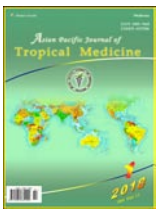

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Effects of aqueous extract of *Notobasis syriaca* on lipopolysaccharide-induced inflammation in rats

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ABSTRACT

Objective: To investigate the effects of a dry aqueous extract of *Notobasis syriaca* (*N. syriaca*) on lipopolysaccharide (LPS)-induced inflammation in rats. **Methods:** Rats were fed the dried extract [500 mg/(kg•d)] for three consecutive days and then were intraperitoneally injected with LPS (1 mg/kg). Two hours after LPS injection, rats were sacrificed and blood and brain regions were collected. Inflammatory mediators' levels in plasma and homogenates of brain regions were determined by ELISA. **Results:** Pretreatment with the *N. syriaca* extract resulted in significant anti-inflammatory effects ($P<0.05$), including: i) attenuated LPS-induced hypothermia; ii) decreased hypothalamus and hippocampus prostaglandin E_2 levels in the LPS-treated rats; and, iii) reduced hypothalamus and hippocampus interleukin-6 and tumor necrosis factor- α levels in the LPS-treated rats. **Conclusions:** These results suggest that *N. syriaca* possesses anti-inflammatory properties. Thus, it is possible that long-term consumption of this plant may result in beneficial pharmacological effects.

1. Introduction

Notobasis syriaca (L.) Cass. (*N. syriaca*) is single species in the plant genus *Notobasis* of the Asteraceae family[1]. It is one of the most widespread plants in the eastern part of the Mediterranean, with spiny, beautiful flowers. In many regions it was used by humans and animals as nutritious food since antiquity. Archeological findings indicate the use of *N. syriaca* as a food source by humans as early as 23 000 years ago[2]. Many of the medicinal and other activities of this plant are unknown, as compared to similarly common plants in Euroasia. For example, the chemical compositions and almost all medicinal properties of *Micromeria fruticosa*[3], *Origanum majorana*[4], *Salvia officinalis*[5] and *Malva sylvestris*[6] are well established. Another notable difference can be easily observed between *N. syriaca* and *Silybum marianum*, a very closely related plant which mostly grows in the same habitats. The activities of *Silybum marianum*-medicinal, anti-inflammatory and others – were extensively studied and its chemical composition is completely known[7,8]. The anti-inflammatory properties of many other plants were summarized in a recently published review[9].

N. syriaca is a well-known plant to traditional medicine. In the Arab society, it has been used for treating headaches, plague, canker sores, vertigo and jaundice, and sometimes it is used to enhance lactation. It is also a component in some bitters formulas and an antioxidant. Fresh stem and leaf base are edible[10]. In many countries *N. syriaca* is used as a food[11]. For example, in Italy, the

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plant is used as a food with wide variety of forms: boiled, fried as vegetables with eggs and in omelets[12].

As mentioned above, *N. syriaca* was partially investigated by modern research. Antimicrobial activity of some extracts of the plant was reported by Ali-Shtayeh *et al*.[13] but they did not indicate the extraction solvents and methods. On the contrary, total phenolic content and antioxidant activity of the plant were investigated by other groups that reported detailed extraction methods and solvents[14,15]. In the study by Alali *et al*.[14] only aqueous extract was used, while in the study of El-Hela *et al*.[15], methanolic and aqueous extracts were reported. Expectedly, the aqueous extract was more active with higher phenolic content. Aqueous extract of *N. syriaca* was found to have prominent antifungal activity[16], and natural products with antimicrobial activity were isolated from *Phomopsis* sp. fungi that lived on the plant[17].

The purpose of the present study was to examine the effects of an aqueous extract of *N. syriaca* on bacterial endotoxin (lipopolysaccharide, LPS)-induced inflammation in rats. Systemic administration of LPS to animals results in an intense inflammatory response including prominent secretion of inflammatory constituents, abnormal changes in body temperature (BT), cardiovascular complications (such as reduced myocardial contractility and a decrease in blood pressure), organs failure (e.g., acute kidney injury), among other pathological findings[18-24]. In mice and rats intraperitoneal (ip) administration of LPS induces a biphasic change in BT – an early decrease in BT (hypothermia) followed by elevation in BT (fever)[18,21,24-26]. The mechanism underlying the hypothermia caused by LPS is not fully understood. Many inflammatory components are thought to contribute to this intricate process; a central one of which is prostaglandin (PG) E_2[18,21,24-26]. LPS-induced variations in BT are accompanied by increased levels of PGE_2 in the hypothalamus[18,21,24-26]. Of note, the thermoregulation zone is located in the hypothalamus in the anteroventral periventricular nucleus. Anti-inflammatory drugs that decrease hypothalamic production of PGE_2 attenuate LPS-induced hypothermia[27]. Moreover, it was found that psychotropic drugs (which are not classic anti-inflammatory drugs) significantly reduced LPS-induced hypothermia in rats, probably due to reduction of hypothalamic PGE_2 levels[24].

The present study tested the effects of pretreatment with an aqueous extract of *N. syriaca* on plasma and brain levels of PGE_2, interleukin (IL)-6 and tumor necrosis factor (TNF)-α in LPS-treated rats. In particular, we examined the levels of PGE_2, IL-6 and TNF-α in three brain regions: hypothalamus (HT), hippocampus (HC) and frontal cortex (FC). PGE_2 affects various crucial physiological functions, including: endocrine system activity, cardiovascular and kidney function, excretion of neurotransmitters, immune-inflammatory activity, regulation of BT, among others[24,28-32]. IL-6 is a cytokine that exerts both anti-and pro-inflammatory activities; it regulates many physiological and pathological processes[33]. TNF-α is a potent pro-inflammatory cytokine but it also confers some anti-inflammatory functions[34-36]. It is involved in numerous physiological as well as pathological conditions in humans and is thus a target for multiple therapeutic interventions[34-36]. In the brain, TNF-α affects the expression of many genes that are crucial for neurons function and survival[34].

2. Material and methods

2.1. Extraction of plant material

Aerial parts of *N. syriaca* were air dried and ground to fine powder, and 500 g of the powder was soaked in distilled water for 24 h at 50 ℃. Then, it was filtered (Whatman 44) and the clear solution was concentrated by a rotary evaporator (50 ℃), yielding about 17 g of dry extract which was stored in deep freeze.

2.2. Animals

Male Sprague-Dawley rats were used all through the studies. Animals (weighing 225–250 g at experiments commencement) were housed three per cage and maintained under controlled environmental conditions (ambient temperature of (22±1) ℃, humidity 55%–60%, photoperiod cycle 12 h light: 12 h dark), fed regular Lab Chow and water *ad libitum*. Only animals with no signs of sickness were included in the studies. The procedures of the study were in accordance with the guidelines of the institutional Committee for the Use and Care of Laboratory Animals.

2.3. Measurement of body temperature

Rectal BT was measured with a plastic-coated thermometer (Anristu Meter Co., Japan). Rats were accustomed to BT measurement during three days before the beginning of the experiments.

2.4. Treatment with N. syriaca extract

After a week of acclimation to housing conditions and BT measurements, rats were orally treated with the dry extract of *N. syriaca* for three successive days. On the evenings before each day of extract treatment, rats were deprived of food from 20:00 pm to 08:00 am. At 08:00, rats were allowed access to special containers containing either 2.5 g per rat of regular food, or, a total of 2.5 g per rat of regular food + dry extract of *N. syriaca* (Extract). The extract was calculated per animal weight to give a dose of 500 mg/kg. Thus, each extract-treated rat received 500 mg/kg extract per day for 3 consecutive days. Rats were allowed access to the regular food or extract-containing containers for 2 h during which the animals consumed all amount of the food in the containers. Thereafter, all animals were allowed free access to regular food again. During the entire experimental procedure rats had free access to water.

2.5. Induction of inflammation by LPS

LPS from *Escherichia coli* was dissolved in sterile NaCl 0.9% solution. Rats were administered (ip) with LPS 1 mg/kg to induce an inflammatory response as described previously[24]. Control rats were injected ip with 0.2 mL NaCl 0.9%. LPS was administered at 2 h after food/extract consumption.

2.6. Blood collection and preparation of brain regions homogenates

BT was evaluated immediately before LPS injection (time zero)

and at about 90 min thereafter. Then, rats were briefly anesthetized (with a mixture of 4% isoflurane in 100% oxygen) and immediately executed by decapitation. Blood was collected for plasma separation and brain regions (HT, HC and FC) were extracted and immediately frozen (–80 ℃). Thereafter, brain regions were weighed and homogenized in a homogenizing buffer (cold phosphate-buffered saline solution containing a cocktail of phosphatase and protease inhibitors purchased from Sigma) and centrifuged at 10 000 *g*, 4 ℃ for 10 min. Supernatants and pellets were separated, collected and immediately transferred to –80 ℃.

2.7. Determination of IL–6, PGE_2 and TNF– α levels

Levels of IL-6, PGE_2 and TNF- α in plasma and brain samples (supernatants of homogenates) were measured using rat DuoSet ELISA kits (R&D Systems; Minneapolis, MN, USA) according to manufacturer's instruction. The detection limits of the assays were as follows: 39–2 500 pg/mL for PGE_2; 62.5–4 000 pg/mL for TNF- α ; and, 125–8 000 pg/mL for IL-6. For determining plasma PGE_2 levels, samples were diluted 100 times to fit the standard curve of the assay. In all ELISA experiments, when the level of the tested inflammatory constituent was under the lower detection limit of the assay, results were marked as "undetectable" and calculated as zero.

2.8. Statistical analysis and presentation of the data

Statistical evaluations were carried out using two-tailed Student's *t*-test. Results are presented as mean±SEM for the sample size as indicated in each table. Values of $P<0.05$ were considered statistically significant. The data express the results of one experiment out of three independent experiments with similar results. Results of plasma IL-6, TNF- α and PGE_2 levels are presented as pg/mL according to ELISA results. Results of IL-6, TNF- α and PGE_2 levels (homogenates of brain samples) were calculated as follows: ELISA result (pg/mL) divided by weight of each brain sample; data are presented as pg/(mL•mg wet weight of brain sample).

3. Results

3.1. Effects of N. syriaca on LPS–induced hypothermia in rats

Administration of LPS led to a significant hypothermia in rats. The BT of the control rats, extract-treated rats, LPS-treated rats and LPS + Extract-treated rats was (0.08±0.13), (0.24±0.14), (-1.16±0.26), and (-0.61±0.12) ℃, respectively. Pretreatment with *N. syriaca* extract alone slightly but significantly increased BT of treated animals ($P<0.05$). Moreover, pretreatment with *N. syriaca* extract significantly attenuated LPS-induced hypothermia in rats ($P<0.05$).

3.2. Effects of N. syriaca on IL–6, TNF– α and PGE_2 levels in the plasma of LPS–treated rats

As seen in Table 1, IL-6 was undetectable in plasma of control and extract (alone)-treated animals. On the other hand, LPS led to a significant elevation in plasma IL-6 levels ($P<0.05$). Pretreatment with *N. syriaca* extract did not significantly decrease LPS-induced elevation in IL-6 levels. Similarly TNF- α was undetectable in plasma of control and extract-treated animals (Table 1) and pretreatment with *N. syriaca* did not significantly alter LPS-induced elevation in plasma TNF- α levels (Table 1). As for PGE_2, its levels did not differ significantly between control animals and those treated with *N. syriaca* extract alone (Table 1). LPS significantly increased its plasma levels ($P<0.05$) and pretreatment with *N. syriaca* led to a non-significant decrease in PGE_2 levels in LPS-treated rats (Table 1).

Table 1
Effects of *N. syriaca* on plasma IL-6, TNF- α and PGE_2 levels rats (pg/mL).

Group	IL-6	TNF- α	PGE_2
Control	UD	UD	1 591.0±179.0
Extract	UD	UD	1 442.0±8.0
LPS	2 419.8±632.4*	615.5±181.3*	2 074.0±143.0*
LPS + Extract	1 331.0±349.4*	795.7±170.6*	1 771.0±150.0

LPS, lipopolysaccharide; IL-6, interleukin 6; TNF- α , tumor necrosis factor- α ; PGE_2, prostaglandin E_2. Rats were fed with regular food or with food containing *N. syriaca* [500 mg/(kg•d)] for 3 consecutive days. On day 3, at 2 h after *N. syriaca* consumption, rats were injected LPS (1 mg/kg, ip). Rats were sacrificed at about 2 h post LPS injection and blood was collected for plasma separation. Plasma IL-6, TNF- α , and PGE_2 levels were determined by ELISA. Values are means±SEM of 12 rats. *$P<0.05$ *vs*. Control. UD denotes undetectable.

3.3. Effects of N. syriaca on IL–6, TNF– α and PGE_2 levels in the hypothalamus of LPS–treated rats

Administration of LPS and pretreatment with *N. syriaca* alone did not significantly alter IL-6 and TNF- α levels in the HT (Table 2). On the other hand, pretreatment with *N. syriaca* significantly decreased IL-6 and TNF- α levels in HT of LPS-treated rats ($P<0.05$) (Table 2). Consistent with previous studies, administration of LPS led to a significant increase in HT PGE_2 levels ($P<0.05$) (Table 2). Pretreatment with *N. syriaca* alone did not alter PGE_2 levels in this brain region, whereas pretreatment with *N. syriaca* significantly decreased HT PGE_2 levels in LPS-treated rats ($P<0.05$) (Table 2).

3.4. Effects of N. syriaca on IL–6, TNF– α and PGE_2 levels in the hippocampus of LPS–treated rats

Administration of LPS did not significantly alter IL-6 and PGE_2 levels in the HC (Table 2). In contrast, LPS significantly decreased HC TNF- α levels ($P<0.05$) (Table 2). Pretreatment with *N. syriaca* significantly reduced IL-6 and TNF- α levels in the HC of control as well as LPS-treated rats ($P<0.05$) (Table 2). Similarly, pretreatment with *N. syriaca* significantly decreased HC PGE_2 levels in LPS-treated rats ($P<0.05$) (Table 2).

3.5. Effects of N. syriaca on IL–6, TNF– α and PGE_2 levels in the frontal cortex of LPS–treated rats

Neither administration of LPS nor pretreatment with *N. syriaca* alone did not affect IL-6, TNF- α and PGE_2 levels in the FC (Table 2). Unexpectedly, pretreatment with *N. syriaca* significantly elevated IL-6 and TNF- α levels in the FC of LPS-treated rats ($P<0.05$) (Table 2) and pretreatment with *N. syriaca* led to a non-significant increase in PGE_2 levels in the FC of LPS-treated rats (Table 2).

Table 2

Effect of *N. syriaca* on hypothalamus IL-6, TNF- α and PGE_2 levels in rats [pg/(mL•mg wet weight of brain sample)].

Group	Hypothalamus			Hippocampus			Frontal cortex		
	IL-6	TNF- α	PGE_2	IL-6	TNF- α	PGE_2	IL-6	TNF- α	PGE_2
Control	100.9±9.8	86.5±6.4	36.9±4.0	300.5±20.8	113.3±8.0	75.5±8.0	180.3±27.8	138.0±16.8	55.4±4.6
Extract	83.7±8.0	94.3±3.7	39.0±5.5	233.2±18.2*	64.5±9.0*	78.8±6.0	189.0±29.0	131.4±13.6	66.4±5.8
LPS	94.9±9.0	106.4±7.2	74.7±13.9*	264.4±18.7	68.9±7.4*	64.7±6.4	202.0±32.0	140.7±11.2	67.1±5.7
LPS + Extract	39.2±10.0*#	83.7±3.8#	26.2±2.1*#	182.8±15.3*#	40.1±4.2*#	46.9±8.5*	300.1±35.0*#	194.8±13.7*#	79.7±15.1

LPS, lipopolysaccharide; IL-6, interleukin 6; TNF- α, tumor necrosis factor- α; PGE_2, prostaglandin E_2. Rats were fed with regular food or with food containing *N. syriaca* [500 mg/(kg•d)] for 3 consecutive days. On day 3, at 2 h after *N. syriaca* consumption, rats were injected LPS (1 mg/kg, ip). Rats were sacrificed at about 2 h post LPS injection immediately after which hypothalamus was removed and further processed as described under "Materials and Methods". Hypothalamic IL-6, TNF- α, and PGE_2 levels were determined by ELISA. Values are means±SEM of 12 rats. *P<0.05 *vs*. Control; #P<0.05 *vs*. LPS.

4. Discussion

To the best of our knowledge, the present study is the first to demonstrate that *N. syriaca* exerts protective effects against LPS-induced inflammation. We found that oral pretreatment with a dry extract of *N. syriaca* for 3 d resulted in significant anti-inflammatory effects in LPS-treated rats (P<0.05), including: i) Attenuation of LPS-induced hypothermia; ii) reduction of hypothalamic and hippocampal PGE_2 levels in LPS-treated rats; and, iii) decrease of IL-6 and TNF- α levels in HT and HC of LPS-treated rats. Nevertheless, pretreatment with *N. syriaca* also led to some pro-inflammatory effects such as elevation of IL-6 and TNF- α in FC of LPS-treated rats.

In general, the results of the present study suggest that *N. syriaca* confers anti-inflammatory effects, and that chronic consumption of the plant may be associated with anti-inflammatory and probably other protective properties. It is worth noting that this study utilized a sub-chronic treatment protocol – animals were treated with the extract once daily for 3 consecutive days. It is possible that a longer treatment-duration would have resulted in more profound anti-inflammatory effects. However, this assumption remains to be confirmed or alternatively refuted. Further study is also necessary to determine the dose-dependent effect of *N. syriaca* on the inflammatory parameters that were examined in this study. Moreover, the choice of oral (in the food) administration of the extract was done purposely to resemble a naturally occurring consumption of the plant materials. We decided against administering the extract through oral lavage or parenteral administration because these routes would not have mimicked a natural consumption of the plant.

The protective effect of *N. syriaca* against LPS-induced hypothermia resembles that of classic anti-inflammatory drugs such non-steroidal anti-inflammatory drugs[27]. These drugs attenuate LPS-induced hypothermia primarily due to inhibition of PGE_2 production in the HT[27]. Consistently, psychotropic drugs, that are not classic anti-inflammatory compounds, were also capable of reducing LPS-induced hypothermia in rats, probably due to decreasing HT PGE_2 levels[24]. Therefore, we speculate that *N. syriaca* also attenuated LPS-induced hypothermia due to inhibition of PGE_2 production in the HT. Importantly, the protective effect of *N. syriaca* against LPS-induced hypothermia was observed in all three experiments that we performed. On the other hand, currently we do not understand the reason for the slight elevation in BT which was seen in rats that were treated only with *N. syriaca*. Although these animals did not show any visible pathological characteristic (anhedonia – as could be featured by reduction in social activity or decrease in food and water consumption, weight loss or diarrhea), this result was seen in two of the three experiments that we performed.

The precise mechanism underlying the protective effects of *N. syriaca* against LPS-induced inflammation is currently not known. We speculated that *N. syriaca* may alter the activity of the transcription factor nuclear factor kappa B (NF- κ B), as was reported for other plant products with anti-inflammatory activities[9]. NF- κ B regulates the activity of numerous cellular pathways such as those involved immune and inflammatory responses, cancer pathophysiology, among others[37-40]. The mammalian NF- κ B pathway involves the activity of a number of proteins such as p50, p65, and RelB, which contribute to various physiological and pathological processes[37-39]. Different stimuli activate the NF- κ B pathway including infectious microorganisms, LPS, cytokines, tissue damage, neurodegeneration and ischemia[37-39]. These stimuli activate the pathway by inducing phosphorylation and subsequent degradation of NF- κ B inhibitor proteins (e.g., I κ B α and I κ B β) which inhibit the translocation of NF- κ B proteins to the nucleus. After dissociation of NF- κ B proteins from I κ B, they undergo phosphorylation by other kinases and then translocate to the nucleus and facilitate transcription of target genes. Therefore, it is recognized that elevated levels of phosphorylated NF- κ B proteins (such as p65) in the nucleus represent increased activity of the pathway[24,37,39]. In this regard, we conducted a preliminary experiment in which we examined the effect of *N. syriaca* on levels of nuclear phosphorylated-p65, as we described previously[24]. We found that pretreatment with *N. syriaca* for 3 d did not at all affect nuclear phosphorylated-p65 levels in HT and HC of LPS-treated rats (data not shown). These preliminary results suggest that the anti-inflammatory effects of *N. syriaca* probably derive from a non-NF- κ B-associated mechanism.

This study demonstrated that an aqueous extract of *N. syriaca* exhibited anti-inflammatory effects in LPS-treated rats. Further study is necessary to elucidate the mechanism underlying the anti-inflammatory effects of *N. syriaca* and the appropriate treatment regimen, including determination of the most efficacious dose and treatment duration. Moreover, further research is warranted to determine the chemical composition of the extract and reveal its most biologically active chemical constituents.

Conflict of interest statement

We declare that we have no conflict of interest.

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