



Investigating Anti-Oxidative and Anti-Inflammatory Effects regarding Ethanol Extract of *Allium Porrum L.* in Rats with Type 2 Diabetes Mellitus

Nahid Najafi; PhD Candidate¹, Seyed Jalil Masoumi; MD, MPH, PhD*^{1,2,3}, Ali Akbar Nekooeian; VMD, PhD⁴, Nader Tanideh; VMD, MPH, PhD^{5,6}, Siavash Babajafari; MD, PhD¹, Hossein Khosravi-Boroujeni; MSc⁷, Najmeh Maayeshi; MSc^{8,9} & Saeed Sherafatmanesh; PhD Candidate^{8,9}

¹ Nutrition Research Center, School of Nutrition and Food Sciences, Shiraz University of Medical Science, Shiraz, Iran; ² Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; ³ Employee Cohort Study, Shiraz University of Medical Sciences, Shiraz, Iran; ⁴ Cardiovascular Pharmacology Research Lab. Department of Pharmacology, Shiraz University of Medical Sciences, Shiraz, Iran; ⁵ Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; ⁶ Pharmacology Department, Shiraz University of Medical Sciences, Shiraz, Iran; ⁷ School of Medicine and Griffith Health Institute, Griffith University, QLD, Australia; ⁸ Research Center for Food Hygiene and Safety, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; ⁹ Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

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*Corresponding author:

masoumi7415@gmail.com
Nutrition Research Center,
School of Nutrition and Food
Sciences, Shiraz University
of Medical Science, Shiraz,
Iran.

Postal code: 7134814336

Tel: +98 71 37251007

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ABSTRACT

Background: Type 2 diabetes mellitus (T2D) is a common endocrine and metabolic disorder worldwide. Meanwhile, *Allium Porrum L.* (AP) has been recognized as one of the most eminent traditional herbal medicines with substantial health benefits. The aim of the present study is to evaluate anti-oxidative and anti-inflammatory effects of ethanol extract of AP in rats with T2D. **Methods:** 70 male Sprague-Dawley rats were randomly divided into 7 groups of 10 animals each. The study groups were as follows: The study groups were as follows: healthy control (HC); healthy control rats receiving 100 mg/kg of AP extract (HC.AP.100); diabetic control (DC); diabetic rats receiving 10 mg/kg metformin (DT.M.10); diabetic animals receiving 50 mg/kg of AP extract (DT.AP.50); diabetic rats receiving 100 mg/kg of AP extract (DT.AP.100); and diabetic animals receiving 200 mg/kg of AP extract (DT.AP.200). **Results:** After 6 weeks of intervention, the level of serum malondialdehyde (MDA) and tumor necrosis factor alpha (TNF- α) were significantly increased ($P=0.02$) and ($P=0.002$), respectively; while superoxide dismutase (SOD) concentration was notably reduced ($P=0.04$) in DC.M.10 in comparison with that of the HC.AP.100 group. Also, compared to DC.M.10 group, all rats treated with metformin and AP showed a significant decrease in the level of MDA and TNF- α , along with an enhancement of SOD concentration (All $P<0.001$). Moreover, in comparison with DT.M.10, DT.AP.50, and DT.AP.100 treatments, the DT.AP.200 group revealed a significantly higher improvement in serum TNF- α concentration ($P=0.04$, $P=0.003$ and $P=0.003$, respectively). **Conclusion:** The findings revealed that the use of AP extract for 6 weeks may have beneficial effects on oxidative stress and inflammatory biomarkers in T2D-induced rat models.

Introduction

Type 2 diabetes mellitus (T2D) is known as a main global chronic endocrine and metabolic illness which is associated with serious pathologic features including insulin resistance and disturbances in blood lipid and glycemic profile (Dilworth *et al.*, 2021). T2D has become much more prevalent in recent decades. Over 171 million people globally had diabetes in 2000, and it is predicted that this number would rise to 366 million by 2030 (Wild *et al.*, 2004). It has been documented that the absence of sufficient compensation of insulin resistance may result in damaged body antioxidant defense system due to higher generation of reactive oxygen species (ROS) during long-term hyperglycemic state (Sanders *et al.*, 2001, Sherafatmanesh *et al.*, 2019, Virally *et al.*, 2007). Elevated ROS levels have been identified as the primary cause of T2D long term-complications such as systemic inflammation, neuropathy, nephropathy, and cardiovascular diseases (Singh *et al.*, 2022).

Presently, pharmaceutical therapy including metformin and glucocorticoids is the common remedy for T2D; however, the effectiveness of these treatments is restricted due to their adverse side effects such as hepatotoxicity, acute pancreatitis, and vitamin B12 alterations (Shurrah and Arafa, 2020). Hence, in order to advance the management of T2D, natural approaches should be replaced with synthetic ones that have less detrimental consequences (Suji and Sivakami, 2003).

Allium porrum (AP; also known as leek), from the alliaceae family, is an eminent bulbous perennial plant with overlapping leaves which is naturally found in different regions of the Middle East, including Iran and Turkey (Al-Snafi, 2013, Aslan *et al.*, 2010, Keusgen, 2002, Zeng *et al.*, 2017). Previous studies have shown that leek leaves may play an important role in improving digestive problems, atherosclerosis, joint pain, respiratory inflammation, and kidney stones. AP is considered a high source of nutritive elements such as potassium, iron, selenium, thiamine, riboflavin, pyridoxine, and vitamin C (Koca and

Tasci, 2015). Furthermore, it contains bioactive components, including saponin, sapogenin, quercetin and kaempferol, which also have been reported to contain high potential therapeutic properties, such as high antioxidant capacity, antihyperlipidemic, immune system regulating, and anti-cancer activities (Benedé *et al.*, 2019, El-Shenawy *et al.*, 2013, Kazemi *et al.*, 2010, Kovarovič *et al.*, 2021, Movahedian *et al.*, 2006). Therefore, in the current study; the authors aim to investigate antioxidative effects of AP extract on T2D-induced rats.

Materials and Methods

Preparation of AP extract

AP leaves were harvested from a farm located in Nourabad Mamasani region, Fars province, Iran. The genus identification (herbarium number: 2196) was certified by plant taxonomy biologist (Dr. Khademian) from the Department of Pharmacognosy, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. Dried leaves were powdered with an electric mill, then, 500 g of the powder was mixed with 70% ethanol. The mixture was blended using a magnetic stirrer (Labinco model L81, DG Breda, Netherlands) at room temperature for 72 h, 300 rpm. At last, the residual content was filtered, and the solvent was isolated by a Rotary concentrator and dried in a desiccator at 37 °C.

Experimental animals

First, 70 male Sprague-Dawley rats weighing 200–220 g were obtained from the central animal house of Shiraz University of medical sciences, Iran. Then, rats were caged under appropriate laboratory conditions (21±2 °C temperature, 12-h dark and light cycle. 50-60% humidity) with free access to similar ad libitum and water for two weeks. The animal procedures in this experiment were conducted in accordance with the ethics stated in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources (US), 1996).

Disease induction

At baseline, rats were randomly divided into

two groups of control and diabetic. The diabetes was induced using an intraperitoneal injection of 65 mg/kg streptozotocin (Zelbio, Germany), along with 100 mg/kg nicotinamide, 10 minutes after the first injection. Subsequently, rats with fasting blood sugar concentration above 126 mg/dl were considered as type 2 diabetic rats, and rats with lower levels were excluded from the study.

Study design

After the disease induction phase, the study animals were divided randomly into seven equal groups of 10 rats each: Healthy control group receiving distilled water (HC); healthy control rats receiving 100 mg/kg of AP extract (HC.AP.100); diabetic control animals receiving distilled water (DC); diabetic rats receiving metformin (Shafa Pharmaceutical Co., Tehran, IRAN) dissolved in normal saline at a level of 10 mg/kg (DT.M.10); diabetic animals receiving 50 mg/kg of AP extract (DT.AP.50); diabetic rats receiving 100 mg/kg of AP extract (DT.AP.100) and diabetic animals receiving 200 mg/kg of AP extract (DT.AP.200). All administrations were conducted by oral gavage at a specific time of the day for 6 weeks.

Blood biochemical measurements

At the end of the experiment, the rats were fasting for 12 hours. They were then euthanized in a container pre-filled with 70% carbon dioxide (CO₂) gas, which according to prior research, was a standard and secure technique for euthanizing experimental animals (Conlee *et al.*, 2005). 5 ml of blood was taken by a disposable syringe injected directly into the heart and centrifuged at 3500 rpm for 10 min to separate the serum. All the serum samples were kept in sterile micro-centrifuge tubes at -80 °C until the study parameters were evaluated. An ELISA kit (Zelbio, Germany) was used to determine the serum superoxide dismutase (SOD) concentrations. The level of malondialdehyde (MDA) was measured spectrophotometrically with the thiobarbituric acid reactive substances (TBARS) method (Kalaivanam *et al.*, 2006). At

last, the concentration of serum tumor necrosis factor alpha (TNF- α) was assessed using the ELISA kit (IBL International, Germany) according to the manufacturer's instructions.

Ethical considerations

The protocol of the study was confirmed by the Institutional Animal Ethics Committee (IAEC) of Shiraz University of Medical Sciences (Shiraz, Iran), following the NIH guidelines for the care and use of animals (NIH publication NO. 85-23, revised in 1996).

Data analysis

All Statistical analyses were done using the Statistical Package for Social Sciences (version 23.0; SPSS Inc). For each variable, the Kolmogorov-Smirnov test was used to assess the normal distribution of the data. One-way ANOVA test was used for comparing the variables between groups, and Tukey test was used for pairwise comparisons. P-values < 0.05 were considered to be statistically significant.

Results

The alterations in serum concentrations of the oxidative stress parameters after six weeks of intervention have been reported in **Table 1**. As the table depicts, the amount of serum MDA ($P=0.02$), and TNF- α ($P=0.002$) were significantly increased, while the SOD concentration ($P=0.04$) was notably reduced in the DC group in comparison with that of the HC group. By the end of the experiment, compared to the DC group, a significant decrease was detected in the level of MDA and TNF- α , along with an enhancement of SOD concentration in all rats treated by metformin and AP (All $P<0.001$). Meanwhile, compared to the DT.M.10, DT.AP.50 and DT.AP.100 treatments, the DT.AP.200 group showed significantly higher improvement in serum TNF- α concentration ($P=0.04$, $P=0.003$, and $P=0.003$, respectively).

Discussion

The results of the present study provided clinical evidence that the consumption of AP in the form of its hydroalcoholic extract may lead to

the improvement of oxidative stress indicators in experimental diabetic rats.

The use of herbal medicine has been globally considered to control and also treat different diseases (Ryan *et al.*, 2001). Reports on diabetic

patients showed that one-third of diabetic patients use herbal remedies and consider these agents to be effective and satisfactory (Ryan *et al.*, 2001, Vats *et al.*, 2002).

Table 1. Serum oxidative and inflammatory parameters after six weeks of intervention.

Groups	HC	HC.AP.100	DC	DT.M.10	DT.AP.50	DT.AP.100	DT.AP.200	P-value*
SOD ($\mu\text{mol/ml}$)	39.13 \pm 17.71 ^a	39.64 \pm 17.87 ^a	37.10 \pm 17.85 ^b	43.54 \pm 17.81 ^c	41.85 \pm 16.20 ^c	42.38 \pm 17.37 ^c	43.01 \pm 16.52 ^c	0.002
MDA (mmol/l)	3.90 \pm 0.15 ^a	3.76 \pm 0.21 ^a	4.77 \pm 0.22 ^b	3.18 \pm 0.26 ^c	3.49 \pm 0.51 ^c	3.34 \pm 0.53 ^c	3.11 \pm 0.15 ^c	<0.001
TNF- α (ng/ml)	182.62 \pm 17.11 ^a	182.23 \pm 19.08 ^a	217.00 \pm 20.09 ^b	168.50 \pm 19.25 ^c	173.71 \pm 22.74 ^c	171.43 \pm 21.67 ^c	159.18 \pm 20.58 ^d	0.004

HC: Healthy control, **DC:** Diabetic control, **DT:** Diabetic treatment, **M:** metformin, **AP:** Allium Porrum, **SOD:** Superoxide dismutase, **MAD:** malondialdehyde, **TNF- α :** Tumor necrosis factor alpha, *: Obtained from One-way ANOVA, P-values < 0.05 were considered to be statistically significant, Data expressed as Mean \pm SD. In each row, figures bearing different superscripts are significantly different at P < 0.05 (Tukey's test).

Various factors are known to be involved regarding the production of free radicals and the occurrence of oxidative stress in diabetic patients. Hyperglycemia is among the most important risk factors, which can increase the oxidation of glucose and lead to the production of free radicals (Qing *et al.*, 2018). Hypoglycemic effects of Allium species have been reported in several studies. It has been documented that AP phytochemicals, including saponin, saponin, and quercetin, which have high potential antioxidant properties, may act as one of the main underlying mechanisms regarding the inhibition of gluconeogenesis, and consequently, glycemic control (Mansour *et al.*, 2002, Radovanović *et al.*, 2015). On the other hand, increased insulin secretion and higher peripheral glucose uptake are among other mechanisms proposed for the effects of AP in order to reduce blood glucose concentrations (Belemkar *et al.*, 2013). In addition, low levels of antioxidants including ascorbate, glutathione peroxidase and SOD have been detected in diabetic patients which can accelerate the production of pre-inflammatory cytokines in body (Zeng *et al.*, 2017). These cytokines are able to stimulate inflammatory

cascade activities in pancreatic beta cells, leading to increased pancreatic beta cells death and inhibition of insulin secretion (Elhagrasi *et al.*, 2019, Vats *et al.*, 2002). In 2011, Adão CR *et al.* (Adão *et al.*, 2011) conducted an experimental study regarding the anti-inflammatory effects of AP in rats. The researchers reported that pro-inflammatory cytokines in the treated rats were significantly decreased compared to the control group, which were in agreement with the results of the current study. Previous investigations demonstrated that saponins, alkaloids, tannins, and quinine acids in plants such as AP may reveal anti-inflammatory and anti-oxidant properties due to improvement in the balance between oxidants and systemic antioxidants in body (Fattorusso *et al.*, 2001, Kleijnen *et al.*, 1989). Therefore, reducing the concentration of inflammatory factors in diabetic patients may be an effective therapeutic goal in health care services (Rochette *et al.*, 2014).

The limitations of the present study should be taken into account when interpreting the findings. Due to the limited funding, the authors could not evaluate other valuable inflammatory biomarkers such as serum interleukin-1 and interleukin-6.

Therefore, future well-designed investigations are required to elucidate other favorable effects of AP consumption in patients with T2D.

Conclusion

Data showed that the use of AP extract for six weeks may have supportive effects on oxidative stress and inflammatory biomarkers in T2D-induced rat models.

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Authors' contribution

All authors read and approved the manuscript. Najafi N was involved with original drafting, data curation, formal analysis, investigation, software, visualization, review and editing; Masoumi SJ carried out conceptualization, data curation, formal analysis, methodology, project administration, supervision, validation, visualization, original draft, review, and editing; Nekooeian AA did supervision, data curation, formal analysis, visualization, review, and editing; Tanideh N performed methodology, formal analysis, validation, visualization, review, and editing; Babajafari S was involved with methodology, data curation, validation, visualization, review and editing; Khosravi-Boroujeni H, Maayeshi N, and Sherafatmanesh S carried out methodology, data curation, validation, visualization, review, and editing.

Conflict of interest

The authors declared no conflict of interests.

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