Advancement in Medicinal Plant Research
Vol. 4(1), pp. 1-10, February 2016
ISSN: 2354-2152
Full Length Research Paper

Teucrium polium significantly lowers blood glucose levels acutely in normoglycemic male Wistar rats: A comparative to insulin and metformin

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Accepted 11 February, 2016

ABSTRACT

Teucrium polium (commonly known as golden germander) is a herb that grows wild in the Mediterranean region. The natives commonly drink it as a tea for its medicinal reputation in alleviating ailments including diabetes mellitus. Some animal studies have supported its glucose lowering activity but the majority of these studies were uncontrolled and did not adequately assess its acute effect on blood glucose concentrations *in vivo*. The current study was undertaken to assess the acute glucose lowering effect of *T. polium* extract *in vivo.* We also aimed to identify some of the phytochemicals in the extract tested. A single dose of either the aqueous extract of *T. polium*, insulin, metformin or vehicle was injected in normoglycemic rats. Blood glucose samples were taken at set time points within the first 3.5 h of administration of each treatment. The glucose lowering potential of *T. polium* was compared to that of insulin and metformin. The total aqueous extract of *T. polium* significantly ($p < 0.01$) lowered blood glucose concentration with an efficacy approaching that of insulin within the first 30 min of its administration. The extract continued to lower blood glucose levels several hours following its administration and overall was more effective than metformin in lowering blood glucose over time. The total aqueous extract of *T. polium* lowers blood glucose levels acutely and potently. Some phytochemicals of bioactive reputation have been identified in this plant. However, the exact bioactive agent(s) and their mechanism(s) of action responsible for the glucose lowering effect are yet to be identified.

Keywords: *Teucrium polium*, golden germander, diabetes mellitus, antidiabetic effect, hypoglycemic potential, acute study, blood sugar levels.

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INTRODUCTION

Teucrium polium is a herb, native to the Mediterranean and Middle Eastern regions. The aerial parts of this herb are commonly made into a tea by boiling the leaves in water. Although the decoction is very bitter, many of the natives drink it for the treatment of a range of ailments including diabetes mellitus (Otoom et al., 2006). The various reported benefits of consuming *T. polium* have been related to its inherent flavonoid, terpenoid and volatile oil constituents (Aburjai et al., 2006; Pacifico et al., 2012; Sharififar et al., 2009; Kawashty et al., 1999; Florentino et al., 2011; Capasso et al., 1983; Tepe et al., 2011; Wassel and Ahmed, 1974a,b). The main chemical constituents identified in water, methanol and ethanol extracts of the aerial parts of *Teucrium polium* L. include apigenin (including dimethoxy apigenin, apigenin 5-galloylglucoside and vicenin-2), luteolin (including luteolin 7-glucoside), poliumoside (caffeic glycoside ester), rutin (quercetin glycoside), sabinene, germacrene D and β-caryophyllene.

The glucose-lowering activity of *T. polium* has been demonstrated in a small but growing number of studies in animals following continual dosing of the extract

(Ardestani et al., 2008; Esmaeili and Yazdanparast, 2004; Gharaibeh et al., 1988). Three animal trials reported on the acute glucose lowering effects of *T. polium* (Table 1), but these showed contradictory results (Afifi et al., 2005; Gharaibeh et al., 1988; Stefkov et al., 2011). The variability in the results can be attributed in part to: the study designs; the animal model used; the mode of administration; the numbers of animals used in each group; the time difference between plant extract administration and blood sampling; the controls (or lack thereof); the plant material used (authentic or not; year and soil); the extract preparation technique as well as the low statistical power in some studies.

In this study, we aimed to compare the acute effect of *T. polium* to known drugs on the market and also identified some of the phytochemicals present in the extract, in particular those of emerging glucose lowering reputation. We therefore monitored the blood glucose lowering effects of *T. polium,* metformin and insulin over an acute time-course in rats. Great care was taken to ensure that the sample size would be adequate to demonstrate a biologically relevant difference *in vivo*. We discuss our findings in context with each of the other studies investigating the potential of *T. polium* as a treatment to lower blood glucose levels in animal models.

MATERIALS AND METHODS

Plant material and extraction procedure

The dry plant material from *T. polium* L (Labiatae) was sourced from Jordan. It was collected from the Al-Salt region (25 km west of Amman) in April to May, 2004. The herb was authenticated by Professor Dawud Al-Eisawi, Department of Biological Sciences, Faculty of Science at the University of Jordan and brought into Australia under quarantine (Australian Quarantine and Inspection Service permit).

The aerial parts (2.5 g) of *T. polium* were extracted for 3 h in 200 ml of 90% methanol using a Soxhlet Extractor at 80 to 100°C (the extraction time was equivalent to three reflux cycles). This process gave an extraction yield from dry plant material of 20.4%. The plant extract was cooled and then concentrated in a rotary evaporator set at 45 ± 1°C for 30 to 45 min. The concentrate was then divided into aliquots calculated to enable single dose delivery of extract from 100 mg of dry starting plant material (20.4 mg final extract weight per animal) following dissolution in vehicle, with pH adjustment and filtration, as described below. The vials were placed in a vacuum oven set at 45 ± 1°C for 3 h to ensure dryness before purging with nitrogen and storage at 4°C. All extracts used in this study were generated from the same single batch of extraction.

Chemical fingerprint profiling of the plant extract was carried out by Dr Adrian Lutz, Metabolomics Australia, School of BioSciences, University of Melbourne. Total ion chromatograms (TIC) were collected using a Liquid Chromatography-Mass Spectrometry (LC-MS) system: Agilent 6520 QTOF MS system (Santa Clara, CA, USA) with a dual sprayer electrospray ionization (ESI) source and attached to an Agilent 1200 series HPLC system (Santa Clara, CA, USA) comprised of a vacuum degasser, binary pump, with a thermostated auto-sampler and column compartment. The MS was operated using the following conditions: nebuliser pressure 45 psi, gas flow-rate 10 L/min, gas temperature 300°C, capillary voltage 4000 V in positive ESI mode (3500 V in negative ESI mode), fragmentor 150 and skimmer 65 V. Tandem MS analysis was conducted at 10, 20 and 40 CE. Instrument was operated in the extended dynamic range mode with data collected in m/z range 70 to 1700 amu.

An Agilent Zorbax Eclipse XDB-C18, 2.1 × 100 mm, 1.8 µm (Agilent) column was used with a flow rate of 400 µl min^{-1}, maintained at 40 ± 1°C, with a 17 min run time. A gradient LC method was used with mobile phases comprised of (A) 0.1% formic acid in deionized water and (B) 0.1% formic acid in acetonitrile. Gradient: 5 min linear gradient from 5% solvent (B) to 30% solvent (B), followed by a 5 min linear gradient to 30% solvent (B) to 100% solvent (B), then a 2 min hold at 100% solvent (B) and a 4 min re-equilibration at 5% solvent (B).

Chemical profiling of the extract using the negative ESI mode (Figure 1) revealed a number of the previously documented secondary metabolites found in *T. polium* (Pacifico et al., 2012; Sharififar et al., 2009; Kawashty et al., 1999). Apigenin, rutin and, in much less quantities, quercetin were identified by matching molecular features against an in-house library and manual retention time. These compounds were further confirmed to be in the plant extract by matching signals in both reverse phase +/- ESI modes against external standards (apigenin, quercetin dihydrate and rutin hydrate purchased from Sigma) for accurate mass, retention time as well as MSMS fragmentation spectra. Other compounds which have been putatively identified with reasonable certainty in the extract by comparison of the LCMS profile run in +ESI mode against a compound library matching with accurate mass and retention time include: diosmetin, luteolin, carnitine, tricin and diosmin.

Total phenolic content on random stored extracts was measured regularly, before and after animal experimentation (30 days), as an estimate of stability. The total phenolic content in the stored extracts decreased from 14.2 to 13.5 mg during the 30 days of animal experimentation. Phenolic content was quantitated according to the method published by the Association of Official Analytical Chemists (1990), which entails colorimetric estimation of phenolic compounds at 760 nm in relation to a tannic acid standard curve. The total phenolic content was measured and used as a crude estimate of stability of the total extract while in storage during the study period. Although there was ~5% reduction in total phenolic content, this did not appear to influence the effectiveness of the extract in reducing blood glucose levels in the rats over the entire period of experimentation. It is worth noting that it is not possible to determine the exact stability of the active component as that compound is yet to be identified.

On the day of animal experimentation, a fresh vial of the stored extract was solubilized in phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, 18 mM KH_2PO_4), pH adjusted to 7.4 and then filtered immediately, using a 0.8 µm syringe filter, before administration. The pH of the extract was adjusted to suit the physiological conditions of the animal blood.

Experimental animals

Male Wistar rats (180 to 190 g) were purchased from the Animal Resource Centre, Murdoch Drive, Murdoch, Western Australia 6150 and were housed at the Animal Facility at Curtin University. Animal experimentation was approved (Approval No: R10/2009) by the Animal Ethics Committee at Curtin University. The research was conducted in accordance with the Australian Code for the Responsible Conduct of Research and in compliance with the Animal Welfare Act 2002 (http://www.slp.wa.gov.au/legislation/statutes.nsf/main_mrtitle_11300_homepage.html).

The rats were fed pelletised commercial chow, allowed free access to water, were caged either individually or in pairs and allowed to acclimatise for 3 days prior to experimentation. All animals were housed under standard conditions and temperature

Table 1. Published studies on the acute effects of *Teucrium polium*.

Reference	Study design			Outcome: Blood glucose levels
Garaibeh et al. (1988)	Male Fisher NG and STZ (65 mg/kg body weight, by ip) induced diabetic rats (200 - 250 g)			
	Oral:			
	Purina laboratory chow *ad lib.*			Oral: Not Significant
		NG	*STZ*	
	Ad lib Tap Water	n = 9	n = 9	
	Ad lib Decoction*	n = 5	n = 17	
	*1 ml ≡ 40 mg of dry starting *Tp* material.			
	Blood samples taken before and 24 h later.			
	Intraperitoneal (ip):			
		NG	*STZ*	ip: Significant ($p < 0.05$) decrease relative to pre-treatment values
	5 ml Saline	n = 9	n = 9	
	5 ml Decoction*	n = 5	n = 17	
	*Equivalent to 1 g of dry starting *Tp* material.			
	Blood samples taken before and 24 h later.			
	Intravenous (iv):			
		NG	*STZ*	iv: Significant ($p < 0.01$) decrease relative to pre-treatment values
	5 ml Saline	n = 9	n = 6	
	5 ml Decoction*	n = 8	n = 8	
	*Equivalent to 1 g of dry starting *Tp (labiatae)* material.			
	Rats were fasted for 4 h. Blood samples taken before and 4 h later.			
Afifi et al. (2005)	Males and female NG & Alloxan (150 mg/kg body weight, by iv) induced diabetic white French rabbits (2.5 to 3.4 kg) – same animals both NG, then Alloxan induced.			
	Intranasal:			
	NG rabbits (n = 10) were administered 0.1 ml extract per kg rabbit body weight, ie equivalent to 25 to 34 mg of dry starting *Tp (labiatae)* material, followed by one week washout. Rabbits then rendered diabetic (Alloxan induced over 7 days) and 0.1 ml extract re-administered.			
	NG			
	Vehicle	n = ?		No difference between extract and control
	0.1mL extract (10 mg/kg)	n = ?		
	Blood sampling taken at 0, 15, 30, 45, 60, 120, 180, 240 and 300 min.			

Table 1. Continues.

	Alloxan		
	Vehicle	n = ?	No difference between extract and control
	0.1 ml extract (10 mg/kg)	n = ?	
	Blood sampling taken at 0, 15, 30, 45, 60, 120, 180, 240 and 300 min.		
Stefkov et al. (2011)	Male Wistar NG and STZ (35 mg/kg body weight, by ip) induced diabetic rats (460 to 500 g) – different animals used for NG from STZ induced.		
	Intragastric:		
	NG		
	5 ml distilled water	n = 6	Significant ($p < 0.001$) decrease with T1 or T2 compared to water.
	5 ml T1* (125 mg/kg)	n = 6	
	5 ml T2** (125 mg/kg)	n = 6	
	5 ml Glibenclamide (2.5 mg/kg)	n = 6	
	Blood glucose measured at 0, 4, 8 and 12 h.		
	STZ		
	5 ml distilled water	n = 6	Significant ($p < 0.05$) decrease with T1 or T2 at day one compared to water.
	5 ml T1* (125 mg/kg/day)	n = 6	
	5 ml T2** (125 mg/kg/day)	n = 6	
	5 ml Glibenclamide (2.5 mg/kg)	n = 6	
	Blood glucose measured at 0, 4, 8, 12, 24 h and per day for 10 days.		†Significant decreases reported with extracts were comparable with the average effect of Glibenclamide.
	Used *Tp (capitatum)*; Extract T1* = spray dried; extract T2** = freeze dried		

NG = Normoglycemic; STZ = Streptozotocin; *Tp* = *Teucrium polium.*

(20 ± 1°C), with a regular 12 h dark and 12 h light cycle. Beddings were changed as necessary and, in particular, just prior to the 18 h fast before administration of the treatment. During their acclimatisation periods, the rats gained weight, on average about 10 g per day, and weighed in the range of 190 to 232 g on the day of experimentation (Table 2).

Animal study design

This current pilot study was carried out to screen for the acute "anti-diabetic" effects of *T. polium* by measuring blood glucose levels in response to intravenous injection of the plant extract in comparison with known drugs (insulin and metformin). Metformin was used since it too is of plant origin and insulin was used as the gold standard; these drugs are representative treatments for type II and type I diabetes mellitus, respectively. The extract concentration used in this trial is in line with that used previously by Gharaibeh et al. (1988). The dose used in our acute study (100 mg/kg) was much less than the reported LD_{50} of 262 mg/kg with chronic use (Khleifat et al., 2002). The concentration of extract used did not negatively affect any of the animals but the concentrations of insulin and metformin needed adjusting to avoid ill-effects to the rats studied.

The animal model used was an extension of that described to investigate insulin in an anesthetized animal model by Schaffer et al. (2003). The anesthetised animal model was chosen as the stress-related variations in blood glucose levels over time with repeated handling is avoided and thus allows for better control of background noise when assessing the acute effects of treatments.

Animals were fasted overnight and then randomly allocated into one of four treatment groups: Vehicle (PBS); Insulin; Metformin or *T. polium*. The rats were anaesthetized with 2 ml of an anesthetic combination delivering ketamine (75 mg/kg) and xylazine (10 mg/kg) as

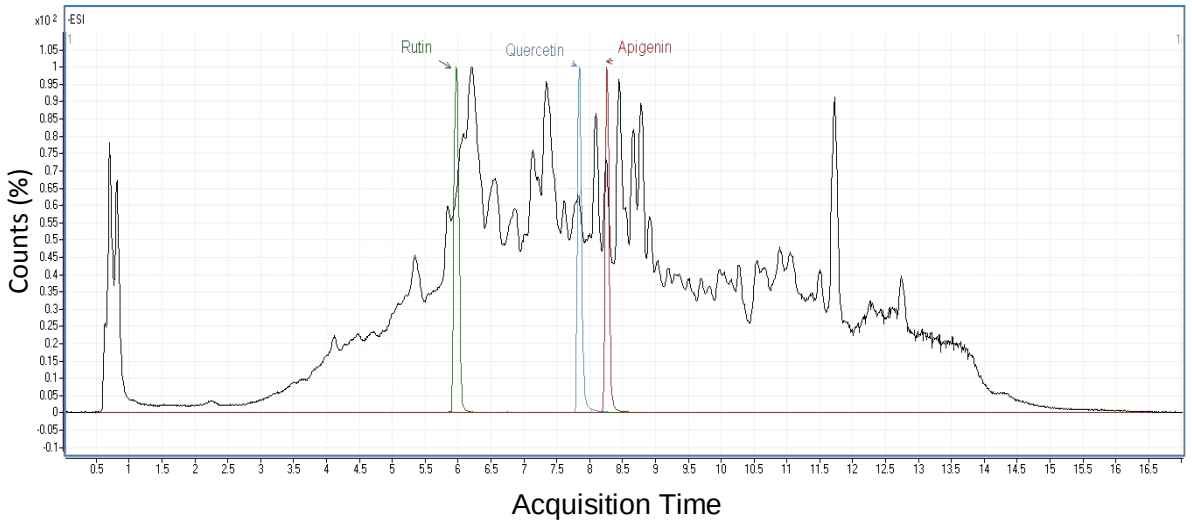

Figure 1. Total ion chromatogram (TIC) scan of the plant extract in -ESI mode. Three compounds were confirmed with reasonable certainty by matching extracted ion chromatogram signals (overlayed and adjusted to 100%) of the plant extract with accurate mass, retention time and MSMS fragmentation of pure compounds. Other compounds which have also been identified with reasonable certainty in the +ESI by comparison of the LCMS profile of the extract against a compound library matching for accurate mass, retention time and MSMS fragmentation profiles include: diosmetin, luteolin, carnitine, tricin and diosmin.

Table 2. Baseline rat weights (grams) and blood glucose concentrations at the specific time points through the trial within each treatment group. The p-values at each time point from 30 min onwards were obtained from a random effects regression model, and show the statistical significance of the change from baseline in the blood glucose concentrations within each treatment.

Experimental Group	Dose in 0.2 ml of PBS	Rat weight (g) Mean (SD) [range]	Blood Glucose Concentrations (mmol/L) Mean (SD)					
			Fasting (0 min)	30 min	60 min	120 min	180 min	210 min
Vehicle (n = 8)	PBS	208.1 (15.0) [190 – 232]	7.60 (0.8)	7.39 (1.3) p = 0.6886	7.16 (1.3) p = 0.3579	6.76 (1.1) P = 0.0669	6.55 (1.3) p = 0.0208	6.28 (1.5) p = 0.0035
Metformin (n = 6)	20 mg/rat	199.3 (9.6) [190 – 215]	6.73 (0.9)	5.82 (0.7) p = 0.1803	6.03 (0.8) p = 0.3597	5.28 (0.6) p = 0.0179	4.35 (1.2) p < 0.0001	4.02 (1.2) p < 0.0001
Plant Extract (n = 8)	100 mg/rat	214.6 (10.8) [200 – 230]	6.39 (0.8)	4.42 (0.3) p < 0.0001	4.58 (1.3) p < 0.0001	4.05 (1.4) p < 0.0001	3.45 (0.6) p < 0.0001	3.11 (0.9) p < 0.0001
Insulin (n = 8)	0.1 Unit/rat	220.5 (7.0) [210 – 232]	7.05 (0.6)	5.08 (1.4) p < 0.0001	4.30 (1.4) p < 0.0001	3.03 (0.3) p < 0.0001	2.21 (0.2) p < 0.0001	1.95 (0.1) p < 0.0001

a priming dose 50 min before test-substance injection, followed by an additional 1 ml/kg of this combination at 40 minute intervals thereafter. This protocol ensured that the blood glucose concentration in each animal was at steady state prior to tail vein injection with the test-substance.

The rats received a single 200 µl intravenous injection (tail vein) of either PBS (n = 8), 0.5 U/kg of Humulin R (insulin) in PBS (n = 8), 100 mg/kg of 1,1-dimethylbiguanide hydrochloride (also known as metformin) in PBS (n = 6) or an equivalent total extract from 100 mg of dry *T. polium* solubilised in PBS (n = 8). Blood samples were obtained by nicking the lateral tail vein with a scalpel (Caccetta and Al Salami, 2013). Baseline blood samples were taken 45 min after the initial priming anesthetic injection (5 min before test substance administration) and this was considered to be the baseline (zero time) measurement. Subsequent blood samples were taken at 10, 20, 30, 45, 60, 90, 120, 180 and 210 min following administration. Whole-blood glucose concentration was immediately determined using Accu-chek Go Glucometer Strips read in an Accu-chek® *Go* Glucometer. Upon completion of experimentation all rats were euthanized by cervical dislocation while under anaesthesia.

Statistical methods

Each rat was weighed on arrival to the facility and at the start of the day of experimentation. The blood glucose data were compared between treatment groups at baseline using an Analysis of Covariance model, with the weight of the rat included as a covariate in the model. A repeated measures analysis was then used to model change in blood glucose from baseline, with covariates of baseline weight and the interaction between time (as a categorical variable) and treatment group. This analysis allowed a different pattern of change over time for each treatment. The correlations in the data due to the multiple measurements on the same rat were taken into account by modelling the rat as a random effect. Finally, a separate regression model was fitted at each time point, in order to make a direct comparison of blood glucose concentration between groups at each time that the blood was taken. Data were transcribed into an Excel spreadsheet, and transferred for analysis in the SAS version 9.2 software (SAS Institute Inc, Cary, NC, USA. 2008). For all statistical tests, a p-value < 0.05 was taken to indicate a statistically significant association.

RESULTS

The acute effects of intravenous administration of vehicle alone, metformin, insulin or *T. polium* on blood glucose levels were compared in fasted normoglycemic rats (Figure 2). Table 2 shows the mean weights of the rats at baseline, and the mean blood glucose levels measured at each time point during the study. The baseline weights appeared to differ between groups ($p = 0.0011$), so this was included as an independent variable in subsequent analyses. The average baseline (time = 0) blood glucose levels varied significantly amongst the four treatment groups ($p = 0.0071$). In order to adjust for these baseline differences, subsequent analyses used the change in blood glucose from baseline as the dependent variable. The first analysis (based on the whole dataset) included the baseline weight, and the interaction between time and treatment as independent variables. This model showed no significant influence of baseline weight ($p = 0.2964$), but very significant interaction between time and treatment ($p < 0.0001$). Table 3 shows p-values indicating the statistical significance of changes from baseline in blood glucose concentrations for each treatment.

Blood glucose levels of animals receiving the vehicle decreased very slowly over the 3.5 h of measurement (Figure 2 and Table 2). The p-values show that the drop in blood glucose for the vehicle group first appeared to significantly differ from baseline at 3 hours, while the group treated with metformin showed a significant change from 2 h onwards. On the other hand, both the insulin and plant extract groups showed significant changes from baseline from the 30 min time point in the study.

Table 3 shows a series of analyses performed at each time point separately. Each of these analyses included the baseline weight of the rat and the treatment group as independent variables, and used the change in blood glucose from baseline as the dependent variable. There appeared to be no significant difference between metformin and vehicle throughout the study (Table 3). In contrast, relative to the vehicle, insulin induced a rapid and sustained decline in blood glucose levels over this time. This effect of insulin was highly significant ($p < 0.0001$) at each time point. Similarly to insulin, the *T. polium* extract also induced a rapid decline in blood glucose over the first 30 min and blood glucose levels continued to decline over the time course of the experiment. Also similarly to insulin, the effect of *T. polium* extract was significantly different from the vehicle treatment group throughout the study ($p < 0.01$ at each time point). Insulin and *T. polium* total extract both appeared to act quickly, influencing the change in glucose from baseline to the 30 min time point (Table 3). For the first 30 min, the decline in blood glucose levels in response to insulin and plant extract was very similar with no significant difference between them, and both decreased blood glucose levels significantly ($p < 0.01$) compared to vehicle and metformin. From 60 min onwards, however, the insulin group experienced a greater drop in blood glucose than all other groups. It was only at the 120 min mark for the first time, that the metformin group appeared to ‘catch up’ to the plant extract group. The performance of the metformin group appeared to lie between the vehicle and plant extract groups at all times; the plant extract group performed better than metformin initially but from 120 min onwards it was no longer significantly different from the metformin group.

DISCUSSION

This is the first study evaluating the acute effects of *T. polium* extract on blood glucose levels *in vivo*. We demonstrated that the plant extract lowers blood glucose levels significantly ($p < 0.0001$) over time. Indeed, the *T. polium* extract was as effective as insulin over the first 30 min of administration and significantly ($p < 0.01$) more

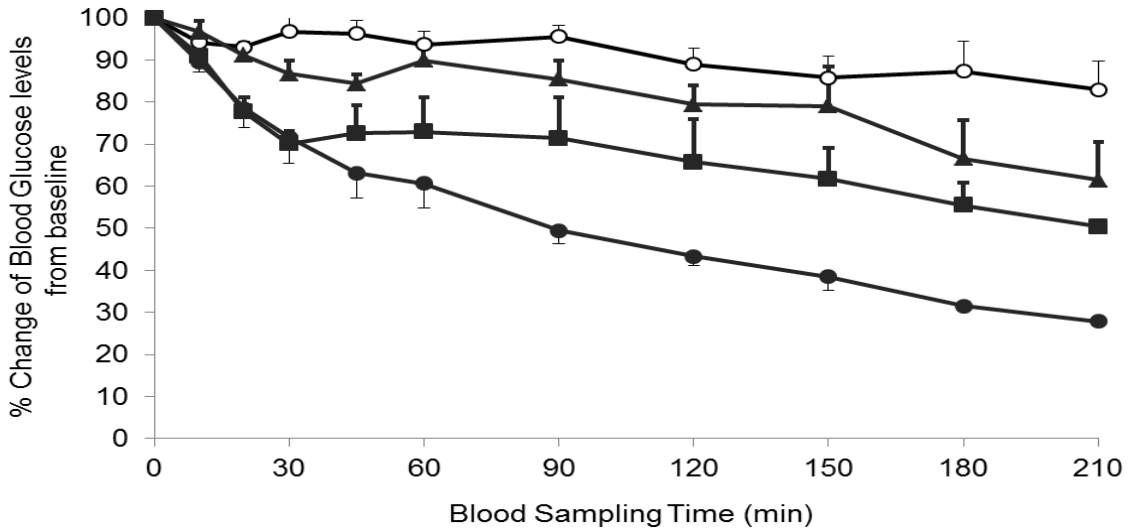

Figure 2. The effect of *Teucrium polium* extract on blood glucose levels in rats. Mean (+/- SEM) blood glucose levels percentage change from baseline over 3.5 h in normoglycaemic fasted rats post injection of vehicle (○; n = 8), metformin (▲; n = 6), insulin (●; n = 8) and *T. polium* (■; n = 8).

Table 3. Regression analysis results comparing changes from baseline in blood glucose level (mmol/L) between treatment groups, calculated at each time point separately.

Timing (min)	Treatment group p-value (overall)	Experimental group	Mean change in blood glucose from baselin (mmol/L)	Pairwise p-values comparing experimental groups		
				Metformin	Plant	Insulin
30	<0.0001	Vehicle	-0.21	0.2749	<0.0001	<0.0001
		Metformin	-0.92		0.0012	0.0009
		Plant Extract	-1.96			0.6484
		Insulin	-1.98			
60	<0.0001	Vehicle	-0.44	0.9787	0.0019	<0.0001
		Metformin	-0.70		0.0079	<0.0001
		Plant Extract	-1.81			0.0269
		Insulin	-2.75			
120	<0.0001	Vehicle	-0.84	0.4717	0.0066	<0.0001
		Metformin	-1.45		0.1014	0.0001
		Plant Extract	-2.34			0.0024
		Insulin	-4.03			

Table 3. Continues.

180	<0.0001	Vehicle	-1.05	0.0817	0.0030	<0.0001
		Metformin	-2.38		0.3657	0.0016
		Plant Extract	-2.94			0.0027
		Insulin	-4.84			
210	<0.0001	Vehicle	-1.33	0.0815	0.0030	<0.0001
		Metformin	-2.72		0.3696	0.0028
		Plant Extract	-3.28			0.0054
		Insulin	-5.10			

The changes shown are the raw (unadjusted) data, while the p-values were obtained from a regression model at each time point. The dependent variable in the regression was the change in glucose from baseline, and the model included a term for the baseline weight of the rat, as well as the treatment group. P-values were obtained from pairwise differences of adjusted mean differences (least squares means) obtained from the model.

effective than vehicle at each time point during the study. Moreover, the *T. polium* extract was more effective than metformin which is a medication of choice used by many diabetics.

Three previous animal trials have measured the early effects of the plant extract but only one study provided data earlier than 4 h post-administration (Table 1). Two of these studies found a significant ($p < 0.05$) decrease in blood glucose levels 4 h after intravenous (Gharaibeh et al., 1988) or intragastric (Stefkov et al., 2011) administration of *T. polium* extracts to normoglycemic and streptozotocin-induced hyperglycemic rats. A third study by Afifi et al. (2005) found no significant effect on blood glucose levels over the first 6 h following intranasal administration of *T. polium* extract to alloxan-induced hyperglycemic rabbits. Only one of these studies used a positive control, glibenclamide (Stefkov et al., 2011).

Several other *in vivo* trials that investigated the hypoglycemic potential of *T. polium* studied its effects in streptozotocin-induced rats following repeated oral gavage of the aqueous extract (Ardestani et al., 2008; Esmaeili and Yazdanparast, 2004; Zal et al., 2001). These investigations assessed the effect of the plant extract on blood glucose levels over days (1 to 12 days). A significant reduction in blood glucose levels was reported in animals treated with the extract compared to untreated animals. Unfortunately, these studies do not clearly describe the interval between dosing with the extract and the blood glucose measurement and so it is difficult to know whether the observations are due to an acute effect or a longer-term, ongoing effect of the plant extract. Moreover, it is concerning that no positive control was used in each of these studies. In another study (Gharaibeh et al., 1988), *T. polium* extract was administered to normoglycemic and streptozotocin-induced hyperglycemic rats in drinking water *ad libidum*, but no effect on blood glucose levels was observed (Table 1). Regrettably, the authors did not measure the amount of water taken by the animals over the duration of the study. As the extract-spiked water is quite acidic with a highly bitter taste, it is quite likely that the rats did not consume much of it.

Many studies investigating the efficacy of *T. polium* in reducing blood glucose levels were carried out on streptozotocin-treated animals. Streptozotocin is widely used to induce experimental diabetes mellitus in animals and this is mediated by the generation of reactive oxygen species in beta cells of the pancreas (Szkudelski, 2001). This process causes destruction of the insulin producing cells and thus streptozotocin treatment leads to a state of insulin-dependency resembling type I diabetes mellitus. Not all animals lose total functionality of their pancreatic beta cells following a given dose of streptozotocin. It is suggested that *T. polium* acts to restore the function of the (partially destructed) beta cells in low dose (generally ≤ 50 mg/kg) streptozotocin-induced diabetic rats (Yazdanparast et al., 2005). Nonetheless our current work reports on the acute glucose lowering effect of *Teucrium polium* extract in normoglycemic rats which suggests that mechanisms (eg, insulin mimetic or insulin secretagogue) other than restoration of beta cell functionality are more likely.

The main chemical constituents of *T. polium* have been identified by different groups (Aburjai et al., 2006; Pacifico et al., 2012; Sharififar et al., 2009; Kawashty et al., 1999; Florentino et al.,

2011) and some of these were detected in our LCMS profile of the extract (Figure 1), but these also exist in many other plants and at higher concentrations. Although some of these pure compounds, notably quercetin or rutin (Vessal et al, 2003; Coskun et al, 2005; Rauter et al, 2010; Torres-Piedra et al, 2010) and apigenin as aglycone or glycosides (Rauter et al., 2010; Cazarolli et al, 2009a, 2009b) have been investigated *in vivo*, at concentrations of 4 to 50 mg/kg, on steptozotocin-induced or alloxan-induced hyperglycemic rats, it is not known if these compounds (singularly or collectively) are responsible for the observed significant glucose lowering effect of the plant extract. In each case, the chemical extraction techniques used will determine the types of compounds isolated and their chemical state. The compounds identified within the extract are further dependent on the analytical techniques used to identify them. Therefore the compiled information about the chemical constituents of any of the extracts is not conclusive and the bioactive constituent/s responsible for the glucose lowering effect of *T. polium* extracts are yet to be confirmed.

CONCLUSION

The intravenous administration of the aqueous extract of *T. polium* significantly decreased blood glucose concentrations acutely in normoglycemic rats and this effect was comparable to maximised concentrations of insulin and metformin. This is the first study that investigated the immediate glucose lowering effects of the plant extract in comparison with premium medications on the market. This initial proof of concept work provides convincing evidence for the further investigation of *T. polium* in the pursuit of identification of active constituents which could end up being lead molecules for alternative glucose lowering medications in the future.

ACKNOWLEDGEMENTS

A.I. ran the pilot study as part of her degree of Master of Pharmacy at Curtin University. R.C., E.H. and A.I. were involved in the design of the study with statistical advice from RP. A.I. conducted the research under the supervision of R.C and E.H. The first draft of the manuscript was written by R.C. who also researched the data, constructed Figure 2, compiled Table 2 and reviewed/edited the manuscript. R.P. statistically analyzed, provided Tables 2 and 3, and reviewed/edited the manuscript. E.H. further contributed to the discussion and reviewed/edited the manuscript. A.I. was supported by an AusAid Scholarship.

Dr Adrian Lutz (Metabolomics Australia) and Mr Giuseppe Luna (School of Pharmacy, Curtin University) are acknowledged for their assistance in the generation of Figure 1. The authors acknowledge the assistance of Dr Beng Chua (Animal facility Manager) and Ms Tammy Dejong (Animal Technician) at Curtin University for their time in assisting and helping with the animal work. Also, the authors acknowledge the assistance of Elias and Farida Abu-Amsha in developing the connections in Jordan to source and import the plant material.

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Citation: Ireng A, Helmerhorst E, Parsons R, Caccetta R, 2016. *Teucrium polium* significantly lowers blood glucose levels acutely in normoglycemic male Wistar rats: A comparative to insulin and metformin. Adv Med Plant Res, 4(1): 1-10.