

Evaluating the effects of *Prunus laurocerasus* seed, fruit and leaf extracts on hyperglycaemia, insulin sensitivity and anti-oxidative activities in experimental diabetes in rat

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Abstract

Although insulin resistance is widely accepted as one of the main characteristics of type 2 diabetes, there are studies suggesting that there is a link between the development of type 1 diabetes and insulin resistance in recent years. Therefore, in this study, it was aimed to investigate the effects of *Prunus laurocerasus* (PL) seeds, fruit and leaf extracts on hyperglycemia, adiponectin and irisin levels which are known to be effective in the mechanisms of insulin sensitivity, and oxidative stress in rats with type 1 diabetes. The study groups were as follows: normoglycaemic (NC), diabetic control (DC), diabetic + seed extract (D+S), diabetic + fruit extract (D+F), diabetic + leaf extract (D+L) and diabetic + insulin (D+I) groups. Fasting blood glucose levels showed significant decreases in the experimental groups starting from the 10th day compared to the DC group. HbA1c (except for D+L group), adiponectin and TNF- α levels decreased, whereas irisin levels increased in the experimental groups compared to the DC group. In addition, it was determined that MDA levels decreased while GSH levels increased in experimental groups compared to DC group. To conclude, especially the fruits and seeds extracts of PL plant have strong lowering hyperglycemia, insulin sensitivity and anti-oxidative effects.

Keywords: Hyperglycaemia, Insulin sensitivity, Oxidative stress, *Prunus laurocerasus*, Type I diabetes

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Introduction

Diabetes is one of the most common diseases worldwide (Shaw *et al.*, 2010) with a rapidly increasing prevalence. According to the International Diabetes Federation, there were 425 million diabetic people in the world in 2017, and this number is estimated to increase to 629 million (a 48% increase) by 2045. The global healthcare cost incurred for the treatment of diabetes and related complications was estimated at USD 727 billion in 2017 (IDF, 2017). Diabetes is a chronic metabolic disease associated with insulin secretion, insulin action or a combination of both and is diagnosed by the presence of hyperglycaemia (American Diabetes Association, 2004). Previously, type 1 diabetes has been widely accepted as a disorder in children and adolescents; In recent years, this opinion has changed and it has been stated that age of onset is not a limiting factor (Atkinson *et al.*, 2014). In addition, although insulin resistance is commonly associated with type 2 diabetes, it has been suggested in recent years that there may be a link between insulin resistance and the development of type 1 diabetes (Greenbaum, 2002; Kaul *et al.*, 2015). Type 1 diabetes is a chronic autoimmune disease in which T lymphocytes destroy insulin producing β cell in the pancreas (Baytop, 1963). In type 1 diabetes, cellular glucose uptake is prevented due to insulin deficiency, thus leading to hyperglycemia. This hyperglycemia, if left untreated, can lead to some complications and even death (Paschou *et al.*, 2018). Notably, reactive oxygen

species (ROS) are significantly increased in diabetes owing to prolonged hyperglycaemia, activation of polyol and hexosamine pathways and effects of cytokines (Vincent *et al.*, 2004). The imbalance between the excessive increase in ROS and body's defence mechanisms against antioxidants often leads to development of oxidative stress. Increased oxidative stress favourably supports the pathogenesis of several chronic complications of diabetes (Wolff, 1993). Moreover, hyperglycaemia negatively affects the whole body, including the liver, eyes, kidneys, nerves, heart and vessels (Paneni *et al.*, 2013).

The most common conditions associated with diabetes are the development of insulin resistance and deterioration of energy metabolism. Adiponectin, tumour necrosis factor- α (TNF- α) and irisin play a significant role in this condition. Adiponectin is a protein-structured hormone that is secreted from many tissues, especially fat and muscle tissues, and increases anti-inflammatory activity and insulin sensitivity (Yamauchi, 2003). TNF- α , a proinflammatory cytokine secreted from adipocytes due to insulin resistance, causes beta cell damage in islets of Langerhans (Spiegelman and Hotamisligil, 1993). Irisin is an essential hormone that is involved in energy metabolism as it increases energy consumption by converting white fat tissue into brown fat tissue (Stengel *et al.*, 2013). It is a proteolytic product of fibronectin type III domain 5 (FNDC5), which is secreted from many tissues, mainly skeletal muscle and fat tissues (Figure 1).

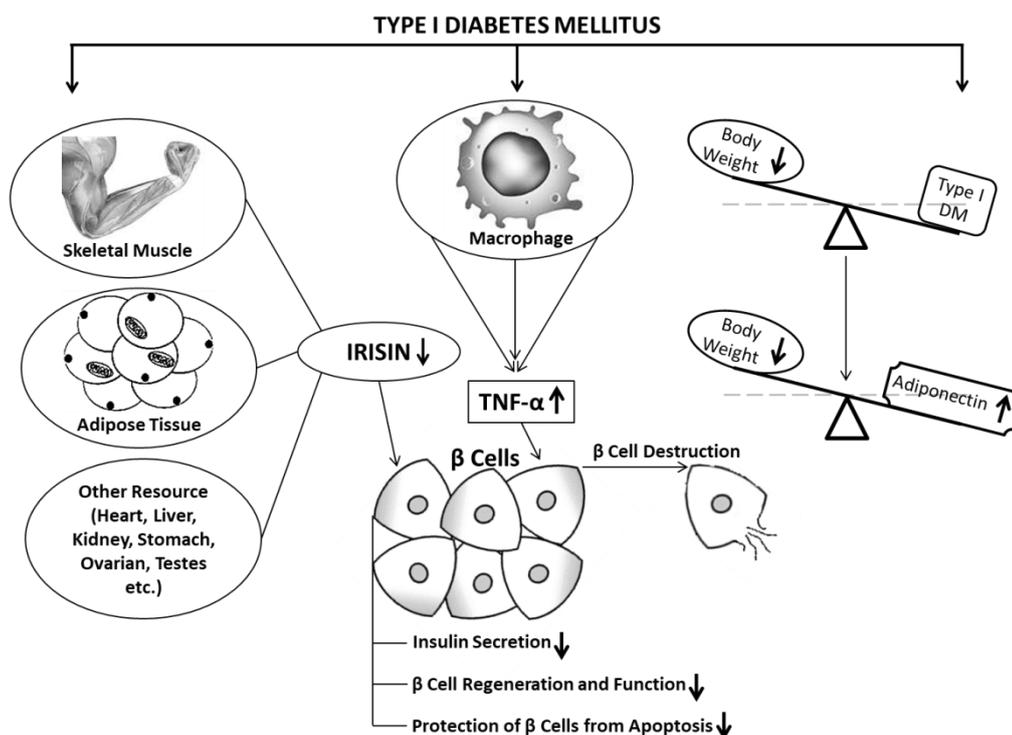


Figure 1 Effects of irisin, TNF- α and Adiponectin in type 1 diabetes. In type I diabetes, decreased insulin secretion from beta cells and increased beta cells destruction were occurred due to the reduction of irisin secretion from skeletal muscle, adipose tissue and some other tissues such as heart, liver and kidney, and the increase in TNF- α secretion from macrophages. In addition, in type 1 diabetes, the body weight decreases and the level of adiponectin increases accordingly.

Nowadays, the lack of a definitive treatment method for diabetes and the fact that existing treatment methods cause various complications steer researchers

to seek new treatment resources, such as *Prunus laurocerasus* L. (PL L.) (Turan *et al.*, 2013). PL L. (syn: *Laurocerasus officinalis* M. Roem) is a member of the

Rosaceae family and is commonly known as cherry laurel or wild cherry (Turan *et al.*, 2013). It is a perennial evergreen herb with white flowers and purplish-black berries. This plant is cultivated in the temperate regions around the world and is generally used as an ornamental plant (Sulusoglu and Cavusoglu, 2013). In Turkey, it is mainly distributed on the Black Sea coast and is locally called Karayemiş, Taflan or Laz kirazı. PL fruits are consumed in various forms – fresh, dried, pickled, preserved, marmalades and beverages (Kolayli *et al.*, 2003). Traditional folk medicine different parts of the PL plant to treat digestive system problems, bronchitis, eczema, asthma, cough, haemorrhoids, headache, acidity and diabetes and its complications. PL has been found to also have diuretic, antispasmodic, narcotic and analgesic effects (Erdemoğlu *et al.*, 2003; Orhan *et al.*, 2015). The PL plant has been found to contain chlorogenic acid, o-coumaric acid, quercetin-3-glucoside, luteolin-7-glucoside, apigenin-7-glucoside, kaempferol-3-glucoside, naringenin, vanillic acid, caffeic acid and rutin, and these bioactive components are thought to be responsible for the high antioxidant activity of PL (Orhan and Akkol, 2011; Karabegović *et al.*, 2014).

The mechanisms of insulin resistance in type I diabetes have not been fully elucidated, and more studies are needed. Therefore, in this study, it was aimed to investigate whether *Prunus laurocerasus* extracts (fruit, leave and seed) used in type I diabetes have antihyperglycemic, antioxidative and insulin sensitivity enhancing effects.

Materials and Methods

Preparation of extract: PL leaves and fruits were collected from Turkey's Trabzon province. The seeds, deseeded fruits and leaves were dried in the shade. The dried seeds, fruits and leaves were then ground into a powder at the mill and dissolved in a mixture of ethanol and water (2:8) in a ratio of 1:5 (plant part:solvent) per the procedure described by Hamza *et al.* (2012). The mixtures were maintained at room temperature for 2 days in a shaking water bath. The mixtures were filtered through filter paper, and an evaporator was used to evaporate the solvent at 50°C; 5.88 g of seed, 24.24 g of fruit and 10.51 g of leaf extract were obtained from 250 ml of maceration.

Experimental design: The Kafkas University Animal Experiments Local Ethics Committee approved this study (2015/023). This study was conducted on 60 Sprague-Dawley male rats, aged 3 months that were divided into 6 groups of 10 animals each. Before the study began, the weights of the rats were determined to be 356 ± 20 g. Type I diabetes was induced using a single-dose (50 mg/kg) intraperitoneal injection of streptozotocin (STZ) in all rats except the normoglycaemic control group. Fasting blood glucose (FBG) levels were determined with blood taken from the tail vein of rats 3 days after STZ injection. The rats were defined as diabetic if the FBG levels were higher than 200 mg/dL. Subsequently, these diabetic animals were included in the experimental groups. After the control and experimental groups were formed as

follows, substance administration was started and continued for 21 days.

The study groups were as follows:

Normoglycaemic Control Group (NC): No treatment was performed in this group.

Diabetes Control Group (DC): Physiological saline solution was administered to provide standardisation with other groups throughout the study.

Diabetes + PL seed extract (D+S): Throughout the study, 500 mg/kg seed extract was administered through oral gavage.

Diabetes + PL fruit extract (D+F): Throughout the study, 500 mg/kg fruit extract was administered through oral gavage.

Diabetes + PL leaf extract (D+L): Throughout the study, 500 mg/kg leaf extract was administered through oral gavage.

Diabetes + Insulin (D+I): Throughout the study, 2 IU insulin (Levemir Flexpen) was given subcutaneously.

Biochemical analyses: FBG levels after 8 h of fasting (on 3rd, 10th, 17th and 24th days of study) were determined (Blood samples were taken from the tail vein) periodically using a glucometer (Contour TS, Bayer). At the end of the 24th day, intracardiac blood samples were taken from rats under 0.4 ml / kg pentobarbital sodium anesthesia into anticoagulant (EDTA) tubes, followed by their euthanasia. Blood samples were centrifuged per the kit procedures, and plasma samples were obtained. The plasma samples were stored at -20°C until use. Glycated haemoglobin (HbA1c), insulin (Lot : FZBTBQK1), irisin (Lot : AK0017MAR13034), adiponectin (Lot : 2TYKBJTW) and TNF- α (Lot : GA2TJXY8) levels were measured in plasma samples using ELISA device (BioTek, USA) with ELISA test kits (Elabscience, USA). Plasma levels of malondialdehyde (MDA) (Placer *et al.*, 1966), glutathione (GSH) (Sedlak and Lindsay, 1968), and Glutathione peroxidase (GPx) (Matkovics *et al.*, 1988), were determined spectrophotometrically per previously described methods.

Statistical analyses: All biostatistical evaluations were performed using SPSS 18 software. One-way analysis of variance was used to determine the differences among the groups. Tukey's multiple range test was used to detect changes between the groups. $p < 0.05$ was considered significant

Results

Initially, the FBG levels of all groups were similar. FBG levels were observed to increase significantly in all groups 3 days after STZ administration ($p < 0.001$). On the 10th, 17th and 24th days of the study, FBG levels were significantly decreased in the D+S, D+F and D+I groups compared with the DC group ($p < 0.001$). Moreover, FBG levels were significantly reduced in the D+L group on the 24th day of the study ($p < 0.05$) (Fig. 2).

HbA1c levels were significantly higher in the DC group than in the NC group ($p < 0.001$). However, HbA1c levels were significantly decreased in the D+S,

D+F and D+I groups ($p < 0.001$). Table 1 shows that insulin levels significantly increased in the DC group compared with the NC group ($p < 0.01$). Plasma insulin levels were found to increase in the D+S group, but this increase was not significant ($p > 0.05$) (Table 1).

The initial body weights of all animals were similar. However, the body weight significantly decreased in all groups 3 days after STZ administration ($p < 0.001$). On the 10th day of the study, no significant difference was observed in the experimental groups compared with the DC group ($p > 0.05$). However, on the 17th day of the study, the body weight was found to be significantly higher in the D+S group than in the DC group ($p < 0.05$). On the 24th day, the body weight in the D+S, D+F and D+I groups was significantly higher than that in the DC group ($p < 0.001$, $p < 0.05$ and $p < 0.05$, respectively) (Fig. 3).

Compared with the NC group, the DC group was found to have decreased irisin hormone levels and significantly increased TNF- α and adiponectin levels (p

< 0.001). Irisin hormone levels were significantly increased in the D+S, D+F, D+L and D+I groups compared with the DC group ($p < 0.01$, $p < 0.01$, $p < 0.001$ and $p < 0.01$, respectively). In contrast, adiponectin hormone levels were significantly decreased in the D+S, D+F, D+L and D+I groups ($p < 0.001$, $p < 0.001$, $p < 0.05$ and $p < 0.001$, respectively). Proinflammatory cytokine TNF- α levels had increased in the DC group and significantly decreased in all experimental groups ($p < 0.001$) (Table 2).

Compared with the NC group, in the DC group, MDA levels were significantly increased ($p < 0.001$), whereas GPx and GSH levels were significantly decreased ($p < 0.001$). Furthermore, compared with the DC group, the groups treated with PL seed, fruit and leaf extracts had significantly decreased MDA levels ($p < 0.001$) but increased GSH levels ($p < 0.01$, $p < 0.01$ and $p < 0.001$, respectively). Compared with the DC group, the rats treated with PL seed extracts showed significantly increased GPx levels ($p < 0.05$) (Table 3).

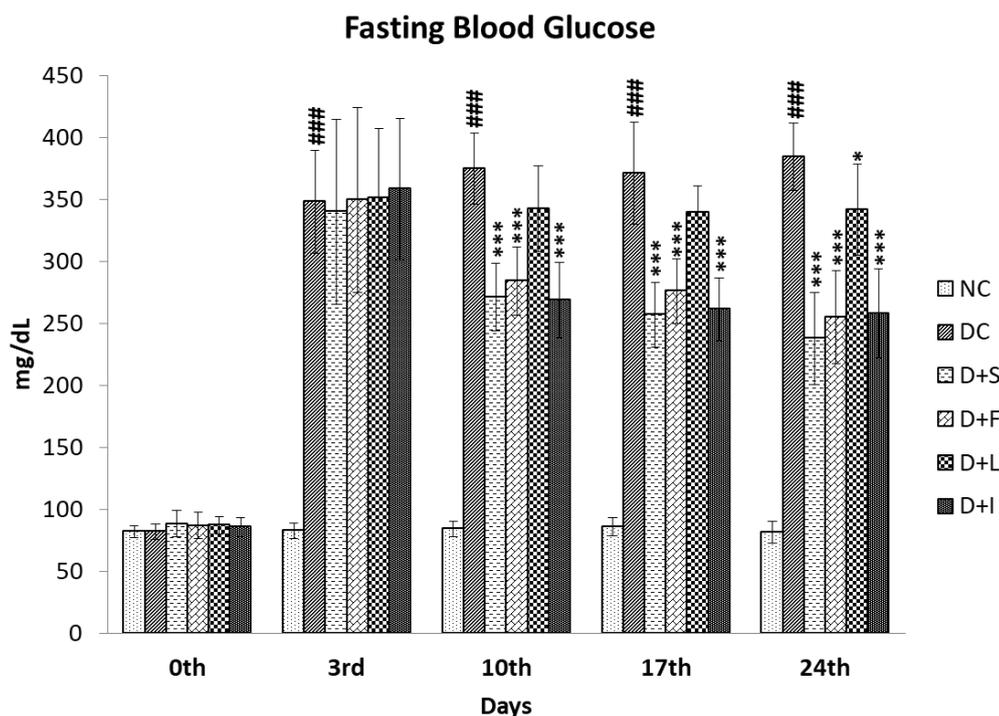


Figure 2 Fasting blood glucose (mg/dL) levels of NC and experimental groups
: $p < 0.001$ as compared with NC group, * : $p < 0.05$, *** : $p < 0.001$ as compared with DC group

Table 1 Plasma HbA1c (ng/mL) and insulin (ng/mL) levels of NC and experimental groups

	NC	DC	D+S	D+F	D+L	D+I
HbA1c	0.16±0.07	0.76±0.14###	0.32±0.07***	0.34±0.10***	0.58±0.13	0.32±0.08***
Insulin	1.07±0.12	0.57±0.09##	0.75±0.32	0.52±0.17	0.52±0.19	0.52±0.14

: $p < 0.01$, ### : $p < 0.001$ as compared with NC group, * : $p < 0.05$, *** : $p < 0.001$ as compared with DC group, (HbA1c $R^2 = 0.9910$, Insulin $R^2 = 0.9777$)

Table 2 Plasma irisin (pg/mL), adiponectin (pg/mL) and TNF- α (pg/mL) levels of NC and experimental groups

	NC	DC	D+S	D+F	D+L	D+I
Irisin	172.5±14.1	75.0±17.7###	123.0±9.1**	131.7±18.1**	135.8±31.4***	126.0±15.6**
Adiponectin	24.1±6.1	69.6±7.0###	31.3±7.7***	40.1±7.0***	56.5±9.4*	39.8±6.6***
TNF-α	160.6±2.3	172.3±6.4###	161.7±2.1***	160.1±2.7***	160.5±2.5***	162.0±2.3***

: $p < 0.001$ as compared with NC group, * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ as compared with DC group, (Irisin $R^2 = 0.9968$, Adiponectin $R^2 = 0.9772$, TNF- α $R^2 = 0.9611$)

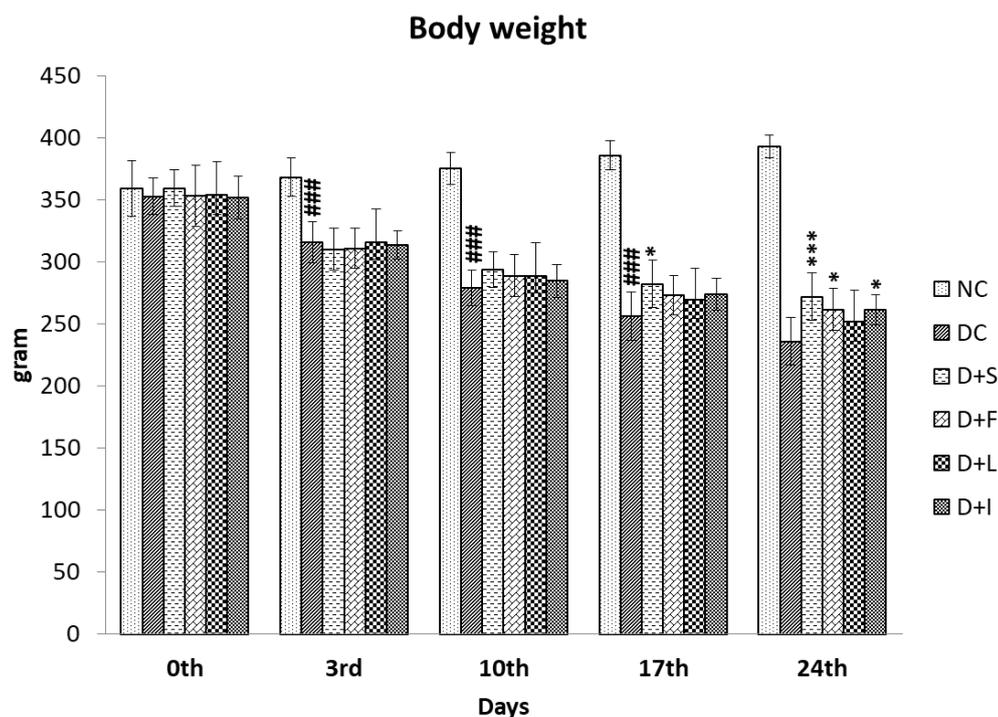


Figure 3 Body weight (g) of experimental animals throughout the study.
: $p < 0.001$ as compared with NC group, * : $p < 0.05$, *** : $p < 0.001$ as compared with DC group

Table 3 Plasma MDA (nmol/mL), GSH ($\mu\text{mol/mL}$) and GPx (U/mL) levels of NC and experimental groups

	NC	DC	D+S	D+F	D+L	D+I
MDA	1.81 \pm 0.15	3.33 \pm 0.32###	2.29 \pm 0.07**	2.32 \pm 0.10**	2.31 \pm 0.09**	2.04 \pm 0.11**
GSH	177.2 \pm 4.6	150.7 \pm 14.9###	172.3 \pm 11.3**	171.8 \pm 6.2*	177.0 \pm 4.9**	178.4 \pm 5.5**
GPx	0.93 \pm 0.14	0.26 \pm 0.02###	0.43 \pm 0.08*	0.41 \pm 0.07	0.40 \pm 0.07	0.38 \pm 0.04

: $p < 0.001$ as compared with NC group, * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$ as compared with DC group

Discussion

Currently, no definitive treatment for type I diabetes is available. Therefore, it becomes imperative to develop new treatment strategies to maintain a normoglycaemic index and improve the wellbeing of individuals with type I diabetes.

In previous studies, it was reported that PL fruit, seed (Orhan *et al.*, 2003) and leaf (Uslu *et al.*, 2018) extracts have very high antioxidant activity. PL is known to contain several phenolic compounds such as chlorogenic acid, o-coumaric acid, quercetin-3-glucoside, luteolin-7-glucoside, apigenin-7-glucoside, kaempferol-3-glucoside, naringenin, vanillic acid, caffeic acid and rutin (Karabegović *et al.*, 2014).

One study reported that PL fruit extract decreased total oxidant status and MDA levels in dimethoate toxicity, whereas it increased total antioxidant status and superoxide dismutase (SOD), catalase and GPx levels (Eken *et al.*, 2017). Another study reported that PL leaf extract decreased the levels of thiobarbituric acid reactive substances (TBARS) and increased those of GSH and SOD (Uslu *et al.*, 2018). Several researchers have indicated that chlorogenic acid – the main active ingredient of PL – is effective in reducing MDA and TBARS levels (Yukawa *et al.*, 2004; Wang *et al.*, 2012). In the present study, it was determined that PL seed, fruit and leaf extracts decreased MDA levels, but

significantly increased GSH levels. However, only the seed extract was found to be effective in increasing GPx levels. Many studies have previously reported that PL extracts have high levels of free radical scavenging activity (Karabegović *et al.*, 2014; Uslu *et al.*, 2018; Orhan and Akkol, 2011). We consider that these antioxidative effects of PL are arise from phenolic compounds, especially chlorogenic acid.

Chlorogenic acid – the major component of PL – has been reported to exhibit anti-hyperglycaemic and insulin resistance-reducing effects (Meng *et al.*, 2013; Xiu-ci *et al.*, 2013). In the present study, it was determined that the seed, fruit and leaf extracts of PL significantly reduced blood glucose levels. Additionally, it was observed that PL seed and fruit extracts were effective in decreasing HbA1c levels in diabetics. However, PL extracts were not effective in increasing plasma insulin levels. Turan *et al.* (2013) were reported that PL fruit extract reduced postprandial blood glucose levels in alloxan-induced type II diabetic rats. Furthermore, Şenaylı *et al.* (2012) have reported that PL seed extract significantly reduces blood glucose levels in type I diabetic rats besides increasing insulin levels. Orhan *et al.* (2015) have stated that the PL seed extract was effective in decreasing blood glucose levels, whereas the fruit extract was not effective. Additionally, some studies

were reported that PL leaf extract do not altered HbA1c levels in type II diabetic patients (Kutlucan *et al.*, 2013) and type I diabetic rats (Uslu *et al.*, 2018).

Weight loss is a well-known significant symptom of hyperglycaemia in type I diabetes mellitus (American Diabetes Association, 2014). In this study, significant reductions in body weights of diabetic rats were detected. However, it was noted that PL seed and fruit extracts were significantly decreased the degree of weight loss in diabetes. This effect of the seed and fruit extracts in maintaining the body weight can be attributed to their mechanism of reduction of hyperglycaemia and prevention of lipolysis.

Several researchers have found a positive correlation between plasma irisin levels and body mass index (Liu *et al.*, 2013; Stengel *et al.*, 2013). Choi *et al.* (2013) were reported that significantly decreased serum irisin levels in patients with new-onset type II diabetes compared with non-diabetic individuals. Liu *et al.* (2013) were also reported that significantly lower plasma irisin levels in patients with type II diabetes than that of non-diabetic individuals. In this study, significantly decreased plasma irisin levels were determined in the DC group compared with the NC group and the levels were increased with PL extract and insulin application.

Notably, adiponectin increases the efficacy of peroxisome proliferator-activated receptor- γ , thereby reducing liver-derived glucose production as well as enhancing glucose uptake and free fatty acid oxidation in muscle tissue (Yamauchi *et al.*, 2003). Frystyk *et al.* (2005) showed that serum adiponectin levels are increased in type I diabetic patients. Another study suggested that fasting plasma adiponectin levels were decreased in individuals with type II diabetes, with no change observed in those with type I diabetes (Perseghin *et al.*, 2003). Many researchers have found increased serum adiponectin levels in individuals with type I diabetes (Imagawa *et al.*, 2002; Majewska *et al.*, 2016). Our observation of increased adiponectin levels in diabetic rats was in accordance with the literature. Conversely, we observed significantly decreased adiponectin levels in all our experimental groups with diabetes.

Furthermore, adiponectin reduces secretion of TNF- α from monocytes, macrophages and foam cells (Ouchi *et al.*, 1999). TNF- α is expressed as a proinflammatory cytokine that is closely related to diabetes and excessive secreted from adipocytes because of insulin resistance (Spiegelman and Hotamisligil, 1993). Additionally, increased TNF- α levels in type I diabetes cause beta cell damage. Some studies, it has been reported that TNF- α levels are significantly increased in type I diabetes (Atila and Yüce, 2016; Uslu *et al.*, 2018). The finding of elevated TNF- α levels in type I diabetes in our study was consistent with the literature. Notably, the PL extracts (fruit, seed and leaf) effectively reduced the proinflammatory cytokine TNF- α levels.

In conclusion, we determined that especially the seed and fruit extracts of PL are highly effective in decreasing FBG levels, regulating the weight change imbalance, preventing oxidative stress and inflammation due to diabetes and potential to increase insulin sensitivity.

Conflicts of Interest Statement: The authors declare no conflict of interest.

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