses used (50 or 500 mg/kg, per os, twice daily) were chosen to simulate the amount of tannins administered in the animals treated

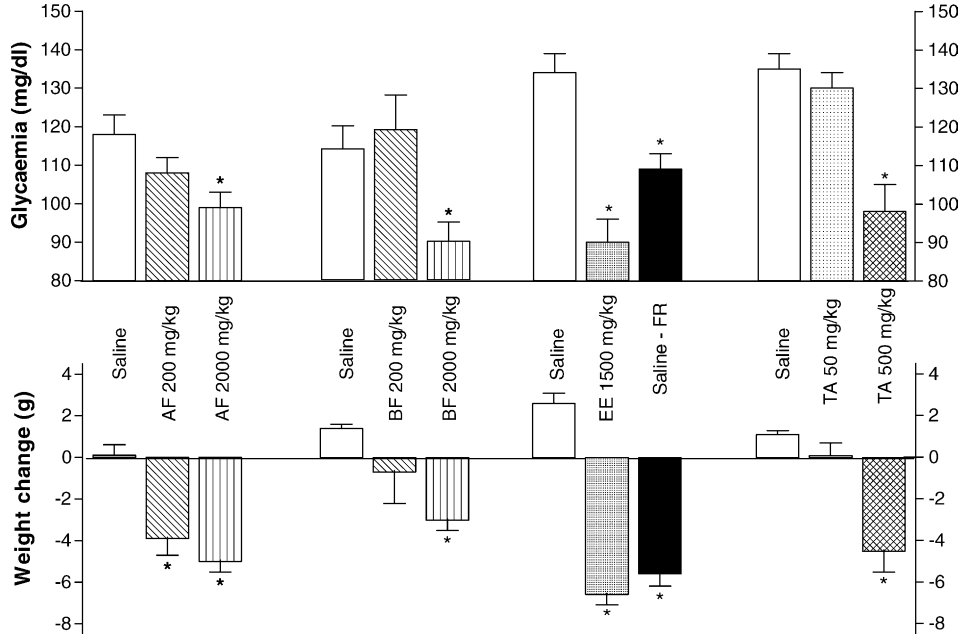

Fig. 1. Effect of a 7-day treatment of non-diabetic mice with crude ethanolic extract (EE), aqueous (AF) or butanolic (BF) fractions of *Syzygium cumini*, tannic acid (TA) and food restriction (FR) on glycaemia and body weight. $^{*}P < 0.05$ as compared with the group treated with saline ($n = 5$–7).

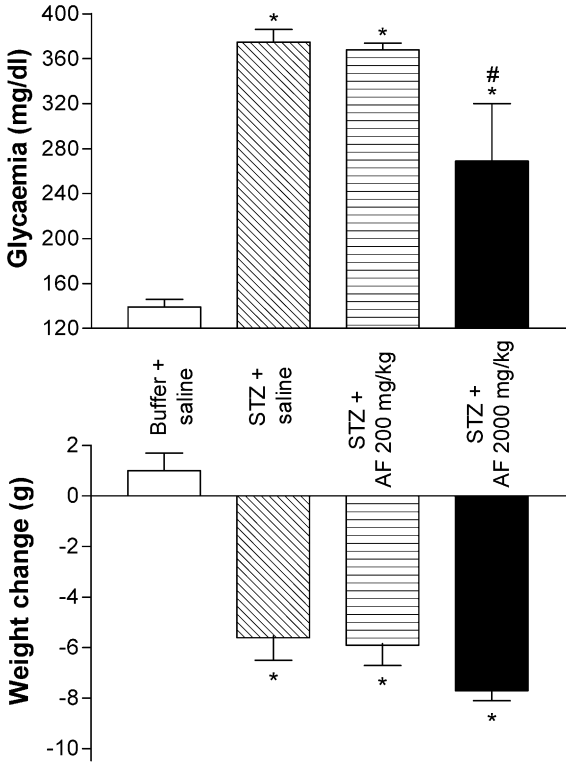

Fig. 2. Effect of a 7-day treatment of streptozotocin-induced diabetic mice with aqueous fraction (AF) of *Baccharis trimera* on glycaemia and body weight. *and #$P < 0.05$ as compared with the group treated with buffer + saline and streptozotocin (STZ) + saline, respectively ($n = 5–7$).

with AF of *Syzygium cumini* (200 or 2000 mg/kg). A 7-day treatment with the highest dose of TA (500 mg/kg) reduced glycaemia, food intake and body weight (Fig. 1).

3.3. Effect of a 7-day treatment with the extracts and fractions of Baccharis trimera and Syzygium cumini on the glycaemia of diabetic mice

The AF of *Baccharis trimera* (2000 mg/kg) significantly reduced glycaemia of streptozotocin-induced diabetic mice. However, no effect was observed after treatment with the EE, AF and BF of *Syzygium cumini* or EE and BF of *Baccharis trimera.* Although streptozotocin reduced the body weight of the mice, the effect of the AF of *Baccharis trimera* was not associated with additional reduction (Fig. 2). Insulin reduced glycaemia by 60–70% in streptozotocin-induced diabetic mice.

3.4. Effect of acute treatment with the extracts and fractions of Baccharis trimera and Syzygium cumini and tannic acid on hyperglycaemia induced by glucose overload in mice

We investigated whether AF (2000 mg/kg) and EE (2000 mg/kg) of *Syzygium cumini*, EE of *Baccharis trimera* (2000 mg/kg) and TA (500 mg/kg) could reduce the hyperglycaemia induced by a glucose overload. In the first protocol, glycaemia of the control animals (88 ± 4 mg/dl) was increased after the per os administration of glucose (119 ± 9 mg/dl), but not reduced by treatment with the EE of *Baccharis trimera* (133 ± 3 mg/dl) or *Syzygium cumini* (125 ± 5 mg/dl). In the second protocol, glycaemia was also increased after administration of glucose (65 ± 6 versus 92 ± 4 mg/dl), but was not changed by treatment with TA (104 ± 2 mg/dl) or AF of *Syzygium cumini* (103 ± 3 mg/dl).

3.5. Chemical constituents of the extracts and fractions

The chromatograms for the dichloromethane fractions of both plants showed peaks for several compounds between Rt 23.00 and 38.00 min, while the BF and AF showed peaks at Rt 12.00–21.00 min. The peaks from the active AF of *Baccharis trimera* were identified by their characteristic bands on the UV spectra as flavonoids and chlorogenic acids. Concentrations of tannins ranged from 20 to 35% in the EE, AF and BF of the leaves of *Syzygium cumini*.

4. Discussion and conclusion

Acute treatment with the fractions and extracts of *Baccharis trimera* and *Syzygium cumini* did not reduce glycaemia of non-diabetic mice. However, a reduction of glycaemia was observed after a 7-day treatment with the EE, AF or BF of *Syzygium cumini.* Noteworthy, this effect was associated with a significant reduction of body weight and food intake, arguing against a genuine hypoglycaemic effect. To better evaluate this possibility, a group of mice was fed with the daily amount of food that was consumed by the group treated with the EE of *Syzygium cumini.* Food restriction significantly reduced glycaemia and body weight, thus suggesting that lower food consumption may be associated with glycaemia reduction. This result raises important considerations concerning experimental protocols used to evaluate the effect of prolonged treatment with plant extracts on some metabolic parameters. Clearly, the effect on food consumption and body weight must be always reported to allow correct interpretation of the results. Several plant extracts present high concentrations of tannins, compounds that may exert an anti-nutritional effect by interfering with gut function (Carbonaro et al., 2001) and reduce the glycaemic response to carbohydrate foods in humans (Thompson et al., 1984; Gin et al., 1999). We hypothesize that these polyphenolic compounds may contribute to the reduction of food intake, body weight and glycaemia. TA reduced glycaemia and body weight, giving further support to this hypothesis.

As there was no evidence of a genuine effect of the extracts and fractions on the glycaemia of non-diabetic mice, we investigated their effect in diabetic mice. In streptozotocin-induced diabetic mice, only the AF of *Baccharis trimera* induced a partial, but significant reduction

of glycaemia. Although the mice had their body weight reduced after treatment with strepotozotocin, the effect of *Baccharis trimera* was not associated with an additional reduction. This effect may be associated with the presence of flavonoids and chlorogenic acids, as their hypoglycaemic activity has been previously demonstrated (Ahmad et al., 2000; Andrade-Cetto and Wiedenfeld, 2001). As far as we know, our results represent the first report of the potential antidiabetic effect of *Baccharis trimera* in experimental animals and justify further investigation.

We found no evidence of an antidiabetic effect induced by extracts and fractions of *Syzygium cumini* leaves, in different experimental models, including non-diabetic, diabetic and non-diabetic animals submitted to a glucose overload. Similar results have been reported by Teixeira et al. (2000) and Pepato et al. (2001) after prolonged treatment of diabetic rats with decoction of the leaves. However, different studies have shown that prolonged treatment with extracts of the seeds reduces glycaemia in diabetic rats (Prince et al., 1998; Grover et al., 2000; Sharma et al., 2003). Altogether, these results indicate that if *Syzygium cumini* presents an antidiabetic effect, this may be associated with compounds found in the seeds, not in the leaves.

In conclusion, the results indicate that the AF of *Baccharis trimera* reduces glycaemia of diabetic mice. However, this result must be weight against the toxicity reported for other species of the genus *Baccharis.* Our results also indicate that researchers must be careful in the interpretation of their results when the plant extracts induce a reduction of food consumption and body weight. These changes may affect not only glycaemia, but also other biochemical, physiologic and behavioural parameters.

References

Ahmad, M., Akhtar, M.S., Malik, T., Gilani, A.H., 2000. Hypoglycaemic action of the flavonoid fraction of *Cuminum nigrum* seeds. Phytotherapy Research 14, 103–106.

Andrade-Cetto, A., Wiedenfeld, H., 2001. Hypoglycemic effect of *Cecropia obtusifolia* on streptozotocin diabetic rats. Journal of Ethnopharmacology 78, 145–149.

Bhatia, I.S., Bajaj, K.L., 1975. Chemical constituents of the seeds and bark of *Syzygium cumini*. Planta Medica 28, 346–352.

British Pharmacopoeia, 1993. Her Majesty's Stationery Office, London.

Carbonaro, M., Grant, G., Pusztai, A., 2001. Evaluation of poliphenol bioavailability in rat small intestine. European Journal of Nutrition 40, 84–90.

Gin, H., Rigalleau, V., Caubet, O., Masquelier, J., Aubertin, J., 1999. Effects of red wine, tannic acid, or ethanol on glucose tolerance in non-insulin-dependent diabetic patients and on starch digestibility in vitro. Metabolism 48, 1179–1183.

Grover, J.K., Vats, V., Rathi, S.S., 2000. Anti-hyperglycemic effect of *Eugenia jambolana* and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. Journal of Ethnopharmacology 73, 461–470.

Grover, J.K., Yadav, S., Vats, V., 2002. Medicinal plants of India with anti-diabetic potencial. Journal of Ethnopharmacology 81, 81–100.

Jarvis, B.B., Wang, S., Cox, C., Rao, M.M., Philip, V., Varaschin, M.S., Barros, C.S., 1996. Brazilian *Baccharis* toxins: livestock poisoning and the isolation of macrocyclic trichothecene glucosides. Natural Toxins 4, 58–71.

Pepato, M.T., Folgado, V.B.B., Kettelhut, I.C., Brunetti, I.L., 2001. Lack of antidiabetic effect of a *Eugenia jambolana* leaf decoction on rat streptozotocin diabetes. Brazilian Journal of Medical and Biological Research 34, 389–395.

Prince, P.S.M., Menon, V.P., Pari, L., 1998. Hypoglycaemic activity of *Syzygium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats. Journal of Ethnopharmacology 61, 1–7.

Ramirez, R.O., Roa Jr., C.C., 2003. The gastroprotective effect of tannins extracted from duhat (*Syzygium cumini Skeels*) bark on HCl/ethanol induced gastric mucosal injury in Sprague-Dawley rats. Clinical Hemorheology and Microcirculation 29, 253–261.

Ravi, K., Sekar, D.S., Subramanian, S., 2004. Hypoglycemic activity of inorganic constituents in *Eugenia jambolana* seed on streptozotocin-induced diabetes in rats. Biological Trace Element Research 99, 145–155.

Sharma, S.B., Nasir, A., Prabhu, K.M., Murthy, P.S., Dev, G., 2003. Hypoglycaemic and hypolipidemic effect of ethanolic extracts of seeds of *Eugenia jambolana* in alloxan-induced diabetic rabbits. Journal of Ethnopharmacology 85, 201–206.

Teixeira, C.C., Pinto, L.P., Kessler, F.H.P., Knijnik, L., Pinto, C.P., Gastaldo, G.J., Fuchs, F.D., 1997. The effect *of Syzygium cumini* (L.) skeels on post-prandial blood glucose levels in non-diabetic rats and rats with streptozotocin-induced diabetes mellitus. Journal of Ethnopharmacology 56, 209–213.

Teixeira, C.C., Rava, C.A., Silva, P.M., Melchior, R., Argenta, R., Anselmi, F., Almeida, C.R.C., Fuchs, F.D., 2000. Absence of antihyperglycemic effect of jambolan in experimental and clinical models. Journal of Ethnopharmacology 71, 343–347.

Thompson, L.U., Yoon, J.H., Jenkins, D.J., Wolever, T.M., Jenkins, A.L., 1984. Relationship between polyphenol intake and blood glucose response of normal and diabetic individuals. American Journal of Clinical Nutrition 39, 745–751.

Varaschin, M.S., Alessi, A.C., 2003. Poisoning of mice by *Baccharis coridifolia*: an experimental model. Veterinary and Human Toxicology 45, 42–44.