ORIGINAL ARTICLE

Effects of a novel formulation of essential oils on glucose–insulin metabolism in diabetic and hypertensive rats: a pilot study

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Background: Insulin resistance and its most severe form type 2 diabetes mellitus are rapidly increasing throughout the world. It is generally recognized that natural products with a long history of safety can increase insulin sensitivity.
Aims: The present investigation examined the ability of various combinations of essential oils such as fenugreek, cinnamon, cumin, oregano, etc. to enhance insulin sensitivity. As a first approximation, we examined the effects of these natural products on Zucker fatty rats (ZFRs), a model of obesity and insulin resistance, and spontaneously hypertensive rats (SHRs), a model of genetic hypertension.
Material and Methods: Water or essential oils were given orally via droplets, and insulin sensitivity was estimated by systolic blood pressure (SBP) changes and circulating glucose and/or insulin concentrations.
Results: We have found that the ability to alter SBP in rat models is the most sensitive early index of insulin sensitivity. The combined essential oils lowered circulating glucose levels and SBP in both ZFRs and SHRs, suggesting that these natural products are enhancing insulin sensitivity. The second series of studies examined two additional combinations of essential oils along with the original formula. The major differences were in the types and proportions of individual oils contributing to a given formula.
Conclusions: Although all the three formulae decreased SBP in ZFRs, one of the formulae was more effective than the others in lowering circulating glucose in the glucose tolerance testing. Accordingly, some essential oils may be added to the long list of natural products that can affect insulin sensitivity.

Keywords: diabetes mellitus, essential oils, insulin resistance, insulin sensitivity, insulin sensitivity, natural products
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Introduction

The prevalence of insulin resistance and its most severe form type 2 diabetes mellitus is rapidly increasing in the USA – even throughout the world [1–3]. The recent increase is attributed, at least to some extent, to the greater occurrence of overweight and obesity that is due mainly to an augmented intake of calories and refined carbohydrates, lesser consumption of fibres and a more sedentary lifestyle [4–6]. Obviously, reversal of these situations should ameliorate the problem. Unfortunately, more is often needed than simply advising lifestyle changes that frequently fail in order to combat insulin resistance and its accompanying perturbations (cardiovascular diseases, obesity, dyslipidemias, diabetes mellitus and premature ageing) [7,8]. Experience shows that the afflicted not infrequently require aids to help with the maintenance of a healthful lifestyle.

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The average doctor would think initially of these aids in terms of drugs. However, drugs in general have not been the solid answer to date. Because many drugs for type 2 diabetes mellitus have inadvertently produced serious adverse side effects [9,10], their use has often been limited to situations where an absolute need is present – certainly not routinely to prevent or even to treat milder forms of insulin resistance. As a result, some caregivers have considered natural products with a long history of safety to aid in the enhancement of insulin sensitivity.

The purpose of the present investigation was to assess the ability of various oils, often referred to as essential oils, to enhance insulin sensitivity. As a first approximation, we examined the effects of these natural products on Zucker fatty rats (ZFRs), a model of obesity and insulin resistance [11], and spontaneously hypertensive rats (SHRs), a model of genetic hypertension [12].

Materials and Methods

The Animal Welfare Board at Georgetown University Medical Center approved the protocol for the investigation. ZFRs and genetically SHRs were obtained from Charles River (Wilmington, MA, USA). The mixtures of essential oils (Diabegone™, North American Herb and Spice, Waukegan, IL, USA) were the generous gift of North American Herb and Spice, Waukegan, IL.

Protocols

Two separate studies were conducted. The ZFRs and SHRs were given orally two to three drops of water (control) or two to three drops of novel combinations of essential oils (test) twice daily. The larger ZFRs were given three drops and the smaller SHRs received two drops at each dosing.

Essential Oil Formulae

We arbitrarily labelled the three formulae EO1, EO2 and EO3. The initial studies were carried out with EO1. In the second series of studies, the two new formulations (EO2 and EO3) were compared to the original tested EO1.

EO1 was composed of pumpkinseed oil, extra virgin olive oil, oregano, cinnamon, fenugreek, cumin and fennel.

EO2 was composed of pumpkinseed oil, extra virgin olive oil, oregano, cinnamon, fenugreek, cumin, myrtle, allspice and ginger.

EO3 was composed of pumpkinseed oil, extra virgin olive oil, oregano, cinnamon, fenugreek, cumin and myrtle.

Study 1

There were two groups of ZFRs and SHRs: (1) water-receiving control, and (2) the test group receiving a combination of essential oils (EO1). Each group contained six rats. In an acute study, ZFRs and SHRs were given the drops of water or essential oils in a single dosing, and SBP was followed over the next 20–30 h. SBP was measured over 20 h in SHRs and 30 h in ZFRs, i.e. until the SBP returned to baseline. In a separate chronic study, SBP was measured at baseline (0), 8, 17 and 25 days in ZFRs and SHRs while the animals were on their respective regimens receiving EO1 twice a day. Four hours after food was removed on day 25, blood was drawn for blood chemistries; and then, a glucose challenge was given.

Study 2

There were four groups of six ZFRs: (1) water control, (2) essential oil mixture EO1, (3) essential oil mixture EO2, and (4) essential oil mixture EO3. SBP and BW were measured weekly over the course of 4 weeks. At the end of study, a glucose tolerance test was performed.

Systolic Blood Pressure (SBP)

SBP was measured by tail plethysmography using an instrument from Narco Biosciences (Houston, TX, USA). This provided an indirect measure of SBP in conscious, slightly warmed rats [13]. In all studies, rats were allowed free access to their diet and water until SBP readings were obtained between 13 and 17 h. Readings on individual rats were taken 0.5–1 min apart. To be accepted, SBP measurements on a given rat had to be virtually stable for at least three consecutive readings. In all studies, weekly readings were recorded.

Body Weight

BW in the second study was estimated by routine scale measurements.

Blood Chemistries

Blood for chemical analysis was obtained at the end of the first protocols following removal of food 4 h earlier. Glucose tolerance tests were performed in both studies at the end. Blood was drawn from the tail of the rats. Biochemical analyses were performed by routine clinical procedures.

Glucose Tolerance Test

The rats received a gavage of 1.0 g of glucose in water after the baseline blood was drawn. Additional bloods were drawn hourly over 4 h.

Statistical Analyses

Results are presented as mean ± s.e.m. SBP and BW were examined by two-way analyses of variance (one factor being group and the second factor being time of examination). Where a significant effect of regimen was detected by ANOVA ($p < 0.05$), the Dunnett *t*-test was used to establish which differences between means reached statistical significance [14]. When the data from two columns of data were analysed at a single time point, the Student's *t*-test was used. Statistical significance was set at a p-value of 0.05 or less.

Results

Study 1

Acute Effects of Essential Oil on SBP (figure 1)

In ZFRs, the single oral presentation of three drops of the combined essential oils (EO1) produced a significant decrease in SBP at 4, 10 and 20 h compared to the ZFRs receiving equivalent drops of water. At 30 h, after this single, acute challenge, the lowering effect had dissipated in the ZFRs, i.e. the SBP of the test group was essentially the same as that of the control. In the case of SHRs, the oral intake of two drops of the novel combination of essential oils resulted in a significant decrease in SBP at 4 h compared to that in the controls receiving plain water. Unlike ZFRs, the effects of the essential oil combination were not found in the SHRs at the measurement made 10 and 20 h after the single challenge with essential oils.

Effects of Essential Oil on SBP over 25 days (figure 2)

The two rat strains were given water or essential oils *per os* via drops twice daily for 25 days. In the ZFRs and SHRs, measurements taken at 8, 17 and 25 days showed a significantly lower SBP in the rats receiving the combination of essential oils (EO1) compared to that in their controls receiving water alone. In ZFRs, the average difference of measured values ranged between 16 and 20 mmHg, whereas in the SHRs, the average differences ranged between 11 and 17 mmHg.

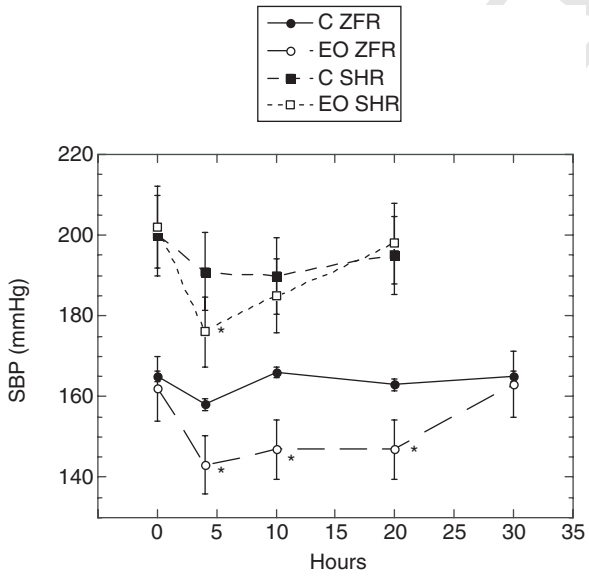

Fig. 1 Systolic blood pressure (SBP) in Zucker fatty rats (ZFRs) and spontaneously hypertensive rats (SHRs) after a single dosing of water (C) or combined essential oils (EOs). Rats were followed until SBP returned to original baseline. Mean ± s.e.m. is shown. The symbol '*' represents that the value is significantly different from control at that time period.

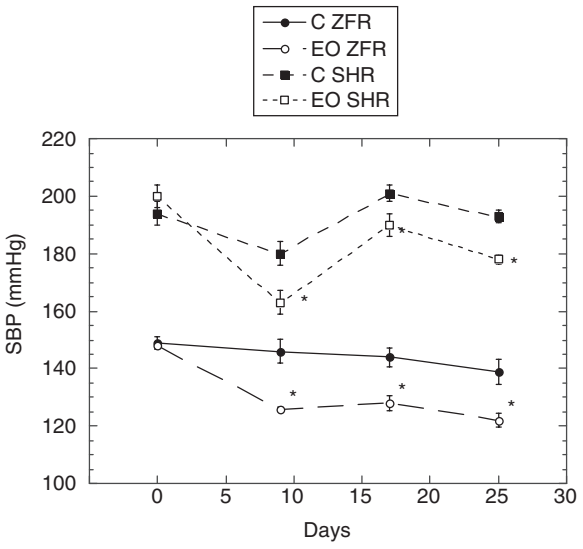

Fig. 2 Systolic blood pressure (SBP) in Zucker fatty rats (ZFRs) and spontaneously hypertensive rats (SHRs) over the course of 25 days. Rats received water (C) or combined essential oils (EOs) orally twice a day. Mean ± s.e.m. is shown. The symbol '*' represents that the value is significantly different from control at that time period.

Blood Chemistries after 25 days of Essential Oils (table 1)

After 25 days of receiving the combined essential oils, the fasting blood glucose levels in the test ZFRs were significantly lower than that in the control ZFRs – 180 ± 5.5 vs. 233 + 12 mg/dl (s.e.m.) ($p < 0.02$). This significant difference in circulating glucose concentrations was not seen in the SHRs. Measurements of cholesterol, ALT, AST, creatinine and BUN were not different between control and test rats in either strain. While the mean triglyceride level was lower in the test group for the SHRs, there was no significant difference seen in the ZFRs.

Glucose Tolerance Tests (figure 3)

After oral challenge with one gram of glucose, the circulating levels of glucose, at 2 and 3 h were statistically significantly lower in the test group of ZFRs, whereas no significant differences were found in the SHRs (figure 3; upper). The mean values between the test and control rats in each rat strain were essentially the same by the fourth hour. Concerning circulating insulin values, no differences were seen in the SHRs (figure 3; lower). However, the circulating insulin levels were consistently lower over the period of challenge in the test ZFRs, although the differences were not statistically significant. The insulin levels in the control and test groups of SHRs were essentially the same.

Table 1 Blood chemistries after 4 weeks on essential oils (EOs) compared to control

Parameter	Control	EO1	p-Value
Zucker fatty rats			
Glucose	233 ± 12.8	180 ± 5.5	<0.02
Cholesterol	138 ± 6.1	123 ± 10.3	Not significant
Triglyceride	343 ± 59.7	333 ± 62.0	Not significant
ALT	67 ± 6.8	52 ± 2.8	Not significant
AST	81 ± 20.9	58 ± 6.5	Not significant
Cr	0.4 ± 0.03	0.4 ± 0.06	Not significant
BUN	14.8 ± 0.2	14.9 ± 1.3	Not significant
Spontaneously hypertensive rats			
Glucose	150 ± 3.4	151 ± 4.9	Not significant
Cholesterol	58 ± 1.5	61 ± 2.0	Not significant
Triglyceride	67 ± 5.9	52 ± 2.8	<0.01
ALT	110 ± 11.9	99 ± 7.5	Not significant
AST	145 ± 3.4	120 ± 8.9	Not significant
Cr	0.6 ± 0.02	0.6 ± 0.04	Not significant
BUN	13.2 ± 0.8	14.0 ± 0.6	Not significant

Mean ± s.e.m. is shown. Glucose, cholesterol and triglyceride levels were higher in Zucker fatty rat and ALT, AST and Cr higher in spontaneously hypertensive rats.

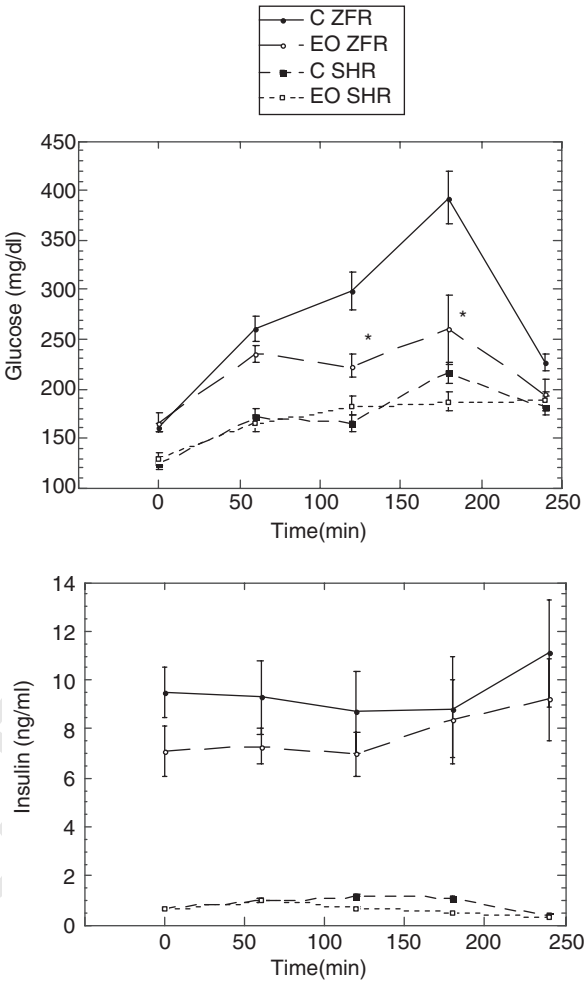

Fig. 3 Results of a glucose tolerance test in Zucker fatty rats (ZFRs) and spontaneously hypertensive rats (SHRs) performed after 25 days of receiving water (C) or combined essential oils (EOs) orally twice a day. Glucose results are shown in upper panel and insulin results in lower panel. Mean ± s.e.m. is shown. The symbol '*' represents that the value is significantly different from control at that time period.

Study 2

Comparing Effects of Three Formulae of Essential Oils (figures 4–6)

In comparing the three variations of essential oil formulae (EO1–3) with a water-receiving control group, it can be seen in figure 4 that the body weights of the ZFRs did not differ significantly after 4 weeks. In contrast, the SBPs of the three essential oil groups were all significantly lower by roughly, 19 mmHg, i.e. the lowering was comparable in the three test groups (figure 5). Blood glucose levels were followed over 3 h after a 1-g glucose

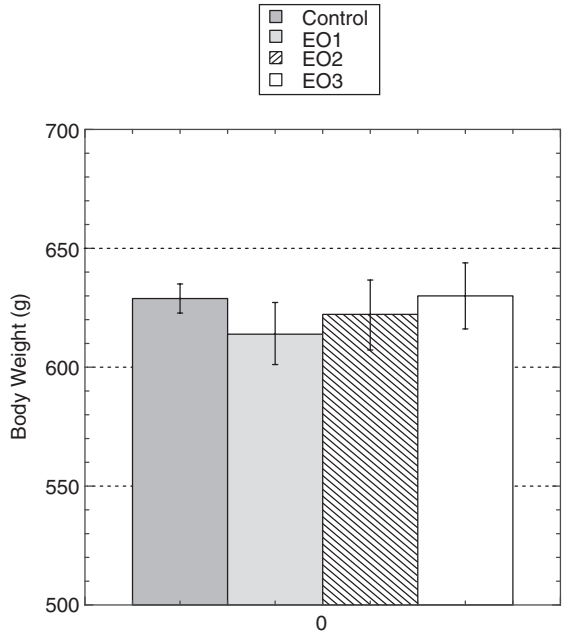

Fig. 4 Bar graft showing weight of Zucker fatty rats after 4 weeks receiving drops of water (control) and three different combinations of essential oils labelled EO1, EO2 and EO3. Mean ± s.e.m. is shown. The symbol '*' represents that the value is significantly different from control at that time period.

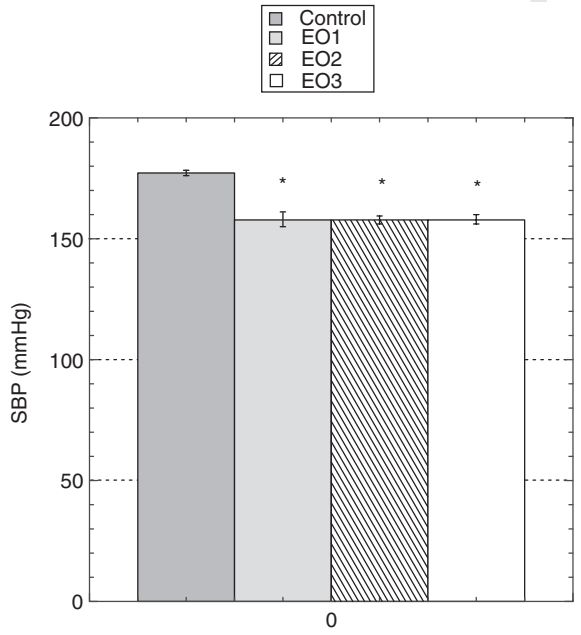

Fig. 5 Bar graft showing systolic blood pressure (SBP) of ZFR after 4 weeks receiving drops of water (control) and three different combinations of essential oils labelled EO1, EO2 and EO3. Mean ± s.e.m. is shown. The symbol '*' represents that the value is significantly different from control at that time period.

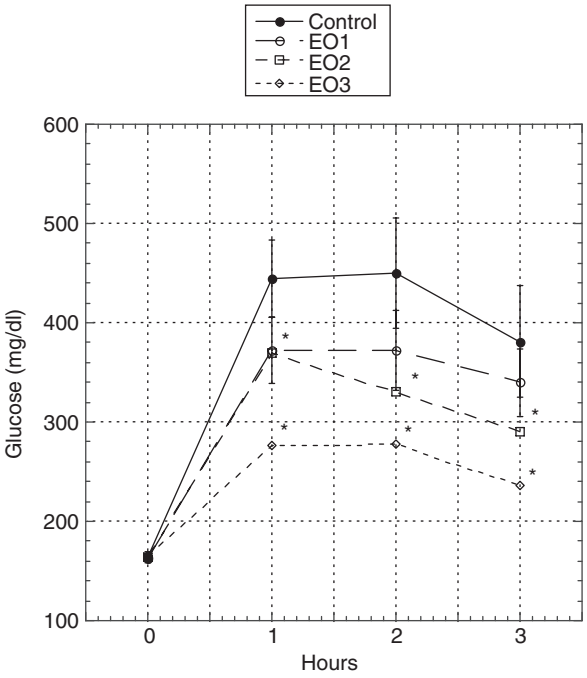

Fig. 6 Results of a glucose tolerance test in Zucker fatty rats performed after 4 weeks of receiving water (control) or combined essential oils (EO1, EO2 and EO3) orally twice a day. Glucose results are shown. Mean ± s.e.m. is shown. The symbol '*' represents that the value is significantly different from control at that time period.

challenge. The measurements in the three groups receiving the formulae of essential oils were consistently lower, with the lowest values seen in the EO3 variant of essential oils.

Discussion

Glucose intolerance and obesity are common findings in the ageing human [1,8,15–17]. Nutritional perturbations, such as excess intake of calories, simple sugars and trans fatty acids coupled with low fibre consumption are prevalent in the western diet and can be linked frequently to augmented insulin resistance and obesity [18,19]. In addition, the lack of proper exercise is a contributing factor [1]. Accordingly, the best means to prevent or ameliorate obesity and insulin resistance is to restrict calories, balance the macronutrient in a manner to reduce the intake of refined carbohydrates and trans fats, increase fibre consumption and engage in a proper exercise program [1,4]. However, the majority of individuals require extra aids in order to carry out the preceding and maintain good future health [1]. Considering this, some caregivers

have found dietary supplements to be useful. For example, supplementary niacin-bound chromium [20,21], garlic [22], soluble fibres [23] and vanadium [24] can overcome, to some extent, many of these perturbations. Thus, natural nutrients/elements have the potential to favourably influence the glucose/insulin system and the onset of age-related chronic disorders with little threat of serious adverse side effects [1]. We should mention that it is possible that proper combinations of these natural products may give even better risk/benefit ratios than single agents alone.

We believe that essential oils may prove useful in the battle against insulin resistance and type 2 diabetes mellitus. Safety-wise, essential oils have been in the food chain for centuries; and various oils have been in the market as therapeutic agents for years without the occurrence of significant adverse health effects [25,26]. As an additional benefit to therapeutic potential, individuals with diabetes are prone to develop a wide range of life-threatening infections [27,28], and many essential oils, especially oregano, are reported to be useful against a variety of fungi, bacteria, and viruses [25,26,29–32]. Thus, essential oils may have a beneficial duel therapeutic role of being anti diabetic and anti microbial.

In our first study, we examined the ability of a combination of essential oils (EO1) to enhance insulin sensitivity. SBPs of both rat strains were significantly lowered in response to the combined essential oils. In the past, we have found that the ability to alter SBP in rat models is the most sensitive early index of insulin sensitivity [33,34]. That the combined essential oils also lowered circulating glucose levels in both ZFRs and SHRs further suggests strongly that this is the case. The second series of studies examined two additional combinations of essential oils (EO2 and EO3) along with the original formula. In addition to adding new essential oils, the major differences were in the proportions of individual oils contributing to a given formula. All the three formulae decreased SBP in ZFRs, but one of the new formulae (EO3) appeared more effective in this small study than the others in lowering circulating glucose in the tolerance testing. Further studies should reveal if EO3, in fact, is superior to the others and if even better formulations are possible.

Of the essential oils tested, fenugreek and cinnamon have had extensive studies devoted to their anti-diabetic properties [35–39]. The major action of fenugreek may be via blockage of glucose absorption [35]. Cinnamon has been shown to have insulin-like action [38] and affect insulin signalling [39]. There is little association of diabetes treatment with cumin, pumpkin seed and oregano [35], although animal studies do suggest that cumin may lower circulating lipid levels in diabetic rats [40]. The long-term safety of these condiments in the food supply suggests the possibility of daily use over the long haul to prevent or ameliorate insulin resistance and perhaps even increase useful lifespan [1,41]. Previous studies with other natural products have suggested this potential [42]. Future studies are necessary to test the veracity of this hypothesis.

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