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# Research Article
# Two Cultivars of *Ocimum basilicum* Leaves Extracts Attenuate Streptozotocin-mediated Oxidative Stress in Diabetic Rats

Lyutha Al-Subhi and Mostafa Ibrahim Waly

Department of Food Science and Nutrition, College of Agricultural and Marine Sciences, Sultan Qaboos University, Muscat, Oman

## Abstract

**Background and Objective:** Leaves of basil plant (*Ocimum basilicum*) are often used for medicinal purposes because of their bioactive constituents, yet the antioxidant properties of this plant are not fully studied in the field of diabetes. The present study investigated the antioxidant property and anti-diabetic effect of two basil cultivars of *Ocimum basilicum,* 'Italian Genovese' and 'Thyrsiflora', in a Streptozotocin (STZ) rat model of diabetes . **Materials and Methods:** Sixty adult Sprague Dawley rats (n = 10/group) were divided into 6 groups: Three non-diabetic and three diabetic groups that either did not receive any supplementation or were supplemented with the leaves extract of one or the other cultivar. After 13 weeks of feeding, all rats were sacrificed, pancreatic tissues were homogenized and used for evaluating oxidative DNA damage and dichlorofluorescein fluorescence (DCF) assay. Blood was collected for the measurements of glucose and insulin. **Results:** The STZ caused oxidative stress in the diabetic group as evidenced by an increase in oxidative DNA damage and also caused DCF production in pancreatic tissues as compared to non-diabetic groups, ($p<0.05$). The STZ treatment resulted in hyperglycemia and low serum insulin level in diabetic rats. Supplementation with extracts of 'Italian Genovese' and 'Thyrsiflora' to the diabetic groups significantly abrogated the STZ-mediated effects ($p<0.05$). **Conclusion:** The results indicated that the extracts from the leaves of the two examined basil cultivars act as potent antioxidants and combat the STZ-mediated diabetogenic effect.

Key words: Diabetes, *Ocimum basilicum*, oxidative stress, streptozotocin, basil cultivars, diabetogenic effect

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Corresponding Author: Mostafa Ibrahim Waly, Department of Food Science and Nutrition, College of Agricultural and Marine Sciences, Sultan Qaboos University, Muscat, Oman Tel: 00968-2414-1229



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Diabetes mellitus is a life-threatening disease that affects millions of people worldwide and its comorbidity with other diseases is considered a global health problem[1]. In experimental animal's models, oxidative stress mediates the pathogenesis of diabetes and hyperglycemia via different mechanisms including the oxidative damage of pancreatic cells[2]. It has been revealed that the development of oxidative stress is largely associated with low intake of dietary antioxidants. Hence, the use of natural plants extracts with antioxidant potential properties could have an added protective benefit[3]. Several experimental studies have investigated the anti-diabetic effect of different basil cultivars; however, the mechanism elucidating their protective effect in combating oxidative stress-associated diabetes mellitus was not well documented[4]. A variety of basil plant species have a long history of their use as medicinal herbs due to their flavonoids and polyphenols content[5]. Basil leaves were extensively used to treat diabetes in traditional medicine and the animal models have previously shown its antidiabetic effects, but without specifying its antioxidant effect on hyperglycemia and insulin secretion[6]. The findings from a randomized placebo-controlled trial of basil leaves have suggested that basil leaves might be prescribed as adjunct to dietary therapy and drug treatment in patients with mild to moderate non-insulin-dependent diabetes mellitus[7]. Numerous laboratory studies have shown the efficacy of Streptozotocin (STZ) drug in inducing diabetes like condition in rodents. The STZ is an oxidizing insult that selectively induces oxidative stress in pancreatic cells with a consequent hyperglycaemia[2,8]. The present study was carried out to investigate the profound antioxidant property and anti-diabetic effect of two basil cultivars of *Ocimum basilicum* (*O. basilicum*) 'Italian Genovese' and 'Thyrsiflora', in a rat model of diabetes.

## MATERIALS AND METHODS

**Study area:** The study was carried out at the Small Animal House and the Department of Food Science and Nutrition, College of Agricultural and Marine Sciences, Sultan Qaboos University, Oman, from January-December, 2019.

**Preparation of basil leaves aqueous extract:** Two basil cultivars of *O. basilicum* 'Italian Genovese' and 'Thyrsiflora' were purchased from the local market at Muscat city, Sultanate of Oman and identified by two expert botanists at Sultan Qaboos University and Oman Botanic Garden. The first is consistently imported to the local food chain outlets and thus was obtained from a supermarket. The second was obtained from a plant nursery and is wildly grown as an ornamental plant in houses where it flourishes and commonly known to be a local basil plant. Both cultivars were obtained at end of June, 2018 (40-45 days old with a heigh range of 20-26 cm). The plant height was measured from the base (top of the soil) to the heights point of the stem. Leaves from each plant were manually separated from the stems, frozen at -40°C for 12 h and then freeze dried for 4 days at 22°C using 200 Pa (Edwards Freeze Drier, UK). The dried basil leaves were ground to powder by an electric grinder (Moulinex AR1043-UK0) and the powder (300 g) was infused for 30 min in 200 mL of distilled water at 100°C followed by filtration. The solution obtained was concentrated (50 g powders per 300 g of fresh basil leaves) in a rotary evaporator under a vacuum at 65°C. The resulting crude extract (50 g dry solids) was suspended in 100 mL sterile distilled water and stored at -20°C till use for subsequent experiments.

**Total polyphenols and flavonoids contents of basil leave extracts:** Total polyphenols content of the two basil leaves extracts were determined according to the Folin–Ciocalteu method as previously reported and was expressed as mg Gallic Acid Equivalents per 100 g dry solids[9]. Flavonoids content was determined as follows: 4 mL distilled water was added to 1 mL of each basil leaves extract. Then, 5% $NaNO_2$ (0.3 mL) was added followed by addition of 10% $AlCl_3$ (0.3 mL). The mixtures were incubated at ambient temperature for 5 min and then 2 mL of NaOH (1 N) was added. Immediately, the mixture volume was topped to 10 mL with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink color was determined at 510 nm. A standard curve was developed by using Catechin as standard. The results were expressed as mg Catechin Equivalents per 100 g dry solids[10].

**Evaluation of the free radical scavenging capacity of basil leaves extracts:** The capacity of each one of the two basil leaves extract to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was performed by a spectrophotometric methodology as previously described[11]. Briefly, 0.5 mL aliquots of basil leave extracts at different concentrations (10-300 mM) were mixed with stable DPPH radical in methanolic solution (0.3 mL of DPPH radical solution 0.5 mM in 3 mL ethanol). When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from

deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 30 min of reaction using a UV-Vis spectrophotometer (DU 800, Beckman Coulter, Fullerton, CA, USA). Controls contained all the reaction reagents except basil leaves extracts or 2,6-di-tert-butyl-4-hydroxytoluene (BHT) and the positive control. The free radical scavenging capacity of different samples were expressed as DPPH inhibition (%), a higher free radical scavenging activity (%) value indicates a higher antioxidant activity and it was calculated as follows[8]:

$$\text{DPPH inhibition (\%)} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

**Experimental animals design:** Sixty male adult Sprague-Dawley rats (200±5 g) were randomly divided into 6 groups (n = 10), three groups as control (non-diabetic) and three as diabetic groups: Non-diabetic rats; non-diabetic rats that received oral extract of 'Italian Genovese' non-diabetic rats that received oral extract of 'Thyrsiflora', STZ-induced diabetic rats, diabetic rats that received oral extract of 'Italian Genovese', diabetic rats that received oral extract of 'Thyrsiflora'. All rats were housed in individual polypropylene cages provided with both, a standard laboratory chow diet (from Oman Mills, Muscat, Oman) and normal tap water *ad libitum*, under standard conditions of temperature (22±2°C), humidity (60%) and 12 h light:dark cycle. This study was conducted under the approval of the Animal Ethics Committee of Sultan Qaboos University (SQU) in accordance to International Laws and Policies (EEC Council directives 86/609, OJL 358, 1 December, 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

The aqueous extracts of the two cultivars of *O. basilicum* 'Italian Genovese' and 'Thyrsiflora' were administrated to the pertinent rats through oral gavage at a dose of 0.5 mg dry solids extract/1 mL water/day. The extracts were given on a daily basis at similar timing, early morning, throughout the 13 weeks of the study period. The used effective dose for the two basil cultivars was based on preliminary screening experiments conducted at the Food Science and Nutrition Department, College of Agricultural and Marine Sciences, SQU.

**Diabetes induction and blood glucose levels:** Diabetes mellitus was induced in the diabetic groups by a single intraperitoneal injection of freshly prepared STZ (Sigma-Aldrich Co., St. Louis, MO, USA) at a dose of 50 mg $kg^{-1}$ b.wt., dissolved in 0.01 M citrate buffer, pH 4.5 as previously reported[2,8]. The non-diabetic rats received a single intraperitoneal injection of 0.1 M citrate buffer solution. After 72 h of the STZ injection, the blood glucose levels were measured in all rats using a portable glucose meter (LifeScan Inc., Milpitas, CA, USA). Briefly, the distal part of the tail was gently snipped, the first blood drop was discarded and the second was absorbed by a test strip inserted in the glucose meter. The glucose level for the non-diabetic rats was ≤5 mmol $L^{-1}$, meanwhile the rats with fasting blood glucose levels higher than 5 mmol $L^{-1}$ were selected for the diabetic groups. Once the study groups were confirmed (diabetic vs. non-diabetics), blood glucose was measured twice a week in all rats throughout the 13 week feeding trial. The blood glucose measurements were always conducted around 8 AM.

**Animal sacrifice, serum separation and tissue homogenates:** At the end of the 13 weeks treatment, all rats were anesthetized with a lethal dose of a cocktail containing ketamine (1 mg), xylazine (5 mg) and acepromazine (0.2 mg). Blood was collected from the heart and the serum was separated by centrifugation and used later for biochemical measurements of glucose by using an automated clinical chemistry analyzer (Olympus AU400 analyzer, Tokyo, Japan) and insulin which was measured by the enzyme linked immunosorbent assay (ELISA) based technique (RAB0327 1KT, Sigma). The pancreas tissue of each rat (~50 mg) was immediately homogenized in 5 mL of 100 mM potassium phosphate buffer (pH 7.2) in a glass Teflon homogenizer with an ice cold jacket and centrifuged at 10,000×g at 4°C for 60 min. The homogenate was then used for determining the levels of protein content, free radical production and DNA oxidative damage.

**Analysis of protein content:** The Lowry's method was used in measuring the protein content of pancreas tissue homogenates and the protein content was expressed as mg $mL^{-1}$ of sample[12].

**DNA oxidative damage using 8-oxo-7,8-dihydro-20-deoxyguanosine (8-oxodGuo) assay:** The DNA was isolated from the pancreatic tissues homogenates and the DNA oxidative damage was measured by using 8-oxo-7,8-dihydro-20-deoxyguanosine (8-oxodGuo) assay as described earlier[2]. The pancreatic tissues were homogenized in 50 mM Phosphate Buffer Solution (PBS) containing 0.1 M dithiothreitol and then centrifuged at 4°C for 20 min at 2000 g. These pellets were used for 8-oxo-7,8-dihydro-20-deoxyguanosine (8-oxodGuo). The pancreatic pellets were re-suspended the DNA was isolated and the purified DNA (about 50 mg) was hydrolyzed with P1 nuclease (10 IU) and alkaline phosphatase (7 IU). The hydrolyzed mixture was filtered by using Micropure-EZ enzyme remover (Amicon, MA, USA) and 50 mL

was injected into High Pressure Liquid Chromatography with Electrochemical Detection (HPLC-ED). The nucleosides were separated by a C18 reverse-phase column (Supelco, 5 mm, I.D. 0.46×25 cm). The 8-oxodGuo and 2dG in the DNA were detected by using an ESA Coulochem II electrochemical detector in line with a UV detector.

**Dichlorofluorescein fluorescence assay:** The dichlorofluorescein fluorescence (DCF) assay was used to measure cellular peroxide production and other reactive species. Aliquots of pancreatic homogenates were added to a medium containing Tris-HCl buffer (0.01 mM, pH 7.4) and dichlorofluorescein diacetate (7 μM). The medium was incubated in dark for 1 h until the fluorescence measurement (excitation at 488 nm and emission at 525 nm, with both slit widths at 1.5 nm). Oxidized DCF was determined by using a standard curve of oxidized DCF and results were expressed as μmol of oxidized DCF/mg protein.

**Statistical analysis:** Statistical analysis was performed by using GraphPad Prism (version 5.03; GraphPad Software Inc. San Diego, CA). The results are expressed as Means±Standard Deviation (SD). The statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's test and a p-value of less than 0.05 was considered significant.

## RESULTS

**Polyphenols and flavonoids measurements:** The total polyphenol contents of 'Italian Genovese' and 'Thyrsiflora' leaves extracts were 128±6 and 245±11 mg Gallic Acid Equivalents/g dry solids, respectively. A similar difference in the contents of flavonoids was observed for both 'Italian Genovese' and 'Thyrsiflora' leaves extracts (95±2 and 314±9 mg Catechin Equivalents /g dry solids, respectively).

**DPPH measurements:** The 'Italian Genovese' and 'Thyrsiflora' leaves extracts inhibited DPPH formation in a dose-dependent manner (10-300 μM) as compared to BHT standard and it was observed that 'Thyrsiflora' leaves extract exhibited a protective effect at a higher rate compared the 'Italian Genovese' leaves extract, although there was no significant difference (p = 0.214) (Fig. 1).

**Body weight gain of animals:** As shown in Fig. 2, the body weight for each rat was recorded weekly for the whole duration of the experiment. The initial body weight increased gradually throughout the experimental period for all the groups. There was no significant difference in the body weight among all the groups (p = 0.525). However, all rats in the diabetes group showed a consistent decrease in body weight throughout week 5 to week 13 as compared to negative control groups (p<0.05). However, the diabetic rats supplemented with either 'Italian Genovese' or 'Thyrsiflora' leaves extracts showed an improvement in compensating the weight loss due to diabetes induction (p<0.05).

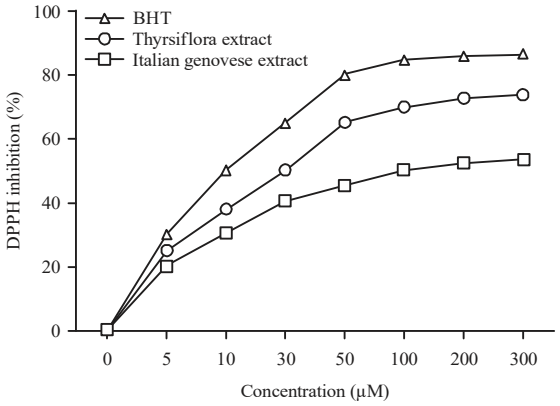


Fig. 1: Scavenging effect of "Italian Genovese" and "Thyrsiflora" leaves extracts and 2,6-di-tert-butyl-4-hydroxytoluene (BHT) against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical formation
Results are Mean±SD of six measurements

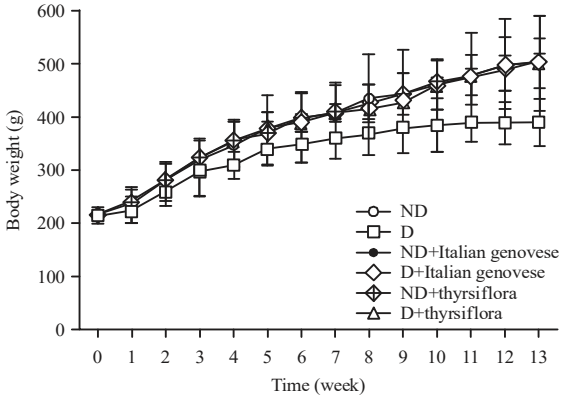


Fig. 2: Effects of "Italian Genovese" and "Thyrsiflora" leaves extracts on body weight of the non-diabetic and diabetic groups
ND: Non-diabetic rats that received chow diet, D: STZ-induced diabetic rats, ND+Italian Genovese: Non-diabetic rats that received chow diet plus oral supplementation of Italian Genovese leaves extract, D+Italian Genovese: Diabetic rats that received chow diet plus oral supplementation of Italian Genovese leaves extract, ND+Thyrsiflora: Non-diabetic rats that received chow diet plus oral supplementation of thyrsiflora leaves extract, D+Thyrsiflora: Diabetic rats that received chow diet plus oral supplementation of thyrsiflora leaves extract

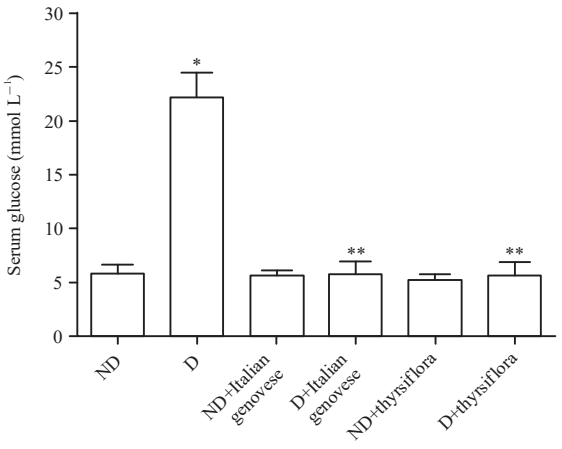


Fig. 3: Effects of "Italian Genovese" and "Thyrsiflora" leaves extracts on blood glucose measurements

ND: Non-diabetic rats that received chow diet, D: STZ-induced diabetic rats, ND+Italian Genovese: non-diabetic rats that received chow diet plus oral supplementation of Italian Genovese leaves extract, D+Italian Genovese: Diabetic rats that received chow diet plus oral supplementation of Italian genovese leaves extract, ND+Thyrsiflora: Non-diabetic rats that received chow diet plus oral supplementation of thyrsiflora leaves extract, D+Thyrsiflora: Diabetic rats that received chow diet plus oral supplementation of thyrsiflora leaves extract, Results are Mean±SD, *Significantly higher than the ND group, (p<0.05), **Significantly lower than the D group, (p<0.05)

**Effects of STZ and basil leaves extracts on serum glucose and insulin levels:** STZ-induced diabetic rats have significantly higher levels of serum glucose as compared to non-diabetic rats p<0.05 (Fig. 3). Nevertheless, the STZ-induced hyperglycemia was suppressed by 'Italian Genovese' and 'Thyrsiflora' leaves extracts supplementation as they had a hypoglycemic effect which was significantly different from the control STZ-induced diabetic group (p<0.05). There were no significant differences in glucose levels among all non-diabetic groups (p>0.05).

As presented in Fig. 4, 'Italian Genovese' or 'Thyrsiflora' leaves extracts restored the STZ induced insulin deficiency to levels that are comparable to the non-diabetic group (p<0.0001). There was no significant difference in serum insulin levels between non-diabetic groups either in the presence or absence of 'Italian Genovese' and 'Thyrsiflora' leaves extracts supplementation (p>0.05).

**Effects of STZ and basil leaves extracts on oxidative stress:** The effects of basil leave extracts on pancreatic DNA oxidative damage are illustrated in Fig. 5. The STZ-induced diabetic groups showed a significant DNA damage as compared to non-diabetic group (p<0.05). Administration of 'Italian

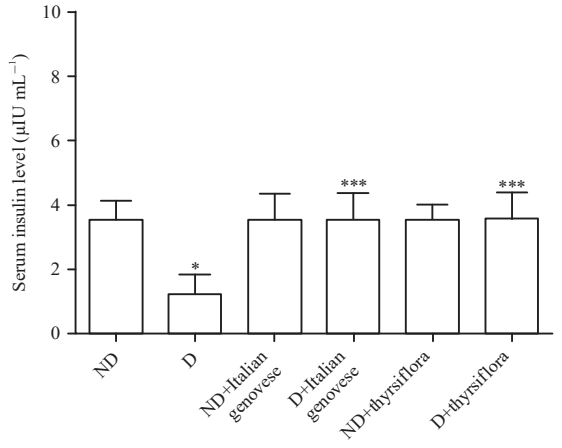


Fig. 4: Effects of "Italian Genovese" and "Thyrsiflora" leaves extracts on serum insulin levels

ND: Non-diabetic rats that received chow diet, D: STZ-induced diabetic rats, ND+Italian Genovese: Non-diabetic rats that received chow diet plus oral supplementation of Italian genovese leaves extract, D+Italian Genovese: Diabetic rats that received chow diet plus oral supplementation of Italian genovese leaves extract, ND+Thyrsiflora: Non-diabetic rats that received chow diet plus oral supplementation of thyrsiflora leaves extract, D+Thyrsiflora: Diabetic rats that received chow diet plus oral supplementation of thyrsiflora leaves extract, Results are Mean±SD, *Significantly lower than the ND group (p<0.05), ***Significantly higher than D group (p<0.0001)

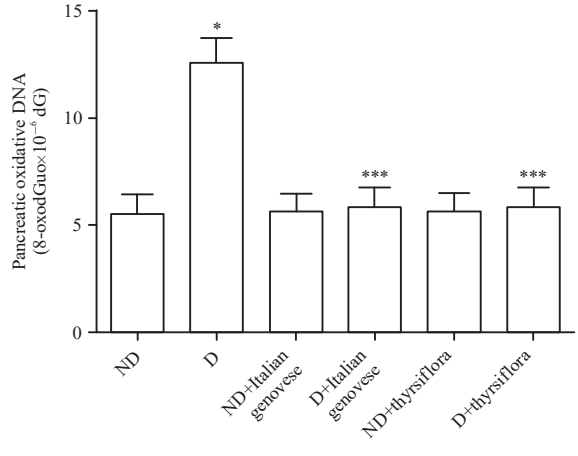


Fig. 5: Effects of "Italian Genovese" and "Thyrsiflora" leaves extracts on pancreatic DNA oxidative damage

ND: Non-diabetic rats that received chow diet, D: STZ-induced diabetic rats, ND+Italian Genovese: Non-diabetic rats that received chow diet plus oral feeding of Italian genovese leaves extract, D+Italian genovese: Diabetic rats that received chow diet plus oral feeding of Italian genovese leaves extract, ND+Thyrsiflora: Non-diabetic rats that received chow diet plus oral feeding of thyrsiflora leaves extract, D+Thyrsiflora: Diabetic rats that received chow diet plus oral feeding of thyrsiflora leaves extract, Results are Mean±SD, *Significantly higher than the ND group (p<0.05), ***Significantly lower than D group (p<0.0001)

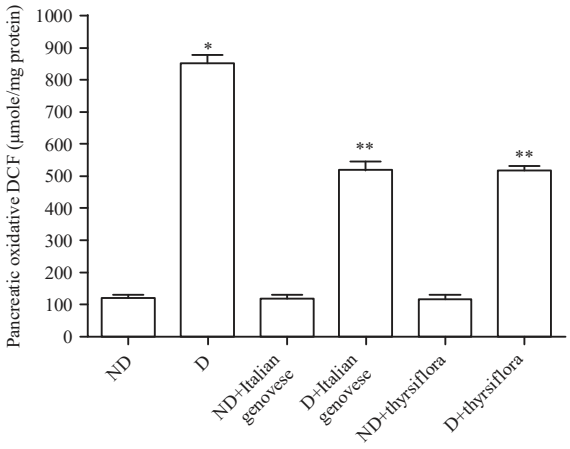


Fig. 6: Effects of "Italian Genovese" and "Thyrsiflora" leaves extracts on pancreatic oxidized dichlorofluorescein fluorescence (DCF) formation

ND: Non-diabetic rats that received chow diet, D: STZ-induced diabetic rats, ND+Italian Genovese: Non-diabetic rats that received chow diet plus oral supplementation of Italian genovese leaves extract, D+Italian Genovese: Diabetic rats that received chow diet plus oral supplementation of Italian genovese leaves extract, ND+Thyrsiflora: Non-diabetic rats that received chow diet plus oral supplementation of thyrsiflora leaves extract, D+Thyrsiflora: Diabetic rats that received chow diet plus oral supplementation of thyrsiflora leaves extract, Results are Mean±SD, *Significantly higher than the ND group ($p<0.05$), **Significantly lower than the D group ($p<0.05$)

Genovese' or 'Thyrsiflora' leaves extracts to the diabetic groups significantly reduced the oxidative DNA damage ($p<0.0001$).

As shown in Fig. 6, the DCF assay was used to measure the oxidized DCF-protein and it was observed that diabetes induction showed a significant increase in oxidized DCF levels as compared to negative (non-diabetic) groups. Meanwhile, treatment with leaves extracts of 'Italian Genovese' and 'Thyrsiflora' prevented the formation of oxidized DCF proteins in the diabetic groups compared to the underrated diabetic group ($p<0.05$).

## DISCUSSION

Oxidative stress is a characteristic of diabetes, due to hyperglycemia and some degree of insulin deficiency that mediates tissue damage and the progression of complications[13,14]. The search for natural sources of hypoglycemic agents is an ongoing endeavor[15,16]. However, many health related positive findings of *O. basilicum* are lacking information about the specific cultivars of the plants[16-19]. Thus, the current study compared the potency of aqueous extracts of two cultivars of *O. basilicum,* 'Italian Genovese' and 'Thyrsiflora' to restore hyperglycemia, insulin deficiency and oxidative damage in STZ-induced diabetic rats.

Higher total polyphenols and flavonoids content were found in 'Thyrsiflora' (locally called "Omani basil") extract, than in 'Italian Genovese'. Such findings could be attributed to cultivation conditions as differences are reported for two other cultivars of *O. basilicum* ('German' and 'Mesten') in the USA[20] and for *O. basilicum* in Pakistan, cultivar not specified[21]. In our study, there was no control for any cultivation factors. Hence, standardizing cultivation conditions is needed to validate the observed differences and the effects. Also, it was only utilized for the leaves of the plants while different parts of basil have been used to extract bioactive compounds[22-24]. The drying and extraction methods could be sources of variation in polyphenols and flavonoids yields. While, some air dried the basil materials under shade[17,25] or used 30-40°C hot air oven[20,21], freeze drying was used in our samples. It was used aqueous extraction (infusion) process for it is a simple yet an effective procedure[26] and it resulted in the highest total phenols in samples of *O. tenuiflorum* compared to chloroform, ethyl acetate and methanol extracts[27].

The STZ caused an increase in the level of oxidative DNA damage in the diabetic groups, which is consistent with earlier findings[2,8]. This STZ mediated oxidative stress was abrogated by the aqueous extracts of "Italian Genovese" and "Thyrsiflora" that are rich in polyphenols (128±6 and 245±11 mg GAE/g dry-solids, respectively) and flavonoids (95±2 and 314±9 mg CE/g dry-solids, respectively). Comparable total polyphenols (146±5.26 mg Catechin/g dry extract) and lower flavonoids (41±2.2 rutin/g dry extract) are reported for aqueous extract of unknown cultivar of *O. basilicum* from Saudi Arabia[16]. It should note that qualification of polyphenols and flavonoids is benchmarked with different standards and to date the conversion between these standards is not clear. As a result, comparison among studies with variable standards is impractical.

A dose dependent inhibition of DPPH was observed with both cultivars; similar finding is reported in *O. basilicum* of unknown cultivar[16]. Comparison with the literature in this regards is limited by the limited studies on specific cultivars of *O. basilicum*. Studies on *O. basilicum* mostly reported on yield and composition of oils from various cultivars[28-30]. Only Beatović *et al.*[25] reported DPPH radical scavenging activity for oil extract of Genovese, while the current study used aqueous extract. Studies from Oman refereed to *O. basilicum* as "Omani basil"[28,29], without a botanic name, when there are a number of cultivars of *O. basilicum* in Oman[31].

The STZ-induced hyperglycemia and insulin deficiency were significantly improved by the two cultivars in our study. As a result, the treated diabetic rates significantly gained more weight compared to the control diabetic rats. Collectively, these findings signify improved cellular glucose uptake and are supported by studies on *O. basilicum* and *O. tenuiflorum*[17,27,32]. The mechanisms by which the extracts decreased blood glucose level may be related to *in vitro* findings as carbohydrate metabolizing enzymes, a-glucosidase and a-amylase were inhibited by aqueous leaves extract of *O. basilicum*[16,17]. Natural flavonoids[33] and phenolics[34] are reported to inhibit these enzymes and thus delay the rate of glucose absorption. Again, the cultivars of *O. basilicum* are not specified in these studies[16,17,32] which renders the comparison unpersuasive and therefore, a need for further research.

## CONCLUSION

It can be concluded that the observed effects of 'Italian Genovese' and 'Thyrsiflora' leaves extracts in the diabetic rats indicate their efficacy in combating the STZ-mediated oxidative damage in pancreatic tissue. However, further research is needed to identify the active constituents of the two cultivars.

## SIGNIFICANCE STATEMENT

Our observation opens new avenues toward the primary prevention of type 2 diabetes. The molecular mechanisms underlying the effect of basil leaves extracts in pancreatic cells under the effect of STZ treatment need to be further investigated.

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## REFERENCES

1. Waly, M.I., M.M. Essa, A. Ali, Y.M. Al-Shuaibi and Y.M. Al-Farsi, 2010. The global burden of type 2 diabetes: A review. Int. J. Biol. Med. Res., 1: 326-329.
2. Suresh, S., M.I. Waly, M.S. Rahman, N. Guizani and M.A.B. Al-Kindi *et al*., 2017. Broccoli (*Brassica oleracea*) reduces oxidative damage to pancreatic tissue and combats hyperglycaemia in diabetic rats. Prevent. Nutr. Food Sci., 22: 277-284.
3. Al-Maskari, M.Y., M.I. Waly, A. Ali, Y.S. Al-Shuaibi and A. Ouhtit, 2012. Folate and vitamin $B_{12}$ deficiency and hyperhomocysteinemia promote oxidative stress in adult type 2 diabetes. Nutrition, 28: e23-e26.
4. Aguiyi, J.C., E.I. Obi, S.S. Gang and A.C. Igweh, 2000. Hypoglycaemic activity of *Ocimum gratissimum* in rats. Fitoterapia, 71: 444-446.
5. Zheljazkov, V.D., A. Callahan and C.L. Cantrell, 2008. Yield and oil composition of 38 basil (*Ocimum basilicum* L.) accessions grown in Mississippi. J. Agric. Food Chem., 56: 241-245.
6. Pattanayak, P., P. Behera, D. Das and S.K. Panda, 2010. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Pharmacogn. Rev., 4: 95-105.
7. Agrawal, P., V. Rai and R.B. Singh, 1996. Randomized placebo-controlled, single blind trial of holy basil leaves in patients with noninsulin-dependent diabetes mellitus. Int. J. Clin. Pharmcol. Ther., 34: 406-409.
8. Waly, M.I., N. Guizani, S. Suresh and M.S. Rahman, 2015. Ginger extract attenuates preliminary steps of streptozotocin-mediated oxidative stress in diabetic rats. Int. J. Nutr. Pharmacol. Neurol. Dis., 5: 151-158.
9. Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult., 16: 144-158.
10. Zhishen, J., T. Mengcheng and W. Jianming, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555-559.
11. Lopez, V., S. Akerreta, E. Casanova, J.M. García-Mina, R.Y. Cavero and M.I. Calvo, 2007. *In vitro* antioxidant and anti-rhizopus activities of lamiaceae herbal extracts. Plant Foods Hum. Nutr., 62: 151-155.
12. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
13. Giacco, F. and M. Brownlee, 2010. Oxidative stress and diabetic complications. Circ. Res., 107: 1058-1070.
14. Wright, Jr.E., J.L. Scism-Bacon and L.C. Glass, 2006. Oxidative stress in type 2 diabetes: The role of fasting and postprandial glycaemia. Int. J. Clin. Pract., 60: 308-314.
15. Chattopadhyay, R.R., 1999. A comparative evaluation of some blood sugar lowering agents of plant origin. J. Ethnopharmacol., 67: 367-372.
16. El-Beshbishy, H.A. and S.A. Bahashwan, 2011. Hypoglycemic effect of basil (*Ocimum basilicum*) aqueous extract is mediated through inhibition of α-glucosidase and α-amylase activities: An *in vitro* study. Toxicol. Ind. Health, 28: 42-50.
17. Ezeani, C., I. Ezenyi, T. Okoye and C. Okoli, 2017. *Ocimum basilicum* extract exhibits antidiabetic effects via inhibition of hepatic glucose mobilization and carbohydrate metabolizing enzymes. J. Intercult. Ethnopharmacol., 6: 22-28.

18. Harnafi, H., M. Aziz and S. Amrani, 2009. Sweet basil (*Ocimum basilicum* L.) improves lipid metabolism in hypercholesterolemic rats. Eur. e-J. Clin. Nutr. Metab., 4: e181-e186.
19. Yacout, G.A., N.M. Elguindy and E.F. El Azab, 2012. Hepatoprotective effect of basil (*Ocimum basilicum* L.) on $CCl_4$-induced liver fibrosis in rats. Afr. J. Biotechnol., 11: 15702-15711.
20. Zheljazkov, V.D., C.L. Cantrell, W.B. Evans, M.W. Ebelhar and C. Coker, 2008. Yield and composition of *Ocinum basilicum* L. and *Ocimum sanctum* L. grown at four locations. HortScience, 43: 737-741.
21. Hussain, A.I., F. Anwar, S.T.H. Sherazi and R. Przybylski, 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem., 108: 986-995.
22. Patra, J.K., G. Das, S. Lee, S.S. Kang and H.S. Shin, 2018. Selected commercial plants: A review of extraction and isolation of bioactive compounds and their pharmacological market value. Trends Food Sci. Technol., 82: 89-109.
23. Prakash, P. and N. Gupta, 2005. Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with a note on eugenol and its pharmacological actions: A short review. Indian J. Physiol. Pharmacol., 49: 125-131.
24. Singh E., S. Sharma, J. Dwivedi and S. Sharma, 2012. Diversified potentials of *Ocimum sanctum* Linn (Tulsi): An exhaustive survey. J. Nat. Prod. Plant Resour., 2: 39-48.
25. Beatović, D., D. Krstić-Milošević, S. Trifunović, J. Šiljegović, J. Glamočlija, M. Ristić and S. Jelačić, 2015. Chemical Composition, antioxidant and antimicrobial activities of the essential oils of twelve *Ocimum basilicum* L. cultivars grown in Serbia. Rec. Nat. Prod., 9: 62-75.
26. Zhang, Q.W., L.G. Lin and W.C. Ye, 2018. Techniques for extraction and isolation of natural products: A comprehensive review. Chin. Med., Vol. 13. 10.1186/s13020-018-0177-x
27. Mousavi, L., R.M. Salleh, V. Murugaiyah and M.Z. Asmawi, 2016. Hypoglycemic and anti-hyperglycemic study of *Ocimum tenuiflorum* L. leaves extract in normal and streptozotocin-induced diabetic rats. Asian Pac. J. Trop. Biomed., 6: 1029-1036.
28. Al Abbasy, D.W., N. Pathare, J.N. Al-Sabahi and S.A. Khan, 2015. Chemical composition and antibacterial activity of essential oil isolated from Omani basil (*Ocimum basilicum* Linn.). Asian Pac. J. Trop. Dis., 5: 645-649.
29. Al-Maskri, A.Y., M.A. Hanif, M.Y. Al-Maskari, A.S. Abraham, J.N. Al-Sabahi and O. Al-Mantheri, 2011. Essential oil from *Ocimum basilicum* (Omani Basil): A desert crop. Nat. Prod. Commun., 6: 1487-1490.
30. Shiwakoti, S., O. Saleh, S. Poudyal, A. Barka, Y. Qian and V.D. Zheljazkov, 2017. Yield, composition and antioxidant capacity of the essential oil of sweet basil and holy basil as influenced by distillation methods. Chem. Biodivers., Vol. 14, No. 4. 10.1002/cbdv.201600417
31. Miller, A.G., M. Morris and S. Stuart-Smith, 1988. Plants of Dhofar: The Southern Region of Oman: Traditional, Economic and Medicinal Uses. Office of the Advisor for Conservation of the Environment, Diwan of Royal Court, Muscat, ISBN-13: 9780715708088, Pages: 361.
32. Mbaoji, F.N., C.O. Okoli and A.C. Ezike, 2014. Preliminary antihyperglycemic activity-guided studies on the leaf extract and fractions of *Ocimum basilicum* L. J. Chem. Pharm. Res., 6: 575-580.
33. Lo Piparo, E., H. Scheib, N. Frei, G. Williamson, M. Grigorov and C.J. Chou, 2008. Flavonoids for controlling starch digestion: Structural requirements for inhibiting human α-amylase. J. Med. Chem., 51: 3555-3561.
34. Asgar, A., 2013. Anti-diabetic potential of phenolic compounds: A review. Int. J. Food Proper., 16: 91-103.