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Anticomplementary Activities of Aqueous Extracts of the Fruits of *Melia azedarach* and *Cotoneaster prostratae* in Rats

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Abstract: The effects of aqueous fruit extracts of *Melia azedarach* (Meliaceae) and *Cotoneaster prostratae* (Rosaceae) on rat complements were examined. Both extracts showed significant anticomplementary activities on rat serum in both classical and alternative pathways. The effect was more pronounced in classical than alternative pathway. The IC 50% obtained for *M. azedarach* and *C. prostrata*e in the alternative pathway was 31.2 and 1.9 mg mL^{-1}, respectively, whereas IC 50% recorded in the classical pathway was 7.8 mg mL^{-1} for *M. azedarach* and 0.9 mg mL^{-1} for the *C. prostratae*. Although both extracts were capable of inhibiting complement activity, total inhibition was achieved at higher *M. azedarach* extract concentrations when compared with those of *C. prostrata*e extract.

Key words: Aqueous fruit extract, melia azeda, anticomplementary

INTRODUCTION

Melia azedarach (Fam. Meliaceae) and *Cotoneaster prostrata*e (Fam. Rosaceae) grow wild in northern districts of Iraq as ornamental trees or shrubs [1,2]. The common name for both plants is Zarur, since they resemble the fruits of fodder Zarur (*Crataegus monogyna*) another member of Rosaceae [2]. Both plants have been widely used in traditional folk medicine in Iraq as antiviral, anthelmintic, antiinflammatory and antirheumatic agents [3-6]. In a previous work, we observed that alcoholic extracts of *M. azedarach* and *C. prostratae* fruits are capable of exerting anti-inflammatory activities by reducing the delayed type of hypersensitivity response and phagocytosis mediated by rat peritoneal exudates cells[7]. *M. azedarach* leaves are associated with the human complement, T-lymphocyte proliferation, phagocytic capability and oxidative metabolism of peripheral blood monocytes and polymorphonuclear leukocytes [8,9]. In the present study we report the effect of *M. azedarach* and *C. prostratae* fruits extracts on rat complement, one of the important components of the immune system closely associated with inflammation and rheumatism.

MATERIALS AND METHODS

Twenty male albino rats, weighing between 150 to 200 g were used for each plant extract. Rats were housed 5 per cage with sterile wood-chip bedding and provided with food pellets and tap water ad libitum. The animal quarter was maintained at 21-24 Celsius with a 10/14 hrs light/dark cycle.

Preparation of plant extracts: Fresh ripe fruits of *M.azedarach* and *C. prostratae* were collected during Winter 2002 from gardens of the University of Mosul, where they were identified and authenticated. The fruits were washed with distilled water and blended with phosphate buffer saline, pH 7.2 (1 g plant material mL^{-1}) using electric blender (Philips HR 2109, England). The crude preparation was filtered through cheesecloth and then the filtrate was further clarified by centrifugation at 10000 rpm for 45 min. The supernatant fluid was obtained and sterilized by filtration through nitrocellulose membranes (pore size 0.22 μ). The filtrates were dried by lyophilization at 40 Celsius, and then stored at -20 Celsius until used [10].

Preparation of anti sheep red blood cell sera (anti- sheep RBC): It was prepared by subcutaneous priming of sheep RBC in rabbits and after an interval of 2 weeks, boosting (weekly for 4 times) with 10^{10} RBC. Two weeks after the last booster, the rabbits were bled for serum [11].

Preparation of sensitized sheep RBC: Freshly obtained blood from sheep was diluted 1:2 with Alsevers solution[12] and used as a source of RBC. The February 27, 2002 concentration of washed RBC was photometrically adjusted to $3x10^8$ cells mL^{-1} (Veronal Saline Buffer, VSB, supplemented with Ca^+ and $Mg^{+)}$. The cells were diluted 1:2 with an appropriate dilution of heat in VSB activated (30 minute at 56 Celsius) antiserum in VSB and incubated under magnetic stirring for 10 min. at room temperature. The antiserum dilution for sheep RBC was 1:400. After incubation, the cells were

washed once and resuspended in VSB to a final concentration of $1.5x10^8$ cells mL^{-1}[12].

Hemolytic assay for rat complement activity: Rats were anesthetized with ether and bled from the retroorbital venous plexus. Serum was separated from blood by centrifugation, pooled and stored at -70 Celsius until use. Alternative (AP) and Classical (CP) complement pathway activities in rat sera were determined by a method using normal rabbit RBC and sensitized sheep RBC as target cells, respectively[12]. All reagents were diluted in VSB or VSB-EGTA (Erthylene-glycol tetraacetic acid) for CP and AP assays, respectively. Optimal serum concentration giving rise to 50% hemolysis of target cells (1 AP 50 or CP 50 Unit) was calculated by the VonKrogh equation [13]. The hemolytic activity of rat serum is expressed in AP 50 or CP 50 units mL^{-1}. For the determination of anticomplementary activity of *M. azedarach* and *C. prostratae* extracts 0.3 mL^{-1} of appropriate rat pooled serum dilution was, incubated for 30 min. at 37 Celsius. Then 0.15 mL^{-1} of the target cells suspension (1% in the appropriate buffer) was added and the mixture was incubated at 37 Celsius for 60 min, or 30 min (CP or AP assays, respectively). The suspensions were centrifuged at 2000 rpm/min for 10 min and the optical density (OD) of the supernatant (I) was measured at 542 nm. Percentage of hemolysis inhibition was calculated according to the following formula: % inhibition = 100–(I-II/III –IV) x 100 , where II refers to the OD at 542 nm of a similarly prepared supernatant but using heat inactivated (56 Celsius, 30 min) serum as the extract was colored this control[11] was done for each extract dilution assayed. III refers to the OD at 542 nm of hydrolyzed RBC (100% hemolysis control) and IV to the OD at 542 nm of supernatants of RBC incubated with buffer (0% hemolysis control) as a positive control on both CP and AP pathways[10]. Different concentrations (0.1-250 mg mL^{-1}) of heparin were employed [14]. The results of anticomplementary activity for heparin and both extracts were compared by analysis of variance, followed by the least significant difference test[15]. The level of significance was at $p<0.05$.

RESULTS

As shown in Table 1, preincubation of rat serum with different concentrations of fruit extracts of *M. azedarach* or *C. prostrata*e caused a concentration-dependent reduction in the hemolytic activities in both CP and AP pathways. When the results were expressed as the extract concentration (mg dried plant material/mL^{-1}) that caused 50% reduction in the hemolytic capacity of rat serum (IC50%), it was shown that the effect was more pronounced in CP than in AP for both plant extracts. Thus the IC50 obtained for *M. azedarach* and *C. prostrata*e in the AP were 31.2 and 1.9 mg mL^{-1}, respectively, whereas the IC50 recorded in the CP were 7.8 and 0.9 mg mL^{-1}, respectively. However, both extracts were capable of inhibiting complement activity, total inhibition was even reached at higher *M. azedarach* extract concentrations as compared with those of *C. prostratae* extracts (Table 1). Preincubation of erythrocytes with the extract did not prevent complement-mediated lysis in both pathways.

As a positive control on both complement pathways, heparin with a known anticomplementary activity, was used. Although this compound was also capable of inhibiting the serum hemolytic activity on both CP and AP complement pathways, this effect was less pronounced than that exerted by *M. azedarach* and *C. prostratae* aqueous fruits extract (Table 1). Heat inactivated serum of rats showed no hemolytic activity.

Table 1: Anticomplementary activities of aqueous extracts of the fruits of *Melia azedarach* and *Cotoneaster Prostratae* as well as heparin on classical (CP) and alternative (AP) pathways in rats

Concentration	*Melia azedarach*		*Cotoneaster prostratae*		Heparin	
mg mL^{-1}	CP	AP	CP	AP	CP	AP
0.1	0	0	0	0	0	0
0.2	0	0	0	0	0	0
0.4	0	0	40±3.4	25±1.3	0	0
0.9	0	0	53±2.3*	41±2.3	0	0
1.9	0	0	75±2.6*	60±2.2*	7±3.6	0
3.9	38±2.8	22±3.1	97±2.1*	89±2.1*	15±3.2	0
7.8	57±2.3*	31±4.3	98±2.0*	96±2.5*	30±3.6	6±1.7
15.6	71±2.1*	43±3.9	98±2.3*	98±2.2*	42±2.9	44±2.3
31.2	87±2.5*	54±3.6*	100±2.5*	96±2.6*	60±3.1	34±3.7
62.5	97±2.2*	80±4.1*	100±2.3*	89±2.4*	75+3.6	52±3.1
125	98±2.1*	98±3.4*	100±2.2*	100±2.1*	78±3.2	69±3.4
250	98±2.3*	98±4.2*	100±2.6*	100±2.7*	89±3.5	80±2.9

Values are expressed as mean ± S.D. of 20 independent measurements. * Significant at $p<0.05$ in comparison with heparin

DISCUSSION

The present study shows that aqueous extracts of the fruits of Melia *azedarach* and *Cotoneaster prostrata*e, two autochthonous plants which grow in northern Iraq, exert inhibitory effects on rat complement activities. These results agree with those obtained for other Meliacea plants such as *Azadirachta indica*[16], Mumroming pumila[17], *Cedrela lillioi*, *C. biflora* and *Trichilia elegans*[6,9,18]. However, limited information exist on the effect of *Cotoneaster* sp. (Rosaceae) on the immunomodulatory activities.

The simultaneous inhibition observed on both complement activation pathways (classical and alternative), suggests a possible effect on some complements of the common terminal route (C3-C9). Nevertheless, the differences seen in patterns of inhibition for both pathways may also indicate that the extracts could affect the first components. Activated complement is involved in inflammatory responses by increasing capillary permeability, degranulating mast cells or promoting neutrophil activation and chemotaxis[8]. The inhibition of complement activation may in part explain the antiinflammatory effects claimed for fruit extracts preparations of *Melia azedarach* and *Cotoneaster prostrata*e in folk medicine.

Further experiments are needed to purify and establish the chemical nature of the principles responsible for the inhibitory activities of the fruits of these plants.

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