



Biological And Immunological Effects of Ammi Visnaga Seeds on Diabetic Rats

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ABSTRACT:

Ammi visnaga seeds have potential immune-modulating effects, where they increase white blood cell counts and enhance antibody production. The seeds also exhibit antioxidant and anti-inflammatory properties, which are key to a healthy immune response.

Aim: This study aimed to determine the biological and immunological effects of Ammi visnaga seeds on diabetic rats.

Materials and Methods: Thirty-six male Sprague Dawley rats were divided into normal (n=6) and diabetic (n=30). The second group was injected with streptozotocin (at a dose of 75 mg/ kg intraperitoneally), and those with blood glucose concentrations more than 250 mg/dL were classified as diabetic. Rats were divided into six groups, (1) non-diabetic group (n=6) received distilled water (2 mL/ day); (2) diabetic control group (n=6) received distilled water (2 mL/ day); (3) diabetic group (n=6) received basal diet +5% Ammi visnaga; (4) diabetic group (n=6) received basal diet +10% Ammi visnaga; (5) diabetic group (n=6) received basal diet +15% Ammi visnaga. (6) diabetic group (n=6) received a basal diet +20% Ammi visnaga. After 28 days, blood was collected, and serum was extracted to determine Blood Parameters (RBC, Hgb, MCV, RDW, HCT, MCH, MCHC, WBC, LYM)

Results: At the end of the experiment, the best group for WBC, LYM, PLT, MID & GRAN was that of the group 6 (20% Ammi visnaga).

Conclusion: Ammi visnaga seeds hold significant potential as a natural aid in boosting immune health and promoting overall well-being. These findings suggest that Ammi visnaga seeds may hold promise for developing new treatments for human diabetes.

Introduction

Ammi visnaga (*A. visnaga*) has immunostimulatory activity, with studies showing it enhances antibody production in mice and possesses immunomodulatory properties. Its extracts, particularly those rich in the furanochromones khellin and visnagin, have shown promise in boosting the immune response. This is supported by *in silico* studies that show a robust affinity among visnagin or khellin and key immunomodulatory proteins. (Beltagy et al., 2015) Ammi visnaga L. (*Visnaga daucoides* Gaertn., Family Apiaceae), commonly referred to as Khella Baldi or toothpick weed, is a biennial or annual herb native to the Mediterranean area of North Africa, Europe, and Asia. The plant has historically been utilized in traditional medicine. Currently, it is utilized in modern medicine to manage numerous ailments, like coronary insufficiency and renal colic, and serves as an antifungal, antioxidant, and antibacterial agent, exhibiting a larvicidal influence

on mosquito larvae. Peer-reviewed investigations indicate that these pharmacological actions result from its valuable chemical contents, primarily important oils, polyphenolic chemicals involving flavonoids, and γ -pyrones, primarily visnagin and khellin (Fatimah et al., 2019). *A. visnaga* is known by various prevalent names in Arabic countries, like Khella, Khella baladi, Khellakl, Khellah, Kella, Chellah, Kammon habashi, Gazar sheitani, Kulla, Bizer Al-Khilla, and Swak Al-Nabi. In Turkey, it is referred to as kilir, hiltan, and disotu. In England, it is referred to as Toothpick and Pick tooth. In France, it is referred to as Herbe aux cure-dents, whereas in Germany, it is called Zahnstocherkraut. The Berber refers to it as Tabellaout (Albrahim et al., 2020). Ammi visnaga is an herbaceous annual or occasionally biennial plant that grows a height of up to 1.3 m and a diameter of the green aerial parts up to 1.2 m. The root is a cylindrical taproot and exhibits a light brown color. The root is



either straight or slightly curved, growing in a vertical direction. The root surface exhibits remnants of secondary fibrous rootlets (Hossain et al., 2020). The chemical parts of Ammi visnaga L are well recognized and were published by multiple researchers in different research over the years. Prior research has documented numerous constituents in Ammi visnaga L, such as γ -flavonoids, pyrones, coumarins, as well as important oils. The quality & quantity of these 2nd metabolites are based on specific plant portion analyzed, & the growing conditions, & the application of any bioregulators. (Kamal et al., 2022). The chemical contents of A. visnaga are extensively studied and were documented via multiple studies in multiple investigations over the years. Prior research has documented numerous chemical compounds in A. visnaga, involving γ -pyrones, flavonoids, coumarins, and important oils. The quantity and quality of these 2nd metabolites are based upon the specific plant part examined, the growing conditions, and the application of any bioregulators (Bdewi et al., 2020). The anti-inflammatory properties of A. visnaga were examined, showing that its visnagin content correlates with a reduction in mRNA expression and the secretion of TNF- α , IFN γ , and IL-1 β . Furthermore, visnagin diminished LPS-induced mRNA levels of IL-6 and MCP-1, indicating that its anti-inflammatory property may result from the suppression of transcription factors like NF- κ B and AP-1. Furthermore, visnagin exhibited a neuroprotective effects by inhibiting kainic acid-induced pathogenesis in the brain, and these neuroprotective effects are related to its anti-inflammatory properties (Aourabi et al., 2018). A. visnaga total and protein extracts exhibited immunostimulatory properties. Extracts have been evaluated utilizing an MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) assay on splenocytes, both with and without activation via concanavalin-A (Con-A), a mitogenic agent utilized as a positive control. This may clarify the conventional application of this plant (Alejandro et al., 2020). Antibody production A. visnaga extract significantly increased. Also, humoral response enhancement, where 60% hydroethanolic extract of A. visnaga enhanced the humoral immune response, increasing the hemagglutination antibody (HA) titer. also, A. visnaga can have immunomodulatory effects, which are linked to its antioxidant properties. Molecular revealed that

khellin and visnagin, compounds found in A. visnaga, have a strong affinity for immunomodulatory proteins like p52, NF- κ B, and TNF- α (Benabderrahmane et al., 2023)

2- AIM OF STUDY: -

This research aimed to define the biological and immunological influences of Ammi visnaga seeds on diabetic rats

3- MATERIALS AND METHODS: -

A- Source of Ammi visnaga : Ammi visnaga were purchased from Al-Baha City, KSA, local market, washed, cleaned, blended, & ground into fine powder utilizing an electric grinder. To reduce oxidation, they were stored in dark-stoppered glass bottles until ready to be used. according to (Russo, 2001).

B-Rats: Thirty-six male Sprague Dawley mice (number=thirty-six) each weighing between 150 and 170 grams, have been attained from the Animal Unit of Egypt's Ministry of Health's at Helwan Farm. For 2 weeks, the mice have been housed individually in plastic cages under controlled conditions, with a temperature of twenty-two degrees Celsius and a twelve-hour light/dark cycle at the Faculty of Home Economics, Menoufia University, Egypt. The mice had free access to water and food. Every experiment followed the National Institutes of Health's Guiding Principles for Animal Care and Use. Following a 2-week of acclimatization period, mice have been weighed and randomly assigned to 1 of 2 groups: diabetic (30 mice) and normal (6 mice)

C- Induction of Diabetes (T1DM): Following a 2-week acclimatization period for the mice, type 1 diabetes mellitus has been caused through intraperitoneal injections of Streptozotocin (STZ) as previously defined. The mice received an intraperitoneal injection of seventy-five milligrams per kilogram of Streptozotocin (Sigma-Aldrich, St. Louis, MO, United States of America). After this, each rat had an 8-hour fast, after which blood samples have been collected from the retro-orbital veins to measure blood glucose levels. The research involved diabetic mice with blood glucose levels exceeding 250 milligrams per deciliter. After excluding mice with blood glucose levels under 250 milligrams per deciliter and those who were died, 24 mice have been involved into the research



and then developed diabetes. Furthermore, diabetic mice

D- Diets:

- **Basal diet:** The basal diet comprises protein (ten percent), corn oil (ten percent), choline chloride (0.2 percent), cellulose (five percent), combination of vitamin (1%), salt combination (four percent) (**Hegsted et al., 1941**), & corn starch (to one hundred percent). in accordance with **AIN (1993)**

E- Experimental Design

The research involved all normal (six mice) and diabetic (thirty mice). As well as the experimental procedure, all mice included in this study received the standard diet. The suggested therapies have been administered orally once daily. **AIN, (1993)** The weights of the mice have been documented and the diabetic mice were separated into experimental groups accordingly. The experimental groups were as follows:

- 1- The non-diabetic group (ND-Gr) comprised six normal mice, each administered two milliliters of distilled water orally once per day.
- 2- The diabetic control group (DC-Gr) comprised six diabetic mice, each administered two milliliters of distilled water orally once per day.
- 3- The diabetic group (DC-Gr), comprising six mice, was administered basal diet + 5% Ammi visnaga
- 4- The diabetic group (DC-Gr), consisting of six mice, was administered basal diet + 10% Ammi visnaga
- 5- The diabetic group (DC-Gr), consisting of six mice, was administered basal diet + 15% Ammi visnaga
- 6- The diabetic group (DC-Gr), consisting of six mice, was administered basal diet + 20% Ammi visnaga

F- Complete blood count (CBC) test

The test is involved WBC count, HB, RBC count, WBC& Platelet count. The outcomes of CBC are produced through greatly automated electronic and

pneumatic analyzers depend on aperture-impedance and/or laser beam cell sizing and counting based on **Jacobs et al., (2001)**.

G- Lymphocyte transformation test

• Separation of lymphocytes

This carried out according to **Boyum (1968) & Burrels and Wells (1977)**, utilizing sterile Pasteur pipettes, the heparinized blood has been layered carefully on the surfaces of lymphocyte separation medium (Ficoll hypaque (1.1) within a 50ml sterile polystyrene centrifuge tube). The tube has been centrifuged for forty minutes at 2400 rpm in a cooling centrifuge at +4°C. The mononuclear leukocytes, which formed a band at the ficoll plasma interface have been carefully aspirated using a sterile Pasteur pipette and transferred into a clean sterile centrifuge tube containing cold RPMI-1640. The separated buffy coat has been rinsed with RPMI-1640 medium three times each for ten minutes at 2400 Rpm, 2000 rpm, and 1500 rpm correspondingly. The last washing was H applied to eliminate any remnants of thrombocytes. Following the last washing, the sediment rinsed lymphocytes have been resuspended in 1 ml RPMI-1640 medium comprising ten percent of foetal calf serum.

H- Statistical analysis:

The Student-Newman-Keuls test has been used to separate the means following a significant main effect was discovered. The information has been investigated via an entirely randomized factorial design [SAS, 1988]. Treatment variances (P0 value less than 0.05) have been deemed significant by the Costat Program. Analyses of biological results have been performed utilizing one-way ANOVA. (**1967, Snedecor and Cochran**)

• Ethical Approval

The Science Research Ethics Committee of the Faculty of Home Economics accepted the protocol of the research #15-SREC-06-2025.



4- RESULTS AND DISCUSSION

• RESULTS

4.1. Immunological results

- Blood Parameters

Table (1) illustrates the influence of various concentrations of Ammi visnaga on RBC, Hgb, MCV, RDW, HCT, MCH and MCHC in diabetic rats.

As shown the mean value of RBC in the positive control was significantly lower (p-value below 0.05) in comparison with the negative control, with values of 1.10 ± 0.18 and 5.30 ± 0.15 m/ul, correspondingly. Whereas the value of all the experimental groups. (5%.10%, 15%, and 20%) Ammi visnaga were significantly greater in comparison with the positive control by mean 1.20 ± 0.03 , 1.38 ± 0.03 ; 1.35 ± 0.02 , and 1.30 ± 0.06 m/ul, respectively.

At the same time Hgb of the positive control group was significantly lower (p-value below 0.05) in comparison with the negative control mice being 10.10 ± 0.37 and 15.52 ± 0.50 g/dl, correspondingly. Additionally, the value of all the experimental groups. (5%.10%, 15%, and 20%) Ammi visnaga were significantly greater in comparison with the positive control mice by mean 15.38 ± 0.54 , 15.98 ± 0.51 ; 15.66 ± 0.57 , and 15.90 ± 0.53 g/dl, respectively.

As demonstrated in table (1) the mean value of MCV in the positive control group was significantly greater (p-value below 0.05) in comparison with the negative control group, with values of 65.96 ± 4.61 and 58.62 ± 3.16 fl, correspondingly. Additionally, the values of all the experimental groups. (5%.10 percent, fifteen percent, and twenty percent) Ammi visnaga were significantly lower in comparison with the positive control being 57.02 ± 2.63 , 49.54 ± 1.36 ; 54.48 ± 2.17 , and 52.82 ± 2.57 fl, respectively.

As demonstrated in table (1) the mean value of RDW in the positive control mice was significantly greater (p-

value below 0.05) in comparison with the negative control 20.42 ± 0.52 and 13.22 ± 0.69 %, correspondingly. Additionally, the values of all the experimental groups. (5%.10%, 15%, and 20%) Ammi visnaga were significantly lower in comparison with the positive control being 12.20 ± 0.72 , 11.34 ± 0.57 ; 11.94 ± 0.91 , and 10.94 ± 0.91 %, respectively.

As demonstrated in table (1) results, the mean value of HCT in the positive control mice was significantly lower (p-value below 0.05) in comparison with the negative control group, 17.00 ± 0.53 and 30.40 ± 1.51 %, correspondingly. Additionally, the values of all the experimental groups. (5%.10%, 15%, and 20%) Ammi visnaga were significantly higher in comparison with the positive control group which were 19.00 ± 1.00 , 18.42 ± 0.52 ; 19.00 ± 1.02 , and 29.00 ± 1.73 %, respectively.

The mean value of MCH in the positive control was significantly lower (p-value below 0.05) in comparison with the negative control, with values of 9.10 ± 0.29 and 17.20 ± 0.22 pg, correspondingly. Additionally, the value of all the experimental groups. (5%.10%, 15%, and 20%) Ammi visnaga were significantly higher in comparison with positive control which were 10.40 ± 0.10 , 14.30 ± 0.26 ; 10.30 ± 0.17 , 14.50 ± 0.17 ; and 13.78 ± 0.23 pg, respectively.

As shown in table (1) the mean value of MCHC in the positive control was significantly lower (p-value below 0.05) in comparison with the negative control rats, with values of 6.40 ± 1.22 and 12.50 ± 0.70 g/dl, correspondingly. whereas, the value of all the experimental groups. (5%.10%, 15%, and 20%) Ammi visnaga were significantly greater in comparison with the positive control by mean 8.50 ± 0.53 , 9.04 ± 0.94 ; 10.30 ± 0.61 , and 12.00 ± 0.43 grams per deciliter, respectively. It might be noticed (Table 1) that the best treatment was that of the group 6 (20% Ammi visnaga), concerning the red cells parameters, this positive effect appeared when the plant percentage increased to 20%.



Table (1): Mean \pm SD values of RBC, Hgb, MCV, RDW, HCT, MCH and MCHC for control positive, control negative of diabetic rats fed on different concentrations of Ammi visnaga

Group Parameter	Control (-ve)	Control (+ve)	5% Ammi visnaga	10% Ammi visnaga	15% Ammi visnaga	20% Ammi visnaga
RBC (m/ul)	5.301 \pm 0.15	1.101 \pm 0.18	1.201 \pm 0.03	1.38 \pm 0.03	1.351 \pm 0.02	1.301 \pm 0.06
Hgb (g/dl)	15.521 \pm 0.50	10.101 \pm 0.37	15.381 \pm 0.54	15.981 \pm 0.51	15.661 \pm 0.57	15.901 \pm 0.53
MCV(fl)	58.621 \pm 3.16	65.961 \pm 4.61	57.021 \pm 2.63	49.541 \pm 1.36	54.481 \pm 2.17	52.82 \pm 2.57
RDW(%)	13.221 \pm 0.69	20.421 \pm 0.52	12.20 \pm 0.72	11.341 \pm 0.57	11.941 \pm 0.91	10.941 \pm 0.91
HCT(%)	30.401 \pm 1.51	17.001 \pm 0.53	19.00 \pm 1.00	18.421 \pm 0.52	19.001 \pm 1.02	29.001 \pm 1.73
MCH(pg)	17.201 \pm 0.22	9.101 \pm 0.29	10.401 \pm 0.10	14.30 \pm 0.26	10.301 \pm 0.17	14.501 \pm 0.17
MCHC(g/dl)	12.501 \pm 0.70	6.401 \pm 1.22	8.501 \pm 0.53	9.041 \pm 0.94	10.301 \pm 0.61	12.001 \pm 0.43

Variances are significant at five percent (P-value below 0.05). Control-Ve: Mice nourished on basal diet.

Control +Ve: Diabetic mice nourished on a basal diet.

-Platelets

Table (2) illustrate the influence of various concentrations of Ammi visnaga for 28 days on PLT and MPV in diabetic rats. As demonstrated in this table, the mean value of PLT in the positive control was greater compared to the negative control, with values of 367.10 \pm 11.19 and 110.50 \pm 3.50 k/ul, respectively. The values of all the experimental groups. (5%.10%, fifteen percent, and twenty percent) Ammi visnaga were significantly lower (p-value below 0.05) in comparison with the positive control, by mean 148.00 \pm 3.61, 147.00 \pm 2.65; 114.40 \pm 2.51, and 113.00 \pm 3.46 k/ul, respectively.

As for MPV, the mean value of the positive control was reduced compared to the negative control, which were 2.00 \pm 0.20 and 3.85 \pm 0.13 fl, correspondingly, as well as the values of all the experimental groups. (5%.10%, 15%, and 20%) Ammi visnaga were greater compared to the positive control, by mean 2.38 \pm 0.11, 2.52 \pm 0.03; 3.38 \pm 0.06, and 3.54 \pm 0.02. fl, respectively. It is evident that the PLT raised while MPV decreased by hyperglycemia, while feeding with herbs diets showed the reverse. The best group was recorded was group 6 (20% Ammi visnaga), concerning the red cells parameters, this positive effect appeared when the plant percentage increased to 20%.

Table (2): Mean \pm SD values of PLT and MPV for control positive, control negative of diabetic rats fed on different concentrations of Ammi visnaga.

Group Parameter	Control (-ve)	Control (+ve)	5% Ammi visnaga	10% Ammi visnaga	15% Ammi visnaga	20% Ammi visnaga
PLT(k/ul)	110.50 \pm 3.50	367.10 \pm 11.19	148.00 \pm 3.61	147.00 \pm 2.65	114.401 \pm 2.51	113.001 \pm 3.46



MPV(fl)	3.85±0.13	2.00±0.20	2.38±0.11	2.52±0.03	3.381±0.06	3.541±0.02
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Cells related to immunity-

Table (3) illustrate the influence of various concentrations of Ammi visnaga on WBC, LYM, MID and GRAN in mice pretreated with Alloxan. The mean value of WBC in the positive control was significantly reduced (p-value below 0.05) in comparison with the negative control, with values of 5.30±0.26 and 6.13±0.06 k/ul, correspondingly. Additionally, The values of all the experimental groups. (5%.10%, 15%, and 20%) Ammi visnaga were significantly greater in comparison with the positive control by means 7.32±0.07, 8.72±0.26; 6.00±0.10, and 9.44±0.05; k/ul, respectively.

As for LYM, the mean value of control positive was lower than control negative, which were 3.94±0.15 and 5.00±0.17 %L, respectively. whereas The values of all the experimental groups. (5%.10%, 15%, and 20%) Ammi visnaga were higher than control positive which were 5.94±0.26, 7.20±0.07; 4.58±0.10, and 8.10±0.20 %L respectively.

Table (3): Mean ± SD values of WBC, LYM, MiD and GRAN for control positive, control negative of diabetic rats fed on different concentrations of Ammi visnaga.

Group Parameter	Control (-ve)	Control (+ve)	5% Ammi visnaga	10% Ammi visnaga	15% Ammi visnaga	20% Ammi visnaga
WBC(k/ul)	6.13±0.06	5.3±00.26	7.32±0.07	8.721±0.26	6.001±0.10	9.441±0.05
LYM(%L)	5.00±0.17	3.941±0.15	5.941±0.26	7.201±0.07	4.581±0.10	8.101±0.20
MID(%M)	0.70±0.010	0.521±0.017	0.901±0.025	1.021±0.072	0.881±0.017	0.881±0.032
GRAN(%G)	0.42±0.013	0.29±0.019	0.561±0.011	0.591±0.010	0.521±0.014	0.591±0.015

• DISCUSSION

Ammi visnaga seeds contain bioactive compounds, such as khellin and visnagin, which have been shown to possess hypoglycemic and immunomodulatory

The same table demonstrated that the mean value of MID in the positive control was significantly lower (p-value below 0.05) in comparison with the negative control, with values of 0.52±0.017 and 0.70±0.010 % M, correspondingly, The values of all the experimental groups. (5%.10%, 15%, and 20%) Ammi visnaga were higher than control positive, which were 0.90±0.025, 1.02±0.072; 0.88±0.017, and 0.88±0.032% M, respectively.

As for GRAN, the mean value of the positive control was significantly reduced (p-value below 0.05) in comparison with the negative control, with values of 0.29±0.019 and 0.42±0.013 %G, correspondingly. Also the values of all the experimental groups. (5%.10%, 15%, and 20%) Ammi visnaga were greater compared to the positive control, by mean 0.56±0.011, 0.59±0.010; 0.52±0.014, and 0.59±0.015% G, respectively. It evident that best group for WBC, LYM, MID & GRAN was that of the group 6 (20% Ammi visnaga).

properties. These compounds may help regulate blood sugar levels by enhancing insulin sensitivity or stimulating glucose uptake in cells. Additionally, their immunomodulatory effects could support the immune system, which is often compromised in diabetic patients



due to chronic inflammation and oxidative stress. (Sherif et al., 2021). Ammi visnaga seeds may impact the immune system by acting as an antioxidant and possessing immunomodulatory properties, where seeds contain compounds like flavonoids that can reduce oxidative stress and have antiviral, antibacterial, and antifungal effects. A study on rats showed that *A. visnaga* extract elevated the WBC count and improved antibody production, though more study is required to completely understand its effects on the immune system. (Ashry et al., 2021). Ammi visnaga seeds contain polyphenols and flavonoids that act as antioxidants, which can help protect against cellular damage and inflammation, thereby supporting immune function (Emad et al., 2023). Ammi visnaga have immunomodulatory properties, meaning it can help regulate the immune response. it elevated the total WBC count and improved antibody production in response to antigens. (Iid et al. 2020). Ammi visnaga extracts demonstrated immunostimulatory properties. The MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) assay has been utilized to assess the extracts on splenocytes, without or with stimulation by concanavalin-A, a mitogenic medication utilized as a positive control. (Nekhla et al. 2023). The chemical composition of Ammi visnaga includes a variety of inorganic and organic compounds found in various plant parts like stems, leaves, flowers, roots, and fruits. These compounds have an essential role in plant medical properties, involving enhancing immunology, anti-diabetic, anti-bacterial. Moreover, they also have a significant effect on the plant's interactions with its environment and other organisms (Sabri et al. 2022). *A. visnaga* total and protein extracts exhibited immunostimulatory properties. Extracts have been tested utilizing an MTT assay on splenocytes, both without or with activation via concanavalin-A, a mitogenic drug utilized as a positive control. This might clarify the conventional application of this plant (Alejandro et al., 2020). Antibody production *A. visnaga* extract significantly increased. Also, humoral response enhancement, where 60% hydroethanolic extract of *A. visnaga* enhanced the humoral immune response. increasing the hemagglutination antibody (HA) titer. also, *A. visnaga* can have immunomodulatory effects, which are linked to its antioxidant properties. Molecular revealed that khellin

and visnagin, compounds found in *A. visnaga*, have a strong affinity for immunomodulatory proteins like p52, NF- κ B, and TNF- α (Benabderrahmane et al., 2023)

5. Conclusion

Ammi visnaga seeds hold significant potential as a natural aid in boosting immune health and promoting overall well-being. These findings suggest that Ammi visnaga seeds may hold promise for developing new treatments for human diabetes. The seeds could potentially improve blood sugar regulation and enhance immune function in diabetic patients. Additional study is necessary to determine their efficiency and safety in humans.

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