تأثير مستخلص الريحان على مستوي الجلوكوز ودهون الدم في فئران التجارب

# Effects of Sweet Basil *(Ocimum basilicum)* Extract on Glucose and Lipids Profile Level in the Experimental

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#### Abstract

Natural products from various sources tend to be potential candidates for drug discovery. Sweet basil (*Ocimum Basillicum L.*) is well–known as a medicinal plant and culinary herb because of its phytochemical contents. Thus, the aim of the present study was to evaluate the possible effect of sweet basile in diabetic rats. Thirty–two adults male Wistar albino rats (150\_180) g were randomly divided into four groups (8 rats each). The hyperglycemic groups were made with a single intraperitoneal dose of STZ (55 mg/kg). Four groups of rats were used; (I) normal control group, (II) negative control group, administrated Sweet Basile extract with standard rats diet (300 mg/kg/day), (III) STZ group, diabetic rats acted as positive control; and (IV) sweet basil extract group, included diabetic rats treated with sweet basil extract (300mg/kg/day).

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After six weeks, treatment of diabetic rats with sweet Basil extract group (IV) markedly exhibited a significant reduction in glucose, cholesterol, triglycerides, and LDL matched with a significant rise at (p<0.05) in insulin and HDL values close to the corresponding values of healthy ones. In conclusion, sweet basil extract exhibits multi-health benefits with promising potentials against STZ-induced diabetes; this behavior may be attributed to its antioxidant and free radical scavenging mechanisms. Therefore, our results contribute to the prospective characterization and development of new anti-diabetic and hypolipidemic therapeutic agents.

Keywords: Sweet basil, lipids profile, Diabetes, Rats.

# Introduction

Diabetes and other associated diseases are major health problems in modern society. Both type 1 and type 2 diabetes comprise abnormalities of insulin action, which include insulin insensitivity and resistance *(El-Atat et al., 2004)* Diabetes mellitus (DM) is a group of heterogeneous, hormonal, and metabolic disorders characterized by Hyperglycemia and glucosuria, with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both *(Seedevi et al., 2020)*. Diabetes mellitus is associated with long-term complications such as retinopathy, nephropathy, neuropathy and angiopathy. Furthermore, DM is considered a major risk factor for cardiovascular disorders, namely, ischemic heart disease, cerebral stroke and peripheral artery disease, leading to increased mortality in patients with diabetes *(Kristova et al., 2008)*.

Some disorders of diabetes are characterized by chronic Hyperglycemia and abnormalities in lipid profiles, such as cholesterol, low-density and high-density lipoproteins and triglycerides, leading to a series of secondary complications (Seedevi et al., 2005). These lyuria, complications include polyphagia, polydipsia, ketosis, retinopathy, and cardiovascular disorders (Kumar et al., 2002), hypoglycemic pharmacologic agents are unable to control tissue damage in diabetes; they can also cause severe side effects. Hence, researchers are motivated to seek remedies in traditional medicine that have milder toxicity than available synthetic drugs. Natural products from various sources, such as plants, animals and microorganisms, tend to be potential candidates for drug development (Alam et al., 2016). Nanotechnology might serve as a tool in the and treatment of diabetes, as well (Alam et al., 2014).

Many spices are often added to foods as additives to enhance organoleptic qualities, such as the aroma and color of Basil. Basil or Sweet Basil is named *Ocmium basilicum L*. which originated from India, and it is also well known as a culinary herb in other countries such as Italy, Thailand, Vietnam, and Taiwan Aldar *(Mahmut et al., 2023)*. Basil, is an important medicinal plant and culinary herb, belongs to the Liliaceae family, which grows in tropical and sub-tropical climates. The basil name is derived from the Greek word basileus, and it means "king." It includes over 150 species *(Blank et .)al., 2004* 

Basil is widespread today in almost every home garden and is a leading spice worldwide, used for culinary purposes both dried and fresh. It is a source of essential oils and other valuable antioxidant tales and phenolic compounds and a medicinal herb with a history of

and lore. In India, basil is considered sacred; in Italy, it is a symbol of love; *(Native Dubai et al., 2020). Ocimum Basilicum L.* is used to cure many types of fever in traditional medicine. In the current era of drug confrontation and adverse outcomes of synthetic drugs, widespread attention on herbal remedies and metabolites of plant extracts has increased among investigators and the general population

#### globally (Rajesh Kumar Josh et al., 2023).

Basil (*Ocimum Basilicum*) is one of the most important crops with essential as well as polyphenols, phenolics, flavonoids and phenolic acids (*Mohamad et al., 2020*). Given these recent findings, the aim of the present study was to evaluate the Hypoglycemic and Hypolipemic effects of sweet basil extract and its effect on blood glucose, insulin levels and lipids profile in experimental diabetic rats.

#### Materials and methods

#### **Materials**

Tow Kilo of dried basil leaves were obtained from a local supplier (Abdul Rahman Haraz, Bab al–Khalq area, Cairo, Egypt). Streptozotocin (STZ, Sigma 85882), Sodium citrate (Sigma C0909) and Citric acid (Sigma C1909) were purchased by Egyptian International Center for Import, 22 AbuZer El–Ghafary St., Nasr City Cairo, Egypt.

#### **Experimental design**

Thirty tow adult male Wistar albino rats weighting (150–180 g) were obtained from The Animal Colony, National Research Centre, Cairo, Egypt. The rats were housed in suitable plastic cages for one week for acclimation. Excess tap water and standard rodent pellets [20.3% protein (20% casein and 0.3% DL–Methionine), 5% fat (corn

oil), 5% fibers, 3.7% salt mixture and 1% vitamin mixture, obtained from Meladco Company, El–Obour City, Cairo, Egypt] were always available. All rats received human care in compliance with the standard institutional criteria for the care and use of experimental rats according to the Faculty of Science, Al–Azhar University, Assiut, Egypt After induction of diabetes, both normal and diabetic ethical committee. rats were rearranged randomly into four groups (8 rats/group).

**Group (I)** : normal control group.

**Group (II)** : negative control group, administrated sweet basile extract with standard rats diet (300mg/kg/day).

**Group (III)** : STZ group, diabetic rats acted as positive control. **Group (IV)**: diabetic rats treated with sweet basil extract (300 mg/kg/day).

#### **Methods**

#### Preparation of sweet basil

Sweet basil *(Ocimum Basilicum)* the aqueous extract of dry powdered leaves was carried out according to the method of *Kamel et al., 2022* the aqueous extraction process was carried out. In brief, 500 g of the powdered herb material was placed in a 100 ml round– bottom quick fit flask, and 1000 ml distilled water was added; the mixture was left for 24 hours and filtered through qualitative Whatman filter paper No.1 (Whatman International Ltd, Maidstone, England). In aroma and Flavoring department, National Research Center, the filtrates were subjected to a Lyophilization process through a drier (Snijders Scientific–, Tilburg, Holland) under pressure (0.1 to 0.5 bar) and temperature (-35 to  $-41^{\circ}$ C) conditions. The dry extract was stored at  $-20^{\circ}$ C until further investigation as fast as possible.

# Determination of total extract yield

The combined extracts were transferred to a quick fit round bottom flask with known weight (W1), freeze dried and weighted again (W2) and finally the yield was from the following formula: Extract yield (g/ g crude herb) = (W2 - W1)/W3Where,

W1 is the weight of a and dry quick fit flask in grams,W2 is the weight of the flask after lyophilization in gramsW3 is the weight of the crude powdered herb in grams

# Determination of radical scavenging (RSA) activity by DPPH assay

An amount of the crude extract was dissolved in methanol to obtain a concentration of 200 ppm. A volume of 0.2 ml of this solution was completed to 4 ml by methanol; then 1 ml DPPH solution (6.09 x 10– 5 mol/L, dissolved in the same solvent) was added. The absorbance of the mixture was measured at 516 nm after 10 min standing. Also, the absorbance of the reference sample or blank (1 ml of DPPH solution and 4 ml methanol) was measured. Triplicate measurements were made and the radical scavenging activity was calculated by the percentage of DPPH that was scavenged according to *Nogala*–

#### Kalucka et al. (2005).

# Determination of total phenolic content

The content of the OAE was performed by dissolving five mg of the extract in a ml mixture of acetone and water (6:4 v/v). Then, a sample of 0.2 ml was mixed with 1.0 ml of Folin–Ciocalteu reagent (10–fold diluted) and 0.8 ml of sodium carbonate solution (7.5%). After 30 min at room temperature, the absorbance was measured at 765 nm using

a100 UV–Vis spectrophotometer. The phenolic compound as catechin equivalents was carried out using a curve *(Jayaprakasha and Rao, 2000)*.

### Induction of diabetes rats

Streptozotocin dissolved in ice cold sodium citrate-citric acid buffer [20ml of sodium citrate (0.1 M) with 30ml of citric acid (0.1M), pH=4.0). The rats were intraperitoneally injected with (60 mg/kg) after 16 hours of followed by oral administration of 2–3 ml sucrose solution 10% (w/v) for one day next.

Then rats were fasted overnight and one drop blood sample was obtained by nicking the rats' tail lateral-vein using sterile surgical scissors. Immediately the blood glucose Levels was determined using Gluco Dr SUPER SENSOR AGM-2200 glucometer (Korea). Rats with blood glucose level above 240 mg/dl were considered to be diabetic *(Kim et al., 2006).* 

#### **Blood sampling**

At the end treatment period (six weeks), rats were weighed and fasted overnight and the glucose level of each rat was determined using GlucoDr set through blood specimens from the rats' tail. Following anesthesia (inhalation with diethyl ether), blood specimens were withdrawn from the retro–orbital plexus using heparinized and sterile glass capillaries; whole blood specimens were cool–centrifuged at 3000 rpm for 10 minutes using and the sera were separated, divided into aliquots and stored at  $-80^{\circ}$ C till biochemical measurements could be carried out as fast as possible.

# **Biochemical determinations**

Blood glucose level was determined using GlucoDr SUPER SENSOR AGM-2200, a glucometer through a sample obtained from the lateral tail vein using sterile surgical scissors. Serum cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol levels were estimated spectrophotometrically using reagent kits obtained from Biodiagnostic, Dokki, Giza, Egypt.

#### Insulin determinations

Using the technique (Dynatech Microplate Reader Model MR 5000), Insulin level concentrations were measured using rats' reagent EL ISA Kits purchased from SinoGeneClon Biotech Co, Hang Zhou, China.

#### Statistical analysis

The obtained data were subjected to ANOVA followed by Duncan multiple post hoc test at the of  $p \le 0.05$  according to **Steel and Torrie** (**1980**) using a statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

### **Ethical statement**

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

# **Results and discussion**

#### Effect of sweet basil extract on glucose and insulin level

The *in vivo* results exhibited a significant decrease in insulin the level coupled with a significant increase in blood glucose in diabetic group compared with the control group. Interestingly, Developer of diabetic rats with sweet basil extract improved insulin and

glucose levels towards normal values as it significantly increased insulin and significantly decreased the glucose level compared to diabetic rats (Table 1).

The results of this study are in line with the research of Mohammed (2007) who used basil species in Africa (Ocimmum basilicum) to test the antidiabetic effects of basil leaves in streptozocin-induced Developer star rats. The results of the study stated that at the 2nd hour of observation, there was a decrease in the group given the extract, but the increase in blood glucose level occurred after the hour of treatment. The group was given basil leaf KgBW was reported to have the most /extract at a dose of 500 mg effective effect with the presentation of glycemic changes of 81.3% for 8 hours of observation. While at doses of 250 mg /kgBW and 1000 mg / kg BW did not show a significant change delete. Basil leaf extract at a dose of 400 mg / kg can increase antidiabetic effects and inhibit hepatic glucose mobilization and metabolism of carbohydrate enzymes for 2 hours of treatment *(Ezeani et al., 2017)*. In addition, basil leaf extract can also increase insulin secretion from the pancreas within 70 minutes of observation (Hannan et al., 2006). Active compounds such as flavonoids have an activity in increasing insulin secretion by increasing the entry of Ca<sup>2+</sup> ions through Ca channels (*Malapermal et al., 2017*). Saponin compounds work by space between words in the intestine by decreasing glucose absorption and modifying carbohydrate metabolism, increasing glucose utilization in peripheral tissues, glycogen storage and increasing sensitivity of insulin receptors in tissues. Tannin also acts as an antihyperglycemic with a mechanism to inhibit intestinal glucose uptake and inhibits adipogenesis and antioxidants s its ability to regenerate

damaged pancreatic  $\beta$  cells (*Suarsana, 2009*). Basil leaf extract has antihyperglycemic activity caused by its phenolic components contained in it which play a role in lowering blood glucose levels (*Kumari and Jain, 2012*).

# Table 1. Effects of Sweet Basile extract on glucose andinsulin Level in Rats

|                 | Control   | OAE        | STZ                   | STZ+ OAE               |
|-----------------|-----------|------------|-----------------------|------------------------|
| Glucose (mg/dl) | 103.1±2.6 | 101.8±1.9  | 502±79.3*             | 195±4.2 <sup>#</sup>   |
| Insulin (ng/ml) | 2.22±0.18 | 2.27± 0.19 | $0.186 \pm 0.008^{*}$ | 2.01±0.03 <sup>#</sup> |

Data are presented as mean  $\pm$  standard error. \* is significantly different from the control group, while # is significantly different from group (STZ) (p $\leq$  0.05) using one way ANOVA. ; OAE: *Ocimum basilicum* extract; STZ: Streptozotocin.

# Effect of sweet basil extract on serum lipids profile in rats

As illustrated in Table (2) and compared to the healthy control group, administration of healthy rats with Sweet Basile extract didn't deteriorate the lipid profile, while intoxication with STZ led to an atherosclerotic initiation; this was achieved from the significant rise of serum total cholesterol, triglycerides and LDL–cholesterol coupled with the marked reduction in HDL–cholesterol. In contrast, post–treatment of diabetic rats with Sweet Basile extract improved all lipids profile parameters.

In the present study, the streptozotocin induced diabetic rats showed significantly higher levels of blood glucose and lower insulin levels compared to normal control rats. These findings are consistent with the recent studies *(Cheng et al., 2019 and Seedevi et* 

*al.,2020).* Diabetes mellitus is a complex metabolic disease caused by impairment of insulin signaling, pathways, and the defect usually results from pancreatic  $\beta$ -cell deficiency and/or a deficiency of insulin

# (Kahn, 2012).

This disease causes many chronic complications such as vascular disease, retinopathy, neuropathy, kidney disease and heart disease. Cardiovascular is associated with profound alteration in the serum lipid and lipoprotein profile with an increased risk of coronary heart disease. Hyperlipidemia is a recognized complication of Diabetes mellitus characterized by elevated levels of cholesterol, triglycerides and phospholipids; and changes in lipoprotein composition *(Saji et al 2019 and Unuofin et al., 2020)*.

Diabetes mellitus is one of the oxidative stress conditions in which free radicals are increased and/or antioxidant mechanisms are inhibited *(Vadivelan et al., 2019)*. Free radicals induce oxidative stress and can lead to injury of the cellular membrane (Urmila et al., 2003). Free radical formation has been reported to be a direct consequence of hyperglycemia *(Tradit et al., 2019)*.

Flavonoids and beta carotene are known to decrease cholesterol levels, triglycerides, LDL and increase HDL levels because they can inhibit 3– Hydroxy–3–methylglutaryl Coenzyme A (HMG– CoA) reductase which functions as a catalyst in the formation of cholesterol. The inhibition of 3– Hydroxy–3 –methylglutaryl Coenzyme A (HMG–CoA) reductase results in cholesterol synthesis, triglycerides

and LDL being slow which results in decreased cholesterol, triglyceride and VLDL formation processes. Flavonoids can also increase the activity of Lechitin Cholesterol Acyl Transferase (LCAT). LCAT is an enzyme that can convert free cholesterol to a more hydrophobic cholesterol ester, so that cholesterol ester can bind to lipoprotein nucleus particles to form new HDL, which will increase serum HDL levels.

Other compounds found in basil extract such as tannins can reduce cholesterol and LDL levels by increasing cholesterol metabolism into bile acids and increase excretion of bile acids through feces *(Shinde et al., 2013)*. Leaves of *Ocimum basilicum* are rich in essential oils; eugenol has been shown to possess significant antioxidant properties leading to inhibition of lipid peroxidation and hypocholesterolemia *(Hurvey and Ferrier. 2011)*. *Ocimum basilicum* treatment decreases lipid peroxidation and increases reduced glutathione content in the blood. *Ocimum basilicum* oil, since contains both linoleic and linolenic acid, could be considered as a drying oil and is expected to behave similarly; accordingly, *Ocimum basilicum* may absorb the oxygen and get itself preferentially oxidized or metabolized thereby inhibiting the oxidation or metabolism of cholesterol *(Singh et al., 2007)*.

# Table 2. Effect of sweet basil extract on serum lipidsprofile in rats

| Control | OAE | STZ | STZ+ OAE |
|---------|-----|-----|----------|
|         |     |     |          |

| СНО         | 146.9±9.09 | 144.05   | 288.22±18.59*      | 176.1±11.16 <sup>#</sup>       |
|-------------|------------|----------|--------------------|--------------------------------|
| (mg/dl)     |            | ±9.86    |                    |                                |
| TRG (mg/dl) | 83.4±21.6  | 83.24    | 195.13±15.91*      | 118.4±7.1 <sup>#</sup>         |
|             |            | ±6.92    |                    |                                |
| LDL-C       | 22 0+1 76  | 33.82    | <u>26 8±1 81</u> * | 22 8±0 5 <i>4</i> <sup>#</sup> |
| (mg/dl)     | 32.9±1.70  | ±0.89    | 20.8±1.81          | 55.6±0.54                      |
| HDL-C       | 66.9±1.23  | 66 ±6.72 | 109.8±5.2*         | 74.9±0.52 <sup>#</sup>         |
| (mg/dl)     |            |          |                    |                                |
|             |            |          |                    |                                |

Data are presented as mean  $\pm$  standard error. \* is significantly different from the control group, while # is significantly different from group (STZ) (p $\leq$  0.05) using one way ANOVA. ; OAE: *Ocimum basilicum* extract; STZ: Streptozotocin.

The yield, total phenolic content (TPC), radical scavenging and activity (RSA) of the *Ocimum basilicum* aqueous extract (OAE) are shown in table (3).

Phenolic compounds are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may contribute directly to their antioxidant action (Javanmardi et al., 2003). Several investigations of the antioxidant activity of plant extracts have confirmed a correlation between total phenolic content and antioxidant activity (Ahmed et al., 2019).

# Table (3): Yield (%), total phenolic content (mg equivalent/ g) and radical scavenging activity (%) of dry

# powdered Ocimum basilicum extract

| Parameter<br>Extract     | Yield (%) | TPC (mg<br>equivalent/ g) | RSA (%)  |
|--------------------------|-----------|---------------------------|----------|
| Ocimum basilicum extract | 10.3±0.12 | 12.2±0.54                 | 59.2±6.1 |
| <b>A</b> 11 1 ( )        |           |                           | 2        |

All values are represented as means  $\pm$  standard error for 3 measurements (M  $\pm$  SE); TPC (total phenolic content, RSA (radical scavenging activity).

In conclusion, the findings from this study revealed that oral treatment with Sweet Basil extract could help improve glycemic status in rats with STZ induced diabetic rats. However, further studies are needed to investigate and elucidate the possible mechanism of action of the active ingredients and evaluate the potential value of the OAE for the management of diabetes and hyperlipidemic. This may be helpful in developing new drugs from OAE for the management of diabetes and hyperlipidemia.

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تأثير مستخلص الريحان على مستوي الجلوكوز ودهون الدم في فئران التجارب

مستخلص

تتجه المنتجات الطبيعية من مصادر مختلفة إلى أن تكون مرشحة محتملة لاكتشاف الأدوية. ويعرف الريحان الحلو بأنه نبات طبي وعشبي بسبب ما يحتويه من مواد كيميائية. ولذلك، كان الهدف من هذه الدراسة هو تقييم تأثير الريحان الحلو على الجرذان المصابة بمرض السكري وارتفاع دهون الدم. تضمنت الدراسة اثنان وثلاثون من ذكور فئران التجارب البيضاء البالغة والتي تزن حوالي (١٥٠\_١٩٠٢مم)، وتم تقسيمها بشكل عشوائي إلي أربعه محموعات (٨ فئران لكل مجموعه). أصيبت الفئران بارتفاع السكر ودهون الدم عن طريق مجموعات (٨ فئران لكل مجموعه). أصيبت الفئران بارتفاع السكر ودهون الدم عن طريق محموعات (٨ فئران لكل مجموعه). أصيبت الفئران بارتفاع السكر ودهون الدم عن طريق مجموعات (٨ فئران لكل مجموعه). أصيبت الفئران بارتفاع السكر ودهون الدم عن طريق دقعها في البطن بجرعة ٥٥ ملجم /كجم استريتوزيتوسن (STZ). المجموعة الأولى: دهون الدم ، تتغذى على الوجبة الغذائية القياسية للفئران مع مستخلص الريحان الحلو بنسبة المجموعة الثالثة: مجموعة مصابة بمرض السكر وارتفاع دهون الحلو بنسبة الدم ، تتغذى على الوجبة الغذائية القياسية للفئران وكانت بمرض السكر وارتفاع دهون الحلو بنسبة المجموعة الثالثة: مجموعة مصابة بمرض السكر وارتفاع دهون الدم ، تتغذى على الوجبة الغائية القياسية للفئران مع مستخلص الريحان الحلو بنسبة المجموعة الثالثة: مجموعة مصابة بمرض السكر وارتفاع دهون الدم ، تتغذى على الوجبة الغذائية القياسية للفئران مع مستخلص الريحان الحلو بنسبة الدم ، تتغذى على الوجبة الغذائية القياسية للفئران وكانت بمثابة مجموعة ضابطة موجبة؛

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المجموعة الرابعة: مجموعة مصابة بمرض السكر وارتفاع دهون الدم والمعالجة بمستخلص الريحان الحلو (٣٠٠ ملجم/كجم/يوم). بعد ستة أسابيع، أظهرت معالجة الحيوانات المصابة بداء السكري مع مستخلص الريحان الحلو (مجموعه ٤) انخفاضًا ملحوظًا في الجلوكوز والكوليسترول والدهون الثلاثية والبروتين الدهني منخفض الكثافة (LDL) يقابله ارتفاع كبير في قيم الأنسولين والبروتين الدهني المرتفع الكثافة (HDL) مقارنة بالمجموعة الضابطة. في الختام، يُظهر مستخلص الريحان الحلو فوائد صحية متعددة مع إمكانات واعدة ضد مرض والنكام، فإن نتائج الدراسة تساهم في التوصيف المحتمل وتطوير عامل علاجي جديد مضاد لمرض السكري وارتفاع دهون الدم

الكلمات المفتاحية: الريحان الحلو ، دهون الدم ، السكري، الفئران.