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Original Article

The Effect of Some Herbal Extracts on Nitric Oxide Production in Endothelial Cells 3T3 Cell Line

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Abstract

Some studies have demonstrated the potential of herbal drugs for the treatment of various diseases associated with impaired vascular nitric oxide (NO). For diagnosis of the mechanism of these herbal plants, it seems necessary to evaluate the herbal extracts on the Endothelium cell. The aim of the study was to investigate the effect of extract of *Trigonella foenum-graecum* (*T.foenum*), *Nigella sativa* (NS), *Allium sativum,* and *Cannabis sativa* on nitric oxide (NO) production by the endothelial cells 3T3 cell line.Ground herbs suspended in water and methanol, were filtered and concentrated under pressure. Remaining contents were dried and obtained powder dissolved in water. The cell line was treated with plant extracts. The clarified supernatant of the culture was used for evaluation of quantitative changes in NO production using Griess test**.**Among the four extracts, only the group treated with *T.foenum* extract, significantly increased NO production compared to the control group. Also, NO concentration in other extract group was lower than *T.foenum* group**.** This study suggested that the *T.foenum* extract can significantly increase NO concentration in cell culture and the increase in nitric oxide production is because of the presence of diosgenin *T.foenum* extracted.

Keywords: Nitric oxide, *Trigonella foenum-graecum, Nigella sativa*, *Allium sativum, Cannabis sativa*, Endothelial cell.

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1. Introduction

Endothelium-derived nitric oxide (NO) is a potent signaling molecule in the cardiovascular system participating in many processes such as vascular relaxation, inhibition of platelet aggregation, regulation of endothelial cell adhesivity, and preservation of the normal vessel wall structure (1).

NO is synthesized from L-arginine by the L-arginine-nitric oxide pathway (2) and is converted to nitrite and nitrate in oxygenated solutions (3). A family of enzymes, termed the nitric oxide synthases (NOS), catalyze the formation of NO and citrulline from L-arginine, O2, and NADPH (4). The constitutive NOS isoforms (NOS-1 and NOS-3) produce low levels of NO as a consequence of increased intracellular Ca^{2+} (5). By contrast, the inducible isoform of NOS (NOS-2 or iNOS) generates large amounts of NO upon stimulation over a prolonged period of time through a Ca^{2+} independent pathway (6). Inducible NOS expression has been observed in many cells, including murine macrophages (7), smooth muscle cells (8), endothelial (9), and cardiac myocytes (10). NO have different roles in many diseases such as headache, hypertension, and other diseases. Evidence is accumulating that NO determines the antiatherosclerotic properties of the endothelium [5]. All major risk factors for atherosclerosis including hypertension, hypercholesterolemia, and smoking have been related with impaired vascular NO synthesis [6]. The underlying mechanisms are thought to involve decreased formation of NO due to a reduce in NOS expression or a limited availability of L-arginine, as well as increased degradation of NO by reaction with oxidized low density lipoproteins or superoxide anions [5, 6].

Many herbal drugs used for treatment of headache, hypertension. For diagnosis of the mechanism of usefulness of this herbal plants need to diagnosis of the herbal extract on the Endothelium cell.

Four herbal plants based on their properties affecting blood pressure were chosen. Also, below studies confirmed our hypothesis about it.

Trigonella foenum-graecum (*T.foenum*) is an important annual medicinal plant of the Leguminosae family and its leaves and seeds have been used in various illnesses and as a health tonic for a very long time. *T.foenum* is known to have antihypertensive (11) hypoglycemic, hypocholesterolemic, antioxidant potency, digestive stimulant action, and hepatoprotective effects (12).

Nigella sativa (NS) has been used for centuries in medicinal and culinary purposes throughout the Middle East and Africa. It is belongs to the Ranunculaceae family. The exact mechanism on how NS reduces blood pressure is not exactly known. The antihypertensive effects of NS may be due to the many active compounds. Previous studies reported that the volatile oil and thymoquinone decreased both the arterial blood pressure and heart rate (13). The cardiovascular protective effects of NS in hypertension are possibly contributed by its multitude actions including cardiac depressant, diuretic, calcium channel blockade (14), and antioxidant properties (15, 16).

Another palant was *Allium sativum (A.sativum),* commonly known as garlic. It belongs to the Amarylidaceae family. *A.sativum* extract is effective in reducing peripheral and central blood pressure in a large proportion of patients with uncontrolled hypertension, and has the potential to improve arterial stiffness, inflammation, and other cardiovascular markers in patients with elevated levels (17). The present study was designed to investigate the mechanisms underlying the effect of 4 plant extracts on endothelial NO synthesis.

Also, *cannabis sativa* belongs to Cannabaceae family. In traditional medicine of India in particular C. sativa has been used as hallucinogenic, hypnotic, sedative, analgesic, and anti-inflammatory agent. Its fractions attenuate elevated blood pressure (hypertension) development in spontaneously hypertensive rats (18).

2. Material and Methods

3T3 Cells were obtained from Pasteur Institute (Tehran, Iran). Cells were maintained at 37 C in a humidified atmosphere (90%) containing 5% CO2. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 5% (v/v) fetal bovine serum, 100 units/ml penicillin, and 100 lg/ml streptomycin. Cells were seeded at 5000/well onto 96-well culture plates, and then incubated with various concentrations of all extract.

*2.1. Preparation of Herbal Extract*s

All four sample of herbals identified by experts at the Pharmacological Research Center of Medicinal Plants, Medical Faculty, Mashhad University of Medical Sciences. To prepare hydro-alcoholic extract, 150 g of the ground herb was macerated in methanol and water (50-50) for four days. Then it was filtered and concentrated under reduced pressure using a rotary evaporator apparatus. Remaining contents were transferred into Petri dishes and were put into an oven with 40ºc temperature to be dried up the extract. The 8mg of powdered extract was weighted and dissolved in 1cc water and used on the endothelial cells.

2.2. Nitric Oxide Measurment

Nitric oxide was measured according to methods of Griess (19). Briefly, the supernatant

of cell culture was deproteinated with zinc sulfate (Zn_2SO_4) after centrifuging in 3000 g, the clarified supernatant used for NO measurement. In 96 well plate 50 µL of the clarified supernatant and the same volume of Sulfanilamide Solution 1% in HCl solution and 0.1% N-1napthylethylenediamine dihydrochloride in water were added. After incubation in 37 ºC the Vanadium trichloride was added to wells for Conversion of NO3 to NO2 and after 2 hr the optical determined in ELISA reader in 545 nm. The same reaction was performed with Nitrite sodium as standard (0.25-50 micro molar). The concentration of NO was calculated according the standard curve.

2.3. Statistical Analysis

The data were expressed as mean ± SEM. delta analysis according to paired T-test and one way ANOVA was run followed by tukey's post hoc comparisons test. The criterion for the statistical significance was $P < 0.05$.

3. Results and Discussion

There are no significant differences between Allium sativa extract and control group Figure (1). There are no significant differences between Cannabis sativa extract and control group (Figure 2). There are no significant differences between Nigella sativa extract and control group (Figure 3). NO concentration in *T.foenum* group was higher than control group (Figure 4) ($P<0.05$). Also, NO concentration in other extract group was lower than *T.foenum* group (Figure 5). ($P<0.05$)

In this study, we compared the effects of several plant extracts *in vitro* on the concentration of NO production. The results of this study showed that *T.foenum* extract induced NO production more than other extract group compared to control group and between extracts.

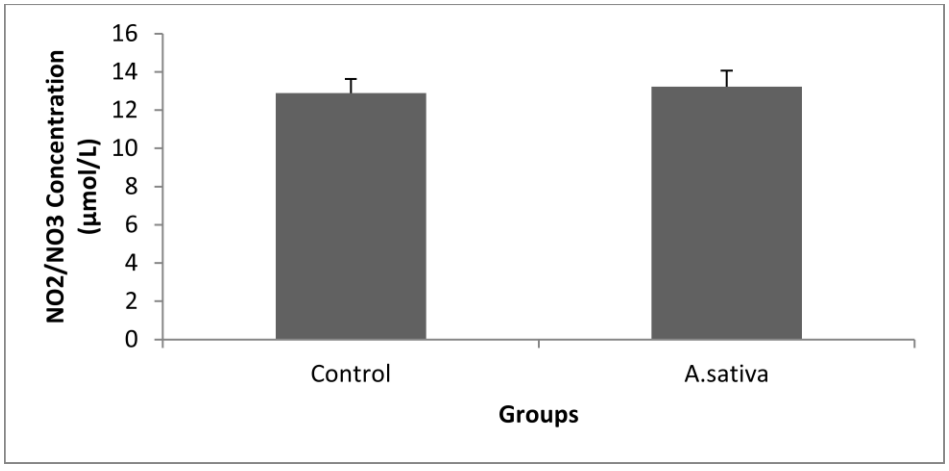

Figure 1. The effect of Allium sativa extract on NO production in endothelial cells 3T3. Data are presented as Mean ± S.E.M. using statistical One- way analysis of variance method and Tukey test was applied for multiple-group comparisons.

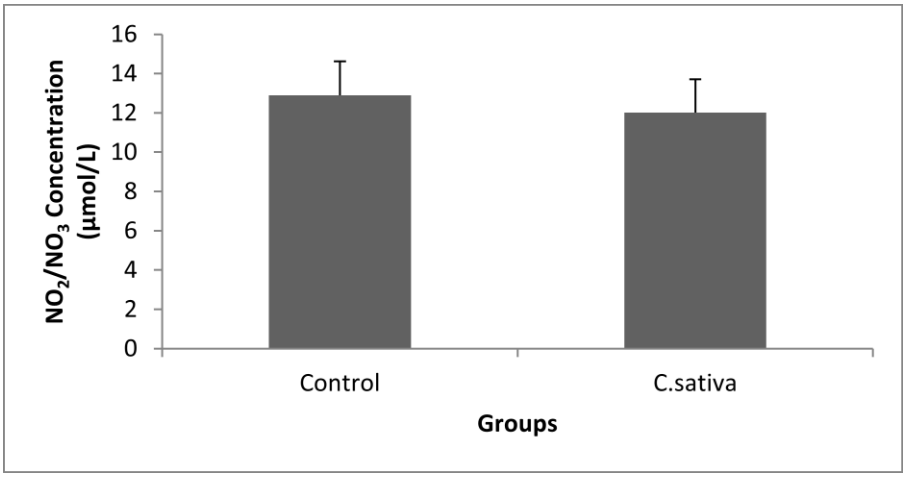

Figure 2. The effect of Cannabis sativa extract on NO production in endothelial cells 3T3. Data are presented as Mean ± S.E.M. using statistical One- way analysis of variance method and Tukey test was applied for multiple-group comparisons.

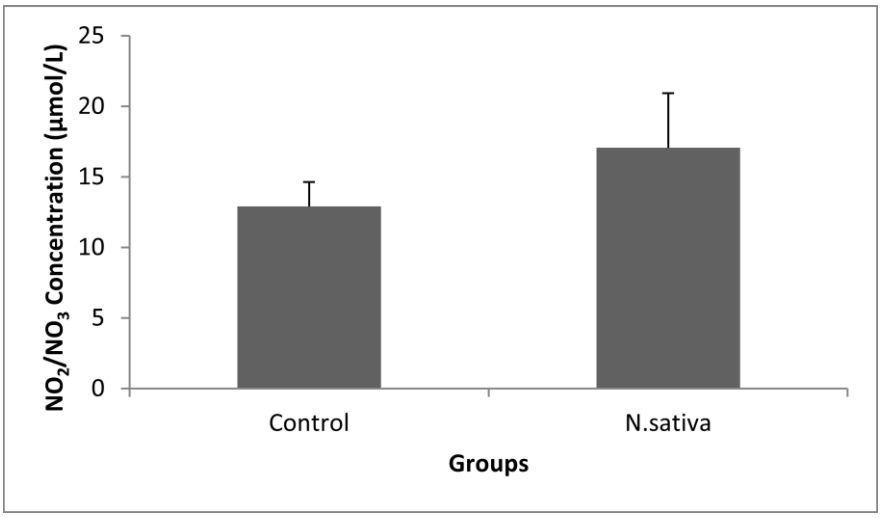

Figure 3. The effect of Nigella sativa extract on NO production in endothelial cells 3T3. Data are presented as Mean ± S.E.M. using statistical One- way analysis of variance method and Tukey test was applied for multiple-group comparisons.

Clinically, endothelial function is most often assessed as a vasodilator response to pharmacological or mechanical stimuli. Numerous studies have shown that the presence of coronary atherosclerotic lesions is associated with impaired endothelium-mediated regulation of vascular tone (20). More importantly, endothelial vasodilator dysfunction has been observed in patients with traditional coronary risk factors, even in the absence of evidence for

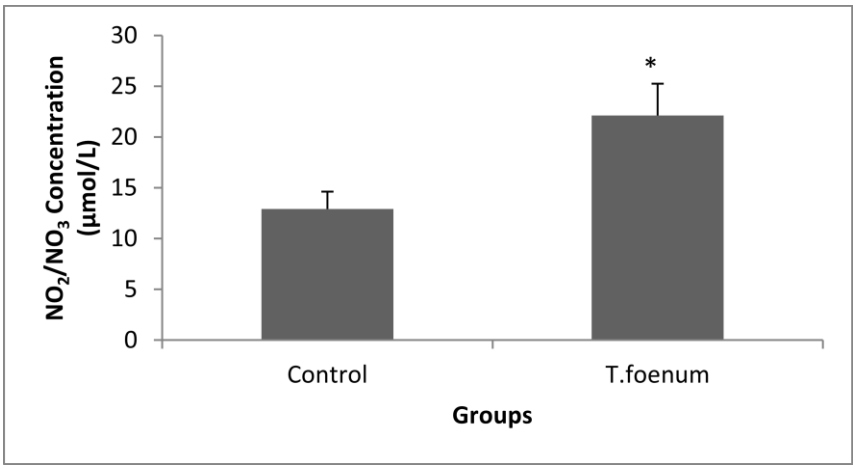

Figure 4. The effect of T.F extract on NO production in endothelial cells 3T3. Data are presented as Mean ± S.E.M. using statistical One- way analysis of variance method and Tukey test was applied for multiple-group comparisons.*, $p<0.05$.

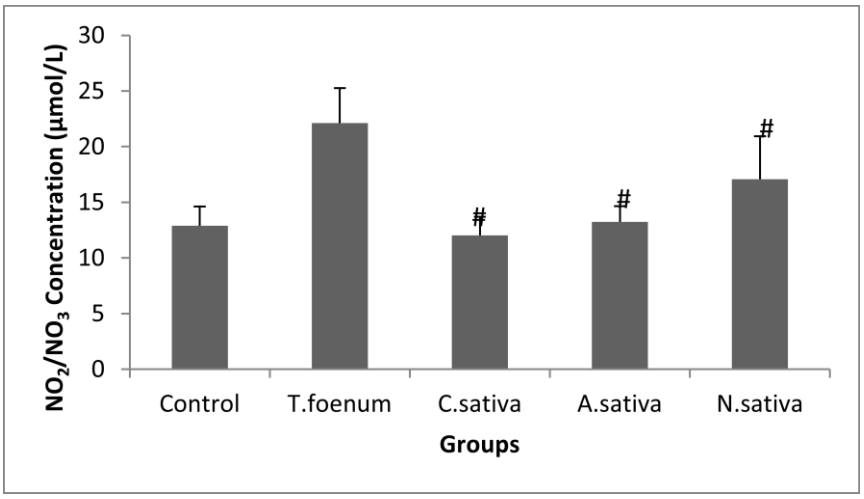

Figure 5. Comparison the different extract groups with T.foenum group as a NO production. Data are presented as Mean ± S.E.M. using statistical One- way analysis of variance method and Tukey test was applied for multiple-group comparisons.#, $p<0.05$.

atherosclerotic lesions, which suggests that the endothelium is both a target and a mediator of atherosclerosis (21). Nitric oxide (NO) is a potent vasodilator that can be synthesized in and released by the vascular endothelium, certain autonomic nerves, and other tissues (22). NO or a closely related compound evokes vasodilation by stimulation of guanosine 3′,5′-cyclic monophosphate pathways in vascular smooth muscle (23). The pharmacological vasodilators

nitroglycerin and sodium nitroprusside both cause vasodilation by donation of exogenous NO or NO-like compounds (24).

So, we decided to find a plant to produce nitric oxide to use it for vasodilator actions and cardioprotective effects in the future. We use some plant extracts in cell culture. The results of this study showed just *T.foenum* extract can significantly increase NO concentration in cell culture. We investigate the major component of *T.foenum* extract. The major component of *T.foenum* extract that was Diosgenin. Diosgenin (25R-spirost-5-en-3β-ol) is a hydrolysate of dioscin found in the rootstock of yam (Dioscorea) and exists widely in the natural plant in the form of glucoside (25). It is a steroidal sapogenin found in *Trigonella foenum-graecum* (26). In addition, diosgenin also increased nitric oxide (NO) levels (27). Many studies showed diosgenin have cardiprotective effects and proposed NO system may be the key players in diosgenin-induced cardioprotective mechanisms (28). Therefore, NO increasing effect of *T.foenum* can be caused by some effective components of *T.foenum* such as diosgenin. Also, in this study *N.sative* has a NO production inducer but lower than *T.foenum* that according to some articles this effect can be attributed to thymoquinone and polyphenols in *N.sativa* extract (29).

4. Conclusion

Based on the results of this study, we propose the *T.foenum* extract induced NO production in cell culture and this NO production may be because of the presence of diosgenin in *T.foenum* extract.

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