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Evaluation of *in vivo* and *in vitro* Biological Activities of Different Extracts of *Cuscuta arvensis*

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In the present study, the potential effects of extracts from the whole plant of *Cuscuta arvensis* were studied in mice using the carrageenan-induced hind paw edema model for antiinflammatory activity and the *p*-benzoquinone-induced writhing reflex for the assessment of antinociceptive activity. In order to obtain the extracts, the whole plant of *C. arvensis* was extracted with different solvents such as *n*-hexane, dichloromethane, ethyl acetate, methanol and distilled water. Antioxidant activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The methanolic and water extracts inhibited the carrageenan-induced paw edema and *p*-benzoquinone-induced writhing reflex, whereas the other extracts showed only mild inhibitory antinociceptive and antiinflammatory activities in these *in vivo* models. Additionally, the methanol and ethyl acetate extracts had higher scavenging ability then the non polar extracts.

Keywords: Anti-inflammatory, antioxidant, antinociceptive, *Cuscuta arvensis*, Convulvulaceae, iksut, kuskut.

Cuscuta (Convolvulaceae) species, commonly known as dodders, are epiphytic vines. Some species are reported as harmful weeds to industrial and vegetable crops. Although they are treated as parasites, *Cuscuta* species are very valuable folk medicines and a source of valuable compounds in rural areas of some developing countries. In traditional and contemporary Chinese medicine, extracts of *Cuscuta* species are used as a tonic to improve the functions of the liver and kidney, to ameliorate sexual functions, to clear blurred vision, and are clinically used to either prevent or delay aging [1]. A decoction or infusion of *C. europaea* L. is used as a laxative, diuretic, spasmolytic, cholagogue and carminative in folk medicine of Bulgaria and Italy [2]. Likewise, a maceration, prepared by incubating the dried flowering *Cuscuta* plant, locally called iksut, in warm water overnight, has been used to heal liver, kidney problems, and jaundice in southeastern Turkey [3].

The fresh whole plant is traditionally applied to the skin to treat sciatica. Also, the juice of *C. chinensis* Lam. is used for the treatment of chronic ulcers, and for healing wounds, inflammation and jaundice in Pakistan, Bangladesh and Nepal [4,5]. Previous studies revealed that *Cuscuta* species have various biological activities. For instance, methanolic extracts of *C. reflexa* stems displayed antibacterial activity against *Staphylococcus aureus*, *S. boydii*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *E. coli* [6]. Phytochemical research, followed by pharmacological analysis, demonstrated that the major compounds of *C. japonica* possess an antihypertensive effect, whereas C3 binding glycoprotein (C3bgp) of *C. europea* has an immunomodulatory effect [7,8]. Moreover, compounds isolated from the most studied species, *C. chinensis*, were shown to possess anticancer, immunostimulant, anti-aging, memory enhancing, hepatoprotective, antioxidant and anti-osteoporotic activities [9-13]. The major active constituents of *C. chinensis* were flavonols, flavonoids, lignans, quinic acid, and polysaccharides [14-17]. Recent study of *C. chinensis* verified that the ethyl acetate extracts have high antioxidant activity that was correlated with the content of flavon derivatives. Reactive oxygen species (ROS), such as hydroxyl radical, hydrogen peroxide and superoxide anion, have a role in oxidative stress that might be the cause of various diseases and symptoms such as heart disease, liver injury, diabetes, cancer and aging [18-22]. The balance between the harmful effects of ROS and their antioxidant capacity could serve as a major mechanism in preventing damage by oxidative stress and aging. Although several *Cuscuta* species have been investigated, no scientific data are available about the anti-inflammatory and antinociceptive activities of the species found in Turkey. Thus, the aim of the present study was to investigate the *in vivo* and *in vitro* pharmacological effects of non-polar (*n*-hexane, dichloromethane) and polar (ethyl acetate, methanol and water) extracts of *C. arvensis*, a species that has not been studied previously.

The whole plants of *C. arvensis* were extracted with *n*-hexane, dichloromethane, ethyl acetate, methanol and water, respectively, and the dried extracts were evaluated

Table 1: Antiinflammatory effect of the extracts from *C. arvensis* on carrageenan-induced hind paw edema in mice.

Test samples	Dose (mg kg^{-1})	Swelling thickness (x10^{-2}mm) ± SEM (inhibition %)			
		90 min	180 min	270 min	360 min
Control		40.2 ± 4.2	44.8 ± 4.7	50.3 ± 4.0	57.1 ± 5.3
n-hexane	100	47.3 ± 5.7	48.5 ± 4.8	60.3 ± 5.1	62.8 ± 4.7
CH_2Cl_2	100	38.3 ± 3.2 (4.7)	41.1 ± 3.9 (8.3)	53.4 ± 4.2	58.9 ± 4.6
EtOAc	100	36.5 ± 3.1 (9.2)	46.5 ± 3.7	52.4 ± 3.7	55.3 ± 3.2 (3.2)
MeOH	100	34.1 ± 3.0 (15.2)	37.3 ± 3.2 (16.7)	39.6 ± 2.8 (21.3)	41.8 ± 2.7 **(26.8)****
H_20	100	35.4 ± 2.9 (11.9)	37.9 ± 3.1 (15.4)	40.2 ± 3.4 (20.1)	45.1 ± 3.4 (21.0)
Indomethacin	10	31.7 ± 2.4 (21.1)	30.8 ± 2.7 **(31.3)****	35.9 ± 2.5 **(28.6)****	34.7 ± 2.9 **(39.2)*****

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$; SEM: standard error mean.

for anti-inflammatory and antinociceptive activities using the carrageenan induced hind paw and *p*-benzoquinone animal models [23]. The radical scavenging activities of the extracts were examined using the DPPH (1,1-diphenyl-2-picryl hydrazine) assay. The results are summarized in Tables 1, 2 and 3.

The antiinflammatory effects of the extracts on carrageenan induced paw edema in mice are shown in Table 1. The results indicate that the polar extracts have significant ($P < 0.0001$) antiinflammatory activity in mice. The methanolic extract exhibited significant inhibition, ranging between 15.2-26.8% in the carrageenan-induced hind paw edema model at 100 mg kg^{-1} dose, and the results were quite comparable with the reference compound, indomethacin (Table 1, 21.1-39.2% inhibition). The carrageenan-induced edema results from the activation of the kinin system, the accumulation of neutrophils and the release of several mediators, such as prostanoids and cytokines. Mast cell amines play a minor role in this inflammatory reaction. The phorbol esters, in acute topical administration, modify the production of pro-inflammatory cytokines, induce neutrophil infiltration in the epidermis and activation of COX-2 [24].

The *p*-benzoquinone-induced writhing test is known to have limitations, as drugs other than analgesics will inhibit writhing; these include antihistamines, sympathomimetics and parasympathomimetics, central nervous system stimulants and adrenergic blockers [25]. Such limitations are reported mainly for the Koster test, in which acetic acid solution is administered to induce writhing. According to the antinociceptive results that we obtained, the methanolic and aqueous extracts produced 29.4% and 24.9% inhibition, respectively, while acetylsalicylic acid (ASA), the reference compound, showed 49.5% inhibition at a dose of 100 mg kg^{-1} (Table 2). In contrast, the other extracts did not demonstrate any inhibitory activity in this model. In order to check the acute toxicity and morbidity, mortality was monitored for a 48 h period after the administration of the extracts to the mice. Neither

Table 2: Antinociceptive effect of the extracts of *C. arvensis* against *p*-benzoquinone-induced writhing in mice.

Material	Dose (mg kg^{-1})	Number of writhings ± S.E.M.	Inhibitory ratio (%)	Ratio of ulceration[a]
Control		40.2 ± 4.7		0/6
n-Hexane	100	43.4 ± 3.6	---	0/6
CH_2Cl_2	100	47.6 ± 4.9	---	0/6
EtOAc	100	38.3± 3.1	4.7	0/6
MeOH	100	28.4 ± 2.8	**29.4****	0/6
H_20	100	30.2 ± 2.6	**24.9***	0/6
ASA	100	20.3 ± 1.9	**49.5*****	4/6
	200	18.4 ± 1.6	**54.2*****	6/6

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$; SEM: standard error mean. [a]:number of stomachs in experimental animals in which gastric lesions had been induced.

Table 3: Antioxidant activity of *C. arvensis* extracts.

Extracts	Antioxidant Activity % at Different Concentrations			
	2000 µg mL^{-1}	1000 µg mL^{-1}	500 µg mL^{-1}	100 µg mL^{-1}
n-Hexane	58.7±0.3	52.1±0.6	43.5±0.5	46.2±0.9
CH_2Cl_2	68.7±0.5	66.0±0.5	61.0±0.6	30.3±0.5
EtOAc	82.3±0.2	78.7±0.2	62.1±0.6	42.1±0.6
MeOH	86.2±0.1	81.4±0.3	78.5±0.4	39.9±0.2
H_20	73.1±0.3	68.0±0.3	58.4±0.3	34.7±0.8
BHA	88.6±0.2	82.8±0.8	77.3±0.3	40.5±0.2

morbidity nor death was recorded and, besides, the extracts did not induce any apparent gastric lesion at the administered dose. The results for the antioxidant activity of the various extracts of *C. arvensis* seeds showed that the methanolic and ethyl acetate extracts demonstrated significantly better and higher effects than the other extracts (Table 3).

In earlier studies, the antioxidant activities of various extracts of the seeds of *C. chinensis* had been analyzed for their DPPH free radical-scavenging, superoxide anion scavenging, anti-superoxide anion formation and anti-lipid peroxidation abilities [12]. In order to analyze the relationship between phytochemical content and antioxidant levels, the flavonol contents of all the test samples were analyzed by high-performance liquid chromatography (HPLC–UV detector). The results show that there is a direct correlation between flavonol content and the antioxidant activities of the extracts and fractions of *C. chinensis*. In addition, the ethyl acetate fraction, in accordance with our results, demonstrated significantly higher antioxidant effects. Furthermore, the same extract had a higher flavonol content than the remaining samples ($P < 0.05$). In the same study, the water fractions exhibited the weakest antioxidant activity and also had a low flavonol content. Besides flavonol heterosides and flavonoids of *C. chinensis*, bioassay-guided fractionation of the ethyl acetate-soluble extract of *C. japonica* presented the phenolic compounds 3,5-di-*O*-caffeoylquinic acid (**1**), methyl 3,5-di-*O*-caffeoylquinate (**2**), 3,4-di-*O*-caffeoylquinic acid (**3**), and methyl 3,4-di-*O*-caffeoylquinate (**4**) [17, 25]. The structures of the compounds were elucidated on the basis of MS and NMR spectroscopic data. *In vitro* activity studies on the ethyl acetate extract demonstrated that compounds **1**-**4** cause

inhibition of the angiotensin I converting enzyme activity in a dose-dependent manner. Therefore, these active compounds might contribute to the antihypertensive action of *Cuscuta* Semen [7].

In line with the preceding studies, polar extracts of *C. arvensis* demonstrated significantly greater activity than the nonpolar ones. For the antiinflammatory and antinociceptive activities it was the methanolic and aqueous extracts, whereas for the antioxidant activity, it was the methanolic and ethyl acetate extracts that were found to have activities that were statistically significantly greater than those of the nonpolar *n*-hexane and dichloromethane extracts. According to the literature review and our knowledge this is the first study showing the pharmacological activities of *C. arvensis*. These findings also support the traditional usage of *C. arvensis*. Ongoing phytochemical studies will yield further information on the compounds responsible for the activities.

Experimental

Animals: Male Swiss albino mice (20-25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health, Ankara, Turkey. The animals, left for 2 days for acclimatization to animal room conditions, were maintained on standard pellet diet and water *ad libitum*. The food was withdrawn on the day before the experiment, but the animals were allowed free access to water. A minimum of 6 animals was used in each group. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals.

Preparation of test samples for bioassay: Test samples were given orally to the test animals after suspending in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those of the test groups, except that the drug treatment was replaced with appropriate volumes of dosing vehicle. Indomethacin (10 mg kg^{-1} and 0.5 mg ear^{-1}) and acetylsalicylic acid (ASA) (100 and 200 mg kg^{-1}) in 0.5% CMC were used as reference drugs.

Acute toxicity: Animals employed in the carrageenan-induced paw edema experiment were observed during 48 h and morbidity or mortality was recorded for each group at the end of the observation period.

Gastric-ulcerogenic effect: After the antinociceptive activity experiment, mice were killed under deep ether anesthesia and the stomach of each mouse was removed. Then the abdomen of each mouse was opened through the greater curvature and examined under a dissecting microscope for lesions or bleeding.

Statistical analysis of data: Data obtained from animal experiments were expressed as the mean standard error (±SEM). Statistical differences between the treated and the control groups were evaluated by ANOVA and Students-Newman-Keuls post-hoc tests. $p < 0.05$ was considered to be significant [* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$].

Collection of plant material and extraction: The plant material was purchased from an akhtar in Mardin. The akhtar showed the host and the *Cuscuta* species which was attached to the host. The collected host was determined as *Lens culinaris* and the *Cuscuta* species was identified as *C. arvensis*. A herbarium specimen was prepared and deposited in the Department of Biology in Kilis 7 Aralik University. The plant samples were powdered and divided into 30 g samples. Samples were extracted separately with *n*-hexane (2 x 300 mL), dichloromethane (2 x 300 mL), ethyl acetate (2 x 300 mL), methanol (2 x 300 mL), and water (2 x 300 mL). The yields of the extracts were 1.2%, 19.3%, 25.16%, 31%, and 20.1%, respectively.

Radical scavenging assay: The antioxidant activity of the extracts was determined by the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH; Sigma-Aldrich D9132-1G Steinheim, Germany) radical. The *n*-hexane extracts were dissolved in methanol, and the others in ethanol (75%). Four dilutions (100, 500, 1000 and 2000 µg L^{-1}) were made containing 77 µL of the corresponding extract and 3 mL of the DPPH solution dissolved in either methanol or ethanol according to the type of extract, while keeping the concentration at 6 x 10-5 mol/L. A synthetic antioxidant, butylated hydroxyanisole (BHA) (Sigma B-1378 Steinheim, Germany), was used as a reference. After incubation in the dark for 15 min, the absorbance was measured at 515 nm using a spectrophotometer (PG Instruments GB). The radical scavenging effect was calculated as follows: antiradical effect (%) = $(A_{blank}-A_{extract})/A_{blank}$ x 100. The blank is the absorbance of the control, which contains all reagents except the extract; $A_{extract}$ is the absorbance of either the test extract or the reference.

References

[1] Zheng HZ, Dong ZH, She J. (**1998**) Modern Study of Traditional Chinese Medicine, 1st Ed., Beijing Xue Yuan Press of the People's Republic of China, Beijing, China. 4110–4120.

[2] Leporatti ML, Ivancheva S. (**2003**) Preliminary comparative analysis of medicinal plants used in the traditional medicine of Bulgaria and Italy *Journal of Ethnopharmacology*, ***87***, 123–142.

[3] Sekeroglu N, Meraler SA, Koca U. (**2011**) Medicinal plants used as traditional medcine in Mardin. *Mardinlife Magazine*. In press.

[4] Qureshi R, Bhatti GR. (**2008**) Ethnobotany of plants used by the Thari people of Nara Desert, Pakistan. *Fitoterapia*, ***79***, 468-473.

[5] Kalpana G, Rishi RB. (**2009**) Ethnomedicinal knowledge and healthcare practices among the Tharus of Nawalparasi district in central Nepal Forest. *Ecology and Management*, ***257***, 2066-2072.
[6] Pal DK, Mandal M, Senthilkumar GP, Padhiari A. (**2006**) Antibacterial activity of *Cuscuta reflexa* stem and *Corchorus olitorius* seed. *Fitoterapia, **77,*** 589–591.
[7] Oh H, Kang DG, Lee S, Lee HS. (**2002**) Angiotensin converting enzyme inhibitors from *Cuscuta japonica* Choisy. *Journal of Ethnopharmacology*, ***83***, 105-108.
[8] Stanilova SA, Zhelev ZD, Dobreva ZG. (**2000**) Preliminary studies on the immunomodulatory effect of the C3 binding glycoprotein isolated from *Cuscuta europea. International Journal of Immunopharmacology*, ***22***, 15-24.
[9] Nisa M, Akbar S, Tariq, M, Hussain Z. (**1986**) Effect of *Cuscuta chinensis* water extract on 7, 12-dimethylbenz[*a*] anthracene-induced skin papillomas and carcinomas in mice. *Journal of Ethnopharmacology*, ***18***, 21–31.
[10] Bao X, Wang Z, Fang J, Li X. (**2002**) Structural features of an immunostimulating and antioxidant acidic polysaccharide from the seeds of *Cuscuta chinensis*. *Planta Medica*, ***68***, 237–243.
[11] Liu JH, Jiang B, Bao YM, An LJ. (**2003**) Effect of *Cuscuta chinensis* glycoside on the neuronal differentiation of rat pheochromocytoma PC12 cells. *International Journal of Developmental Neuroscience*, ***21***, 277–281.
[12] Yen FL, Wua TH, Lin LT, Cham TM, Lin CC. (**2008**) Concordance between antioxidant activities and flavonol contents in different extracts and fractions of *Cuscuta chinensis. Food Chemistry*, ***108***, 455–462.
[13] Yang L, Chen, Q, Wang F, Zhang G. (**2011**) Antiosteoporotic compounds from seeds of *Cuscuta chinensis Journal of Ethnopharmacology*, ***135***, 2, 553-560.
[14] Du XM, Kohinata K, Kawasaki T, Guo YT, Miyahara K. (**1998**) Components of the ether-insoluble resin glycoside-like fraction from *Cuscuta chinensis*. *Phytochemistry*, ***48***, 843–850.
[15] Wang Z, Fang, JN, Ge DL, Li XY. (**2000**) Chemical characterization and immunological activities of an acidic polysaccharide isolated from the seeds of *Cuscuta chinensis* Lam. *Acta Pharmacologica Sinica*, ***21***, 1136–1140.
[16] Ye M, Li Y, Yan Y, Liu H, Ji X. (**2002**) Determination of flavonoids in *Semen Cuscuta* by RP-HPLC. *Journal of Pharmaceutical and Biomedical Analysis*, ***28***, 621–628.
[17] Ye M, Yan Y, Guo DA. (**2005**) Characterization of phenolic compounds in the Chinese herbal drug Tu-Si-Zi by liquid chromatography coupled to electrospray ionization mass spectrometry. *Rapid Communications in Mass Spectrometry*, ***19***, 1469–1484.
[18] Giordano FJ. (**2005**) Oxygen, oxidative stress, hypoxia, and heart failure. *The Journal of Clinical Investigation*, ***115***, 500–508.
[19] Rolo AP, Palmeira CM. (**2006**) Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicology and Applied Pharmacology*, ***212***, 167–178.
[20] Jaeschke H. (**2000**) Reactive oxygen and mechanisms of inflammatory liver injury. *Journal of Gastroenterology and Hepatology*, ***15***, 718–724.
[21] Klaunig JE, Kamendulis LM. (**2004**) The role of oxidative stress in carcinogenesis. *Annual Review of Pharmacology and Toxicology*, ***44***, 239–267.
[22] Bokov A, Chaudhuri A, Richardson A. (**2004**) The role of oxidative damage and stress in aging. *Mechanisms of Ageing and Development*, ***125***, 811–826.
[23] Yeşilada E, Küpeli E. (**2007**) *Clematis vitalba* L. aerial part exhibits potent anti inflammatory,antinociceptive and antipyretic effects. *Journal of Ethnopharmacology*, ***110***, 504-515.
[24] Vinegar R, Schreiber HR. (**1969**) Biphasic development of carageenin oedema in rats. *Journal of Pharmacology and Experimental Therapeutics*, ***166***, 96-103.
[25] Björkman R, Hallman KM, Hedner J, Hedner T, Henning M. (**1994**) Acetaminophen blocks spinal hyperalgesia induced by NMDA and substance P. *Pain*, ***57***, 259–264.