LEX LOCALIS-JOURNAL OF LOCAL SELF-GOVERNMENT
ISSN:1581-5374 E-ISSN:1855-363X
VOL. 23, NO. S6(2025)

THE EFFECT OF SOME ACTIVE COMPOUNDS OF THE AQUEOUS EXTRACT OF BASIL ON SOME BIOCHEMICAL PARAMETERS OF MALE RATS INDUCED WITH DIABETES

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Abstract
Diabetes mellitus is a collection of metabolic disorders that raise blood sugar by decreasing pancreatic beta cell insulin production or weakening its processes. It's one of the oldest diseases diagnosed by humans. The World Health Organization has divided diabetes into two types: insulin-dependent and non-insulin-dependent. The American Council on Diabetes Studies and Research has also added another type: gestational diabetes. Due to the expenses associated with the chemicals used to treat this ailment, there has lately been a transition towards the utilization of herbal treatments. These medicines contain active compounds that play a significant role in treating this disease. One such plant is basil. Its aqueous extract contains many active compounds such as flavonoids, saponins, alkaloids, and others that play an effective role in treating diabetes. Therefore, the study aimed to use the aqueous extract of basil leaves to treat diabetes induced in laboratory rats using the drug alloxan. (40) laboratory rats were used in this study, aged (10-12) weeks and weighing (200-250) grams. Then they were divided into four treatments. The first treatment was a control treatment without treatment. The second treatment was alloxan (150) mg/kg live weight, and the third was alloxan and 250 mg/kg basil leaf extract daily for a month. The fourth was injected with alloxan and given 500 mg/kg living weight basil leaf extract daily for a month. It examined glucose, cholesterol, triglycerides, creatinine, urea, and liver enzymes (AST, ALT). Data indicates a considerable increase in glucose and cholesterol levels ($p<0.05$) after alloxan injection. These levels returned to control group levels after basil aqueous extract treatment. Significantly increased liver enzyme values (ALT, AST) were observed ($p<0.05$) compared to the untreated control group. These two enzymes returned to near-normal levels after one month of water-based basil leaf extract therapy. The infusion of alloxan significantly increased creatinine and urea levels (+) ($p<0.05$). Later, water-based basil leaf extract restored concentrations.

Key words : Diabetes, basil, alloxan, Male Labratory rats

Introduction:

Diabetes mellitus is characterized by a group of metabolic disorders that lead to high blood sugar due to a partial or complete decrease in insulin secretion from specialized cells in the pancreas (beta cells) or a weakening of the hormone's mechanisms of action. These metabolic disorders include impaired carbohydrate, fat, and protein metabolism, which can lead to death (Daisy et al., 2009).

Diabetes is one of the oldest diseases diagnosed by humans, as it was described in the oldest Chinese, Egyptian and Indian manuscripts (Khardori & Pauza, 2003). The World Health Organization divided diabetes into type 1, which is insulin-dependent, and type 2, which is non-insulin-dependent (Organization, 1980).

In 1977, the American Diabetes Association (ADA) divided diabetes into two types: Type 1, Type 2, and gestational diabetes. As many patients suffer from the high cost of chemical medications, research has turned to the use of natural and plant-based products to treat certain diseases, including diabetes. One such plant is basil (Ocimum basilicum), which belongs to the mint family. It has multiple uses in traditional medicine due to its nutrient-supporting properties, such as calcium and antioxidants. Basil is used to control blood sugar levels, especially its leaves, due to their ability to improve and develop pancreatic beta cells, which are responsible for insulin

secretion. It can also improve glucose absorption by muscle cells, thus reducing blood sugar levels (Brazier, 2021).

Insulin is a peptide hormone produced by pancreatic beta cells. It regulates the body's metabolism. It is responsible for converting glucose absorbed into various tissues into glycogen, fat, or both (Cobry et al., 2010) (Cryer, 2013) (Saydah, 2021). The aqueous extract of basil leaves contains many active ingredients, such as flavonoids, saponins, alkaloids, glycosides, and steroids. It has been widely used in some studies as an effective treatment for diabetes. In addition, it contains antioxidants that play an effective role in maintaining the body's natural immunity (Joshi et al., 2023) (Okwu & Morah, 2004). It was also crucial in elevating the concentrations of certain biochemical and hematological variables in the blood of mice used in experiments.

A glucose level of 101.8 mg/dL was observed in mice that were given an aqueous extract of basil leaves. A level of 103 mg/dL was obtained by the control group. Low levels of cholesterol and triglycerides were also seen. In contrast to the control group's levels of 46 and 83.4 mg/dL, respectively, the aqueous extract treatment group reported 144 and 83.2 mg/dL. When mice were given oral dosages of aqueous basil extract many times, (Zeggwagh et al., 2007) found that glucose, cholesterol, and triglyceride levels decreased significantly.

The group that consumed persimmons—which contain active chemicals comparable to those in water-based basil leaf extracts—also had significantly lower glucose levels, liver enzymes (ALP, GPT, GOT), creatinine, and urea, according to Khater et al. (2015).

Thus, the purpose of this research was to find out how an aqueous basil leaf extract affected the levels of lipids, blood sugar, liver enzymes, urea, and creatinine in rats that had been artificially made diabetic using alloxan.

Materials and Methods :

Fourty male laboratory rats, aged 12-15 weeks and weighing (200-250) were used. They were then randomly divided into four groups, with ten rats per group, as follows:

1. **Group 1**: Control group, administered a physiological saline solution.
2. **Group 2**: Diabetes was induced using alloxan at a dose of 150 mg/kg live weight via intraperitoneal injection.
3. **Group 3**: Diabetes was induced using alloxan, and the animals were administered an aqueous extract of basil leaves at a dose of 250 mg/kg live weight one month after induction.
4. **Group 4**: Diabetes was induced using alloxan, and the animals were administered an aqueous extract of basil leaves at a dose of 500 mg/kg live weight one month after alloxan injection.

5 ml blood samples were drawn from rats using a sterile syringe after the animals were anesthetized. Blood was drawn directly from the heart, and samples were stored in anticoagulant-free vials for serum separation. Samples were stored at refrigerator temperature until serum separation was performed using a centrifuge for 15 minutes at 4000 rpm. The serum was stored in sterile tubes at -20°C until required analyses. Glucose, cholesterol, triglycerides, liver enzymes (ALT, AST), urea, and creatinine were measured using a Cobas C111 chamber. Data were statistically analyzed using SPSS version 26 (2019). The least significant difference test was performed between means at a probability level of ($p<0.05$).

Results and Discussion:

1_ Changes in Glucose Levels

The results in Table 1 showed a significant increase ($p<0.05$) in glucose concentration in the groups treated with alloxan compared to the untreated control group. The table also shows that

treating diabetic rats with an aqueous extract of basil leaves at doses of (250-500) mg/kg live weight caused a significant decrease ($p<0.05$) in blood sugar levels compared to the control group treated with the extract and not treated with the extract. There was no significant effect of the duration of treatment with the extract, which was (15-30) days.

The effect of alloxan on pancreatic beta cells occurs through the destruction of these cells (Eteng et al., 2008). It also disrupts insulin secretion through unstable oxygen molecules, which form free radicals (Kim et al., 2006). The significant decrease in the two treatments that were dosed with the aqueous extract of basil leaves (500, 250) mg/kg live weight is due to the fact that basil belongs to the group of plants that contain glycosides and saponins that lead to a reduction in blood sugar levels (Abdel-Hassan et al., 2000). The flavonoids present in the extract also lead to an increase in insulin secretion in the blood (Bhushan etat, 2010).

2_ Changes in Cholesterol Concentration

Table 2 shows a significant effect ($p<0.05$) of diabetes induction in rats. The second, third, and fourth treatments outperformed the first, non-induced treatment. Cholesterol levels recorded a significant increase in these treatments. This is due to the fact that alloxan administration led to increased intestinal cholesterol absorption due to increased activity of the cholesterol acyltransferase enzyme, which subsequently led to an increase in cholesterol levels (Hori et al., 2004). The table above shows that treating rats with an aqueous extract of basil leaves at doses of 250,500 mg/kg live weight resulted in a significant decrease ($p<0.05$) in cholesterol levels. The third and fourth treatments recorded a significant decrease ($p<0.05$) compared to the second treatment when treated with the extract during the different dosing periods. This decrease may be due to insulin inhibiting the action of lipase in fat cells, which leads to a decrease in the breakdown of fats into other compounds (Roche et al., 2005).

3_ Changes in triglyceride concentration:

Table 3 indicates that treating rats with alloxan (treatments two, three, and four) resulted in a significant increase ($p<0.05$) in triglyceride concentration compared to the control group. The table above shows that treating diabetic rats with an aqueous extract of basil leaves at doses of 250,500 mg/kg live weight caused a significant decrease ($p<0.05$) in triglyceride levels compared to the first (control) group and the second (not treated with the extract). The reason for the increased triglyceride concentration in diabetic animals is due to a decrease in the lipoprotein lipase enzyme in adipose tissue. The presence of insulin is necessary for the enzyme to be effective, which breaks down triglycerides under normal conditions (Jasmine & Daisy, 2007). Treating animals with an aqueous extract of basil leaves improved insulin secretion from pancreatic cells, thus restoring the lipoprotein lipase enzyme to its normal function and effectiveness in analyzing triglycerides, thus returning triglyceride levels to their normal levels (Cho et al., 2008).

4_ Changes in liver enzyme concentrations (AST, ALT):

Tables 4-5 indicate a significant increase ($p<0.05$) in the concentration of liver enzymes (AST, ALT) when rats were treated with alloxan (treatments two, three, and four) compared to the control group. This increase is attributed to the formation of free radicals. These radicals play a role in the destruction of hepatic cells, thus releasing enzymes into the blood. These radicals also lead to cirrhosis of hepatic cells and the loss of enzyme receptors located in the cells lining the bile, which results in the release of enzymes outside the cells (Al-Wabel et al., 2008). Giving the aqueous extract of basil leaves to rats (treatments three and four) reduced the formation of free radicals, thus reducing the leakage of the enzymes into the blood and returning to normal levels compared to the second group, not treated with the extract (Prakasam et al., 2004).

5_ Changes in Creatinine and Urea Concentrations (Kidney Function)

Tables 6, 7 show a significant increase ($p<0.05$) in creatinine and urea concentrations when rats in the second, third, and fourth treatments were injected with alloxan compared to the control treatment (the first). This increase is due to complications caused by diabetes, one of which is diabetic nephropathy, which is characterized by changes in kidney function resulting in elevated creatinine and urea concentrations (Bartošíková et al., 2003). Impaired kidney function is one of the most important characteristics associated with diabetes. When animals in the third and fourth groups were dosed with an aqueous extract of basil leaves at 250, 500 mg/kg live weight, there was a significant decrease ($p<0.05$) in creatinine and urea concentrations (Tables 6, 7). This may be due to the active compounds (flavonoids and terpenes) found in the leaves, which cause a decrease in lipid oxidation, an increase in antioxidants, and a reduction in accumulated fat and blood glucose levels. Which in turn leads to improvement in kidney tissue (Abd-Alla et al., 201

Treatment periods	G1	G2	G3	G4	treatments Mean
Before diabetes induction	a 97.82 ± 8.80 A	b 98.53 ± 0.87 A	C 99.17 ± 8.92 A	C 100.13 ± 9.01 A	C 98.91 ± 8.90
15 days after diabetes induction with alloxan	a 98.28 ± 8.84 B	a 313.20 ± 25.19 A	a 310.25 ± 24.82 A	a 326.91 ± 26.15 A	a 262.16 ± 23.65
One month after diabetes induction with alloxan	a 98.15 ± 8.83 B	a 325.42 ± 26.03 A	a 323.72 ± 25.89 A	a 329.17 ± 26.33 A	a 269.16 ± 24.22
15 days after administration of the extract	a 98.25 ± 8.84 C	a 321.95 ± 25.75 A	b 182.17 ± 14.57 B	b 162.73 ± 13.03 B	b 191.275 ± 17.21
One month after administration	a 97.93 ± 8.81 D	a 341.15 ± 31.29 A	b 174.13 ± 13.93 B	b 153.18 ± 12.25 C	b 186 .60 ± 16.79
Periods mean	98.09 ± 8.83 C	280.05 ± 22.40 A	217.89 ± 17.43 B	214.42 ± 17.15 B	

Table 1 Effect of the active compounds of the hot aquatic basil leaf extract on glucose concentration (mg/dl) in the blood serum of rats in different treatments (mean ± standard error)

Capital letters in the horizontal direction indicate significant differences ($p<0.05$) between treatments.
Lowercase letters in the vertical direction indicate significant differences ($p<0.05$) between periods.
G1: First treatment, administered physiological saline only (negative control).
G2: Second treatment, induced diabetes using alloxan (positive control).
G3: Third treatment, induced diabetes, administered orally with 250 mg of hot aqueous basil leaf extract. Mg/kg body weight
G4: The fourth treatment induces diabetes and is administered orally with a hot aqueous extract of basil leaves at 500 mg/kg body weight.
Table 2 Effect of the active compounds of the hot aquatic basil leaf extract on cholesterol concentration (mg/dl) in the blood serum of rats in different treatments (mean ± standard error)

Treatment periods	G1	G2	G3	G4	treatments Mean
Before diabetes induction	a 88.41 ± 7.96 A	b 87.91 ± 7.91 A	b 86.15 ± 7.75 A	b 86.83 ± 7.81 A	C 87.33 ± 9.61
15 days after diabetes induction with alloxan	a 88.93 ± 8.00 B	a 152.13 ± 12.17 A	a 154.16 ± 12.33 A	a 150.97 ± 12.10 A	a 136.55 ± 15.02
One month after diabetes induction with alloxan	a 87.83 ± 7.90 B	a 155.72 ± 12.46 A	a 155.32 ± 12.43 A	a 153.72 ± 12.30 A	a 138.15 ± 15.19
15 days after administration of the extract	a 86.82 ± 7.81 B	a 153.52 ± 12.28 A	b 94.55 ± 7.56 B	b 97.15 ± 7.77 B	b 108.01 ± 11.89
One month after administration	a 88.30 ± 7.95 B	a 156.42 ± 12.51 A	b 90.13 ± 8.11 B	b 88.13 ± 7.93 B	105.75 ± 10.58
Periods mean	88.06 ± 7.93 C	141.14 ± 11.29 A	116.06 ± 9.28 A	115.36 ± 9.23 B	

Treatment / periods	G1	G2	G3	G4	treatments Mean
Before diabetes induction	a 90.73 ± 8.16 A	b 93.60 ± 8.42 A	C 89.73 ± 8.10 A	C 89.96 ± 8.10 A	C 91.51 ± 9.15
15 days after diabetes induction with alloxan	a 92.14 ± 7.37 B	a 140.13 ± 11.21 A	a 142.16 ± 11.37 A	a 147.62 ± 11.81 A	a 130.51 ± 11.25
One month after diabetes induction with alloxan	a 89.94 ± 7.19 B	a 145.15 ± 11.61 A	a 144.72 ± 11.58 A	a 146.75 ± 11.24 A	a 131 .64 ± 11.85
15 days after administration of the extract	a 90.54 ± 7.24 B	a 141.71 ± 11.34 A	b 120.12 ± 9.61 A	b 102.33 ± 8.19 B	a b 113.68 ± 10.23
One month after administration	a 93.05 ± 7.44 B	a 139.87 ± 11.19 A	a 108.63 ± 8.69 B	bc 94.92 ± 7.59 B	bc 109.12 ± 9.82
Periods mean	91.28 ± 8.22 C	132.09 ± 11.89 A	121.47 ± 10.93 AB	116.32 ± 10.47 B	

Capital letters in the horizontal direction indicate significant differences ($p<0.05$) between treatments.

Lowercase letters in the vertical direction indicate significant differences ($p<0.05$) between periods.

G1: First treatment, administered physiological saline only (negative control).

G2: Second treatment, induced diabetes using alloxan (positive control).

G3: Third treatment, induced diabetes, administered orally with 250 mg of hot aqueous basil leaf extract. Mg/kg body weight

G4: The fourth treatment induces diabetes and is administered orally with a hot aqueous extract of basil leaves at 500 mg/kg body weight.

Table 3 Effect of the active compounds of the hot aquatic basil leaf extract on triglyceride concentration (mg/dl) in the blood serum of male rats in different treatments (mean ± standard error)

Treatment periods	G1	G2	G3	G4	treatments Mean
Before diabetes induction	a 71.33 ± 6.42 A	b 75.22 ± 6.77 A	C 70.19 ± 6.32 A	C 74.32 ± 6.70 A	C 72.77 ± 7.28
15 days after diabetes induction with alloxan	a 74.61 ± 6.71 B	a 129.15 ± 10.33 A	a 130.55 ± 10.44 A	a 128.54 ± 10.28 A	a 115.71 ± 11.57
One month after diabetes induction with alloxan	a 70.92 ± 6.38 B	a 134.31 ± 10.74 A	a 138.15 ± 11.05 A	a 131.42 ± 10.51 A	a 118.70 ± 11.87
15 days after administration of the extract	a 72.84 ± 6.56 C	a 132.69 ± 10.62 A	b 107.21 ± 8.58 B	b 104.92 ± 8.39 B	ab 104.42 ± 10.44
One month after administration	a 75.12 ± 6.76 C	a 128.73 ± 10.29 A	b 97.83 ± 7.82 B	b 89.63 ± 7.17 B	b 97.83 ± 9.78
Periods mean	72.96 ± 7.30 B	120.02 ± 12.00 A	102 .79 ± 10.88 A	105.77 ± 10.58 A	

Capital letters in the horizontal direction indicate significant differences ($p<0.05$) between treatments.

Lowercase letters in the vertical direction indicate significant differences ($p<0.05$) between periods.

G1: First treatment, administered physiological saline only (negative control).

G2: Second treatment, induced diabetes using alloxan (positive control).

G3: Third treatment, induced diabetes, administered orally with 250 mg of hot aqueous basil leaf extract. Mg/kg body weight

G4: The fourth treatment induces diabetes and is administered orally with a hot aqueous extract of basil leaves at 500 mg/kg body weight.

Table 4 Effect of the active compounds of the hot aquatic basil leaf extract on ALT enzyme concentration (mmol/L) in the blood of male rats in different treatments (mean ± standard error)

Treatment periods	G1	G2	G3	G4	treatments Mean
Before diabetes induction	a 85.14 ± 7.66 A	b 87.32 ± 7.86 A	C 83.92 ± 7.55 A	C 89.22 ± 7.14 A	C 86.4 ± 8.61
15 days after diabetes induction with alloxan	a 89.13 ± 8.02 B	a 145.11 ± 11.61 A	a 149.71 ± 11.98 A	a 140 .93 ± 11.22 A	a 131.22 ± 13.12
One month after diabetes induction with alloxan	a 87.63 ± 7.89 B	a 148.65 ± 11.89 A	a 142.93 ± 11.43 A	a 142.14 ± 11.37 A	a 130.34 ± 13.03
15 days after administration of the extract	a 85 .73 ± 7.72 C	a 140.87 ± 11.27 A	b 128.52 ± 10.28 AB	b 121.69 ± 9.73 B	b 119.20 ± 11.92
One month after administration	a 90.42 ± 8.14 C	a 150.41 ± 12.03 A	b 117.84 ± 9.43 B	b 114.88 ± 9.19 B	b 118.39 ± 11.84
Periods mean	87.61 ± 8.76 B	134.47 ± 13.45 A	124.58 ± 12.44 A	121.77 ± 12.18 A	

Capital letters in the horizontal direction indicate significant differences ($p<0.05$) between treatments.
Lowercase letters in the vertical direction indicate significant differences ($p<0.05$) between periods.
G1: First treatment, administered physiological saline only (negative control).
G2: Second treatment, induced diabetes using alloxan (positive control).
G3: Third treatment, induced diabetes, administered orally with 250 mg of hot aqueous basil leaf extract. Mg/kg body weight
G4: The fourth treatment induces diabetes and is administered orally with a hot aqueous extract of basil leaves at 500 mg/kg body weight.

Table 5 Effect of the active compounds of the hot aquatic basil leaf extract on AST enzyme concentration and (mmol/L) in the serum of male rats treated with different treatments (mean ± standard error)

Capital letters in the horizontal direction indicate significant differences ($p<0.05$) between treatments.
Lowercase letters in the vertical direction indicate significant differences ($p<0.05$) between periods.
G1: First treatment, administered physiological saline only (negative control).
G2: Second treatment, induced diabetes using alloxan (positive control).
G3: Third treatment, induced diabetes, administered orally with 250 mg of hot aqueous basil leaf extract. Mg/kg body weight
G4: The fourth treatment induces diabetes and is administered orally with a hot aqueous extract of basil leaves at 500 mg/kg body weight
Table 6 Effect of the active compounds of the hot aqueous basil leaf extract on the serum creatinine concentration (mg/dl) of male rats treated with different treatments (mean ± standard error)

Treatment / periods	G1	G2	G3	G4	treatments Mean
Before diabetes induction	a 1.93 ± 0.17 A	b 1.95 ± 1.18 A	C 1.89 ± 0.17 A	b 1.97 ± 0.18 A	b 1.94 ± 0.19
15 days after diabetes induction with alloxan	a 1.89 ± 0.17 B	a 2.84 ± 0.23 A	a 2.95 ± 0.24 A	a 2.92 ± 0.23 A	a 2.65 ± 0.27
One month after diabetes induction with alloxan	a 1.97 ± 0.20 B	a 2.95 ± 0.24 A	a 2.98 ± 0.21 A	a 2.96 ± 0.29 A	a 2.72 ± 0.30
15 days after administration of the extract	a 1.94 ± 0.19 C	a 2.90 ± 0.13 A	b 2.41 ± 0.19 B	b 1.96 ± 0.14 C	b 2.30 ± 0.25
One month after administration	a 1.93 ± 0.22 C	a 2.94 ± 0.29 A	b 2.19 ± 0.22 B	b 1.95 ± 0.19 C	b 2.25 ± 0.27
Periods mean	1.93 ± 0.23 B	2.72 ± 0.33 A	2.48 ± 0.27 A	2.35 ± 0.26 A	

Capital letters in the horizontal direction indicate significant differences ($p<0.05$) between treatments.
Lowercase letters in the vertical direction indicate significant differences ($p<0.05$) between periods.
G1: First treatment, administered physiological saline only (negative control).
G2: Second treatment, induced diabetes using alloxan (positive control).
G3: Third treatment, induced diabetes, administered orally with 250 mg of hot aqueous basil leaf extract. Mg/kg body weight
G4: The fourth treatment induces diabetes and is administered orally with a hot aqueous extract of basil leaves at 500 mg/kg body weight.

Treatment / periods	G1	G2	G3	G4	treatments Mean
Before diabetes induction	a 29.84 ± 2.70 A	b 31.21 ± 2.81 A	C 31.92 ± 2.87 A	C 30.55 ± 2.75 A	b 30.88 ± 3.10
15 days after diabetes induction with alloxan	a 31.42 ± 2.83 B	a 50.72 ± 4.06 A	a 53.11 ± 4.25 A	a 51 .63 ± 4.13 A	a 46.72 ± 4.67
One month after diabetes induction with alloxan	a 32.72 ± 2.94 B	a 52.47 ± 4.19 A	a 51.24 ± 4.10 A	a 53.41 ± 4.27 A	a 47.46 ± 4.75
15 days after administration of the extract	a 30.53 ± 2.75 B	a 53 .92 ± 4.31 A	b 46.32 ± 3.71 A	b 42.65 ± 3.65 A	a 44.11 ± 4.85
One month after administration	a 31.75 ± 2.54 C	a 50.88 ± 3.56 A	b 40.22 ± 3.22 B	b 41.11 ± 3.29 B	a 40 .99 ± 4.51
Periods mean	a 31.25 ± 3.75 B	47.84 ± 5.26 A	44.56 ± 4.90 A	44.47 ± 4.45 A	

Table 7 Effect of the active compounds of the hot aqueous basil leaf extract on the serum urea concentration (mg/dl) of male rats treated with different treatments (mean ± standard error)

Capital letters in the horizontal direction indicate significant differences ($p<0.05$) between treatments.
Lowercase letters in the vertical direction indicate significant differences ($p<0.05$) between periods.
G1: First treatment, administered physiological saline only (negative control).
G2: Second treatment, induced diabetes using alloxan (positive control).
G3: Third treatment, induced diabetes, administered orally with 250 mg of hot aqueous basil leaf extract. Mg/kg body weight
G4: The fourth treatment induces diabetes and is administered orally with a hot aqueous extract of basil leaves at 500 mg/kg body weight.

Conclusions

Diabetes mellitus is one of the dangerous diseases that raised blood sugar levels by decreasing Insulin Insulin hormone hormone secretion from beta cells in Pancreas. The low secretion of Insulin effected in many biochemical blood traits such as reducing some traits and raised others.
Using herbal supplements which contain active compounds play an affective role in treating this disease. One of this herbal is basil that it's aqueous extract contains many active compounds effects in treated diabetes .
The safety using for this extract helps Pancreas to return back to secret Insulin with levels near normally by renovat the beta cells in Pancreas

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