

Full Length Research Paper

Baccharis dracunculifolia with high levels of phenol compounds reduces blood glucose in healthy human

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The present study aimed to analyze the amount of total phenolics compounds in the extract *Baccharis dracunculifolia* and evaluate glycemia in healthy humans. The study group consisted of 16 healthy individuals, aged 21±1.4 years. The cardiovascular parameters of blood pressure, heart rate and blood glucose was analyzed before and after treatment with or without *B. dracunculifolia* (20 mg/kg). *B. dracunculifolia* intake did not lead to significant changes in the cardiovascular variables, but, its ingestion reduced blood glucose levels in 25% (control group: 99 mg/dL and treated Group: 68 mg/dL, P<0.05). In the extract of *B. dracunculifolia* was identified high levels of phenolic compounds (methanol extract: 46.2±0.4 mg GAE/L; acetate extract: 70.5±0.5 mg GAE/L; ethanol extract: 30.3±0.21 mg GAE/L), as well as an efficient capacity for blood glucose reduction after oral intake.

Key words: Blood glucose, phenolic compounds, essential oils, medicinal plants.

INTRODUCTION

Natural medicine is gaining more space in the control of diseases, and the effects of various substances derived from plants are found in many natural treatments. The drugs of origin in plants constituted a principal alternative therapy in the mid 20th century and the interests in this type of therapy are growing again (Castilho et al., 2007). Currently, about 25% of drugs used in developed countries contain one or more ingredients extracted from plants, thereby reinforcing the importance of natural therapy. In the last decade, approximately 80% of the world population uses medicinal plants as the only way to access their basic health needs (Barnes et al., 2008).

The objective of research in ethnopharmacology is related with purpose of used medicinal plants in order to provide tools that can assist in the discovery of new drugs (Albuquerque and Andrade, 2002). The pharmacological effects on glycemic control bring various substances in their formulas arising of plants from different regions. In the study by Thakur et al. (2012), was reported that the extract of *Gymnema sylvestre* may control blood glucose (BG) levels by glycemic reducing.

Baccharis dracunculifolia DC, known as the “alecrim-do-campo” is represented by over 350 species, distributed principally in tropical countries in South America such

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as Brazil, Argentina, Colombia, Chile and Mexico, occupying the higher regions (Cestari et al., 2009). The chemical compounds found in the extract of *B. dracunculifolia* are characterized by caffeic acid, coumaric acid, cinnamic acid, aromadendrin, isosakuranetin and artepelin C (Guimarães et al., 2012). Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) is one of the principal phenolic compounds found in Brazilian green propolis (Choi et al., 2011). Although biological effects of artepillin C, such as antimicrobial (Aga et al., 1994), antioxidant (Feresin et al., 2003) and antitumor (Shimizu et al., 2006) activities, however, its effects on glucose metabolism are currently little studied.

The compounds found in *B. dracunculifolia* could make part in human diet directly and indirectly through green propolis intake, and thus a possible effect on blood glucose (BG), effect of the extract currently little studied, that could be of great relevance in the control of hyperglycemia. The aim of this study was to analyze the effect of acute administration of *B. dracunculifolia* on the glycemic response of healthy individuals during rest.

MATERIALS AND METHODS

Subjects

The study group consisted of 16 healthy individuals, aged 21 ± 1.4 years. Informed consent was obtained for the study in accordance with Resolution of the National Council of Health, which was approved by Ethics Committee (p.63830/2012).

Inclusion and exclusion criteria

Included in the study were patients without medical diagnostic of pathologies which were limited of metabolic homeostasis. All subjects but one were considered non-caffeine users as defined by the consumption of < 2 caffeinated coffee or tea beverages and/or < 5 caffeine-containing soft drinks per week.

Plant

The leaves of plant *B. dracunculifolia* was collected during July, 2012, at the Middle West State University - UNICENTRO, at coordinates $25^{\circ} 32' 07.24''$ south latitude and $50^{\circ} 39' 42.39''$ west longitude.

Extraction and isolation

The drying method employed was as described in the Brazilian Pharmacopoeia (1988), starting from 4 g of weighed sample. A sample of the leaves (2 kg) of *B. dracunculifolia* was cut in small pieces and refluxed with 60% aqueous ethanol for two times, each for 2 h. The sample was heated to 100°C for 3 days, after this period was weighed again. Samples of 50 g of ground leaves for every 220 ml of methanol were used, which remained on the shaker for a week, totaling 1100 g plant spray. Subsequently, the solution was filtered. The filtrate was then evaporated by rotary evaporator initially at a controlled temperature, and then in a water bath (3 days) with temperature controlled to complete removal of the solvent (methanol). After, the extract was diluted in a solution of

distilled water. The preparation method was based on the metabolic extract *B. dracunculifolia* by drying processes, spraying, orbital shaking of the extract in methanol, filtering using a rotary evaporator and addition of toxicity test of the extract on *Artemia salina*.

Total phenolics assay

Total phenolic content were determined by using the Folin-Ciocalteu assay, according to the procedure of Singleton and Rossi (1965). Absorbance measurements were made at 750nm in a spectrophotometer (UV-VIS lambda 40, Perkin Elmer, USA) and the results were calculated using a pre-prepared gallic acid calibration curve (0–100 mg/L). Determinations of the extracts and the calibration curve were carried out with three replicates, and the phenolics content was expressed as mg equivalents of gallic acid equivalents (GAE). The total polyphenol content were quantified in the extract of the *Baccharis dracunculifolia* plant (methanolic, ethanolic and acetone extracts).

Procedures

Eight healthy individuals were randomly divided in two groups, four for the treatment group (TG) and four subjects in control group (CG). The TG received 1 mg/kg of maltodextrin and before 30 min was been administrated orally in a solution of 20 mg/kg *B. dracunculifolia*. The CG received orally only 1 mg/kg of maltodextrin. During 90 min, the variables of HR, BP and BG were observed each for 10 min. The maltodextrin was dissolved in distilled water. Both CG and TG were fasted 8 h before supplementations. In addition, they were instructed to follow a diet with nocaffeine-containing products and alcohol, and avoid strenuous physical activity two days before the experiments. The cardiovascular parameters of blood pressure (BP) (mercury column) and heart rate (HR) (Polar – T-61) were measured in rest and after treatment administration (were checked every 10 min). Capillary blood samples (25 μl) were used to determine the BG in rest and after treatment administration of substances (checked every 10 min) using a digital glucosimeter (ACCU – CHEK Performa, Roche®). The subjects were fasted 12 h before all test protocols.

Statistical analysis

Data are expressed as mean \pm standard error of mean (SEM). Statistical significance was determined using an unpaired Student's t-test. Differences were regarded as statistically significant when the $P < 0.05$.

RESULTS AND DISCUSSION

Polyphenols Content

The results (Table 1) showed that *Baccharis dracunculifolia* plant is a rich source of phenolics compound and that ethanol and methanol were the better extracting solvent (46.2 mg/GAE/L and 70.5 mg GAE/L, respectively) Figure 1.

Phenolic compounds are antioxidant agents and their bioactivities may be related to their capacity to scavenge free radicals, chelate metals and inhibit lipoxygenase

Table 1. Cardiovascular and glycemia responses before and after treatments (n = 8).

Variables	Mean ± SEM			
	Control (CG)		<i>B. dracunculifolia</i> (TG)	
	Fasting	Post-Treatment	Fasting	Post-Treatment
HR (bpm)	60 ± 5	60 ± 6	60 ± 4	64 ± 8
SBP (mm/Hg)	107 ± 2	105 ± 5	105 ± 1	112 ± 7
DBP (mm/Hg)	65 ± 4	62 ± 4	60 ± 3	70 ± 5
BG (mg/dL)	70 ± 2	99 ± 3	71 ± 2	68 ± 4

Data are presented as means ± SEM. (unpaired Student's t-test). Abbreviations: HR: heart rate, SBP: systolic blood pressure; DBP: diastolic blood pressure.

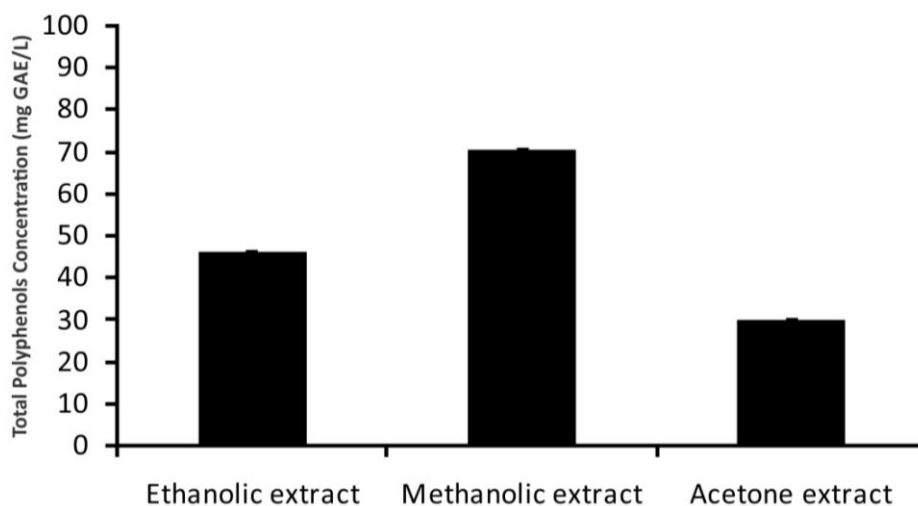


Figure 1. Blood glucose behavior during supplementations. The values are presented as mean ± SEM (n = 8). (*) Statistically different compared to inter-groups ($P < 0.05$; unpaired Student's t-test).

(Sahu and Saxena, 2013). The high contents of phenolic compounds observed in the solution of extract applied on the biological model suggests that these compounds contribute to the high antioxidant activity as well as others biological properties.

Cardiovascular analyzes

B. dracunculifolia intake did not lead to significant changes in the cardiovascular variables (Table 1). Treatment with artemisin C, the principal compound found in *B. dracunculifolia* extract may have anti-inflammatory action by inhibition of prostaglandin E2, and also increase the action of nitric oxide (NO) (Paulino et al., 2008). Studies show that increasing NO is directly involved in lowering blood pressure (Stojanovic et al., 1996; Vanhoutte, 2003). However, our results show that *B. dracunculifolia* raises cardiovascular scores and may be involved with other components of the extract. In the study reported by Hata et al. (2012), the artemisin C has acute effects on the transient receptor potential ankyrin 1 (TRPA1), which is non-selective Ca^{2+} -permeable

channel, thereby increasing the Ca^{2+} intracellular (Story et al., 2003). Also, the activation of TRPA1 could lead to elevation of blood pressure and heart rate, which could explain the findings of this study.

Glycemic analyses

Ingestion of *B. dracunculifolia* reduced glucose basal levels in 25% (control group: 99 mg/dl and treated Group: 68 mg/dl) (Figure 1). The *B. dracunculifolia* is commonly used in natural medicine, and many positive effects were reported. The composition of the extract has compounds with biological activities that may be interesting in diseases control, but that information is scarce, requiring further study. A recent study indicated attenuation of BG with treatment by Brazilian green propolis extract (Choi et al., 2011), showing that there is a relationship between the *B. dracunculifolia* extract and green propolis, both containing large amounts of artemisin C.

There is no high precision liquid chromatography (HPLC) finger print containing artemisin C, discussion about this compound is therefore not relevant to the

experimental result, showing that ingestion of *B. dracunculifolia* did not lower heart rate, systolic/diastolic blood pressure. HPLC analysis of this plant was previously reported by de Souza et al. (2009). The reduction in blood glucose levels after treatment with *B. dracunculifolia* shown in our study can be explained by increased expression of the glucose transporter 4 (GLUT4) in cells expressing the transporter, such information can be observed in study. Choi et al. (2011), which showed higher glucose uptake by administration of artemillin C is related with increased the mRNA and protein expression of GLUT1 and GLUT4 themselves. Thus, treatment with *B. dracunculifolia* can lead to control blood glucose levels, which could aid in the control of diseases related to metabolic disorders.

In a study by Paulino et al. (2008) demonstrated increased nitrite *in vitro* test by artemillin C, which could be involved in the production of NO, occurring like increased glucose uptake (McConell et al., 2006). Furthermore, there is a relationship between increase of Ca²⁺ and increase in NO (Balon and Nadler, 1994), and this increase of Ca²⁺ could be assisting in the cascade of GLUT4 expression and consequent increase glucose uptake. Thus, glycemic control by the extract of *B. dracunculifolia* could help reduce hyperglycemia and the development of diabetes, as the consequences of clinical condition in microvascular pathology, renal disease and a variety of debilitating neuropathies.

Conclusion

Through the analysis performed in this study, it was concluded that the treatment with *B. dracunculifolia* in individuals improve healthy glucose uptake.

Abbreviations

CG, Control group; **TG**, treatment group; **BG**, blood glucose; **SBP**, systolic blood pressure; **DBP**, diastolic blood pressure; **HR**, heart rate.

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