STUDIES ON THE ACTIVITY OF INDIVIDUAL PLANTS OF AN ANTIDIABETIC PLANT MIXTURE

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An extract of a mixture of *Nigella sativa* seeds and gums (myrrh, assafoetida, aloe and olibanum) used by Kuwaiti diabetics was found to improve glucose tolerance in both streptozotocin diabetic and normal rats[1]. We have reported that this effect was neither mediated through the stimulation of insulin secretion nor through the suppression of the intestinal absorption of glucose. In the present report the blood glucose lowering action of the extracts of individual components of this mixture was studied in order to identify the active component(s) responsible for this effect.

MATERIALS AND METHODS

Normal and diabetic male Wistar albino rats (200-250 g) were used. Diabetes was induced by the i.p. administration of streptozotocin (60 mg/kg body weight freshly dissolved in 0.01 mol/l citrate buffer, pH 4.5)[5]. Subsequently, rats received a daily s.c. dose of 2 IU protamine zinc insulin for 7 days to protect against streptozotocin toxicity and the acute phase of diabetes. One week after insulin withdrawal, diabetes was confirmed by the demonstration of glucosuria (glucose indicator strips, Rapignost, Hoechst, FRG) and of hyperglycemia by determining the blood glucose concentration by the glucose oxidase method (Bio Merieux, Maryl, Charbonnieres Lesbains, France)[6] with the aid of a Model-25 spectrophotometer (Beckman, Geneva, Switzerland). Animals were allowed free access to tap water and laboratory chow (E. Dixon and Sons Ware Ltd., Ware, England).

Preparation of extracts and treatment of animals - Suspensions (5% w/v) were prepared for each of *Nigella sativa*, Linn (seeds, family *Ranunculaceae*); *Commiphora myrrh*, Eng (gum-resin, family *Burseraceae*); *Ferula assafoetida*, Linn (gum-resin, family *Umbrelliferae*); *Aloe vera*, Linn (dried juice of leaves and pulp, family *Liliaceae*), and olibanum (gum-resin, family *Burseraceae*). Each suspen-

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sion was boiled separately for 10 min and then filtered through 4 layers of surgical gauze.

Groups of diabetic and normal animals received a daily intragastric 10 ml/kg body weight dose of an extract for a period of one week. They were then fasted for 24h prior to performing the oral glucose tolerance test (OGTT). The OGTT was performed under anesthesia (phenobarbitone sodium 40-45 mg/kg body weight, i.p.[3], and animals were maintained at 37 °C in multichambered, thermostated restraining cages. Initially, blood was collected from the tail vein for the determination of the fasting glucose level, then glucose (3 g/kg body weight) was administered by gastric intubation and blood samples were collected at 30-min intervals for 3h. Twenty µl blood aliquots were used for the determination of glucose concentrations as described earlier.

For the study of acute effects of these extracts on blood glucose concentration, a mixture of 1 g of each of the plants was extracted (5% w/v) and filtered as described earlier. These mixtures were administered intragastrically (10 ml/kg body weight) to diabetic rats under anesthesia. Untreated diabetic rats under anesthesia were used as controls.

Statistical analysis · Data were expressed as the mean ± SEM of the absolute changes from the fasting blood glucose levels both for controls and treatment groups. Significance of differences from the appropriate group of controls was tested by Student's *t*-test.

RESULTS

Effect of individual extract on OGTT · Treating normal rats for one week with extracts of myrrh or aloe gum lowered the fasting blood glucose level significantly from 4.6 ± 0.3 to 3.4 ± 0.4 and 3.4 ± 0.2 mmol/l, respectively. Other extracts did not affect the fasting blood glucose level in normal rats. It has been reported that the sum of the fasting, 1-h and 2-h blood glucose values from the OGTT was an index of glucose tolerance[3]. Using this index revealed that only the myrrh extract was effective in improving glucose tolerance in normal animals (20.3 ± 1.3 mmol/l *vs* 16.1 ± 2 for the untreated and treated

group	0.5h	1h	2h	3h
control	4.0 ± 0.9	4.4 ± 0.6	2.2 ± 0.4	0.3 ± 0.3
olibanum	3.4 ± 0.5	3.0 ± 0.3	1.4 ± 0.2[a]	0.3 ± 0.1
sativa	4.3 ± 0.7	4.9 ± 0.8	1.7 ± 1.1	0.7 ± 0.9
myrrh	5.7 ± 0.3[a]	4.2 ± 0.4	2.5 ± 0.6	1.7 ± 0.5[b]
assafoetida	4.8 ± 0.3	3.9 ± 0.4	0.9 ± 0.7	0.9 ± 0.5
aloe	3.6 ± 0.4	5.2 ± 0.3	2.9 ± 0.5	2.1 ± 0.4[d]

Tab. 1 · Absolute increments in blood glucose during the OGTT in normal rats treated with plant extracts. Normal rats were treated for one week with extracts of the plants shown. Data represent the net increase in blood glucose above the fasting level of the corresponding group of rats; they are expressed in mmol/l and are the mean ± SEM of 5 observations in each group. The statistical significance of the difference from the control group is denoted by: [a] $p < 0.05$; [b] $p < 0.02$; [d] $p < 0.005$.

group	n	0.5h	1h	2h	3h
control	8	4.1 ± 1.9	5.7 ± 3.1	6.0 ± 4.2	0.4 ± 3.0
olibanum	5	8.1 ± 2.5	11.0 ± 1.9	7.9 ± 1.4	4.2 ± 2.3
control	5	9.8 ± 2.5	15.3 ± 3.7	7.2 ± 4.2	5.6 ± 3.8
sativa	5	9.2 ± 2.5	11.5 ± 1.6	7.7 ± 1.8	5.9 ± 1.7
control	6	14.0 ± 2.5	16.1 ± 1.7	11.1 ± 1.9	4.8 ± 3.0
myrrh	6	8.5 ± 3.0	9.1 ± 3.2[a]	8.0 ± 2.8	2.4 ± 1.9
control	6	3.3 ± 2.3	5.0 ± 3.9	2.2 ± 3.5	1.7 ± 3.4
assafoetida	7	9.0 ± 1.3[b]	12.3 ± 2.0	6.7 ± 1.4	2.5 ± 1.5
control	5	12.8 ± 2.9	5.5 ± 2.2	2.1 ± 2.7	−0.6 ± 3.0
aloe	5	10.4 ± 2.6	11.7 ± 1.7[b]	10.9 ± 1.4[c]	5.7 ± 1.3[a]

Tab. 2 - Absolute increments in blood glucose during the OGTT in diabetic rats treated with plant extracts. Diabetic rats were treated for one week with extracts of the plants shown. Data represent the net increase in blood glucose above the fasting level of the corresponding group of rats; they are expressed in mmol/l and are the mean ± SEM of the number of observations (n) in each group. The statistical significance of the difference from the appropriate control group is indicated by: [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.01$.

groups, respectively). Tab. 1 shows the calculated absolute increments over fasting blood glucose of the various groups of normal rats treated for one week with plant extracts. Again, myrrh caused the largest absolute increase in blood glucose during the first 30 min of the OGTT and, as with aloe gum, maintained a significantly large increase in blood glucose at 3h.

The responses of diabetic rats to treatment with the individual extracts for a week are shown in tab. 2. Myrrh extract decreased the absolute increment of blood glucose over the fasting level at all times of the OGTT but the difference was significant only at 1h. Extract of *Nigella sativa* was without effect. Extracts of gum olibanum, gum assafoetida and of aloe gum consistently increased the absolute increments of blood glucose over the fasting level at all times of the OGTT.

Table 3 demonstrates the relative changes in blood glucose concentration in diabetic rats following the acute administration of the extract of mixed plants. Lowering of blood glucose was noted as early as 1h after the admin-

group	n	0h	0.5h	1h	2h	3h	4h	5h
untreated	10	16.1 ± 1.3	15.8 ± 1.3	15.8 ± 1.2	14.1 ± 2.1	11.5 ± 1.9	9.7 ± 0.2	10.1 ± 0.4
(p)			n.s.	n.s.	n.s.	n.s.	< 0.001	< 0.001
treated	5	13.5 ± 1.4	9.2 ± 1.5	7.3 ± 1.7	4.6 ± 0.4	5.4 ± 1.0	4.7 ± 1.0	5.1 ± 1.0
(p)			n.s.	< 0.025	< 0.001	< 0.001	< 0.001	< 0.005

Tab. 3 - Acute effects of oral administration of an extract of a mixture of plants on the fasting blood glucose in diabetic rats fasted for 24h. An extract of equal parts of 5 plants was administered by gastric tube to a group of anesthetized diabetic rats and their blood glucose determined. Untreated diabetics received saline instead of the extract. Results are in mmol/l and represent the means ± SEM of the observations (n) in each group. The fasting blood glucose level in each group served as the reference for statistical comparison of the changes observed.

istration of the extract and was maintained for at least 5h. In contrast, untreated controls showed a decrease of blood glucose concentration only after 4h.

DISCUSSION

Extracts of gum olibanum, *Nigella sativa* and gum assafoetida were ineffective in improving glucose tolerance of both normal and diabetic rats, and may be considered redundant components of the mixture of plant extracts shown to lower blood glucose in diabetics[1]. The increase in glucose tolerance observed in both normal and diabetic rats treated for one week with extracts of either myrrh gum or aloe gum can be explained in terms of the significant lowering of the fasting blood glucose levels in both groups of animals. Since these animals were fasted for 24h prior to the OGTT, it is assumed that glycogenolysis did not contribute significantly to the level of fasting blood glucose. Therefore this decrease is interpreted as reflecting inhibition of gluconeogenesis. During the OGTT, the persistent decrease in the absolute increment of blood glucose over the fasting level in diabetic rats treated with myrrh extract implies action on peripheral glucose utilization, because we have previously shown that myrrh extract did not alter the intestinal absorption of glucose[1]. In contrast, aloe gum extract improved glucose tolerance only by decreasing the fasting blood glucose level, and hence gluconeogenesis.

There was significant lowering of fasting blood glucose level in diabetic rats 1h following the administration of an extract of a mixture of the five plants including aloe gum. This is in contrast with a report that aqueous extracts (25% w/v) of aloe leaves lowered blood glucose by 6% in diabetic mice 4h following oral administration[4]. The earlier response in the present study implies that different parts of the aloe plant may lower blood glucose by different mechanisms. We may therefore conclude that the overall improvement of glucose tolerance reported in our previous study[1] could be the result of the additive effects of extracts of the gums of myrrh and aloe, both of which decrease gluconeogenesis and, in addition, the former increases the peripheral utilization of glucose in diabetic rats. Work is in progress further to fractionate extracts of myrrh and aloe gums in an attempt to isolate active blood glucose lowering components in order to study their mechanism(s) of action.

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SUMMARY

A blood glucose lowering extract of a mixture of five plants in use by Kuwaiti diabetics was studied for the identification of its active component(s). Only the extracts of myrrh and aloe gums effectively increased glucose tolerance in both normal and diabetic rats. The remaining components, gum olibanum, *Nigella sativa* seeds and gum assafoetida were without effect.

REFERENCES

1. AL-AWADI F. M., KHATTAR M. A., GUMAA K. A.: On the mechanism of the hypoglycemic effect of a plant extract · Diabetologia *28*, 432, 1985.
2. BHAGAVAN N. V.: Carbohydrates. In: BHAGAVAN N. V. (Ed.): Biochemistry. 2nd edition. G. B. Lippincott, Philadelphia-Toronto, 1978; p. 350.
3. GREEN C. J.: Rat-recommended anesthetic techniques. In: GREEN C. J. (Ed.) Laboratory animal handbooks. 8. Animal anesthesia. Laboratory Animal Ltd., London, 1979; p. 155.
4. MOSSA J. S.: A study on the crude antidiabetic drugs used in Arabian folk medicine · Int. J. crude Drug Res. *23*, 137, 1985.
5. SRIVASTAVA L. M., BORA P. S., BHATT S. D.: Diabetogenic action of streptozotocin · TIPS *3*, 376, 1982.
6. TRINDER P.: Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen · J. clin. Pathol. *22*, 158, 1969.

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