In vitro Anti-inflammatory Effect of *Carthamus lanatus* L.

Saima Jalil[a], Bozhanka Mikhova[b*], Rilka Taskova[c], Maya Mitova[b], Helmut Duddeck[d], Muhammad Iqbal Choudhary[a], and Atta-ur-Rahman[a]

[a] H. E. J. Research Institute of Chemistry, International Center for Sciences, University of Karachi, Karachi-75270, Pakistan
[b] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria. Fax: ++3592/8700225. E-mail: bozhana@orgchm.bas.bg
[c] Institute of Botany, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[d] Institute of Organic Chemistry, University of Hannover, 30167 Hannover, Germany

* Author for correspondence and reprint requests

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The anti-inflammatory activity of four total extracts, their fractions and two main constituents (α-bisabolol β-D-fucopyranoside and luteolin 7-*O*-glucoside) of *Carthamus lanatus* L. aerial parts, were assessed *in vitro* by determining the inhibitory effects on induced human neutrophils. The dichloromethane extract and its water-alcoholic part exhibited the most significant inhibitory effects.

Key words: Carthamus lanatus, Anti-inflammatory Activity, Neutrophils

Introduction

Inflammation is the reaction of living tissues to injury, infection or irritation. Bacterial infections cause an increased number of neutrophils, which produce an oxidative burst at the site of microbial invasion. The uncontrolled release of reactive oxygen species is assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis. Administration of agents, which decrease the neutrophil accumulation in inflamed areas, might be a remedy in these cases. *Carthamus tinctorius* L. (Asteraceae) was shown to possess anti-inflammatory activity (Kasahara *et al.*, 1994). The present paper deals with the *in vitro* anti-inflammatory effects of four total extracts (dichloromethane, methanol, 50% methanol, water extracts), their fractions and two main constituents (α-bisabolol β-D-fucopyranoside and luteolin 7-*O*-glucoside) from *Carthamus lanatus* aerial parts.

Experimental

Plant material

C. lanatus aerial parts were collected in July, 2001 at the Losen region, Sofia, Bulgaria and identified by Dr. Rilka Taskova. A voucher specimen (SOM 156639) is deposited in the Herbarium of the Institute of Botany, Sofia.

Extraction and isolation

Dichloromethane extract. Air-dried and powdered aerial parts of *C. lanatus* (500 g) were extracted with dichloromethane. The residue of the dichloromethane extract (9.5 g) was partitioned between upper and lower layer (dry residue 4 g) of hexane/methanol/water (19:19:2, v/v/v). α-Bisabolol β-D-fucopyranoside (0.9 g) was isolated from the water/alcoholic phase of the dichloromethane extract by column chromatography on silica gel with hexane and hexane/ethylacetate (20:1 to 1:10).

MeOH extract. C. lanatus (500 g); MeOH extract (53 g). The residue of the methanol extract was extracted subsequently with diethyl ether (6 g), ethyl acetate (1.3 g) and butanol (3 g); water soluble part (37 g). Luteolin 7-*O*-glucoside (0.18 g) was isolated from the butanol fraction by droplet counter current chromatography (DCCC), descending mode, with chloroform/methanol/water/propanol (9:12:8:1, v/v/v/v).

50% MeOH extract. C. lanatus (500 g); 50% MeOH extract (70 g).

Water extract. C. lanatus (100 g); H_2O extract (15 g).

α-Bisabolol β-D-fucopyranoside and luteolin 7-*O*-glucoside were identified by reported spectral data and comparison with reference compounds (Feliciano *et al.*, 1990; El-Shaer *et al.*, 1998).

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Isolation of human neutrophils

Human neutrophils were isolated by the modified method of Siddiqui *et al.* (1995). Briefly fresh heparinized blood collected from healthy volunteers was diluted with equal volume of Hanks balance salts solution (HBSS). After leaving 20 min at room temperature, the upper leukocyte layer was collected, layered over Ficoll paque (Pharmacia Biotech., Uppsala, Sweden) and centrifuged at 1500 rpm for 30 min. Cells were lysed with lysis buffer for 10 min, centrifuged and diluted with HBSS. After centrifuging the pellet was resuspended in HBSS with $CaCl_2$, $MgSO_4 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$. Cell counts were done using the improved Neubaur chamber. The viability of cells determined by the Trypan Blue method was above 97%.

Anti-inflammatory assay

The anti-inflammatory effect was determined by the modified assay of Tan and Berridge (2000): total volume of 250 μl modified Hank's Solution (MHS) with pH 7.4 containing $0.5-1.0 \times 10^4$ neutrophils/ml, 500 μM WST-1 [1,2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt (Dojindo Laboratories, Kumamoto, Japan)] and various concentrations of the test samples. The control contained only buffer, neutrophils and WST-1. All samples were equilibrated at 37 °C and the reaction initiated by adding Zymosan-activated serum ZAS (Sigma Chemicals, St. Louis, USA) for 30 min. Indomethacine was used as reference (IC_{50} 0.27 mg/ml). Absorbance was measured at 450 nm using Spectra MAX 340-plus microplate reader (Molecular Devices, Sunnyvale, California, USA). IC_{50} values were calculated by comparing with DMSO as blank and expressed as percent inhibition of superoxide produced.

Statistical analysis

Data are presented as the means ± s.e.m. Statistical analysis was performed using EziFit 5.0 Windows based software after analysis of variance. P-values < 0.05 were considered to be significant. IC_{50} values were calculated by regression line.

Results and Discussion

The anti-inflammatory effects of the test samples were assessed *in vitro* using the modified cell-based assay of Tan and Berridge (2000) based on reduction of the highly water-soluble tetrazolium salt WST-1 in the presence of activated neutrophils. The obtained data are summarized in Table I. IC_{50} values of the most active fractions were calculated. The analysis revealed that the effect of the dichloromethane extract exceeded that of the more polar methanol, 50% methanol and water extract. The dry residues of the dichloromethane extract and its water/methanolic part showed the highest inhibitory effects: 70.5% and 81.3% with IC_{50} values 0.69 mg/ml and 0.47 mg/ml, respectively. The water/alcoholic fraction is a complex mixture consisting of sesquiterpene fucosides, sterols, triterpenes, dehydroabietic acid, lipids and other constituents (Feliciano *et al.*, 1990; Mitova *et al.*, 2003). α-Bisabolol β-D-fucopyranoside, being one of the main constituents of this fraction, showed 41.9% inhibitory effect and consequently, other constituents attribute to a higher extent to the total effect of this fraction. The active compounds of this fraction might be the sterols and triterpenes as shown for *C. tinctorius* (Kasahara *et al.*, 1994). Furthermore, it is interesting to note that the significant anti-inflammatory effect of the

Table I. Inhibitory effect of extracts, fractions and individual constituents of *C. lanatus* on induced human neutrophils.

Sample	Inhibition of O_2^- produced (%)[a]
CH_2Cl_2 extract	70.5
Hexane part	6.3
Water/methanolic part	81.3
MeOH extract	28.9
Diethyl ether fraction	52.2
EtOAc fraction	37.3
BuOH fraction	17.7
Water fraction	19.0
50% MeOH extract	15.7
H_2O extract	11.2
α-Bisabolol fucopyranoside	41.9
Luteolin-7-*O*-glucoside	31.5
Indomethacine	72.2
Control	0

[a] At a dose of 1 mg of dry residue or constituent per ml.

water/alcoholic fraction is in correlation with the observed high antibacterial and cytotoxic activity of this fraction (Taskova *et al.*, 2002).

The total methanol extract was more inhibitory than the 50% MeOH extract and the water extract as observed for *C. tinctorius* flowers (Kasahara *et al.*, 1994). The effect of the methanol extract is probably due to compounds present in the diethyl ether fraction (52.2%; IC_{50} 0.94 mg/ml), which respectively are available also in the water/alcoholic part of the dichloromethane extract. The effects of the ethyl acetate fraction (37.3%) and its main constituent, luteolin 7-*O*-glucoside (31.5%) were similar.

The present results show anti-inflammatory effect of *C. lanatus* and prove the pharmaceutical importance of this plant.

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