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A chloroform extract obtained from a decoction of *Ficus carica* leaves improves the cholesterolaemic status of rats with streptozotocin-induced diabetes

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The antidiabetic effects of *Ficus carica* leaf extracts have been reported previously. From the aqueous decoction of fig leaves, after treatment with HCl, centrifuging, treatment with sodium hydroxide (NaOH) and extraction with chloroform ($CHCl_3$), the administration of the organic phase rats with streptozotocin-induced diabetes led to a decline in the levels of total cholesterol and an decrease in the total cholesterol/HDL cholesterol ratio (with respect to the control group), together with a reduction of the hyperglycaemia.

Keywords: *Ficus carica*, glycaemia, cholesterol, diabetes

The search for the active principle or principles responsible for the hypoglycaemic, hypotriglyceridaemic, and hypocholesterolaemic activities of a *Ficus carica* leaf aqueous extract has led to its phytochemical extraction, finding various fractions that maintain these effects. While the acid soluble and insoluble powder fractions lead to reductions in plasma levels of glucose, triglycerides and total cholesterol (TC) [(data in press: 2) 4], the basic fraction was also observed to act at the HDL-cholesterol (HDL-C) level, reducing the atherogenic ratio (TC/HDL-C) [data in press: 2].

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We here present a new fraction that we were able to obtain using a modification of Lock's method [3], and its effects on the hyperglycaemic and hypercholesterolaemic status of the diabetic animal.

Materials and methods

Animals

A group of 30 female Wistar rats, weighing approximately 230 g, were housed in the Animalarium of the University of Extremadura, at 24 °C, with lighting from 8 a.m. to 8 p.m. The animals had free access to water and food (B. K. Universal, Barcelona, Spain).

Diabetes was induced by intraperitoneal administration of streptozotocin (65 mg/kg body weight). The diabetic status was confirmed by testing for glycosuria using indicator-strips four days after the streptozotocin injection and for hyperglycaemia and hypertrigliceridaemia by measuring blood levels seven days after the injection. Animals with glycaemia or triglyceridaemia below 27.5 mM or 2.82 mM, respectively, were rejected.

Plant material

The leaves of *Ficus carica* L., Sp. Pl. 1059 (1753) were collected during May–October in the region of Extremadura (South-West of Spain). The leaves were separated and dried in an air forced oven at 50 °C. They were then ground into a powder and stored until use in closed bags.

Preparations of extracts and treatment of animals

For each treated animal, 10 g of the powdered dried leaf were boiled for 30 minutes in 100 ml water, filtered at 50 °C and acidifed with 30 ml 1% HCI. After centrifuging for 5 minutes at 3000 rpm, the pellet was discarded. The supernatant was treated with sodium hydroxide (NaOH) until reaching pH=12, followed by two extractions with 50 ml chloroform ($CHCl_3$). The organic phase was completely dried in an oven at 50 °C for 24 h. The aqueous phase was discarded.

The experiment was carried out by dissolving this dried extract (which we shall call the chloroform fraction) in 0.5 ml olive oil.

The diabetic animals were divided into two groups: untreated diabetic rats (D n=10) and diabetic rats treated with the extract (TD n=10). The animals, in unfasted

status, were injected intraperitoneally: TD received the *Ficus carica* fraction; D, used as control group, received only pure olive oil. Blood samples were taken from every animal at 0 min (just before the injection) and 60 min and 24 h after the intraperitoneal administration.

Blood sampling and biochemical parameters

Approximately 0.3 ml blood was taken from the tail vein without anaesthesia via a small and nearly painless incision. All samples were immediately centrifuged (3000 rpm, 15 min, room temperature) and analysed for glycaemia (glucose oxidase method), triglycerides (GPO, POD method), total cholesterol (CHOD, POD method), with commercial kits (Trace) in a Coulter CPA analyser.

Statistical analysis

The results are expressed as mean ± SD. Statistical analyses were carried out using the Mann-Whitney or Wilcoxon tests. Differences were taken as significant for $p<0.05$.

Results

Figure 1 shows the plasma glucose values obtained in the group injected with the fraction and in the corresponding control group. The control group values were: 40.26± 10.01 mM, basal; 38.11±9.68 mM, 60 min; 42.35±9.07 mM, 24 h. In the treated group, the values were: 38.22±3.63 mM, basal; 33.55±4.12 mM, 60 min ($p<0.001$ compared to the basal value); 36.08±3.85 mM, at 24 h ($p<0.05$ compared to the 60 min value).

No modifications were observed in the plasma triglyceride levels as an effect of the treated with the chloroform fraction.

Figure 2 shows the plasma total cholesterol levels. The values in the treated group were: 2.80±0.28 mM, basal; 2.64±0.41 mM, 60 min; 2.25±0.26 mM, 24 h ($p<0.001$ compared to the basal and $p<0.01$, when compared to the 60 min value). The control group values were: 2.54±0.28 mM, basal; 2.67±0.34 mM, 60 min; 2.75±0.41 mM, 24 h.

There were no changes in the HDL-cholesterol levels as a result of the chloroform fraction injection.

Figure 3 shows the atherogenic ratio (total cholesterol/HDL-cholesterol). The values in the controls were: 2.10±0.35 basal ($n=10$) and 3.40±0.59 at 24 h ($n=10$, $p<0.05$). The values in the treated group were: 2.14±0.39 basal and 2.11±0.32 at 24 h ($p<0.0001$ compared to the control group).

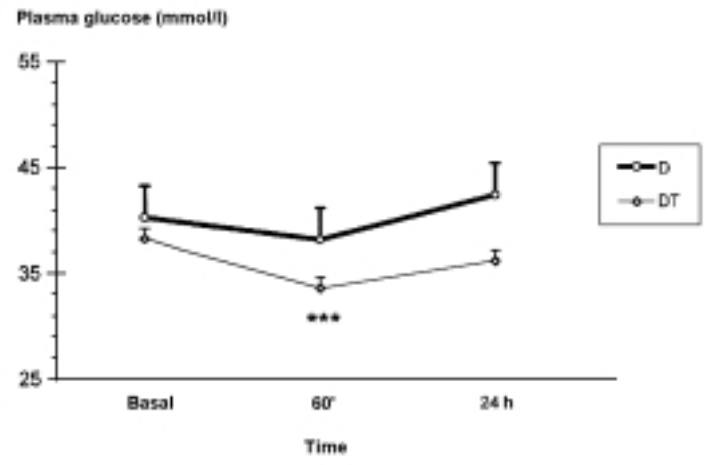

Fig. 1. Plasma glucose levels in controls (D, n=10) and in rats treated with the chloroform fraction (DT, n=10) just before and 60' and 24 h after the administration
*** $p<0.001$ vs basal levels

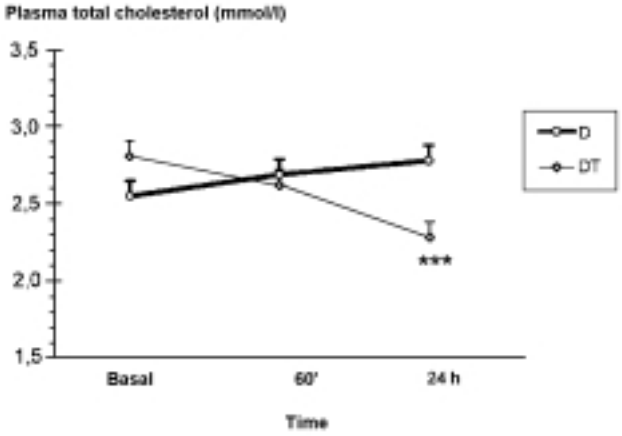

Fig. 2. Plasma total cholesterol levels in controls (D, n=10) and in rats treated with the chloroform fraction (DT, n=10) just before and 60' and 24 h after the administration
*** $p<0.001$ vs basal levels; $p<0.01$ vs 60' level

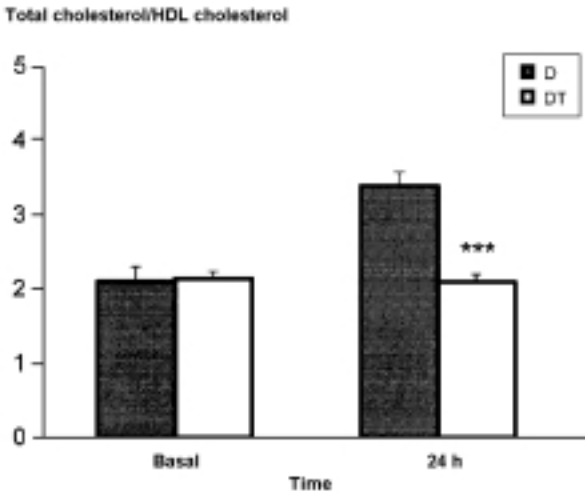

Fig. 3. Total cholesterol – HDL cholesterol levels ratio in controls (D, n=10) and in rats treated with the chloroform fraction (DT, n=10) just before and 24 h after the administration
*** $p<0.0001$ vs 24 h control group levels

Discussion

The intraperitoneal administration of a new fraction obtained by modified Lock's method to streptozotocin diabetic rats [3] showed a significant reduction of the plasma glucose levels, as can be seen in Fig. 1. These data coincide with those found on administering the fig leaf decoction orally or intraperitoneally [1, 5]. The results are also similar to those obtained on administering the fractions obtained previously with this method of phytochemical separation [data in press: 2, 4]. This fall in glycaemia values in the animals who received the chloroform fraction was statistically significant at 60 min, as it was also found with the administration of the basic fraction [data in press: 2]. At 24 h, however, the levels of glycaemia are similar to those found in the basal state, unlike the situation when other forms of administering the fig leaf fractions were used [1, 5].

The administration of the chloroform fraction did not improve the hypertriglyceridaemic status of the diabetic animals. In all the forms of administering the fig leaf extracts, a significant reduction had been found in the animals' plasma triglyceride levels. This inclines us to believe that there must exist at least two groups of active principles in the initial decoction – one responsible for the effect on the

hyperglycaemia, and the other acting at the level of the hypertriglyceridaemia. It would be the latter that is absent from the chloroform fraction but present in the total decoction and in the acid soluble, insoluble powder, and basic fractions.

As it can be seen in Fig. 2, the chloroform fraction leads to a significant decline in the total cholesterol levels at 24 h compared to the basal levels. There is also a noticeable improvement in the general cholesterol metabolism, as it is shown in the representation of the total cholesterol/HDL-cholesterol ratio in Fig. 3.

The chloroform fraction of the *Ficus carica* leaf possesses clear effects on the hyperglycaemia and on the disturbances in cholesterol metabolism found in streptozotocin diabetic animals. The results of investigations into the antidiabetic activity of *Ficus carica* leaves seem to show the presence of different active principles acting on hyperglycaemia, hypertriglyceridaemia, and hypercholesterolaemia associated with diabetes. In the chloroform fraction, only those principles responsible for the effects on glucose and cholesterol are present.

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