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Antihyperglycemic activity of *Arbutus unedo*, *Ammoides pusilla* and *Thymelaea hirsuta*

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The effect of the water extract (WE) of three medicinal plants used as antidiabetic medication in Eastern Morocco (*Arbutus unedo:* Au, *Ammoides pusilla:* Ap and *Thymelaea hirsuta:* Th) was tested in rats with the Oral Glucose Tolerance Test (OGTT) and Intravenous Glucose Tolerance Test (IVGTT). In the OGTT the rats received water, glibenclamide (2 mg/kg) or WE (500 mg/kg for Au and 250 mg/kg for Th and Ap) 30 min before glucose loading (glucose: 1 g/kg). The WE of Au, Ap and Th produced a significant decrease in glycemia after glucose loading. In the IVGTT the WE of Ap and Th produced a significant decrease in glycemia 60 min after i.v. glucose loading (0.5 g/kg). The addition of the WE of Au (500 mg/kg), Ap or Th (250 mg/kg) induced a significant inhibition of jejunal glucose absorption, (31.6%, 28.5% and 40.5% respectively). This effect could explain in part the significant antihyperglycemic effect observed in the OGTT model but it does not exclude other effects on glucose homeostasis, particularly for Ap and Th. Toxicity tests (high LD_{50} value) suggest no adverse effect of the use of these plants.

1. Introduction

Diabetes is a metabolic disease, treated by conventional medicine (insulin for type I diabetes and oral hypoglycemic agents for type II diabetes). In parallel, phytotherapy is a common and very old practice used by the Moroccan population to treat diabetes and several other diseases. According to our recent review of herbal medicine, more than 88 medicinal plants are commonly used to treat diabetes in Morocco (Bnouham et al. 2002). About 40% of this number are commonly used in eastern Morocco (Ziyyat et al. 1997). The object of the present study is to test the antihyperglycemic effect of some medicinal plants believed to have antidiabetic properties (*Arbutus unedo*, *Ammoides pusilla* and *Thymelea hirsuta*).

2. Investigations, results and discussion

Many medicinal plants are commonly used to treat diabetes. We have chosen three medicinal plants. *Arbutus unedo* possesses many medicinal uses; it is used as an antidiabetic (Ziyyat et al. 1997; Bnouham et al. 2002), diuretic (Ziyyat and Boussairi 1998), hypotensive (Ziyyat et al. 1997; Ziyyat et al. 2002), urinary antiseptic, astringent, anti-inflammatory and depurative, (Ziyyat et al. 1997; Bruneton 1987) and it possesses antioxidant activity (Pabuccuoglu et al. 2003). *Thymelea hirsuta* is used as an antidiabetic (Bnouham et al. 2002; Ziyyat et al. 1997), *Ammoides pusilla* is hypoglycemic (Bnouham et al. 2002; Ziyyat et al. 1997), hypotensive and aromatical and it is used against influenza (Ziyyat et al. 1997).

The three medicinal plants were effective in the OGTT which is a model of transient hyperglycemia. Plasma glucose level reached a peak of 1.52 ± 0.11 g/l at 1 h after oral glucose loading (1 g/kg) in control rats (Table 1). The water extract of Au at a dose of 500 mg/kg produced a decrease of glycemia at 1 h and 3 h after glucose loading (21.1%, $p < 0.05$ and 14.1%, $p < 0.05$, respectively). The water extract of Ap produced a 28.3% decrease of glycemia at 1 h ($p < 0.01$), and the glucose lowering effect persisted until 3 h after glucose loading. The water extract of Th produced a significant decrease of glycemia 2 h and 3 h after glucose loading (20.4%, $p < 0.05$ and 16.4%, $p < 0.05$ respectively).

In the IVGTT experiment WE of Ap and Th produced a slight decrease of glycemia 60 min after glucose loading ($p < 0.05$) while WE of Au was without significant effect (Table 2).

The addition of the water extract of Au (500 mg/kg), Ap (250 mg/kg) or Th (250 mg/kg) to the perfusing solution produced a significant inhibition of intestinal glucose absorption, respectively: 31.6% ($p < 0.02$), 28.5% ($p < 0.05$) and 40.5% ($p < 0.02$); Fig. The decrease of glucose absorption could explain in part the antihyperglycemic effect of these plants in the OGTT model but it does not exclude other effects on glucose homeostasis, particularly for Ap and Th which were effective in the IVGTT model. This last effect means that these plants may also act by modulation of peripheral glucose utilization.

Our recent phytochemical study on *Arbutus unedo* has shown that tannins and flavonoids are abundant in the roots

Table 1: Effect of water extract of *Arbutus unedo* (500 mg/kg), *Ammoides pusilla* (250 mg/kg) and *Thymelaea hirsuta* (250 mg/kg) and glibenclamide (2 mg/kg) on plasma glucose level (g/l) of oral glucose loaded healthy rats

Treatment (p.o.)	Plasma glucose level (g/l)				
	Basal value	Time after glucose administration			
		60 min	120 min	180 min	240 min
Controls (n = 7)	1.08 ± 0.12	1.52 ± 0.11	1.47 ± 0.11	1.35 ± 0.05	1.31 ± 0.13
A. unedo 500 mg/kg (n = 6)	0.95 ± 0.06	1.20 ± 0.04*	1.24 ± 0.04[c]	1.17 ± 0.04*[b]	1.16 ± 0.05[b]
A. pusilla 250 mg/kg (n = 6)	0.92 ± 0.05	1.09 ± 0.06**	1.11 ± 0.05*[b]	1.16 ± 0.06*[b]	1.07 ± 0.04[b]
T. hirsuta 250 mg/kg (n = 6)	1.14 ± 0.12	1.26 ± 0.08	1.17 ± 0.04*[b]	1.16 ± 0.05*[b]	1.04 ± 0.07[a]
Glibenclamide 2 mg/kg (n = 6)	1.04 ± 0.11	1.03 ± 0.1**	0.77 ± 0.09***	0.78 ± 0.09***	0.69 ± 0.09**
F values	0.83	5.94	11.55	12.28	7.03
P values	0.52	0.02	0.000	0.000	0.000

Blood glucose levels in groups were expressed as mean ± standard error of mean (SEM). The data were statistically analysed by Student's t-test and one-way ANOVA P values less than 0.05 were considered significant
Rats of all groups were loaded with glucose (1 g/kg p.o.) 30 min after water extract, glibenclamide or water (p.o.)
*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ (versus control value) at the same time
a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$ (versus glibenclamide group) at the same time

Table 2: Effect of water extract of *Arbutus unedo* (500 mg/kg), *Ammoides pusilla* (250 mg/kg) and *Thymelaea hirsuta* (250 mg/kg) and glibenclamide (2 mg/kg) on plasma glucose level (g/l) of intravenous oral glucose loaded healthy rats

	Plasma glucose level (g/l)			
	Basal value	Time after glucose administration		
		30 min	60 min	120 min
Controls (n = 6)	1.08 ± 0.06	1.97 ± 0.33	1.36 ± 0.07	1.23 ± 0.06
Glibenclamide (2 mg/kg) (n = 3)	1.04 ± 0.05	1.53 ± 0.27	0.80 ± 0.09**	0.78 ± 0.08**
A. pusilla (250 mg/kg) (n = 3)	0.94 ± 0.09	1.77 ± 0.21	1.05 ± 0.07*	1.13 ± 0.02[a]
A. unedo (500 mg/kg) (n = 3)	1.12 ± 0.4	1.94 ± 0.15	1.25 ± 0.08[b]	1.1 ± 0.01[a]
T. hirsuta (250 mg/kg) (n = 3)	1.06 ± 0.08	2.45 ± 0.53	1.1 ± 0.04*[a]	1.01 ± 0.10
F values	1.01	1.16	3.22	7.08
P values	0.444	0.380	0.056	0.004

Blood glucose levels in groups were expressed as mean ± standard error of mean (SEM). The data were statistically analysed by Student'st-test and one-way ANOVA. P values less than 0.05 were considered significant. The rats of all groups were loaded with glucose (0.5 g/kg i.v.) 30 min after water extract, glibenclamide or water (p.o.).
*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ (versus control value) at the same time
a: $p < 0.05$; b: $p < 0.01$ (versus glibenclamide group) at the same time

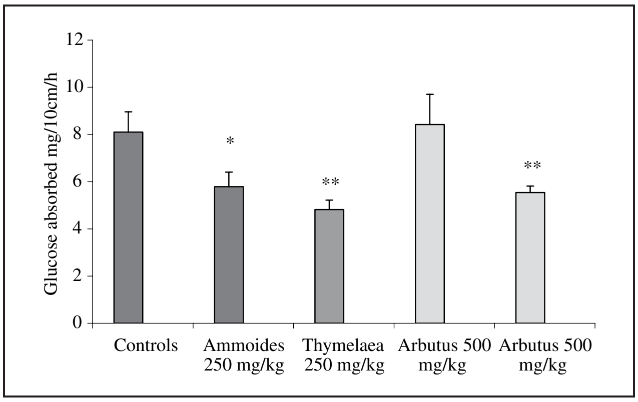

Fig.: Intestinal absorption of glucose (mg/10 cm) before and after addition of water extract of *Ammoides pusilla* (250 mg/kg), *Thymelaea hirsuta* (250 mg/kg) and *Arbutus unedo* (250 mg/kg and 500 mg/kg).
Values presented as mean ± ESM. *$p < 0.05$; **$p < 0.02$ versus control

of this plant. Some fractions contain polyphenol compounds, particularly epicatechin, catechin and catechin gallate (Legssyer et al. 2004). The antihyperglycemic activity of *Arbutus unedo* is likely due to these compounds which are known for their antidiabetic effect (Chakravarthy et al. 1982; Bone et al. 1985). *Thymelaea hirsuta* contains terpene and coumarins (Waltner-Law et al. 2002). The hypoglycemic effects of the plant extracts may be due, in part, to their terpenoid and/or coumarin contents. *Ammoides pusilla* contains flavonoids and tannins which possess antidiabetic activity (Pérez del Castillo et al. 1997).

These results support the beneficial use of these plants in the treatment of type 2 diabetes. The three extracts were effective, perhaps even better than glibenclamide since they were not hypoglycemic but only antihyperglycemic. Moreover, the LD50 value for Th was no less than 4 g/kg. The WE of Au and Ap were without side effects even at a dose of 6 g/kg (at this dose no death was noted). These results support the absence of adverse effects of these plants.

In conclusion, the three extracts have a beneficial effect particularly in the OGTT model. They reduce jejunal glucose absorption. Other studies are desirable to identify the active compounds.

3. Experimental

Plants were bought in a traditional market and have a Voucher code deposited in the Faculty of Sciences, Oujda, Morocco. After they were oven dried at a temperature of 40 °C for 48 h, they were prepared according to traditional methods. 50 g of dried *Ammoides pusilla* (also known as *Ptychotis verticillata*) were infused in 1 l of boiled water for 20 min (yield: 23.2 %). *Thymelaea hirsuta* was prepared by the same method as Ap (100 g in 1 l of distilled water, yield: 4.4%). Dried roots of *Arbutus unedo* (200 g) were boiled in 2 l of water for 2 h (yield: 4.75%). The liquid extracts were filtered and evaporated in vacuo (Laborota 4000, Heidolph).

The Wistar rats (250–300 g) and Swiss mice of both sexes (18–22 g) used were purchased from the animal house of the Faculty of Sciences (Oujda, Morocco). They had free access to pellets and water *ad libitum* in a room at a temperature of 23 ± 2 °C and with a 12/12 light/dark cycle (light 07 h 00–19 h 00).

Seven male rats were fasted for 16 h before the OGTT. Glucose (1 g/kg) was administered orallly 30 min after oral administration of water extract of *Arbutus unedo*, *Ammoides pusilla* or *Thymelaea hirsuta*. Results were compared with those with glibenclamide (2 mg/kg) and controls (water 2 ml/300 g). Blood glucose level was assessed by the glucose oxidase method each hour for 4 h after glucose loading.

The intravenous glucose tolerance test (GTT) was performed after overnight fasting, a 0-min blood sample being taken from the tip of the tail vein from all the groups of rats. Glucose solution at a dose of 0.5 g/kg body weight was administered intravenously through the tail vein of the rats under light ether anaesthesia. Blood samples were taken from the tail vein at 30, 60 and 120 min after glucose administration.

The effect of the extracts obtained from Au, Ap or Th on intestinal glucose absorption was tested in a segment of jejunum (10 cm) by the *in situ* perfusion technique (Ponz et al. 1979) in rats fasted for 36 h and anaesthetized with sodium pentobarbital (50 mg/kg, i.m.). The composition (in g/l) of the perfusing solution was as follows: 7.37 NaCl, 0.20 KCl, 0.065 $NaH_2PO_4 \cdot 6\,H_2O$, 1.02 $CaCl_2$, 0.6 $NaHCO_3$, and 1 glucose, at $pH = 7.5$. The water extract of the plant was added to the perfusing solution at a final concentration of 250 mg/kg for Th and Ap and 500 mg/kg for Au. The system was set at 37 °C, and the perfusion rate was 0.53 ml/min for 1 h. The controls were perfused under the same conditions with the solution without plant extract.

The lethal dose (LD_{50}) of the 3 plant extracts was assessed using groups of 8 mice (4 males and 4 females). Different doses of the extracts were administered by the intra-peritoneal route.

Results are expressed as means ± SEM for n separate experiments. The data were statistically analysed by Student'st-test and one-way ANOVA. P values less than 0.05 were considered significant.

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