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Effect of *Kochia scoparia* on carrageenan-induced paw edema in an experimental model of metabolic syndrome

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Metabolic syndrome (MS) is a global health issue affecting a significant part of the world population. Low-grade inflammation is one of the specific features of MS. *Kochia scoparia* is an annual plant used in the traditional Chinese medicine. The present study aimed to examine the effect of an aqueous infusion of *K. scoparia* seeds on the acute carrageenan-induced rat paw inflammation in an experimental model of MS. Forty male Wistar rats were allocated into four groups: MS, MS+1.5KS, MS+3.0KS and MS+6.0KS, all receiving a high-fat high-fructose diet. The drinking fluid of MS group was 10% fructose in water while the other three groups were drinking 10% fructose in aqueous *K. scoparia* (KS) seeds infusion of different strengths (prepared from 1.5, 3.0 and 6.0 g KS seeds in 100 ml of boiling water, respectively). At the end of the 10 weeks of dietary intervention, carrageenan (1 mg as 0.1 ml solution) was injected in the left hind paw. The paw edema was measured on the 30th, 60th, 120th, 180th, 240th and 300th minute after the injection. On the 30th minute, *K. scoparia* seeds infusion caused a significant dose-dependent decline in the paw edema. No further effect of the infusion was observed during the next time intervals. In conclusion, the results from the present study showed that the chronic administration of the aqueous *K. scoparia* seeds infusion decreased the carrageenan-induced paw inflammation in rats with metabolic syndrome but the anti-inflammatory effect appeared to be weak and short-lived.

Keywords: rats, carrageenan, paw edema, *Kochia scoparia*, metabolic syndrome

INTRODUCTION

Inflammation is an immune system response to harmful stimuli activated in order to enhance the healing process. Commonly triggered are the inflammatory NF-κB, MAPK and JAK-STAT pathways [1]. The primary inflammatory response is characterized by the production of mediators such as C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukins (IL-1, IL-6) and interferon-gamma (IFN-γ). On tissue level inflammation is characterized by swelling, redness, pain and impaired function.

Metabolic syndrome (MS) is a serious global problem affecting half quarter of the world's population. It is a constellation of impaired glucose tolerance, visceral adiposity, arterial hypertension and dyslipidemia (hypertriglyceridemia, increased non-esterified free fatty acids and decreased HDL-cholesterol) [2]. There is strong evidence that MS is accompanied by low-grade chronic inflammation [2]. Patients with MS have elevated CRP, interleukins, TNF-α and prostaglandins [3]. Furthermore, weight loss is accompanied by a decrease in the levels of the proinflammatory markers.

Kochia scoparia is an annual leafy ornamental plant. The fruits of *K. scoparia* are rich in triterpenoids, saponins and alkaloids [4, 5]. In China, Korea and Japan its fruits are used in the treatment of dysuria and skin diseases and the Bulgarian traditional medicine recommends the seed infusion in liver disease. An ethanol extract of *K. scoparia* fruits possesses anti-inflammatory and antiallergic effects [6, 7]. In MS, the triterpenoid momordin Ic, found as a major constituent in *K. scoparia* fruit, has shown anticancerogenic effect [8].

Carrageenan is a mucopolysaccharide extract, discovered by the British pharmacist Stanford in 1862. When injected subcutaneously, carageenan induces acute inflammatory response due to the release of bradykinin, histamine, tachykinins, reactive oxygen and nitrogen species [9]. This leads to edema due to extravasation of fluid and protein and neutrophil infiltration of the inflammatory site. Carrageenan-induced paw edema in rats is a broadly used model for testing the anti-inflammatory activity of various substances [10].

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The present study aimed to examine the effect of an aqueous infusion of *K. scoparia* seeds on the acute carrageenan-induced rat paw inflammation in an experimental model of MS.

EXPERIMENTAL

Animals and experimental protocol

Forty male Wistar rats (initial body weight 160-200 g) were included in the experiment. The animals were kept under a 12/12 hours light-dark cycle, an ambient temperature of 20-25°C and had free access to food and water. They were allocated into 4 groups (10 rats in a group): MS, MS+1.5KS, MS+3.0KS, MS+6.0KS. All rats received high-fat high-fructose (HFHF) diet which consisted of lard (17%), fructose (17%) and regular rat chow. The MS group serving as a control received 10% fructose in the drinking water while the remaining three groups received 10% fructose added to the aqueous infusion of *K. scoparia* seeds of different strengths. The infusions were prepared by soaking 1.5, 3.0 and 6.0 g of *K. scoparia* (KS) seeds, respectively, in 100 ml boiling water and subsequent percolation upon cooling down. The duration of the dietary intervention was 10 weeks. The experimental protocol is given in Table 1.

Table 1. Experimental protocol; MS – metabolic syndrome, HFHF – high-fat high-fructose, KS – *K. scoparia*

Groups	Diet	Drinking
MS	HFHF	10% fructose in the drinking water
MS+1.5KS	HFHF	10% fructose in KS seed infusion (1.5 g/100 ml)
MS+3.0KS	HFHF	10% fructose in KS seed infusion (3.0 g/100 ml)
MS+6.0KS	HFHF	10 % fructose in KS seed infusion (6.0 g/100 ml)

All procedures concerning animal treatment and experimentation were conducted in conformity with the national and international laws and policies (EU Directive 2010/63/EU for animal experiments) and were approved by the Bulgarian Food Safety Agency (Document 177/07.07.2017).

Induction of edema

At the end of the dieting period, carrageenan at a dose of 1 mg as 0.1 ml of freshly prepared solution in 0.9% saline was injected into the plantar surface of the left hind paw of the animals to induce acute inflammation. The paw volumes (ml) were measured before the injection and at the 30^{th}, 60^{th}, 120^{th}, 180^{th}, 240^{th}, and 300^{th} min after that. The digital plethysmometer LE7500 Panlab, Barcelona, was used to make the measurements. The difference between the paw volumes before and after the carrageenan injection was considered a marker of the intensity of the inflammation induced.

The paw edema (%) was calculated using the formula:

$$(V_s\text{-}V_0) \times 100,$$

where: V_s – the average paw volume measured at the six time intervals after the carrageenan injection, V_0 – the average initial paw volume.

Statistical analysis

The results were analyzed by one-way ANOVA and the post-test for linear trend. Graphpad Prism 5 Software was used. The data are presented as means ±SEM and $p<0.05$ was considered to indicate statistical significance.

RESULTS

The carrageenan injection induced acute local inflammation in the rats demonstrated by edema of the left hind paw. On the 30^{th} minute, the mean paw edema (%) of the rats from the control MS group was 12.15 % while the same parameter of the animals from MS+1.5KS, MS+3.0KS and MS+6.0KS groups were respectively 9.31, 7.37 and 5.28. The mean paw edema values in the subsequent time points are given in Table 2.

Table 2. Mean paw edema after carrageenan injection; MS – metabolic syndrome, KS – *K. scoparia*

Mean paw edema (%)	MS	MS+1.5KS	MS+3.0KS	MS+6.0KS	ANOVA	Post-test for linear trend
30^{th} min	12.15	9.31	7.37	5.28	p>0.05	p< 0.05
60^{th} min	12.60	12.54	13.43	9.88	p>0.05	p>0.05
120^{th} min	28.58	27.71	30.89	20.74	p>0.05	p>0.05
180^{th} min	31.34	38.24	34.44	35.65	p>0.05	p>0.05
240^{th} min	46.26	46.61	52.65	44.35	p>0.05	p>0.05
300^{th} min	45.42	47.47	43.69	36.30	p>0.05	p>0.05

DISCUSSION

In the present study we tested the potential of *K. scoparia* seed infusion to exert anti-inflammatory effect. On the 30th minute, a slight linear decline in paw edema from the MS group towards MS+6KS group was noted ($p<0.05$). During the next time periods there was only a non-significant reduction in the paw edema of the MS+6KS group. Our study demonstrated the presence of an early and weak, nevertheless dose-dependent anti-inflammatory effect of *K. scoparia* seed infusion. It occurred at the onset of the inflammatory process and gradually disappeared with time.

The results from our study are partially in line with a number of studies which indicate an anti-inflammatory potential of *K. scoparia*. In their study Jo *et al.* [11] found methanol extract of *K. scoparia* dried fruit to decrease ear swelling in a model of 1-fluoro-2,4-dinitrofluorobenzene (DNFB)-induced contact dermatitis in mice. They suggested that the anti-inflammatory effect is due to suppression of Th1 skewing reactions (decreased production of IL-6, IFN-γ). Another study [12] describes the anti-inflammatory and antiallergic effect of the *K. scoparia* ethanol extract on ovalbumin-induced murine asthma model, where attenuation in the levels of interleukins (IL-4 and IL-5) in a dose-dependent manner has been demonstrated. Similarly, Matsuda *et al*. [13] showed that 70% ethanol extract of *K. scoparia* fruits inhibits the rise of vascular permeability induced by acetic acid, prevents the increase of paw edema induced by carrageenan, histamine, serotonin or bradykinin and ear swelling induced by arachidonic acid and these have been translated into anti-nociceptive effects. Momordin Ic has also been shown to exhibit an inhibitory effect on carrageenan-induced edema. These results indicate that *Kochia* fruits exert a peripheral anti-nociceptive effect mediated by an anti-inflammatory action, and that these activities can be partially attributed to momordin Ic. Evidence that momordin Ic is the most potent anti-inflammatory ingredient in *Kochia scoparia* fruit, acting by inhibiting the production of inflammatory cytokines TNF-α and IL-6 and prostaglandin E_2 is presented in the work of Yoo *et al.* [14].

The majority of the studies focusing on the pharmacological activity of *K. scoparia* use alcohol extracts of the fruits of *Kochia*. More information is also available on the nature of the biologically active substances present in the fruits, but little or nothing is known about the phytochemical content of the *Kochia* seeds. Therefore, it is not surprising that the present results appear to be only a weak reminiscence of what has been found as activity from other parts of the plant. It is not unusual that different bioactive components reside in different anatomical parts of medicinal plants. We have, for example, found some other potentially beneficial effects of *Kochia* seed aqueous infusion (unpublished data).

Our study indicates, opposite to the expectations, a weak and short-lived anti-inflammatory potential of *K. scoparia* seed infusion on carrageenan-induced paw edema in the experimental model of MS. The lack of a more pronounced anti-inflammatory effect could be due to the use of a different form of extract originating from a different part of the Kochia plant compared to those cited in the literature. A further work is needed to identify the presence of phyto-active components in the *K. scoparia* seed infusion when trying to elucidate its potential health benefits.

In conclusion, the **c**hronic administration of the aqueous infusions of *K. scoparia* seeds decreased the paw edema early during the carrageenan-induced inflammation in the experimental rats with metabolic syndrome. However, the anti-inflammatory effect under the experimental circumstances in this study appeared to be weak and short-lived.

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