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Experimental diabetes treated with ficus carica extract: effect on oxidative stress parameters

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Abstract Parameters related to oxidative stress were studied in rats divided into 4 groups: streptozotocin-induced diabetic rats (n=10), diabetic rats who received a single dose of a basic fraction of *Ficus carica* extract (n=14), diabetic rats who received a single dose of a chloroform fraction of the extract (n=10), and normal rats (n=10). Compared to normal animals, the diabetic animals presented significantly higher values for erythrocyte catalase normalized to haemoglobin levels (1.5±0.15 vs. 0.96±0.18 μg/mg) and for plasma vitamin E (73.4±43.9 vs. 12.0±1.6 mg/l), monounsaturated fatty acids (0.219±0.118 vs. 0.067±0.014 mg/ml), polyunsaturated fatty acids (PUFA, 0.567±0.293 vs. 0.175±0.040 mg/ml), saturated fatty acids (0.779±0.262 vs. 0.401±0.055 mg/ml), and linoleic acid (0.202±0.086 vs. 0.106 ±0.014 mg/ml). Both *Ficus carica* fractions tended to normalize the values of the diabetic animals' fatty acids and plasma vitamin E values. On studying the ratios of vitamins E and A to PUFA (129.4±77.5 diabetic and 68.8±9.1 μg/mg normal; 37.5±20.8 vs. 108.0±43.6 μg/mg) and to C18:2 (259.9±65.8 vs. 161.0±21.3 μg/mg; 68.3±37.9 vs. 252.7±102.1 μg/mg), we found statistically significant differences as a function of diabetes, with the vitamin E/C18:2 ratio being normalized by the administration of the chloroform fraction (to 152.1±80.3 μg/mg) and the vitamin A/C18:2 ratio being raised relative to the untreated diabetic rats by the administration of the basic fraction (91.9±14.5 μg/mg). Our work confirms that antioxidant status is affected in the diabetes syndrome, and that *Ficus carica* extracts tend to normalize it.

Key words Stress • Diabetes • Oxidative parameters • *Ficus carica*

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Introduction

The current interest in natural therapies and traditional medicine has motivated the investigation into plants that have traditionally been used in relation to diabetes. Articles of major importance have been published in the research literature in recent years citing a great number of plants used around the world as antidiabetics [1].

Great importance is presently being given to free radicals as the causes of many disorders in general, and of diabetes in particular, and there has been a great deal of work done to see whether the beneficial effect of some plant extracts may play a role against free radical production. For some plant species the results have been interesting. Examples are: *Gingko biloba* [2], shown to help protect erythrocyte membranes from attack by hydrogen peroxide [3]; *Thymus vulgaris* [4], which possesses a potent inhibitor of mitochondrial peroxidation; and *Rosmarinus officinalis* [5], from which diterpenoids have been obtained that inhibit superoxide anion production. The flavonoids are a family of compounds that generally present an antioxidant action [6], of great interest being those obtained from *Vaccinium myrtillus* [7] because of its inhibitory effect on LDL oxidation.

Living organisms generate or make use of a great variety of hydro- and liposoluble antioxidant compounds (e.g. vitamins A and E, glutathione), and also synthesize a series of antioxidant enzymes (e.g. catalase, superoxide dismutase, glutathione peroxidase) which are responsible for deactivating the reactive intermediaries of oxygen. But antioxidant substances and enzymes are not wholly effective in preventing oxidative damage [8].

In recent years, the diabetes syndrome has been associated with an increase in lipoperoxidation, a circumstance which may contribute to tissue damage in the long term [9]. In our present work, we studied the possible influence of *Ficus carica* extracts, previously shown to have hypoglycaemic [10] and hypolipaemic [11] activities, on the parameters that indicate protection against free radicals, such as the erythrocyte catalase enzyme and vitamins A and E. We checked whether they had any effect on one of the secondary metabolites of lipoperoxidation, malondialdehyde, in experimental diabetes.

Materials and methods

Animals

We used 44 female Wistar rats, with a weight of 230 g, divided into 4 groups: normal (n=10), diabetic (n=10), diabetic treated with the basic fraction (n=14), and diabetic treated with the chloroform fraction (n=10). Water and food were supplied ad libitum. The food was commercial feed from B.K. Universal (Barcelona, Spain). Diabetes was induced by an intraperitoneal injection of streptozotozin at a dose of 65 mg/kg body weight, with the animals not fasted. Principles of laboratory animal care (NHI publication no. 83–25 revised 1985) were followed as well as specific Spanish and European laws, where applicable.

Fig leaf processing

As *Ficus carica* is deciduous, the leaves were collected from the end of spring until the beginning of autumn. The collecting and drying processes were carried out industrially by A.S.A.C. Pharmaceutical International (Alicante, Spain).

From among the various methods that have been described to analyze the chemical constituents of a plant sample, we chose to work on the basis of that proposed by Lock de Ugaz [12] with some modifications: 10 g fig leaf was weighed out per animal. The leaves were boiled in water for 30 minutes and the extract was filtered twice. The extract was then treated with 3 ml 1% HCl per gram of leaf. It was centrifuged for 10 minutes at 3000 rpm, and the supernatant was filtered again. It was neutralized with NaOH and was concentrated to approximately 50 ml. It was alkalinized with NaOH, extracted twice with chloroform and centrifuged to obtain two phases: a basic (aqueous) fraction and a chloroform (organic) fraction. The basic fraction was concentrated to approximately 50 ml, its pH was adjusted to 7.4, and it was then oven-dried at 50° C. The dry extract was dissolved in 0.5 ml distilled water per animal. The chloroform extract was oven-dried at 50° C, and the dry extract was dissolved in 0.5 ml olive oil per animal.

Extract administration

The respective extracts were injected intraperitoneally and in a single dose, since a previous study [11] showed the action to be prolonged over several days.

Blood processing

After 24 hours, the animals were anaesthetized with 65 mg/kg sodium pentobarbital, and 6 ml blood was slowly drawn from the aorta. The blood sample was immediately centrifuged at 3000 rpm at 4° C for 10 minutes. The plasma was divided into aliquots and frozen at -80° C. For the erythrocyte assays, a pre-treatment of rinsing and haemolysis was performed, 0.5 ml of the red blood cells was taken, 2 ml distilled water was added (1:5 dilution of haemolysed erythrocytes) and, after dividing into aliquots, the result was stored at -80° C.

Biochemical analyses

Fatty acid extraction was performed by means of the method of Folch, as modified by Castela et al. [13]. The composition was determined by gas chromatography using a Hewlett-Packard model 6890 analytical chromatograph with autoinjector and BPX70 50-m capillary column. The results for each fatty acid are expressed as a percentage of total fatty acids.

Catalase activity was assessed following Aebi [14]. This method is based on the decomposition of hydrogen peroxide by catalase. The decrease in absorbance at 240 nm was measured at room temperature. The blood catalase activity was expressed as micrograms enzyme per milligram haemoglobin.

Malondialdehyde (MDA) assays were performed on erythrocytes by high performance liquid chromatography (HPLC) according to a technique of Esterbauer et al. [15].

Vitamins A and E were assayed by HPLC as described by Shearer [16].

Plasma glucose (hexoquinase-peroxidase glucose) and triglycerides (oxidase-peroxidase lipase-glycerate) were measured in diabetic and normal rats using a Coulter C.P.A. autoanalyzer. Haemoglobin determination was by the technique of Zijlstra and Karpen [17], using spectrophotometry.

Statistical analysis

The ratios between vitamin and lipid values were calculated from the individual values expressed in the same unit of volume in the plasma. All values are given as mean and standard deviation. Statistical analysis was performed using the Mann-Whitney test. A difference was taken as statistically significant if $p<0.05$.

Results

We studied parameters of oxidative stress in normal and streptozotocin-induced diabetic rats, as well as in diabetic rats treated with extracts of *Ficus carica* leaves. Plasma glucose and lipid concentrations were significantly higher in all groups of diabetic animals compared to control rats, and were partially reversed by treatment with *Ficus carica* extract (Table 1). There were no significant variations in the levels of erythrocyte haemoglobin in the four groups of animals: normal group, 59.8±9.9 mg/dl, diabetic group: 64.3±9.6 mg/dl; diabetic group injected with the basic fraction of *Ficus carica* extract, 73.1±19.9 mg/dl; diabetic group injected with the chloroform fraction, 66.9±12.1 mg/dl. The values found by dividing the catalase levels by milligrams of haemoglobin were: normal group, 0.96±0.18 µg/mg; diabetic group, 1.50±0.15 µg/mg ($p<0.05$ with respect to normal); diabetic group injected with the basic fraction, 1.00±0.19 µg/mg ($p<0.01$ with respect to the diabetic group); diabetic group injected with the chloroform fraction, 0.99±0.15 µg/mg ($p<0.05$ with respect to the diabetic group).

The plasma levels of vitamin E were significantly different in the four groups of animals. In the normal group they were 12.0±1.6 mg/l; in the diabetic group, 73.4±43.9 mg/l ($p<0.0001$ with respect to normal); in the basic fraction group, 28.6±17.3 mg/l ($p<0.01$ with respect to normal and $p<0.005$ with respect to the diabetic group); in the chloroform fraction group, 17.5±9.2 mg/l ($p<0.0001$ with respect to normal and $p<0.0001$ with respect to the diabetic group). The ratio between the plasma values of vitamin E and the levels of triglycerides (Fig. 1a) were: in the normal group, 10.8±1.4 µg/mg; in the diabetic group, 5.8±3.4 µg/mg ($p<0.005$ with respect to normal); in the basic fraction group, 4.5±2.7 µg/mg; in the chloroform fraction group, 5.5±2 9 µg/mg. The results of dividing the vitamin E levels by the levels of polyunsaturated fatty acids (Fig. 1b) were: 68.8±9.1 µg/mg in the normal group; 129.4±77.5 µg/mg in the diabetic group ($p<0.05$ with respect to normal); 87.5±52.9 µg/mg in the basic fraction group; 71.7±37.9 µg/mg in the chloroform fraction group. The results of dividing the plasma levels of vitamin E by those of linoleic acid (Fig. 1c) were: 161.0±1.3 µg/mg in the normal group; 253.9±65.8 µg/mg in the diabetic group ($p<0.05$ with respect to normal); 166.3±100.5 µg/mg in the basic fraction group; 152.1±80.3 µg/mg in the chloroform fraction group ($p<0.05$ with respect to the diabetic group).

The plasma levels of vitamin A found in the four groups of animals were: in the normal group 18.9±7.6 mg/l; in the diabetic group, 21.2±11.8 mg/l; in the basic fraction group, 15.8±2.5 mg/l; in the chloroform fraction group, 12.1±6.1 mg/l ($p<0.05$ with respect to normal and $p<0.05$ with respect to the diabetic group). In the ratios between the plasma values of vitamin A and the levels of triglycerides there were significant differences between the groups of animals (Fig. 2a). The ratios were: in the normal group, 17.0±6.9 µg/mg; in the diabetic group, 1.7±0.9 µg/mg ($p<0.0001$ with respect to normal); in the basic fraction group, 2.5±0.4 µg/mg ($p<0.05$ with respect to the diabetic group); in the chloroform fraction group, 3.8±1.9 µg/mg ($p<0.05$ with respect to the diabetic group). Dividing the plasma levels of vitamin A by the plasma levels of polyunsaturated fatty acids (Fig. 2b)

Table 1 Biochemical parameters in normal and diabetic rats, and in diabetic rats treated with basic fraction or chloroform fraction of *Ficus carica* leaves

			Diabetic with *Ficus carica* extract	
	Normal (n=10)	Diabetic (n=10)	Basic fraction (n=14)	Chloroform fraction (n=10)
Glycaemia, mg/dl	142 (12)	814 (108)**	637 (77)††	656 (70)††
Triglycerides, mg/dl	111 (43)	1272 (798)**	639 (361)†	317 (228)††
Linoleic acid (C18:2), mg/ml	0.075 (0.018)	0.311 (0.160)***	0.172 (0.075)†	0.115 (0.072)†
MUFA, mg/ml	0.067 (0.014)	0.219 (0.118)**	0.130 (0.042)†	0.086 (0.049)††
PUFA, mg/ml	0.175 (0.040)	0.567 (0.293)**	0.327 (0.116)†	0.244 (0.117)††
SFA, mg/ml	0.401 (0.055)	0.779 (0.262)*	0.586 (0.085)†	0.451 (0.100)†
NEFA, mg/ml	0.644 (0.102)	1565 (0.699)****	1111 (0.699)†	0.707 (0.142)††
Haemoglobin, mg/dl	59.8 (9.9)	64.3 (9.6)	73.1 (19.9)	66.9 (12.1)
Catalase, µg/mg Hb	0.96 (0.18)	1.50 (0.15)*	1.00 (0.19)†	0.99 (0.15)†
Serum MDA, µM	2.9 (2.9)	2.3 (1.4)	2.5 (0.7)	1.9 (0.7)
Red cell MDA, µM	14.1 (3.5)	15.3 (9.6)	12.5 (9.1)	10.7 (6.9)

MUFA, monounsaturated fatty acids; *PUFA*, polyunsatured fatty acids; *SFA*, saturated fatty acids; *NEFA*, nonesterified fatty acids; *MDA*, malondialdehyde; *Hb*, haemoglobin

*$p<0.05$; **$p<0.001$; ***$p<0.0005$; ****$p<0.0001$ versus normal rats

† $p<0.05$; †† $p<0.005$ versus diabetic rats

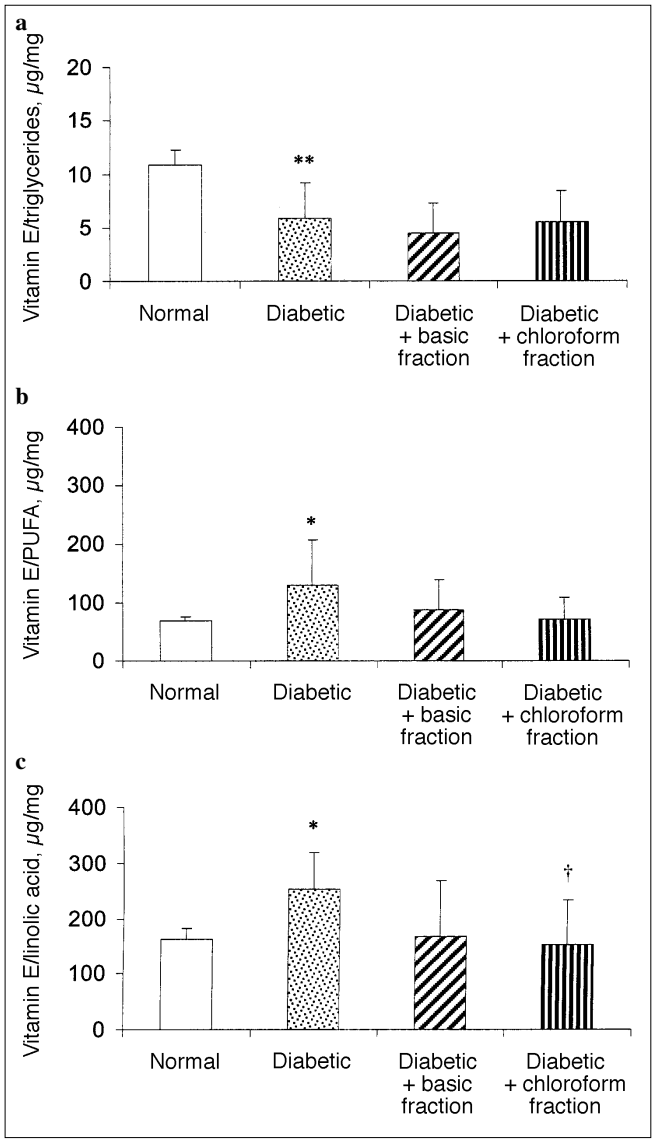

Fig. 1a-c Ratios of vitamin E levels to those of blood lipids in normal and diabetic rats, and in diabetic rats treated with the basic or chloroform fraction of *Ficus carica* extract. **a** Vitamin E/triglyceride ratio. **b** Vitamin E/polyunsaturated fatty acid (PUFA) ratio. **c** Vitamin E/linoleic acid (C18:2) ratio. *p<0.05; **p<0.005 versus normal; † p<0.05 versus diabetic

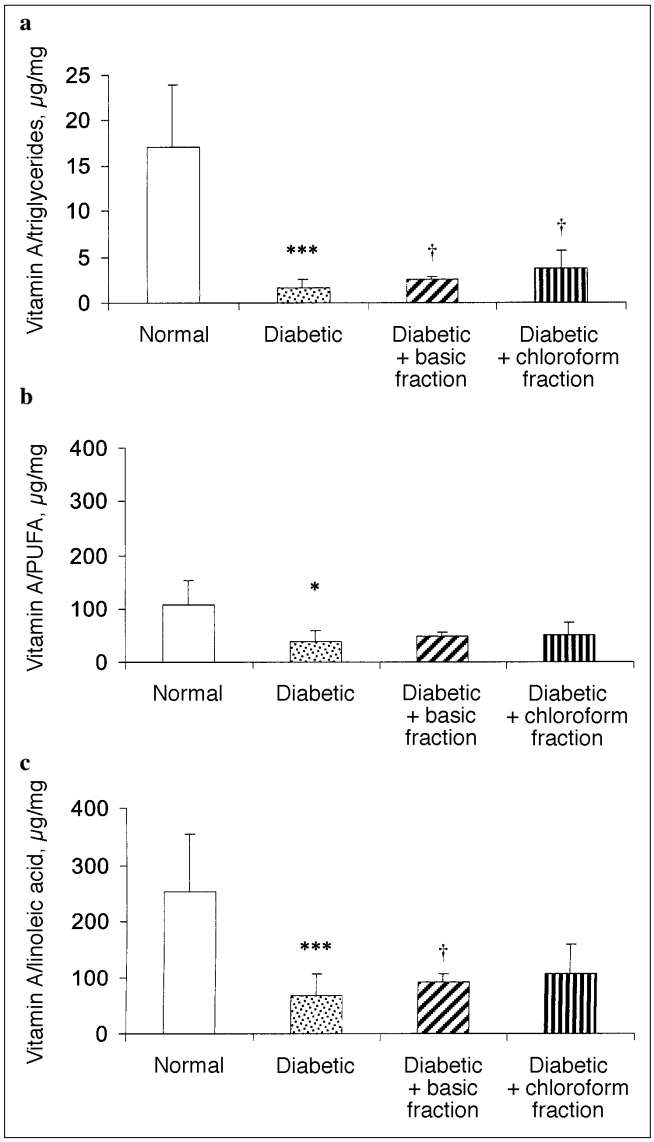

Fig. 2a-c Ratios of vitamin A levels to those of blood lipids in normal and diabetic rats, and in diabetic rats treated with the basic or chloroform fraction of *Ficus carica* extract. **a** Vitamin A/triglyceride ratio. **b** Vitamin A/polyunsaturated fatty acid (PUFA) ratio. **c** Vitamin A/linoleic acid (C18:2) ratio. *p<0.05; ***p<0.001 versus normal; † p<0.05 versus diabetic

yielded: 108.0±43.6 μg/mg in the normal group; 37.5±20.8 μg/mg in the diabetic group (p<0.05 with respect to normal); 48.3±7.6 μg/mg in the basic fraction group; 49.7±25.1 μg/mg in the chloroform fraction group. The results of dividing the plasma vitamin A levels by those of linoleic acid (Fig. 2c) were: 252.7±102.1 μg/mg in the normal group; 68.3±37.9 μg/mg in the diabetic group (p<0.0001 with respect to normal); 91.9±14.5 μg/mg in the basic fraction group (p<0.05 with respect to the diabetic group); 105.4±53.2 μg/mg in the chloroform fraction group.

Discussion

Our results show that aqueous (basic) and organic (chloroform) extracts of *Ficus carica* leaves have similar effects in reducing hyperglycaemia in diabetic rats, whereas the chloroform extract has a greater effect in reducing fatty acid levels. As of now, we know neither the mechanism of action nor the chemical composition of the extracts.

There is evidence that oxidative stress plays a major role in the aetiology of diabetic complications [18]. Several studies have shown an increase in lipoperoxidation products in diabetic patients [19], while others have not shown any such differences [20], or an increase only in diabetic patients with complications [21] or who were poorly controlled [22]. Balashova et al. [23] reported that catalase activity rises by approximately 50% in insulin-dependent patients with angiopathies. The antioxidant status is fairly poor in both glucose intolerance and non-insulin-dependent diabetes, and it is possible that antioxidant therapy mitigates or retards the progress of glucose intolerance [24]. In studies on non-insulin-dependent diabetics who underwent insulin therapy for three days, there was a slight decline in their high levels of malondialdehyde and a rise in the levels of vitamin E, but there were no modifications in the activities of catalase or superoxide dismutase [25].

In our present work, the erythrocyte catalase activity in diabetic animals was significantly greater than that of the control group. This indicates a greater activation of the oxidative defences, in agreement with the suggestion of Ohrvall et al. [26].

One way of comparing different individuals is to consider the ratio between the erythrocyte catalase activity and the haemoglobin content. Our data regarding this ratio still indicate a greater enzyme activity in the diabetic animals relative to the normal controls. On considering, however, the values for the animals who received the basic and the chloroform fractions, we found a decline with respect to the values for the diabetic animals, there being no such difference relative to the normal animals. It is not easy to draw clear conclusions from these data: given the erythrocyte half-life, we cannot explain how a single intraperitoneal injection of a *Ficus carica* extract can change either the total erythrocyte catalase content or its activity, although, in the absence of other data, the latter seems the more plausible cause.

There are many free radical generating mechanisms, but their presence is translated into a series of lipid peroxidation products which include malondialdehyde, a protein-modifying agent [27]. This compound is most commonly used as the marker of the degree of lipoperoxidation. Despite the importance of oxidative stress, its in vivo determination has not led to conclusive results: the interpretation of the relationship between measurements of the products of lipoperoxidation and diabetes has led to certain contradictions in the literature [9]. There are also conflicting results concerning alpha-tocopherol and diabetes, since one may find articles describing it as increased, unchanged, or decreased [28]. A possible explanation of the different conclusions reached in different studies may be that not only is there a rise in free radicals in diabetes, but that there is also an enhancement of the organism's vitamin and enzyme defences.

Some studies detected a greater level of malondialdehyde in diabetic patients who also presented arteriosclerotic symptoms than in those without such symptoms. It may be that lipoperoxidation occurs in such places as the atheromal plaque, and only with time and the progress of the lesions do the molecules of malondialdehyde diffuse into the blood plasma in amounts that are sufficient for their detection [29]. We found no variation in plasma or erythrocyte malondialdehyde levels between the four groups of rats studied (controls, diabetic, diabetic treated with basic fraction, and diabetic treated with chloroform fraction). In a day-by-day study of a numerous group of people, it was found that the great variations in MDA call into question the usefulness of this parameter as an indicator of lipoperoxidation [30].

Some studies have shown the control of diabetes to improve with the administration of high doses of vitamin E to patients, since it protects the fatty acids of the cell membrane and thereby preserves their functions with respect to insulin [31]. Daily administration of 600 mg vitamin E for 3 months to a group of non-insulin-dependent diabetic patients decreased in basal insulin levels [32]. Insulin-dependent patients had a concentration of total plasma vitamin E similar to that of controls, but the ratio of vitamin E to lipids was lower than in normal subjects [33]. The absolute levels of vitamin A are significantly less in insulin-dependent patients than in normal subjects [33].

In studies with well-controlled non-insulin-dependent patients, both vitamin A and vitamin E levels are significantly higher than in healthy subjects taken as controls, and this is also the case for diabetic patients with hyperlipidaemia.

Our results show that diabetic animals present a concentration of vitamin E that is far greater than that in normal animals. We also observed a fall in vitamin E levels relative to the diabetic group's values in the animals who received the *Ficus carica* extracts. However, when we considered the ratio of vitamin E to triglycerides as a measure of the concentration of the liposoluble vitamin in its natural medium, we observed the greatest values of the ratio in normal animals; the ratio was unmodified in animals that received the respective fraction. When we normalized vitamin E levels to those of polyunsaturated fatty acids and especially of linoleic acid, which the vitamin protects from peroxidation, the values for animals that received the extracts were similar to that of the controls. The levels of total vitamin A showed no differences between normal and diabetic animals. When we considered the vitamin A/triglyceride ratio, we observed lower levels in diabetic than in normal rats and with the administration of either *Ficus carica* extract to diabetic animals, the ratio increased. The ratio of vitamin A to PUFA levels was unchanged in diabetic animals who received the extracts. The vitamin A/linoleic acid ratio, however, was significantly raised relative to the untreated diabetic rats.

We conclude that the administration of the basic and chloroform extracts of *Ficus carica* affects the oxidative stress in diabetes, with particular significance regarding the vitamin E/C18:2 ratio when the chloroform fraction is administered, and the vitamin A/C18:2 ratio with the basic fraction.

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