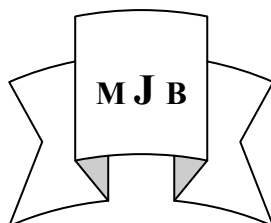


A Study on the Hypoglycemic Effect of *Ficus carica* L. Leaves Aqueous Extract against Alloxan-Induced Diabetes in Rabbits

Kawther M. Ibrahim Nada N. Al-Shawi Rasha Abdulatif Al-Juboury*
College of Pharmacy, University of Baghdad/Baghdad, Iraq
* Al-Yarmouk Teaching Hospital, Baghdad, Iraq.



Abstract

The hypoglycemic effect of orally administered aqueous extract of *Ficus carica* (F.C.) leaves against alloxan-induced diabetes in rabbits was studied. Animals were treated daily with 0.3 gm/kg F.C. aqueous leaves extract alone, or 10 IU/Kg body weight (B.wt) insulin alone, or a combination of 0.3 gm/kg B.wt F.C aqueous leaves extract and 10 IU/Kg B.wt insulin, each started one week after alloxan injection and continued for 6 weeks period for reversing the change in glucose level in diabetic rabbits. In addition, diabetic rabbits were randomly selected and received a combination of different doses of insulin with *Ficus carica* leaves aqueous extract to evaluate the extent of *Ficus carica* leaves aqueous extract in reducing insulin dose compared with diabetic rabbit treated with insulin alone (10 I.U. /Kg). Blood samples were collected weekly from the caudal ear vein of rabbits for the assessment of blood glucose level. Analysis of data obtained from the first experiment showed that, 0.3 gm/kg B.wt. of aqueous extract of F.C. leaves given alone or in combination with insulin caused an improvement of blood glucose level in diabetic rabbits compared to diabetic non-treated group. The results from the second experiment showed that, there were no significant differences between 8 U/kg insulin and 0.3gm/kg *Ficus carica* leaves aqueous extract group compared to 10 U/kg insulin, thus there were a reduction in insulin dose to almost 20% produced by *Ficus carica* leaves aqueous extract. In conclusion, *Ficus carica* leaves aqueous extract has the ability for decreasing glucose level and may have additional effect to insulin, and could be used as a supplement with insulin in order to improve its effect and/or reduced its doses and hence reduces its suspected side effects.

Address correspondence: Dr. Nada N. Al-Shawi, Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad. Email: nadaalshawi@yahoo.com.

الخلاصة

لدراسة تأثير المستخلص المائي لاوراق التين في خفض مستوى ارتفاع السكر في الارانب المصابة بداء السكري المستحدث بمادة الالوكسان، تم تقسيم مجاميع الارانب بحيث تم اعطاء المستخلص المائي يوميا لوحده بجرعة 0.3 gm/kg عن طريق الفم، او مع 10 U/kg انسولين تحت الجلد والمجموعة الاخرى من الارانب اعطيت 10 U/kg انسولين لوحده كل من هذه الجرعة قد اعطي بعد اسبوع من جرعة الالوكسان 100 mg/kg واستمرت لمدة ٦ أسابيع لدراسة مدى قابلية المستخلص المائي لاوراق التين في اعادة مستوى السكري الى المستوى الطبيعي للارانب المصابة بداء السكري. كما تضمنت الدراسة اعطاء الارانب المصابة بداء السكري المستحدث بالالوكسان بجرعة 0.3 gm/kg للمستخلص المائي لاوراق التين مع جرعة مختلفة من الانسولين لدراسة قابلية المستخلص المائي لاوراق التين في خفض جرعة الانسولين بالمقارنة مع 10 U/kg انسولين. من قياس مستوى السكر في الدم أظهرت نتائج التجربة الاولى ان مجموعة الارانب المصابة بداء السكري المستحدث بالالوكسان والتي للمستخلص المائي لاوراق التين لوحده أو مع الانسولين قد خفض بصورة معنوية مستوى السكر في الدم مقارنة مع مجموعة الارانب المصابة بالسكري. كما أظهرت النتائج عدم وجود اختلاف معنوي بين مجموعة الارانب المصابة بالسكري والتي اعطيت جرعة المستخلص المائي لاوراق التين مع 8 U/kg انسولين مقارنة بالمجموعة التي أعطيت انسولين لوحده وجرعة 10 U/kg مما يدل على ان المستخلص المائي لاوراق التين قد خفض جرعة الانسولين بمقدار ٢٠ %. نستنتج من النتائج أعلاه أن المستخلص المائي لاوراق التين له القابلية على خفض مستوى

السكر في الدم لدى الارانب المصابة وقد يكون له تأثير مضاف لتأثير الانسولين حيث من الممكن استعماله كمكمل غذائي مع الانسولين لغرض تحسين عمل الانسولين أو لتقليل جرعة الانسولين وفي النهاية تقليل اعراض الانسولين الجانبية.

Introduction

Diabetes mellitus (D.M) is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. Various complications develop because of the metabolic derangements in diabetes, many of these results to diseases of blood vessels either large (macrovascular disease) or small (microangiopathy). [1] Dysfunction of vascular endothelium is an early and critical event in the development of vascular complications [2]. Macrovascular disease consists of accelerated atheroma which is much more common and severe in diabetic patients. [3] Microangiopathy is a distinctive feature of diabetes mellitus and particularly affects the retina, kidney and peripheral nerves [4,5]; thus, treatments of diabetes mellitus is necessary to achieve as near normal blood glucose levels as possible either through Diet by increase consumption of soluble fibers, unrefined carbohydrate, or through administration of oral hypoglycemic drugs, or Insulin. Currently an attempt was made to collect traditional medicine concerning the treatments of diabetes, as part of this effort, multitudes of plants were being studied world wide to check their possible hypoglycemic effect [6-7] such as turmeric root, fenugreek seeds, bitter melon, green tea, bay leaves [8].

Ficus carica plant (fig tree) belongs to a moraceae or (mullberry family) [6]. Its fruit part has high sugar content, with a low acid complement, and a rich flavor. The list of beneficial vitamins and minerals found in *Ficus carica* is impressive; notably vitamin A, vitamin C, calcium and iron are all present; while leaves contain psi-

taraxasteryl ester, rutin, sapogenin and coumarins [9-10]. Each part of fig tree has several medicinal uses especially the fruit part where it has mild laxative and emollient effects; normalizing the secretion of gastric juices; has a remarkable antidepressant effect; in addition to its disinfectant and expectorant effect, the milky latex used in the treatment of eczema. Moreover, the dried leaves are used to treat red and painful conjunctivitis and sometimes smoked to treat asthma[11-13].

In this study attempts were made to investigate the anti-diabetic effect of the aqueous extract of *Ficus carica* leaves in diabetic rabbits.

Materials and Methods

Ficus carica leaves were collected from Baghdad in autumn 2005 and shed dried at 25 C°. A voucher specimen of the plant leaves was deposited to be identified and authenticated at the National Herbarium of Iraq Botany Directorate in Abu-Ghraib. An aqueous extract of the dried powder of leaves has been prepared by boiling 240 gm of leaves in 500 ml water for 15 min followed by filtration by Bukhner fennel through Wattman paper No. 1. The filtrate was collected and dried at 40 C° by a rotary electrical drier. The residual extracts were dissolved in normal saline whenever used in the experiments [14].

Animals

A total of forty eight healthy adult male rabbits of a local strains weighing between 950-1565 gm were obtained from and housed in the Animal House/College of Pharmacy, University of Baghdad under standard laboratory conditions were selected

The animals were housed under controlled temperature and fed with vegetables. Animals that described as fasted were deprived of food for at least 16 hours, but allowed free access to tap water.

Thirty-six rabbits were allocated into 6 groups of 6 animals each, to study the ability of 0.3 gm /kg B.wt of *Ficus carica* extract given daily by gavage tube given for six weeks after alloxan-induced diabetic rabbits to investigate the ability of the extract for reversing the changes in blood glucose, and glycemic control as follows:

1. Six rabbits were given tap water, served as control group.
2. Six rabbits treated with 2 ml of 0.3gm/ Kg B.wt aqueous extract of F.C leaves, given orally once daily for 6 weeks.
3. Six rabbits rendered diabetics by intraperitoneal (I.P.) injection of 100 mg/kg B.w. Blood

glucose level more than 200 mg/dl after one week of alloxan injection were considered as a diabetic rabbits [15].

4. Six diabetic rabbits treated with 2 ml F.C. leaves aqueous extract (0.3 gm/Kg B wt.) administered as an oral dose daily started one week after alloxan injection and continued for 6 weeks period.
5. Six diabetic rabbits treated with subcutaneous (S.c.) injection of insulin (10 IU/Kg B.wt.) once daily started one week after alloxan injection and continued for 6 weeks period.
6. Six diabetic rabbits treated with both F.C. leaves aqueous extract (0.3 gm/Kg B. wt.) and insulin (10 IU/Kg B.wt.) started one week after alloxan injection and continued for 6 weeks period.

Another twelve alloxan-induced diabetes in rabbits were randomly selected and divided into two groups

(6 animals each) to receive a combination of insulin with *Ficus carica* leaves aqueous extract and the other group were received insulin only. The aim of this experiment was to evaluate the extent of *Ficus carica* leaves aqueous extract in reducing insulin dose as compared with diabetic rabbit treated with insulin alone (10 I.U. /Kg). The blood glucose levels were estimated after 1:30 minute of insulin injections.

Blood samples were collected every week from the caudal ear vein of rabbits and transferred immediately into EDTA tubes for the assessment of blood glucose [16].

Statistical analysis of data was performed utilizing student's t-test. Multiple group comparisons were made using analysis of variance (ANOVA), P values less than 0.05 was considered significant for all data showed in our results.

Results

The results of this study demonstrated that, there was a significant decrease in blood glucose for control treated group with *Ficus carica* leaves aqueous extract (0.3g/kg) as compared to control non-treated group at the end of 6th week experiment (159 ± 4.25) vs. (188 ± 5.23) and in diabetic rabbit treated with *Ficus carica* leaves aqueous extract (0.3g/kg) (337 ± 16.5) vs. diabetic non-treated group (517 ± 29.6) ($P < 0.05$). On other hand, a significant difference in the level of blood glucose ($P < 0.05$) starts in control group treated with *Ficus carica* leaves aqueous extract at 3rd week as compared to 1st week (170 ± 8.4) vs. (184 ± 6.4) and 5th week in case of diabetic treated with *Ficus carica* leaves aqueous extract group (341 ± 12.5) vs. 1st week (379 ± 29) (Table 1 and Figure 1).

Table 1 Effect of F.C. leaves aqueous extract of 0.3 gm /kg /day for 6 weeks, 10 U/kg insulin and a combination of 0.3 gm /kg /day of F.C. leave aqueous extract and 10 U/kg insulin, respectively on blood glucose level (mg/dl).

Groups	n	1 week	2 week	3 week	4week	5week	6 week
Control not treated	6	^a 189 ^A ± 4.2	^a 187 ^A ± 4.5	^a 182 ^A ± 6.2	^a 185 ^A ± 5.79	^a 190 ^A ± 5.16	^a 188 ^A ± 5.23
Control treated with F.C. (0.3 g/kg)	6	^a 184 ^A ± 6.4	^b 179 ^A ± 6.5	^b 170 ^B ± 8.4	^b 162 ^C ± 7.47	^b 160 ^C ± 6.14	^b 159 ^C ± 4.25
Diabetic not treated	6	^b 398 ^A ± 27.9	^c 415 ^A ± 25.9	^c 435 ^A ± 37.2	^c 469 ^B ± 25.7	^c 496 ^B ± 29.0	^c 517 ^B ± 29.6
Diabetic treated with F.C. (0.3 g/kg)	6	^b 379 ^A ± 29	^d 367 ^A ± 11	^d 356 ^A ± 25.1	^d 349 ^A ± 16.0	^d 341 ^B ± 12.5	^d 337 ^C ± 16.5
Diabetic treated with insulin (10U/kg)	6	^c 210 ^A ± 16.6	^e 195 ^A ± 18.1	^e 188 ^B ± 6.9	^e 176 ^C ± 7.3	^e 169 ^C ± 8.9	^e 145 ^D ± 5.5
Diabetic treated with Insulin (10U/kg) + F.C. (0.3 g/kg)	6	^c 194 ^A ± 22.5	^f 175 ^A ± 16.5	^f 169 ^B ± 8.81	^f 153 ^C ± 6.2	^f 147 ^C ± 4.18	^f 138 ^D ± 4.2

-Values are presented as mean ±SD

n=number of rabbits.

-vertical values with different small letter superscripts (a, b, c, d, e, f) were considered significantly different (P<0.05).

-horizontal values with different capital letter superscripts (A, B, C, D) were considered significantly different (P<0.05).

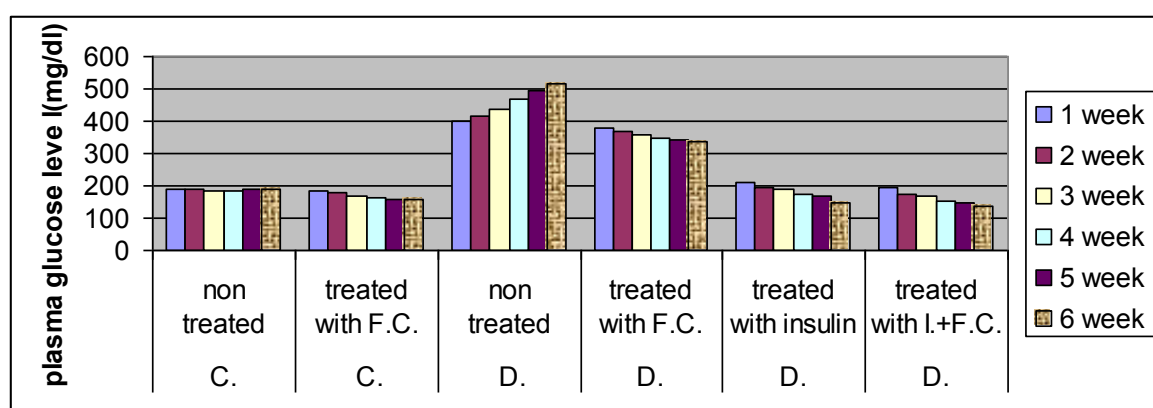


Figure 1 compares the effect of all groups during the treatment period on blood glucose level (mg/dl) in control and diabetic rabbits. C= control rabbits; D= diabetic rabbits; F.C. = *Ficus carica*.

Treatment of diabetic rabbits with 10 U/kg insulin produce a significant

decrease in the level of blood glucose at the end of 6th week as compared to

diabetic non- treated animals (145 ± 5.5) vs. (517 ± 29.6) ($P < 0.05$). On the other hand, a significant difference in the level of blood glucose ($P < 0.05$) starts at 3rd week of treatment with insulin 10 U/kg when compared to 1st week (188 ± 6.9) vs. (210 ± 16.6) (Table 1). Furthermore, diabetic rabbits treated with a combination of insulin (10 U/kg) and *Ficus carica* leaves aqueous extract (0.3 g/kg), showed a significant decrease in blood glucose level at the end of 6th week as compared to diabetic non-treated animals (138 ± 4.2) vs. (517 ± 29.6) ($P < 0.05$) (Table 1). On the other hand, a significant difference in blood

glucose level ($P < 0.05$) starts at 3rd week of treatment with a combination of insulin (10 U/kg) and *Ficus carica* leaves aqueous extract (0.3 g/kg) when compared to 1st week (169 ± 8.81) vs. (194 ± 22.5) (Table 1 and figure 1).

Table 2 showed that, there were no significant differences between insulin (8 U/kg) and *Ficus carica* leaves aqueous extract (0.3gm/kg) group compared to 10 U/kg insulin. This means that; 8U/kg insulin + 0.3gm/kg F.C. gave almost the same hypoglycemic effect of 10 U/kg insulin, i.e. there was about two units reduction in the dose of insulin used.

Table 2 Effect of *Ficus carica* (F.C.) leaves aqueous extract on insulin dose when given together in diabetic rabbits

Days	Dose of insulin U/Kg B.wt.	Blood glucose of insulin group animals(n=6) (mg/dl)	Doses of Insulin (U/Kg) +F.C (gm/kg)	Blood glucose of insulin+ F.C. group of animals (mg/dl) (n=6)
1 st day	10	218 ± 10.1	10 U.+0.3	201 ± 12.6
2 nd	10	207 ± 4.88	8 U.+ 0.3	203 ± 7.18 N.S.
3 rd	10	194 ± 8.19	6 U.+0.3	226 ± 6.8
4 th	10	192 ± 6.8	4 U.+0.5	297 ± 14.4

N.S. = Non-significant differences between insulin group and (insulin + F.C.) group.

Discussion

The data obtained from this study demonstrate that, *Ficus carica* leaves aqueous extract showed a significant hypoglycemic effect in diabetic rabbits (Table 1, Figure 1). A finding which confirm the results of C. Perez et al (2000) [17]. Moreover, exogenous administration of insulin produces a significant hypoglycemia in diabetic animals and when it is given in combination with *Ficus carica* leaves aqueous extract the results showed more potent hypoglycemic effect.

However, another beneficial effect was observed when comparison was made between insulin treatment group (10 U/Kg) with insulin + *Ficus carica* leaves aqueous extract (8 U/Kg+0.3 gm / Kg) group since no significant difference occurs between them, which means that, there was a decrease in total insulin dose about (20%) when it is given in combination with *Ficus carica*, these results is also in agreement with the study of Serracarla et al (1998) (18) since they got 15.5% reduction in total daily insulin dose.

The hypoglycemic effect of *Ficus carica* leaves extract may be due to one or more of the following mechanisms:-

1- It could have insulin like effect by acting peripherally through stimulation glucose utilization by skeletal muscles (i.e. extra-pancreatic effect) rather than stimulating insulin release. especially that; Serracclara et al in 1998 [18] found a non significant differences in c-peptides [which are a good indicators of endogenous insulin secretory capacity results from pro-insulin hormone which is cleaved to release insulin in equimolar amounts to inert c-peptide [17] level after administration of *Ficus carica* aqueous extract, this suggestion is also in agreement with C. Perez et al (2000) [19]. *Ficus carica* leaves aqueous extract could be also useful in type II diabetic patient through its mechanism of facilitating glucose uptake at peripheral level in addition to its beneficial effect to type I-diabetic patient when given as a supplement to breakfast before administration of exogenous insulin to decrease (20%) of the total daily insulin dose.

2- The increase in sensitivity of tissue to available endogenous insulin released from remaining pancreatic β -cell this effect may be due to the effect of saponin which exerts its hypoglycemic effect [20]. An extra-pancreatic mechanism includes increased insulin- receptor binding, and increases trans membrane passage of glucose across cells membranes of most body cell which promotes the conversion of glucose to glycogen through induction of glucokinase and glycogen synthase [21].

In conclusion, the results of this study demonstrated that, the mechanism (s) responsible for improvement of glycemic control of *Ficus carica* leaves aqueous extract in diabetic rabbits could possibly be

related to its insulin like effect through its additional effect when given in combination with insulin, and decreased its dose to almost 20% and / or increase in sensitivity of tissue to release insulin from the remaining unchanged B-cells. Therefore, *Ficus carica* leaves extract could be used as a supplement with insulin in order to increase the duration of action improve its effect and/or reduce its doses and hence reduces its suspected side effects.

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