EVALUATION OF THE PREVENTIVE EFFECT OF *AMMODAUCUS LEUCOTRICHUS* COSS. & DUR. FRUITS AQUEOUS EXTRACT AGAINST ALLOXAN-INDUCED DIABETES IN MICE

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Abstract

Ammodaucus leucotrichus aqueous extract has been shown an antihyperglycemic effect by inhibiting α-glucosidase, α-amylase and intestinal glucose absorption.

This study aims to evaluate the pancreatic protective effect of *Ammodaucus leucotrichus* aqueous extract (AEAL) against experimental alloxan-induced diabetes in mice. Also, antioxidant activity, total phenolic and flavonoids content were evaluated.

The preventive effect was performed on Swiss albino mice treated with the AEAL (150 and 300 mg/kg) before and after a single intraperitoneal alloxan injection. Percent surviving, fasting blood glucose, and body weight were measured. Furthermore, pancreatic tissues were collected for histopathological analysis to evaluate alloxan-induced tissue alterations. The antioxidant activity was determined by using three methods: measuring 2,2-diphenyl-1-picrylhydrazyl free radical scavenging, oxidation of β-carotene/linoleic acid, and ferric reducing antioxidant potential assay. Total phenolic content was determined by Folin–Ciocalteu colorimetric method and total flavonoid content was measured by aluminum chloride colorimetric assay, and phytochemical screening was determined using standard qualitative methods.

The administration of the AEAL at two doses of 150 and 300 mg/kg significantly attenuated alloxan-induced death and hyperglycemia in treated mice. Moreover, histopathological investigations revealed that the AEAL protected islets of Langerhans against alloxan-induced alterations. Moreover, AEAL revealed significant antioxidant activity and contains polyphenols, flavonoids, and betacyanins.

The antidiabetogenic effect of *A. leucotrichus* could be due to their polyphenol and flavonoid components, which could react alone or in combination to scavenge the free radicals created by the alloxan.

Keywords: Alloxan prevention. *A. leucotricus* aqueous extract. diabetes mellitus. polyphenol. flavonoid. antioxidant.

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1. Introduction

Diabetes mellitus is a prevalent health problem, and according to the latest report by the International Diabetes Federation (IDF), the prevalence is expected to increase to 640 million by 2030 (Cho et al., 2018). Several risk factors are responsible for the development and the progression of diabetes and its complication, especially genetic predisposition, behavioral or environmental. Reactive oxygen species (ROS) are chemical oxygen species involved in the etiology and development of vascular complications in diabetes (Son, 2012).

Many studies have highlighted the strong relationship between redox imbalance and the development of diabetes as a metabolic disease caused by hyperglycemic state (Dröge, 2002; Oberley, 1988). The high cellular uptake of glucose enhances intracellular oxidative stress and thus becomes a source of the overproduction of ROS responsible for redox imbalance or an intolerable rate of cellular free radicals (Schulze et al., 2004). Therefore, during hyperglycemia, there is a high risk of initiating the production of different reactive free radical precursors, which degrade the antioxidant systems, by the destruction of many biomolecules, and the rise of lipid peroxidation in diabetes (Gupta et al., 2002).

In general, the increasing prevalence of diabetes mellitus requires effective preventive intervention (Danaei et al., 2011), and resorting to phytotherapy is receiving more attention in the search for therapeutically effective and safe antioxidants. In particular, the consumption of natural antioxidants specifically those found in plants, might be effective in combating oxidative stress-induced illnesses like diabetes.

Ammodaucus leucotrichus is a medicinal plant widely used as sugar regulator for diabetics (Mohammedi et al., 2018). Several studies that have been performed on this plant have shown that it has a potential antioxidant activity that can be beneficial in diabetes mellitus management (El-Ouady & Eddouks, 2019; Sebaa et al., 2018). In addition, a previous study has shown that *A. leucotrichus* has inhibitory effects on α-glucosidase, α-amylase, and intestinal glucose uptake (Bouknana et al., 2022). The acute toxicity evaluation of the aqueous extract of *A. leucotrichus* confirms that its usage is safe and does not lead to any harmful side effects (Bouknana et al., 2022). To our knowledge, no studies have provided information on the protective effect of aqueous extract of *A. leucotrichus* aqueous extract against alloxan (Allx) induced diabetes in mice. Therefore, this study aims to assess the protective effect of *Ammodaucus leucotrichus* aqueous extract (AEAL) on the pancreas in an experimental model of alloxan-induced diabetes in mice, and search for the potential mechanism underlying this effect by examining the antioxidant activity of the extract, as well as determining its qualitative and quantitative phytochemical composition.

1. Materials and methods

1.1. Chemicals

Ascorbic acid, glucose oxidase/peroxidase reagent kit, Folin–Ciocalteu, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, β-carotene, linoleic acid, Tween 40, Potassium ferricyanide K3[Fe(CN)6], Trichloroacetic acid (TCA), Ferric chloride FeCl3, Chloroform, and methanol were purchased from Sigma-Aldrich, Alloxan monohydrate (Allx monohydrate 98%, ACROS Organics). All other reagents and chemicals used are of analytical grade.

1.2. Plant identification

Ammodaucus leucotrichus Coss. & Durieu fruits were obtained from an herbalist in Oujda (Morocco). A botanist performed the authentication. A voucher specimen (HUMPOM432) was deposited in the Herbarium, Department of Biology, Faculty of Sciences, Oujda, Morocco.

1.3. Preparation of the aqueous extract of *Ammodaucus leucotrichus* (AEAL)

Infusion of *A. leucotrichus* fruits was prepared as described in our previous study (Bouknana et al., 2022). In brief, a 10 g quantity of fruit powder was suspended in 100 mL of boiled water for 30 minutes with continuous agitation. After filtration, the filtrates recovered and evaporated using a rotary evaporator under reduced pressure. The dry residues obtained are stored at + 4 °C.

1.4. Effect of AEAL on Allx-induced diabetes in mice

Animals

Swiss albino mice (19 weeks old) of both sexes (♀/♂) were used for this study. They were maintained in environmental conditions cages under standard laboratory prescriptions at the animal house of the Faculty of Sciences, Mohammed First University, Oujda, Morocco. All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, US National Institutes of Health (NIH Publication No. 85-23, revised in 1985) under the certification number 15/19- LBBEH-06.

Diabetes induction

Mice were made diabetic with an intraperitoneal injection of a single dose of alloxan monohydrate (100 mg/kg of body weight) (Berraaouan et al., 2014). The diabetogenic agent was prepared freshly in phosphate-citrate buffer with a pH value of 4.5 and the injection was carried out on overnight fasted mice.

Experimental design

To evaluate the effect of AEAL treatment on the incidence of Allx-induced diabetes, the mice were divided into five groups (n = 6; ♂/♀=1): Control group, Allx group, Allx + AEAL (150 mg/kg), Allx + AEAL (300 mg/kg) treated group, and Allx + Asco (2 mg/kg) group.

For a period of three days, treated animals received orally the AEAL (150 and 300 mg/kg) and ascorbic acid (2 mg/kg) to their respective groups, followed by a single dose (100 mg/kg). The injection of alloxan took place within a one-hour time interval following the completion of the three-day treatment period. Following the administration of alloxan, the treatment was continued for a duration of seven days. The control group and positive control group were given oral doses of distilled water (10 mL/kg) and intraperitoneal injections of phosphate-citrate buffer and alloxan, respectively. Blood samples were collected from the tail vein, and glycemia was measured at the start and at the end of the study by the glucose oxidase–peroxidase method using a commercial kit. Bodyweight measurements were taken prior and after the treatment period. At the end of the treatment, the percentage of surviving animals was determined as an additional evaluation metric (Berraaouan et al., 2014).

Histological analysis of pancreatic tissues

Mice pancreas were subjected for microscopic examination to observe any lesions. All pancreatic tissues were immersed in a 10% buffered formalin solution for 48 hours. Following the preservation process, the tissues were embedded in paraffin and sliced into thin sections measuring 4 to 5 µm using a rotating microtome (microtomeleitz 1512). Hematoxylin and eosin staining were used to stain pancreas slices. Under optical microscopy (Optika Microscopes, Italy), stained sections of the pancreas were examined and viewed subjectively (morphologically), and photographed by an Infinity camera microscope with a magnification of ×40.

1.5. Antioxidant activity

DPPH free radical scavenging activity

The assessment of the free radical scavenging ability of *A. leucotrichus* assay was carried out according to the methodology outlined by de la Rosa et al. 2011 (de la Rosa et al., 2011). Various concentrations (0.016, 0.031, 0.063, 0.125, 0.25, 0.500 mg/mL) of AEAL were used. In brief, 0.5 mL of AEAL solution was mixed with 1 ml of methanol solution of DPPH (0.004 %). After 30 minutes of incubation in darkness and at ambient temperature. The absorbance was measured at 517 nm. Ascorbic acid was employed as a positive control. All measurements were performed in triplicate. The radical scavenging ability was determined using the following equation:

% of radical scavenging activity = (Abs control- Abs sample/Abs control) ×100

β-Carotene bleaching test

To prepare the β-carotene solution, 2 mg of β-carotene was dissolved in 10 mL of chloroform. This solution was then added to a flask containing 0.02 mL linoleic acid and 200 mg of Tween-80. The chloroform was subsequently removed from the solution by heating it to 40 °C. Afterward, 100 mL of distilled water was vigorously shaken with the solution to create an emulsion.

A 2 mL of this emulsion transferred into different test tubes, and each tube received a 0.5 mL sample of the AEAL solution containing the sample AEAL solution (0.5 mL). The tubes were then incubated in a water bath (50 °C) for 2 hours with continuous shaking. BHA was used as a standard. The initial absorbance of the samples (t0) was recorded immediately after the addition of the emulsion, and then, after 2 hours, the absorbance was measured again at 470 nm. All measurements were performed in triplicate.

Bleaching inhibition (%) = $100-[(\frac{(\text{initial }\beta-\text{carotene})(t0)-(\text{final }\beta-\text{carotene})(t120\text{ min})}{(\text{initial }\beta-\text{carotene})(t0)})\times 100]$

Determination of ferric reducing power assay

AEAL were tested for ferric reducing activity using the method given by Dehpour et al. 2009 (Dehpour et al., 2009), based on the reduction of Fe3+ present in the potassium ferricyanide [K3Fe(CN)6] complex in Fe2+. Different concentrations (0.016, 0.031, 0.063, 0.125, 0.25, 0.5 mg/mL) of the extract were prepared. To perform the assay, 0.5 ml of sample extract was mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of K3Fe (CN)6 (1% w/v). The mixture was incubated at 50 °C for 20 min. After cooling to ambient temperature, the reaction was stopped by adding 1.25 mL of Trichloroacetic acid (10% w/v). The resulting mixture was then centrifuged at 3000 rpm for 10 minutes. An aliquot of 1.25 mL of the supernatant solution was combined with 1.25 mL of bidistilled water and 0.25 mL of a solution of ferric chloride (FeCl3) (0.1% w/v). The absorbance was determined at 700 nm against a blank containing bidistilled water. Ascorbic acid was used as a reference standard, such that the absorbance was quantified under the same conditions as the extract. All measurements, including those for the extract and ascorbic acid, were conducted in triplicate. An increase in absorbance corresponds to an enhancement increase in the reducing power of the tested extract.

1.6. AEAL preliminary phytochemical screening

AEAL was tested for quinones, terpenoids, saponins, alkaloids, tannins, and cardiac glycosides using standard

qualitative methods as described by Sofowora (1996) (Sofowora, 1996).

Test of tannins

A volume of 2 mL of 5% ferric chloride (FeCl3) was added to 1 mL of plant extract. The presence of tannins was revealed by the formation of greenish-black.

Test of saponins

The plant extract (2 mL) was combined with distilled water (2 mL) in a graduated cylinder and shaken lengthwise for a duration of 15 minutes. The presence of saponins was determined by the observation of a foam layer measuring approximately 1 cm in thickness.

Test for alkaloids

The Dragendorff reagent has been added to the AEAL; if a black precipitate forms, it indicates the presence of alkaloids.

Test for betacyanin

To 1 mL of 2M sodium hydroxide was added 2 mL of plant extract and heated for 5 minutes at 100oC. The presence of betacyanin was shown by the formation of a yellow color.

Test for quinones

A volume of 1 mL concentrated sulphuric acid was added to 1 mL extract. The detection of quinones was confirmed by the appearance of a distinct red color.

Test for cardiac glycosides

Initially, 2 mL of glacial acetic acid and a few drops of 5% ferric chloride solution were added to 0.5 mL of the extract. Subsequently, 1 mL of concentrated sulfuric acid was introduced into the mixture. The formation of a brown ring at the interface between the two layers indicated the presence of cardiac glycosides.

Test for coumarins

To 1 mL of 10% sodium hydroxide was added 1 mL of extract. The presence of coumarins was detected by the formation of a yellow color.

1.7. Determination of the total phenols of the AEAL

The dosage of total AEAL phenols was determined using the Folin-Ciocalteu colorimetric method (Aquino et al., 2001). Briefly, 1 mL of Folin-Ciocalteu reagent (0.2 N) was added to 0.2 mL of AEAL. After 5 minutes of incubation at room temperature, 0.8 mL of the aqueous sodium carbonate solution (7.5%) was added to the mixture. All samples were carefully agitated, and the absorbance was read after 1 hour at 760 nm against a blank containing 0.2 mL of methanol, 1 mL of Folin-Ciocalteu reagent and 0.8 mL of sodium carbonate (7.5%). Gallic acid (standard) was used to perform a calibration curve at concentrations of 0, 25, 50, 75, 125, 250, 500 µg/ml. was quantified and expressed as "g gallic acid equivalent per 100 g dry extract". All measurements were made in triplicate.

1.8. Determination of total flavonoids

The total flavonoid assay was conducted following the methodology described by Chen et al. 2015 (Chen et al., 2015). Briefly, 1 mL of distilled water and 50 µL of sodium nitrate (NaNO3, 5% w/v) were added to 0.2 mL of AEAL (0.5 mg/mL). After 6 minutes, 120 µL of aluminum chloride (AlCl3, 10% w/v) was added to the mixture. After 5 minutes, 400 µL of NaOH (1 M) was added to the mixture. Absorbance was determined by 430 nm spectrophotometer against 0.2 mL distilled water blank, 50 µL NaNO3 (5%) and 120 µL AlCl3 (10%). Quercetin was employed as a standard to obtain the calibration curve. The total flavonoid content was expressed in “g quercetin equivalent per 100 g dry extract”. All measurements were taken in three copies.

1.9. Statistical Analysis

The obtained results were analyzed using GraphPad Prism 8 software. The data were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using One-way ANOVA. A p-value of less than 0.05 was considered statistically significant.

2. Results

2.1. Effect of AEAL on Allx-induced diabetes in mice

Effect on body weight

Diabetes mellitus has been linked to body-weight loss (Pupim et al., 2005). The variation of body weight in the different groups were represented in Fig. 1. Allx induced a significant decrease in body weight in the Allx group ($p<0,05$), compared to the control group. However, the oral intake of the AEAL (150 and 300 mg/kg) inhibited the body weight loss compared to the Allx group.

Effect on survival rate

In Fig. 2, the survival rate after alloxan injection (100 mg/kg) is shown. The pretreatment with AEAL at doses 150 and 300 improved the survival rate, with a percentage of 75% and 85.74 % respectively, compared to the Allx-group which had a survival rate of 50% when treated with Allx alone. Additionally, the administration of ascorbic acid prevented mortality due to Allx administration, as observed in the Allx group.

Effect on blood glucose level

Fig. 3, the effect of AEAL on blood glucose levels is presented. The injection of Allx led to a significant ($p<0.001$) increase in fasting blood glucose levels in the diabetic control group compared to the normal control group. However, the administration of the AEAL at two doses, 150 and 300 mg/kg with Allx in mice resulted in a significant ($p<0.001$) reduction in the impact of Allx-induced hyperglycemia compared in comparison with the group treated only with Allx. Moreover, the administration of ascorbic acid at 2 mg/kg reduced significantly ($p<0.001$) glycemia compared to the group treated only by Allx. These findings suggest that the treatment with AEAL effectively attenuated the development of the diabetes mellitus induced by Allx in mice.

Histological analysis of pancreatic tissues

Under light microscopy, the pancreas slices from the normal control group exhibited a typical architecture and appearance of the islets of Langerhans, as well as intact acini and islet cells. No structural alterations or

abnormalities were observed, indicating a healthy pancreatic tissue (Fig. 4A). On the other hand, the islet cells of the Allx group displayed notable pathological changes in both the exocrine and endocrine regions of the pancreas. These alterations were characterized by abnormal morphology and a significant reduction in the number of cells (Fig. 4B). The oral administration of AEAL showed a positive appearance of beta cells, and looked to be identical to the control. Furthermore, the majority of the Langerhans islets remained unaltered and necrotic alterations of the islets of Langerhans were absent (Fig. 4C, 4D). Similarly, ascorbic acid reduced the severity of degenerative alterations in the Langerhans islets (Fig. 4E).

2.2. Antioxidant Activity

DPPH-Radical Scavenging Activity

The antioxidant effect of AEAL was evaluated based on a range of concentrations, and the results of the trapping effect of DPPH were illustrated in Fig.5. The AEAL showed significant antioxidant activity, with an IC_{50} value of 0.239 ± 0.039 mg/mL. However, the IC_{50} of ascorbic acid was 0.032 ± 0.003 mg/mL and is lower than AEAL. Furthermore, the AEAL antioxidant capacity increased in proportion to its concentration.

β-carotene bleaching assay

The ability of the AEAL to inhibit the bleaching of β-carotene is presented in Fig. 6. Butylated hydroxyanisole (BHA) was used as the standard drug for the evaluation of antioxidant activity. The IC50 value indicated that AEAL possessed an increased inhibition in a concentration-dependent way (IC_{50}= 0.074 ± 0.006 mg/mL). Moreover, the IC_{50} of BHA was 0.023 ± 0.003 µg/mL.

Reducing Power

The ability of the AEAL to reduce Fe+3 ions into Fe+2 ions was evaluated in Fig. 7. This antioxidant activity of the AEAL was found to be weaker compared to reference compound (ascorbic acid) across concentrations ranging from 0.016 mg/mL to 0.500 mg/mL.

AEAL preliminary phytochemical screening

The phytochemical analysis indicated that the AEAL contains flavonoids and betacyanin. On the other hand, alkaloids, saponins, tannins, quinones, cardiac glucosides, and coumarins are absent in this extract.

Total phenolic and flavonoid contents of AEAL

The content was determined by calculating their concentrations using the regression equation obtained from the gallic acid calibration curve (Y= 0.004983x - 0.06015, R2 = 0.9953), and quercetin (Y= 0.004774x + 0.009726, R2 = 0.9845) respectively. The content of phenolic compounds in the AEAL was 193.5 ± 26.19 mg GAE/g, while the total flavonoid content was 24.39 ± 1.43 mg QE/g.

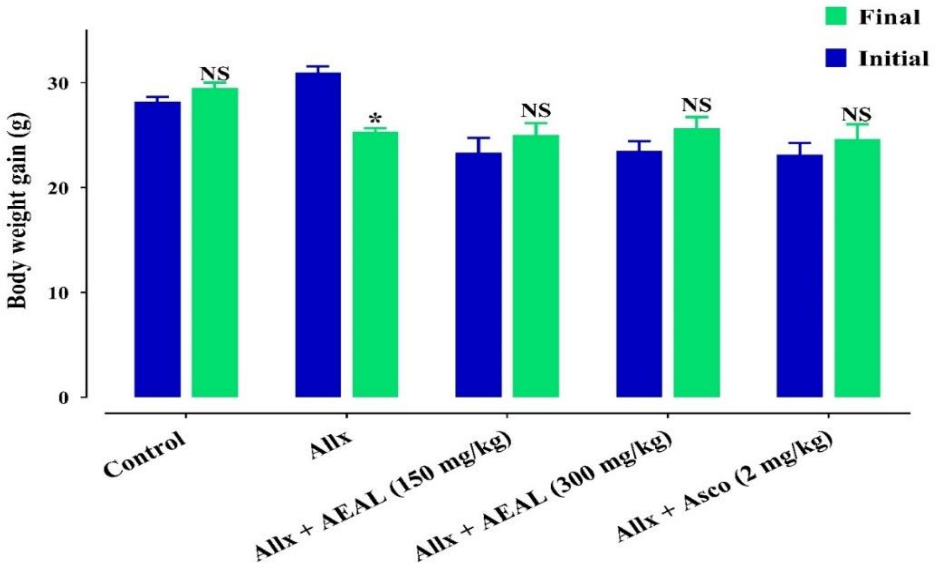

Figure 1. Effect of oral administration of the AEAL and the ascorbic acid on body weight level before and after Allx intraperitoneal injection in mice. The values are presented in mean ± SEM. n = 6, ∗p<0.05 compared to the control group.

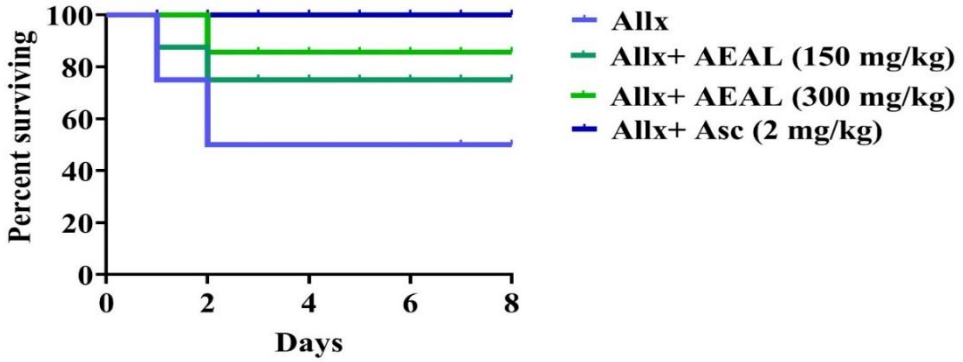

Figure 2. Effect of AEAL on survival rate of Swiss albino mice after alloxan administration.

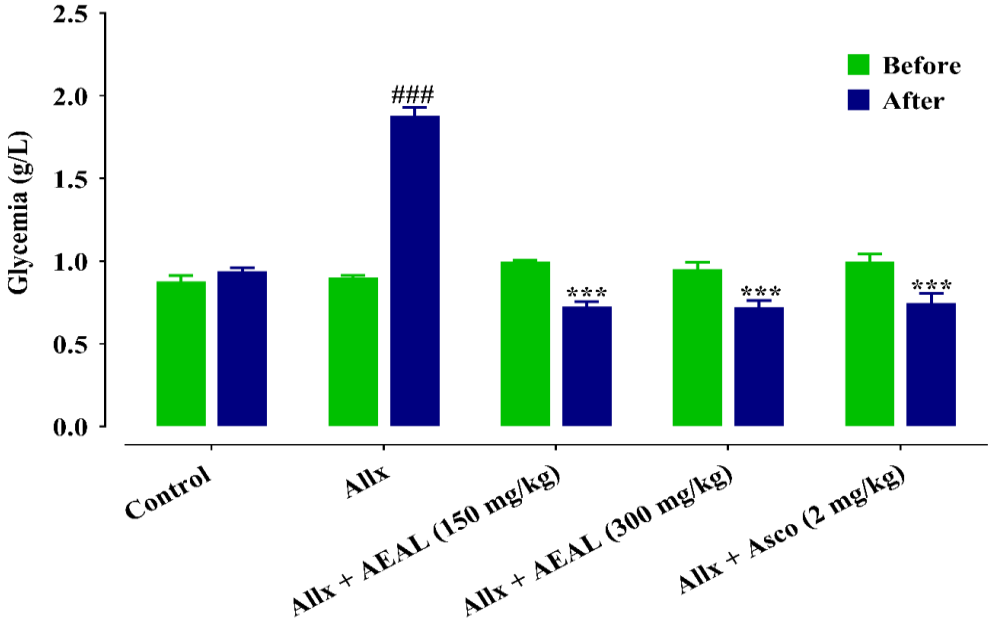

Figure 3. Effect of oral administration of the AEAL and the ascorbic acid on blood glucose level before and after Allx intraperitoneal injection in mice. The values are presented in mean ± SEM. n = 6, ###p < 0.001 compared to the control group and ***p < 0.001 compared to the Allx control group.

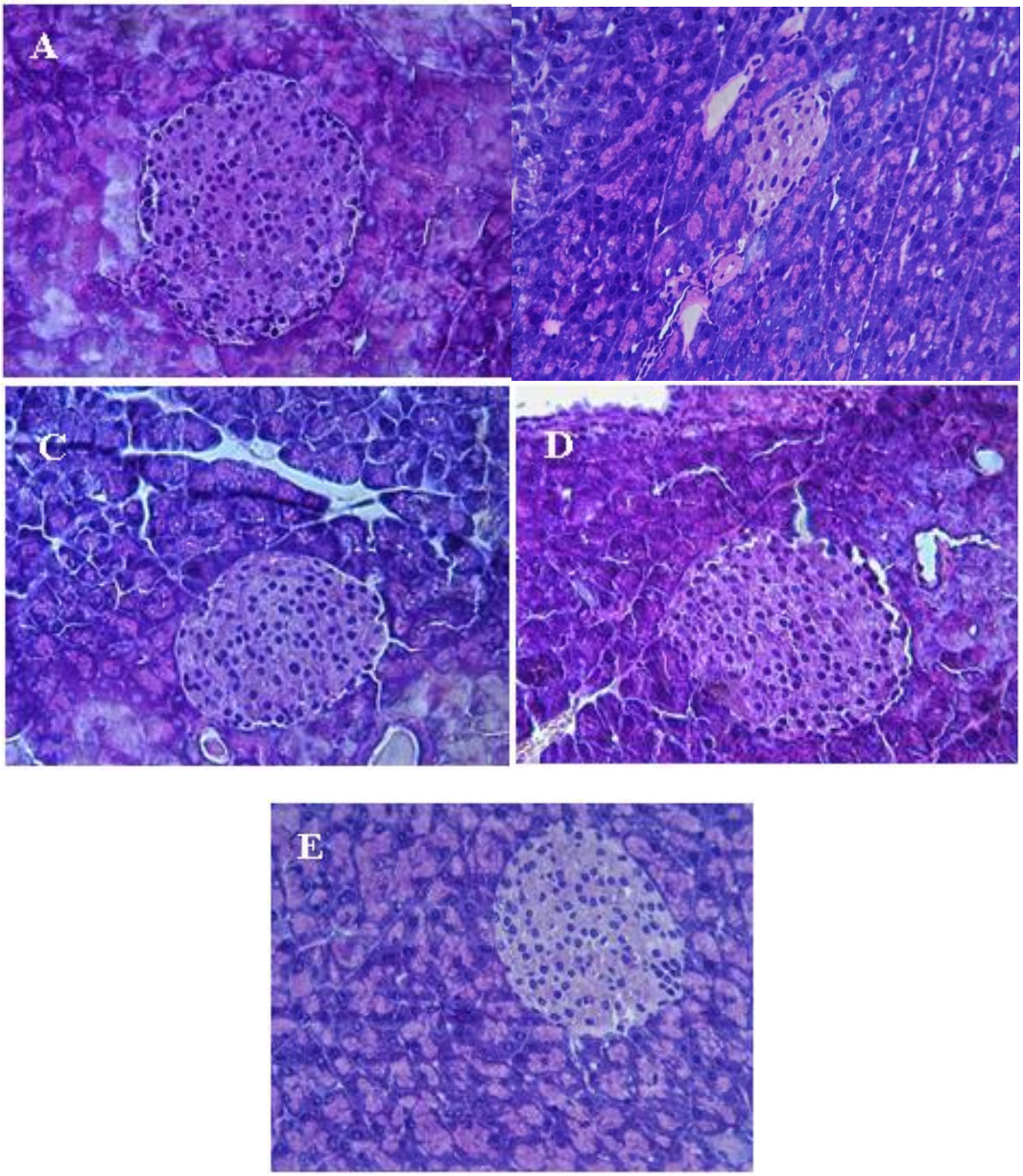

Figure 4. Histopathological observations of pancreatic islets of Langerhans. (A) normal mice, (B) control Allx group, (C) and (D) Allx+ AEAL (150 and 300 mg/kg), (E) Allx + ascorbic acid (2 mg/kg). : Langerhans Islets

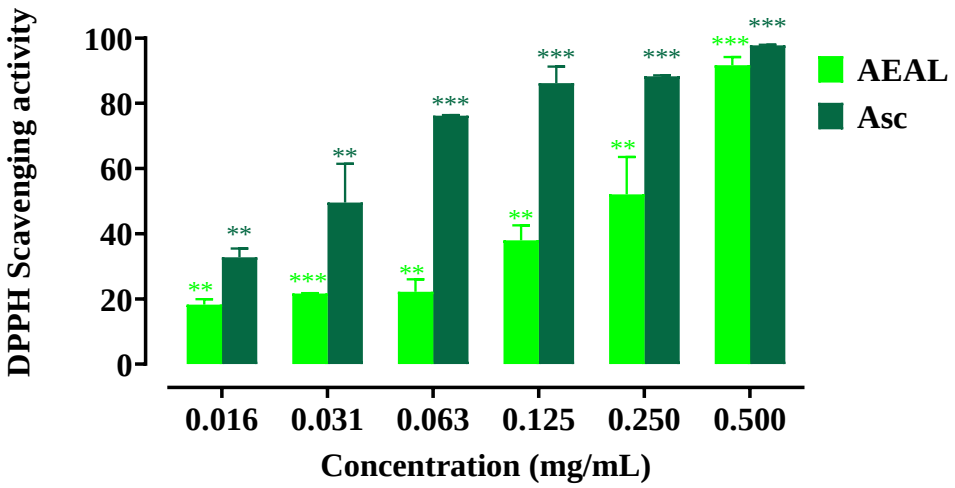

Figure 5. Effect on DPPH scavenging by AEAL and ascorbic acid at various concentrations, (n = 3); AEAL: aqueous extract of *A. leucotrichus*, Asc: ascorbic acid.

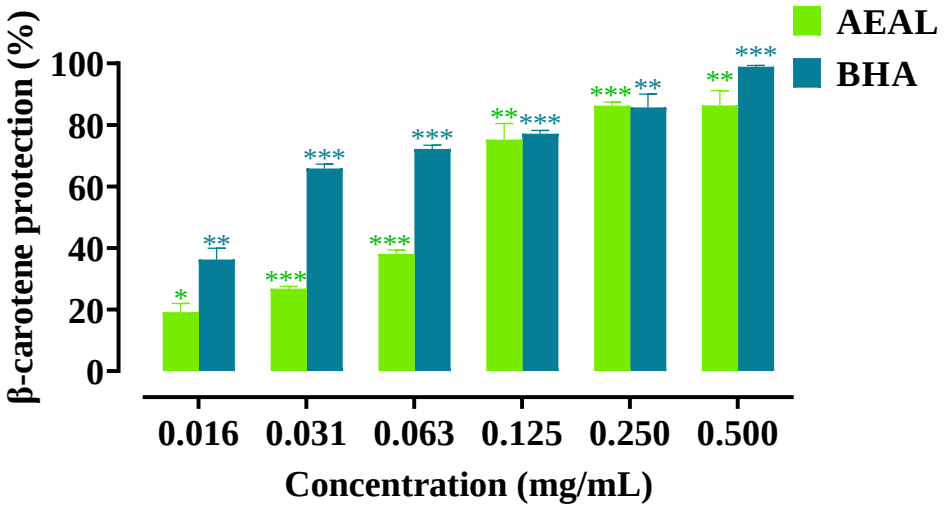

Figure 6. Effect of AEAL on β-carotene bleaching activity and BHA at various concentrations, (n = 3); AEAL: aqueous extract of *A. leucotrichus*, BHA: butylated hydroxyanisole.

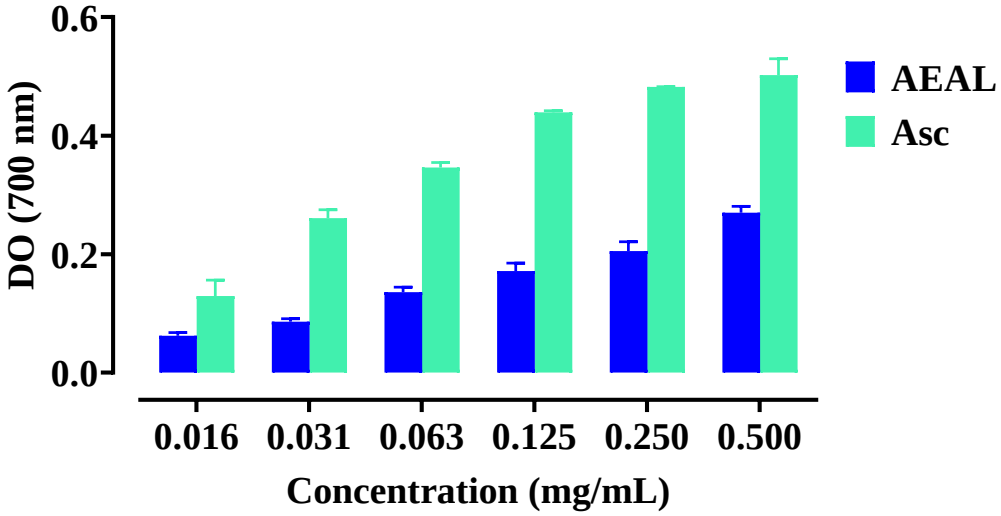

Figure 7. Effect on ferric reducing power by AEAL and ascorbic acid at various concentrations, (n = 3); AEAL:

aqueous extract of *A. leucotrichus*, Asc : ascorbic acid.

3. Discussion

The oral administration of AEAL has demonstrated a preventive effect against alloxan-induced diabetes in albino mice. According to several scientific studies, alloxan causes increased insulin release after injection (Szkudelski et al., 1998). Therefore, the mice subsequently died of hypoglycemic seizures (Waisbren, 1948). Whereas, mice treated with the AEAL survived after receiving the injection of alloxan compared to the diabetic group (Allx). In addition, diabetic control mice showed a reduced body weight related to hyperglycemia caused by the inability of the body to use accumulated glucose in the blood or the use of fats and structural proteins as alternative energy sources (Ludwig & Ebbeling, 2018). However, the administration of the AEAL has reduced body weight loss. This finding asserts the preventive property of the AEAL against the development of alloxan-induced diabetes mellitus in mice. The study demonstrated that the injection of alloxan in mice resulted in an immediate increase in blood glucose levels. This rise in glucose level is due to the oxygen reactive species (ROS) induced by alloxan; Then the resulting imbalance of the redox balance causes the destruction of pancreatic islets (Adeyi et al., 2012; Etuk & Muhammed, 2010). However, the treated mice with AEAL presented reduced blood glucose levels induced by alloxan injection. These results suggest that treatment with AEAL has a preventive effect against alloxan diabetes in mice. In fact, several models of diabetic animals can be used in the study of diabetes, including Alloxan. Alloxan is a chemical compound used in the laboratory to induce diabetic animal models (Dhanabal et al., 2007). It is an unstable molecule that is responsible for the necrosis of pancreatic β-islet cells (Lenzen, 2008), which is also confirmed by our islet analysis (Figure 4B). The observed effect can be explained by the fact that alloxan, upon infiltration into the β cells of the pancreatic islets, undergoes reduction to dialuric acid. Thus, the presence of Allx and dialuric acid promotes the production of superoxide radicals (O2•) (El-Alfy et al., 2005). Then, a dismutation of the superoxide radicals results in the formation of hydrogen peroxide (H_2O_2) which is able to react with iron (Fe^{2+}) to produce hydroxyl radicals (OH•), a high oxidizing agent (Szkudelski, 2001). The imbalance in the redox balance, caused by the diabetogenic effect of alloxan, leads to necrosis and death of pancreatic β cells. This occurs through the oxidation process initiated by the production of free radicals (Lenzen, 2008). Indeed, oxidative stress plays a significant role in the development of various disorders, including diabetes (Zhang et al., 2020). In fact, compounds with antioxidant activity have shown potential in slowing the progression of diabetes (El-Alfy et al., 2005), so that islets of Langerhans remain protected from toxicity caused by free radicals. Concerning the antioxidant activity of *A. leucotrichus* aqueous extract, it has been evaluated using different assays. The *in vitro* antioxidant activity of AEAL as assessed by the DPPH assay, demonstrated significant antiradical properties that increased with higher concentrations of the extract. This dose-dependent response suggests that AEAL contains bioactive compounds with potent antioxidant effects among them phenolic compounds.

In addition, AEAL demonstrated a stronger antioxidant capacity in the β-carotene bleaching test. These findings imply that AEAL has the potential to protect β-carotene from oxidation. While the FRAP activity of the AEAL was medium. These results are consistent with several published studies proving the high antioxidant properties of A. leucotrichus (Dahmane et al., 2017; El-Ouady & Eddouks, 2019; Z Louail et al., 2016; Manssouri et al., 2020; Sebaa et al., 2018). In this study, AEAL showed significant antioxidant activity. The preventive effect may be due to its antioxidant effect. The dosage of total phenols and flavonoids in the AEAL showed that it is rich in antioxidant compounds. A study by Karakaya et al. showed a significant linear correlation between total phenolic concentration and antioxidant dose values (Karakaya AA Taş, S, 2001). *A. leucotrichus* is abundant in phenolic compounds, which are renowned for their potent antioxidant properties. These compounds contribute to the high antioxidant activity exhibited by *A. leucotrichus*. Besides, Luteolin-7-O-glucoside and luteolin-O- (malonyl-hexoside), the major phenolic compounds identified in *A. leucotrichus* extracts, there are phenolic acid derivatives and flavones (apigenin and luteolin derivatives) (Ziani et al., 2019), and other compounds that are capable of donating hydrogen to a free radical, leading to the elimination of an odd electron. This characteristic makes it valuable in addressing various pathological conditions associated with free radicals indicating its usefulness in various radical-related pathological conditions; an inhibition pattern being similar at variable concentrations due to its high radical scavenging activity. Several studies have stressed the importance of phenolic compounds as free radical scavengers. Also, their redox characteristics, that act as reducing agents, hydrogen donors, and singlet oxygen quenchers, are primarily responsible for their antioxidant activity. They may also have metal chelating properties (Gülçin et al., 2010; Mradu et al., 2012). In addition, the antidiabetic, antioxidant, and anti-inflammatory effects of luteolin and luteolin-7-O-glucoside were proved (Park et al., 2016; Rauter et al., 2010; Zang et al., 2016).

On the other hand, a study conducted by Louail et al. demonstrated the detection of ferulic acid in both the aqueous and ethanolic extracts derived from fruits (Louail et al., 2020). This compound displays many beneficial effects such as anticarcinogenic, cardioprotective, neuroprotective, hepatoprotective, and anti-inflammatory activities (Jo et al., 2019). Studies have reported that ferulic acid exhibits protective properties, particularly in diabetic rats. It exerts its effects by modulating oxidative stress, influencing the expression of pro-inflammatory cytokines, and regulating apoptosis (Roy et al., 2013). In addition, ferulic acid has demonstrated both antioxidant and antidiabetic effects, leading to the amelioration of liver, kidney, and pancreas damage induced by alloxan-induced diabetes. These beneficial effects are believed to be attributed to its ability to inhibit the pro-inflammatory

factor NF-κB (Ramar et al., 2012).

4. Conclusion

Allx causes redox cycling, which produces reactive oxygen species, causing necrosis in the pancreas. Apoptosis in the pancreas is a symptom of severe oxidative stress, and AEAL stopped it. AEAL lowers the risk of hyperglycemia. The observed beneficial effect of AEAL is believed to be attributed to the synergistic interaction among its naturally occurring bioactive compounds. AEAL may be beneficial in reducing the progression of diabetes mellitus.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Author Contributions

The ideology and design of the study were done by Saliha Bouknana and Mohamed Bnouham. The experimental procedures were done by Saliha Bouknana under the guidance of Mohamed Bnouham. The statistical data and interpretation of the results were done by Saliha Bouknana, Mounia Driouech and Fatima Zahra Lafdil. Abderrahim Ziyyat, Hassane Mekhfi, and Abdelkhaleq Legssyer contributed to preparation of the manuscript.

5. References

Adeyi, A. O., Idowu, B. A., Mafiana, C. F., Oluwalana, S. A., Ajayi, O. L., & Akinloye, O. A. (2012). Rat model of food-induced non-obese-type 2 diabetes mellitus: comparative pathophysiology and histopathology. *International Journal of Physiology, Pathophysiology and Pharmacology*, *4*(1), 51.

Aquino, R., Morelli, S., Lauro, M. R., Abdo, S., Saija, A., & Tomaino, A. (2001). Phenolic constituents and antioxidant activity of an extract of anthurium v ersicolor leaves. *Journal of Natural Products*, *64*(8), 1019–1023.

Berraaouan, A., Ziyyat, A., Mekhfi, H., Legssyer, A., Sindic, M., Aziz, M., & Bnouham, M. (2014). Evaluation of antidiabetic properties of cactus pear seed oil in rats. *Pharmaceutical Biology*, *52*(10), 1286–1290.

Bouknana, S., Daoudi, N. E., Bouhrim, M., Ziyyat, A., Legssyer, A., Mekhfi, H., & Bnouham, M. (2022). Ammodaucus leucotrichus Coss. & Durieu: Antihyperglycemic activity via the inhibition of α-amylase, α-glucosidase, and intestinal glucose absorption activities and its chemical composition. *Journal of Pharmacy and Pharmacognosy Research*, *10*(1), 94–103. https://doi.org/10.56499/jppres21.1141_10.1.94

Chen, G.-L., Chen, S.-G., Xie, Y.-Q., Chen, F., Zhao, Y.-Y., Luo, C.-X., & Gao, Y.-Q. (2015). Total phenolic, flavonoid and antioxidant activity of 23 edible flowers subjected to in vitro digestion. *Journal of Functional Foods*, *17*, 243–259.

Cho, N., Shaw, J. E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohlrogge, A. W., & Malanda, B. (2018). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research and Clinical Practice*, *138*, 271–281.

Dahmane, D., Dob, T., Krimat, S., Nouasri, A., Metidji, H., & Ksouri, A. (2017). Chemical composition, antioxidant and antibacterial activities of the essential oils of medicinal plant Ammodaucus leucotrichus from Algeria. *Journal of EssEntial Oil REsEarch*, *29*(1), 48–55.

Danaei, G., Finucane, M. M., Lu, Y., Singh, G. M., Cowan, M. J., Paciorek, C. J., Lin, J. K., Farzadfar, F., Khang, Y.-H., & Stevens, G. A. (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2· 7 million participants. *The Lancet*, *378*(9785), 31–40.

de la Rosa, L. A., Alvarez-Parrilla, E., & Shahidi, F. (2011). Phenolic compounds and antioxidant activity of kernels and shells of Mexican pecan (Carya illinoinensis). *Journal of Agricultural and Food Chemistry*, *59*(1), 152–162.

Dehpour, A. A., Ebrahimzadeh, M. A., Fazel, N. S., & Mohammad, N. S. (2009). Antioxidant activity of the methanol extract of Ferula assafoetida and its essential oil composition. *Grasas y Aceites*, *60*(4), 405–412.

Dhanabal, S. P., Raja, M. K. M. M., Ramanathan, M., & Suresh, B. (2007). Hypoglycemic activity of Nymphaea stellata leaves ethanolic extract in alloxan induced diabetic rats. *Fitoterapia*, *78*(4), 288–291.

Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews*.

El-Alfy, A. T., Ahmed, A. A. E., & Fatani, A. J. (2005). Protective effect of red grape seeds proanthocyanidins against induction of diabetes by alloxan in rats. *Pharmacological Research*, *52*(3), 264–270.

El-Ouady, F., & Eddouks, M. (2019). Glucose lowering activity of aqueous Ammodaucus leucotrichus extract in diabetic rats. *Cardiovascular & Hematological Disorders Drug Targets*.

Etuk, E. U., & Muhammed, B. J. (2010). *Evidence based analysis of chemical method of induction of diabetes mellitus in experimental rats*.

Gülçin, İ., Huyut, Z., Elmastaş, M., & Aboul-Enein, H. Y. (2010). Radical scavenging and antioxidant activity of tannic acid. *Arabian Journal of Chemistry*, *3*(1), 43–53.

Gupta, S., Chough, E., Daley, J., Oates, P., Tornheim, K., Ruderman, N. B., & Keaney Jr, J. F. (2002). Hyperglycemia increases endothelial superoxide

that impairs smooth muscle cell Na+-K+-ATPase activity. *American Journal of Physiology-Cell Physiology*, *282*(3), C560–C566.

Jo, E.-R., Youn, C. K., Jun, Y., & Cho, S. Il. (2019). The protective role of ferulic acid against cisplatin-induced ototoxicity. *International Journal of Pediatric Otorhinolaryngology*, *120*, 30–35.

Karakaya AA Taş, S, S. N. El. (2001). Antioxidant activity of some foods containing phenolic compounds. *International Journal of Food Sciences and Nutrition*, *52*(6), 501–508.

Lenzen, S. (2008). Oxidative stress: the vulnerable β-cell. *Biochemical Society Transactions*, *36*(3), 343–347.

Louail, Z, Kameli, A., Benabdelkader, T., Bouti, K., Hamza, K., & Krimat, S. (2016). Antimicrobial and antioxidant activity of essential oil of Ammodaucus leucotrichus Coss. & Dur. seeds. *Journal of Materials and Environmental Science*, *7*(7), 2328–2334.

Louail, Zineb, Djemouai, N., Krimate, S., Bouti, K., Bouti, S., Tounsi, H., & Kameli, A. (2020). *Biological activities of different extracts of Ammodaucus leucotrichus subsp. leucotrichus* Cosson & Durieu from Algerian Sahara.

Ludwig, D. S., & Ebbeling, C. B. (2018). The carbohydrate-insulin model of obesity: beyond "calories in, calories out." *JAMA Internal Medicine*, *178*(8), 1098–1103.

Manssouri, M., Znini, M., & Majidi, L. (2020). Studies on the antioxidant activity of essential oil and various extracts of Ammodaucus leucotrichus Coss. & Dur. Fruits from Morocco . *Journal of Taibah University for Science*, *14*(1), 124–130. https://doi.org/10.1080/16583655.2019.1710394

Mohammedi, H., Idjeri-Mecherara, S., Menaceur, F., Azine, K., & Hassani, A. (2018). Chemical compositions of extracted volatile oils of ammodaucus leucotrichus L. fruit from different geographical regions of Algeria with evaluation of its toxicity, anti-inflammatory and antimicrobial activities. *Journal of Essential Oil Bearing Plants*, *21*(6), 1568–1584.

Mradu, G., Saumyakanti, S., Sohini, M., & Arup, M. (2012). HPLC profiles of standard phenolic compounds present in medicinal plants. *International Journal of Pharmacognosy and Phytochemical Research*, *4*(3), 162–167.

Oberley, L. W. (1988). Free radicals and diabetes. *Free Radical Biology and Medicine*, *5*(2), 113–124.

Park, M., Zhu, Y. X., Pae, H., & Park, S. H. (2016). In Vitro and In Vivo α-Glucosidase and α-Amylase Inhibitory Effects of the Water Extract of Leaves of Pepper (Capcicum Annuum L. Cultivar Dangjo) and the Active Constituent Luteolin 7-O-Glucoside. *Journal of Food Biochemistry*, *40*(5), 696–703.

Pupim, L. B., Heimburger, O., Qureshi, A. R., Ikizler, T., & Stenvinkel, P. (2005). Accelerated lean body mass loss in incident chronic dialysis patients with diabetes mellitus. *Kidney International*, *68*(5), 2368–2374.

Ramar, M., Manikandan, B., Raman, T., Priyadarsini, A., Palanisamy, S., Velayudam, M., Munusamy, A., Prabhu, N. M., & Vaseeharan, B. (2012). Protective effect of ferulic acid and resveratrol against alloxan-induced diabetes in mice. *European Journal of Pharmacology*, *690*(1–3), 226–235.

Rauter, A. P., Martins, A., Borges, C., Mota-Filipe, H., Pinto, R., Sepodes, B., & Justino, J. (2010). Antihyperglycaemic and protective effects of flavonoids on streptozotocin–induced diabetic rats. *Phytotherapy Research*, *24*(S2), S133–S138.

Roy, S., Metya, S. K., Sannigrahi, S., Rahaman, N., & Ahmed, F. (2013). Treatment with ferulic acid to rats with streptozotocin-induced diabetes: effects on oxidative stress, pro-inflammatory cytokines, and apoptosis in the pancreatic β cell. *Endocrine*, *44*(2), 369–379.

Schulze, P. C., Yoshioka, J., Takahashi, T., He, Z., King, G. L., & Lee, R. T. (2004). Hyperglycemia promotes oxidative stress through inhibition of thioredoxin function by thioredoxin-interacting protein. *Journal of Biological Chemistry*, *279*(29), 30369–30374.

Sebaa, A., Marouf, A., Kambouche, N., & Derdour, A. (2018). Phytochemical composition, antioxidant and antimicrobial activities of Ammodaucus leucotrichus fruit from Algerian Sahara. *Oriental Journal of Chemistry*, *34*(1), 519.

Sofowora, A. (1996). Research on medicinal plants and traditional medicine in Africa. *The Journal of Alternative and Complementary Medicine*, *2*(3), 365–372.

Son, S. M. (2012). Reactive oxygen and nitrogen species in pathogenesis of vascular complications of diabetes. *Diabetes & Metabolism Journal*, *36*(3), 190–198.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, *50*(6), 537–546.

Szkudelski, T., Kandulska, K., & Okulicz, M. (1998). Alloxan in vivo does not only exert deleterious effects. *Physiological Research*, *47*, 343–346.

Waisbren, B. A. (1948). Alloxan diabetes in mice. *Proceedings of the Society for Experimental Biology and Medicine*, *67*(2), 154–156.

Zang, Y., Igarashi, K., & Li, Y. (2016). Anti-diabetic effects of luteolin and luteolin-7-O-glucoside on KK-A y mice. *Bioscience, Biotechnology, and Biochemistry*, *80*(8), 1580–1586.

Zhang, P., Li, T., Wu, X., Nice, E. C., Huang, C., & Zhang, Y. (2020). Oxidative stress and diabetes: antioxidative strategies. *Frontiers of Medicine*, *14*(5), 583–600.

Ziani, B. E. C., Rached, W., Bachari, K., Alves, M. J., Calhelha, R. C., Barros, L., & Ferreira, I. C. F. R. (2019). Detailed chemical composition and functional properties of Ammodaucus leucotrichus Cross. & Dur. and Moringa oleifera Lamarck. *Journal of Functional Foods*, *53*, 237–247.