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Defatted Hydroethanolic Extract of *Ammodaucus leucotrichus* Cosson and Durieu Seeds: Antidiabetic and Anti-Inflammatory Activities

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Abstract: The seeds of *Ammodaucus leucotrichus* Cosson and Durieu have been used in the North African Sahara as a traditional medicine to treat diabetes. The present study investigates the antidiabetic, antihyperglycemic, and anti-inflammatory properties of the defatted hydroethanolic extract of *Ammodaucus leucotrichus* (DHEAM). The antidiabetic and the antihyperglycemic studies were assessed on alloxan-induced diabetic with orally administered doses of DHEAM (100 and 200 mg/kg). At the same time, its anti-inflammatory propriety was evaluated by measuring edema development in the Wistar rats paw induced with carrageenan. Treatment of diabetic mice with DHEAM for four weeks managed their high fasting blood glucose levels, improved their overall health, and also revealed an excellent antihyperglycemic activity. Following the anti-inflammatory results, DHEAM exhibited a perfect activity. HPLC results revealed the presence of seven molecules (chlorogenic acid, 3-p-coumaroylquinic acid, gallic acid, ferulic acid, myricetin, quercetin, luteolin). This work indicates that the DHEAM has an important antidiabetic, antihyperglycemic, and anti-inflammatory effect that can be well established as a phytomedicine to treat diabetes.

Keywords: diabetes mellitus; *Ammodaucus leucotrichus* Cosson and Durieu; anti-inflammatory; antidiabetic; alloxan monohydrate; glibenclamide

1. Introduction

Diabetes mellitus is a complicated metabolic disease that induces elevated blood sugar levels, mainly correlated to insulin action, which is a hormone responsible for the management of blood sugar and use of energy in the body. In diabetes, the body loses the ability to produce insulin normally or fails to use it correctly and efficiently [1]. There are mainly two types of diabetes. The first diabetes type is caused by an immune system attack towards the insulin producing cells in the pancreas (five percent of all types of diabetes), impacting insulin's normal development. In contrast, Diabetes's second type is the consequence of a progressive diminution of cells's sensitivity to insulin action, resulting in excessive amounts of sugar building up in the body blood circulation [2].

The IDF (International Diabetes Federation) estimates that the occurrence of diabetes has grown unbelievably, from about 420 million individuals worldwide in 2017 to more than 700 million by 2015 [3]. The condition of diabetes comes up with several challenging changes in the body. By triggering the oxidative stress via free radicals production, the amount of antioxidants in the body is decreased, leading to cell damage, apoptosis, and activation of the inflammatory process, all of which contribute to very severe health problems [4]. Researchers have come up with several synthetic medications over the years that can help restore blood glucose levels on one side and avoid problems on the other. With chronic use, adverse effects such as hypoglycemia, obesity, hepatopathy, and others have led the researcher's focus to shift and look into new, safer alternatives [5,6].

Medicinal plants tend to be a primary source of curative and preventive human therapy preparations, often used to obtain important bioactive compounds [7]. More than 80 percent of the global population, particularly in third-world countries, relies on conventional medicines and health products daily, which, according to them, are as effective as classic medications with almost no side effects [8]. Among the colossal plant diversity, one endemic plant from the North African Saharan and Sub-saharan countries, *Ammodaucus leucotrichus* Cosson and Durieu (Apiaceae, Umbelliferae)—locally known as "Kamounn es-sofi" in Morocco—is a medicinal herb with various culinary uses by native peoples against many diseases [9].

Most research and studies on *A. leucotrichus* seeds activities are associated with the essential oil and apolar compounds [10], however, few research experiments on polar compounds and their functional properties have been carried out. This study investigates the antidiabetic, antihyperglycemic, and anti-inflammatory activities of the defatted hydroethanolic extract of *Ammodaucus leucotrichus* Cosson and Durieu on different in vivo models.

2. Materials and Methods

2.1. Animals

Wistar rats (180 and 230 g) and Swiss albino mice (21 and 26 g) of both sexes were acquired from the department of biology of the FSDM, Fez. They were kept in comfortable and adequate cages with unrestricted access to food and water. Experiments regarding animals followed internationally accepted guidelines for the use, care, and handle of laboratory animals [11], And the institutional animal ethical committee instructions (02/2020/LBEAS).

2.2. Plants

The seeds of *Ammodaucus leucotrichus* Cosson and Durieu were purchased from a local herbalist and were taxonomically identified by a qualified botanist (professor Amina Bari, FSDM, Fez, Morocco). a voucher specimen (BPRN12) was deposited in the herbarium of the LBEAS Laboratory.

2.3. Preparation of Defatted Hydroethanolic Extract

The seeds of *A. leucotrichus* were vigorously washed and air-dried at room temperature, then reduced by an electric grinder to a ground powder. Before embarking on the extraction, the seeds were defatted by washing each 10 g of the seeds powder with 30 mL Hexane 3 times. In an ultrasound-assisted apparatus, the extraction begins by adding 20 g of plant powder to 200 mL of 70% ethanol for 40 min (frequency: 35 kHz) then filtered, concentrated and stored at 4 °C until future use. The doses of 100 and 200 mg/kg were chosen after multiple toxicity tests and pharmacological investigation, indicating that those doses are the lowest most effective doses.

2.4. Extract Phytochemical Analysis

HPLC-DAD was performed to analyze the hydroethanolic extract of *A. leucotrichus*. The apparatus used was a 1260 infinity II (Agilent) coupled to a UV detector at 280 nm. The separation column was a C18 zorbax eclipse plus (5 µm, 4.6 × 150 mm). The mobile phase was composed of A: acidified water

(Formic acid) 0.1% and B: acetonitrile. In the first 0 to 40 min, the gradient used was: 50% A, 50% B; from 40 to 45 min: 40% A, 60% B; and from 45 to 60 min: 100% B. The flow rate was set at 1 mL/min, and the injection volume was 10 µL.

2.5. Evaluation of Antidiabetic Activity

The DHEAM antidiabetic subacute study (28 days) was assessed on alloxan-induced diabetic mice. The extract antihyperglycemic activity was also evaluated. The bodyweight measurements were taken weekly, and biochemical parameters were measured at the end of the study. The animals were monitored for any toxicity or side effect signs and to estimate the effect of the extract on their overall diabetic status.

2.5.1. Induction of Experimental Diabetes

Diabetes was induced experimentally following a modified version of Berraaouan et al. protocol [12] by an injection of alloxan monohydrate. The animals were fasted 12 h prior to injection and alloxan monohydrate was freshly prepared and injected intraperitoneally at the dose of 180 mg/kg. A glucose preparation (0.2 mL/4 g/L concentration) was given orally to the mice after the injection to avoid hypoglycemic shock following the alloxan action. On the 4th day after the induction, the FBG (Fasting blood glucose) was measured, and animals with a FBG above 450 mg/dL were selected.

2.5.2. Groups Repartitions and Design of the Experiment

The experiment design included five different groups with five mice each.

Group 1: Mice treated with distilled water (0.2 mL/day)
Group 2: Diabetic mice treated with distilled water (0.2 mL/day)
Group 3: Diabetic mice treated with glibenclamide (2 mg/kg/day)
Group 4: Diabetic mice treated with DHEAM (100 mg/kg/day)
Group 5: Diabetic mice treated with DHEAM (200 mg/kg/day)

During the 28 days, treatments were administered daily to the test mice by intragastric gavage. On days 1, 7, 14, 21 and 28, after 12 h fast, the fasting blood glucose level was estimated using a glucometer (Accu-chek) (Blood was collected from the tail vein).

On the last day of the study, weekly weight measurements were performed, and mice were sacrificed by cervical decapitation following anesthesia to collect blood to estimate biochemical parameters.

2.5.3. Antihyperglycemic Activity Assessment

The treatments were given directly after the first assessment on the 1st day and the groups' distribution. After 1, 1.5, 3, 6 and 12 h of treatment administration, a series of blood glucose level measurements were carried out for the groups taking DHEAM and glibenclamide to see how these treatments can control the hyperglycemic state of mice during this time.

2.6. The Potential Anti-Inflammatory Evaluation

Following winter et al. protocol on paw edema induction with carrageenan [13], the anti-inflammatory activity was assessed. Edema was caused by a subplantar injection of a 100 µL prepared solution of carrageenan (1%) into each rat's right-hind paws. The rats were split into five groups, each having five rats.

Group 1: Normal control rat without induced edema treated with distilled water (0.2 mL)
Group 2: Negative control rat with induced edema treated with distilled water (0.2 mL)
Group 3: Positive control rat with induced edema treated with diclofenac (15 mg/kg)
Group 4: Rat with induced edema treated with DHEAM (100 mg/kg)
Group 5: Rat with induced edema treated with DHEAM (200 mg/kg)

All groups received their treatments orally one hour prior to carrageenan injection and after 12 h of food deprivation. Before the carrageenan injection, the thickness of the rat paw was measured and taken as a reference. Measurements were taken after injection at the 1st, 2nd, 3rd, 4th and 6th hour. The paw edema development is calculated based on the paw thickness reference and the thickness at the respective hours.

The extract's anti-inflammatory effect was calculated as a percentage decrease in the edema formation compared to the negative control group.

2.7. Statistical Analysis

Graph Pad Prism version 8.0 for Windows was used to perform all the statistical analyses. Values are expressed as the mean ± SD, and the difference between groups was assessed by one-way analysis of variance (ANOVA).

3. Results

3.1. HPLC Analysis

Peak detection is initially achieved by comparing the unknown component's retention time to that of the standard. Seven compounds were revealed during the HPLC-DAD analysis of the hydroethanolic extract of *A. leucotrichus* (Figure 1 and Table 1): Chlorogenic acid, 3-p-coumaroylquinic acid, gallic acid, ferulic acid, myricetin, quercetin, luteolin. Those compounds all belong to the polyphenol family.

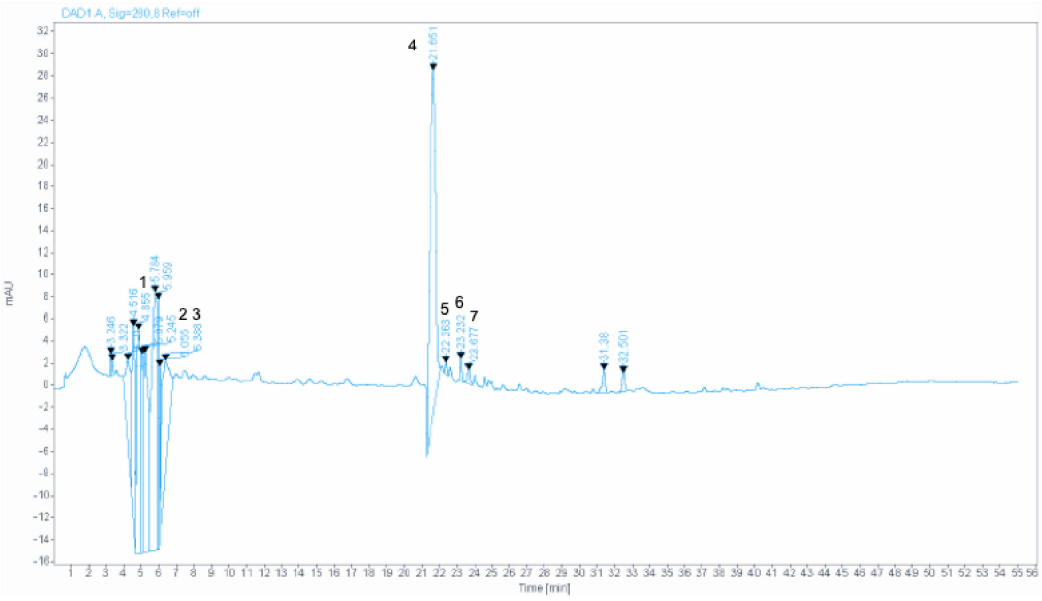

Figure 1. HPLC-DAD chromatogram of hydroethanolic extract of *Ammodaucus leucotrichus* Cosson and Durieu. (1) Chlorogenic acid, (2) 3-p-coumaroylquinic acid, (3) gallic acid, (4) ferulic acid, (5) myricetin, (6) quercetin, (7) luteolin.

Table 1. DHEAM revealed compounds and their retention time.

Compounds	Formula	Standards Retention Time (min)
Chlorogenic acid	$C_{16}H_{18}O_9$	4.85
3-p-Coumaroylquinic acid	$C_{16}H_{18}O_8$	6.00
Gallic acid	$C_7H_6O_5$	6.645
Ferulic acid	$C_{10}H_{10}O_4$	21.63
Myricetin	$C_{14}H_{28}O_2$	22.27
Quercetin	$C_{15}H_{10}O_7$	23.35
Luteolin	$C_{15}H_{10}O_6$	23.70

3.2. Subacute Antidiabetic Study

Figure 2 shows the effect of prolonged administration of DHEAM (100 and 200 mg/kg per day) on the FBG level of alloxan-induced diabetic mice.

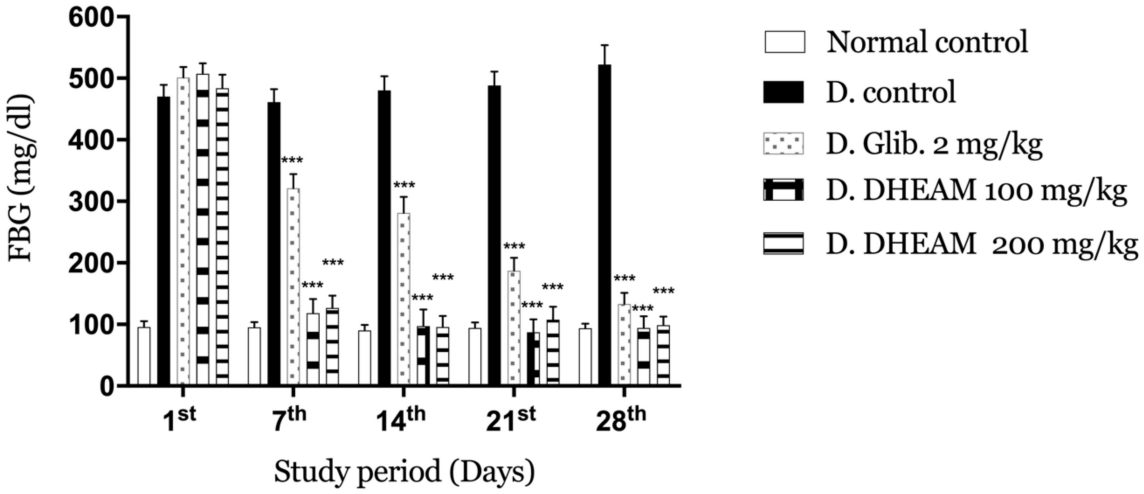

Figure 2. The effect of DHEAM on mice FBG (Fasting blood glucose) during the four weeks of the study. Values are expressed as mean ± SD (n = 5 mice). *** $p < 0.001$ compared to diabetic control.

From the first week of the study, the two doses of DHEAM successfully controlled the diabetic status, prevented hyperglycemia, and sustained a rapid and substantial reduction ($p < 0.001$) (day 7: 36.36%, 74.40%, 72.67%; day 14: 41.46%, 79.79%, 80.20%; day 21: 61.68%, 82.17%, 78.48%; day 28: 74.52%, 82.00%, 81.22%, for glibenclamide, doses 100 and 200, respectively) calculated in comparison to the negative control group.

Table 2 outlines the evolution of bodyweight and the biochemical parameters of the different study groups. Throughout the experimental period, a significant decrease in bodyweight was observed in the untreated diabetic group as opposed to the normal group, and at the end of the fourth week, this drop was very significant ($p < 0.001$). The diabetic groups treated with DHEAM and glibenclamide (2 mg/kg) for four weeks significantly improved in their bodyweight compared to the diabetic control group ($p < 0.001$).

The ASAT (aspartate aminotransferase) and ALAT (alanine aminotransferase) levels were highly elevated in diabetic control mice compared with the normal control group. The effect of the administration of DHEAM (100 and 200 mg/kg bodyweight) to alloxan-induced diabetic mice for 28 days decreases their activities significantly compared to the diabetic control group. An increased level of urea and creatinine were also noted for the alloxan-induced non treated diabetic mice after four weeks as compared to the normal control group. However, the treatment of diabetic mice with DHEAM significantly reduced their creatinine and urea levels compared to the diabetic group.

Figure 3 demonstrates the DHEAM's antihyperglycemic activity. Compared to the positive control (Glibenclamide), the first hour didn't show a significant reduction in BGL (Blood glucose level). Starting from the hour and a half, the extract BGL reached the positive control values until the sixth hour (281 ± 17.8 mg/dL (Positive control) 300 ± 23.0 mg/dL for DHEAM at 100 mg/kg and 254 ± 26.0 mg/dL for DHEAM at 200 mg/kg). After 12 h, the positive control blood glucose values increased against a significant decrease ($p < 0.001$) in those with DHEAM doses, which reflect the long-term effect and the power of the extract.

Table 2. Effect of DHEAM on bodyweight development and biochemical parameter.

Treatment	Bodyweight Development (g)					Biochemical Parameter			
	1st Day	1st Week	2nd Week	3rd Week	4th Week	ASAT(U/L)	ALAT(U/L)	Urea (g/L)	Creatinine (mg/L)
Normal control	23.4 ± 2.3	24.7 ± 2.5 *	25.2 ± 2.4 ***	26.9 ± 2.3 ***	27.2 ± 2.5 ***	253 ± 23.73	45 ± 7.58	0.24 ± 0.04	3.2 ± 0.44
Diab. Control	23.8 ± 2.1	21.7 ± 3.2	20.1 ± 2.7	19.2 ± 2.4	17.7 ± 2.2	502 ± 38.85	134 ± 11.26	0.63 ± 0.06	5.8 ± 0.83
Diab. Glib. 2 mg	24.3 ± 2.6	23.1 ± 1.6	24.2 ± 2.3 **	25.8 ± 2.5 ***	25.9 ± 1.77 ***	298 ± 24.66 ***	77 ± 8.80 ***	0.28 ± 0.04 ***	4.2 ± 0.44 **
Diab. DHEAM 100 mg/kg	22.8 ± 1.7	23.1 ± 1.6	24.3 ± 1.7 **	25.7 ± 1.8 ***	27.2 ± 1.77 ***	223 ± 21.42 ***	51 ± 6.58 ***	0.35 ± 0.05 ***	3.80 ± 0.46 ***
Diab. DHEAM 200 mg/kg	23.6 ± 1.9	23.5 ± 2.3	24.4 ± 1.9 **	25.4 ± 2.2 ***	26.4 ± 2.43 ***	286 ± 25.01 ***	45 ± 6.92 ***	0.34 ± 0.04 ***	3.70 ± 0.32 ***

Values are expressed as mean ± SD (n = 5 mice). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to diabetic control.

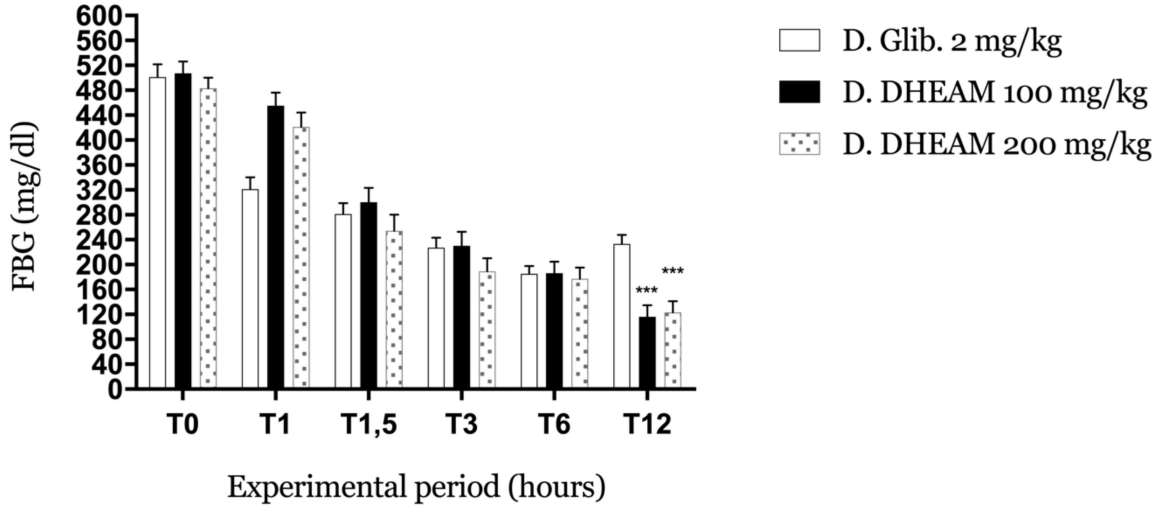

Figure 3. DHEAM effect over the study duration of 12 h on the hyperglycemic state of Alloxan-induced diabetic mice. Values are expressed as mean ± SD (n = 5 mice). *** $p < 0.001$ compared to diabetic control.

3.3. The Anti-Inflammatory Potential of DHEAM

A progressive edema formation is due to the injection of carrageenan into the rat's hind paw, reaching its maximum within the first 60 min. The inhibition rate of the treatments is shown in Figure 4. Within the first hour, the inhibition rate reached 21% for the group of rats treated with DHEAM at 100 mg/kg, 39% for the group treated with a dose of 200 mg/kg against 42% marked for the groups treated with diclofenac. The inhibition rate continues to rise, reaching at the 3rd hour 36%, 66% and 57% for groups treated with DHEAM at 100, 200 mg/kg and diclofenac, respectively; those numbers set on a final inhibition rate of 76%, 84 and 95% at the end of the test.

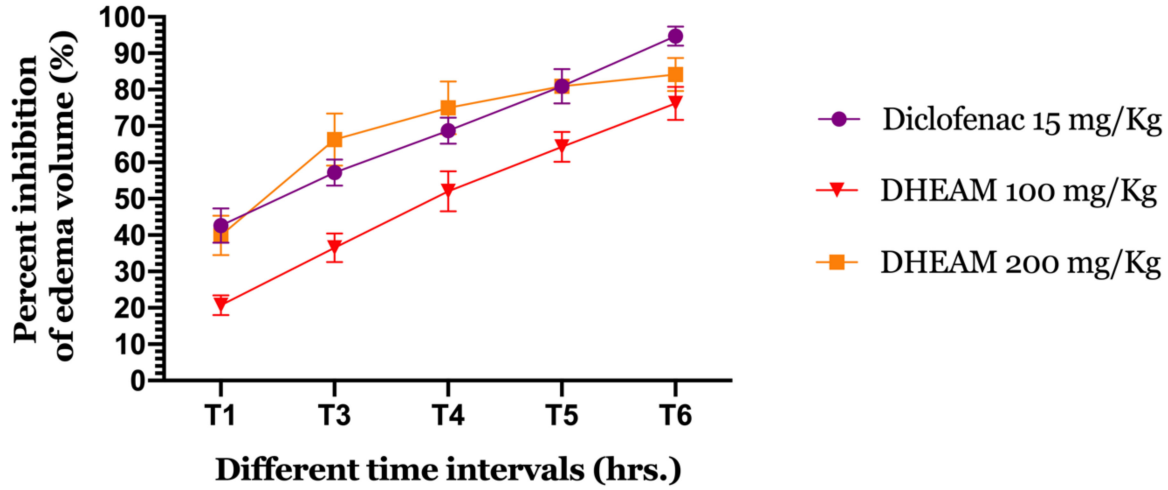

Figure 4. Effect of DHEAM on edema volume inhibition in carrageenan-induced paw edema on Wistar rats.

4. Discussion

As the essential oil, the nonpolar fraction of *Ammodaucus leucotrichus* Cosson and Durieu was the most exploited, this study was intended to focus on the polar part of the plant. The aerial parts of *A. leucotrichus* are known to have cytotoxic activity against multiple tumor cell lines: NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), and MCF-7 (breast carcinoma) [14] and also antioxidant proprieties [15].

The HPLC-DAD analysis revealed the presence of seven polyphenolic compounds; some of them are well known for their antidiabetic and anti-inflammatory activity, namely chlorogenic acid [16], gallic acid [17,18], ferulic acid [19], myricetin [20], quercetin [21], luteolin [22]. Polyphenols are popular in food, drinks, and herbs [23], with over thousands of well-known compounds [24]. All of them are recognized for their wellness benefits. They serve many roles, including anti-cancer, antiviral, anti-allergic, antibacterial, and anti-inflammatory activities [25]. They exert their antidiabetic action via numerous pathways and multiple moleculer targets by controlling the signaling, secretion, and action of insulin, regulating the digestion of carbohydrates, and the control of glucose level in the body. They also regulate the activity of β-cells by promoting their proliferation and insulin secretion [26,27].

Polyphenols in the ingested matrix must proceed into the circulatory system from the gut lumen in order to be absorbed. Until absorption may take place, the attached sugar must be removed. Typically, in the small intestine and liver, most polyphenols undergo sulfation, methylation, and glucuronidation [28], and conjugated metabolites can be found in the plasma after ingestion [29].

Polyphenols can cross biological membranes according to their size, hydrophobicity, and intracellular reactions that facilitate diffusion through the preservation of a gradient of concentration.

The pharmacokinetics of polyphenols vary considerably across various groups, as well as amongst other conjugates of the same compound in a given class, and depend on different parameters. Some may be processed very fast, and some may be very slow, affecting bioavailability.

This research represents the first study on the antidiabetic activity of the defatted hydroethanolic extract of *A. leucotrichus* on alloxan-induced diabetic mice. DHEAM administration for diabetic mice over four weeks resulted in a considerable drop and a stabilization in the FBG compared to the untreated diabetic mice and glibenclamide (positive control), the standard drug used in experimental diabetes studies. The antihyperglycemic activity of DHEAM extract was observed over the next 12 h following the first administration of the treatments to determine the effect during the time of the extract. The results indicate a long-lasting effect of the DHEAM extract, suggesting excellent management of the blood glucose level and glucose utilization by the body tissues.

Diabetic mice showed a substantial drop in bodyweight related to hyperglycemia as a result of the body's inability to use the glucose accumulated in the bloodstream and the usage of the fat and structural proteins instead as a source of energy [30]. That shows the vital role of insulin in regulating energy utilization by the body and protein synthesis and proteolysis [31]. Oral administration of DHEAM and glibenclamide to diabetic mice for 28 consecutive days increased their bodyweight, demonstrating better regulation of their hyperglycemic state. ASAT and ALAT high levels indicate a hepatic cell leakage and loss of its functional membrane integrity, which implies hepatocellular injury [32]. In the present study, elevated serum levels of ASAT and ALAT were noticed in the diabetic control group regarding the normal ones. However, diabetic groups treated with DHEAM reported a substantial decrease in ASAT and ALAT, indicating of potential hepatoprotective effect exhibited by the DHEAM extract. High levels of urea and creatinine in the blood are known as renal injury indicators [33]. Diabetic mice also demonstrated high serum levels of urea and creatinine that may indicate a renal function impairment. Administration of DHEAM to diabetic mice significantly decreased creatinine and urea levels, suggesting a possible preventive effect of the extract against kidney damage.

The anti-inflammatory activity of DHEAM is proved in carrageenan-induced hind paw edema. It is a standard experimental model for the study of acute inflammation by using carrageen to induce inflammatory mediators' release. According to vinegar et al. [34], carrageenan causes biphasic reactions during the formation of edema. The first phase is marked by histamine release, serotonin, and kinins in the first hour after injection. In the second phase, prostaglandins are released over the next two to three hours. Prostaglandins are the main culprit responsible for acute inflammation [35]. DHEAM reveals a promoting anti-inflammatory effect as it exhibited a good activity in the first phase as well as the second phase of the inflammatory process. The results were in accordance with a previous work of

Ziani et al. [14], indicating that the plant extract reduced the expression of ant-inflammatory enzymes and nitric oxide (NO) levels.

5. Conclusions

We conclude that hydroethanolic extract of *Ammodaucus leucotrichus* Cosson and Durieu seeds has a remarkable antidiabetic, antihyperglycemic, and anti-inflammatory activity, having a major role in managing diabetes and its complication. This research further encourages using this plant in conventional antidiabetic preparations and the formulations in a well-established antidiabetic phytomedicine.

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