

Anti-inflammatory action in drugs of natural origin: *Apis mellifica* L., *Arnica montana* L., *Digitalis purpurea* L., *Sambucus nigra* L., *Thuja occidentalis* L., *Urtica urens* L. and *Arctostaphylos uva-ursi* L.

Farah-Saeed^{1*}, Mansoor Ahmad²

¹Department of Pharmacognosy, Dow College of Pharmacy, Dow University of Health Sciences, Karachi-Pakistan;

²Research Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Karachi, Karachi, 75270-Pakistan.

Abstract: The purpose of the current research work was to explore anti-inflammatory activity of the extracts of *Apismellifica* L., *Arnica montana* L., *Digitalis purpurea* L., *Sambucusnigra* L., *Thujaoccidentalis* L., *Urticaurens* L. and *Arctostaphylosuva-ursi* L. Anti-inflammatory activity was assessed by carrageenan induced paw edema method. *A. mellifica* (500mg/kg) inhibited 25.64% paw edema in 3.5 hours. Maximum paw inhibition 25.71% was observed in *A. montana* at the dose of 500 mg/kg at the end of 4.5 hours. *D. purpurea* (300 mg/kg) exhibited 28.57% carrageenan induced paw inhibition at 4.5 hours. *S. nigra* (500 mg/kg) revealed maximum paw inhibition 27.42% at 4.5 hours. *T. occidentalis* (500 mg/kg) exhibited 25.64% maximum paw inhibition in 3.5 hours. 30.76% paw inhibition was exhibited by *U. urens* (500 mg/kg) at 3.5 hours. *A. uva-ursi* (500mg/kg) showed maximum inhibition of paw edema (25.64%) in 3.5 hours. The standard drug, Aspirin exhibited maximum anti-inflammatory activity 22.22% at 1.5 hour. This research work has significance as it revealed dose and time dependent anti-inflammatory effect of seven crude extracts.

Key words: Anti-inflammatory, carrageenan, insect extract, plant extract.

I. INTRODUCTION

Inflammation is a part of a complex biological response involving various mediators like platelet activating factor, leukotrienes, prostaglandins, kinins of vascular tissue to deleterious stimuli like heat, cold, trauma, chemicals, infectious and immunological agents. It is the natural healing process initiated in any living organism for curing of any wound or infection [1,2]. In developing country like Pakistan drugs of natural origin are used in integration with conventional medicines for the treatment of inflammation associated with various pathologies. Apart from this factor due to unsatisfactory outcomes of allopathic drugs along with serious side effects the drug industry started exploring natural sources in the quest to develop effective and safe anti-inflammatory drugs with minimum adverse or toxic effects [3,4]. Therefore, we have selected seven very popular drugs, *A. mellifica*, *A. montana*, *D. purpurea*, *S. nigra*, *T. occidentalis*, *U. urens* and *A.uva-ursi*, of natural origin for evaluation and investigation.

II. MATERIAL & METHOD

2.1. Preparation of crude extracts

A. mellifica, *A. montana*, *D. purpurea*, *S. nigra*, *T. occidentalis*, *U. urens* and *A. uva-ursi* mother tinctures drugs (sealed packs from William Schwabe, Germany) were purchased from local supplier. Extracts were concentrated under reduce pressure on a rota evaporator (Buchi-Rotary Evaporator, Switzerland, model # B490) at 40°C. The obtained extracts were stored in cool, dry place for further studies.

2.2. Experimental animals

Sprague Dawley rats (120-150 g) were obtained from Animal House, Dow University of Health Sciences, Karachi, Pakistan. Animals were kept in Animal House, DUHS, Karachi. They were kept in a climate and light controlled room at least 7 days before initiating neuro-pharmacological activities on them and were provided with food and water *ad libitum*. Animal studies were carried out according to the NIH guide for the care and use of laboratory animals [5].

1.3. Anti-inflammatory activity

The anti-inflammatory activity was evaluated by the carrageenan induced paw edema method. The rats were divided into nine groups of six animals each. Freshly prepared aqueous suspension of carrageenan (2%) was injected in the *plantaaponeurosis* of the right hind paw of each rat. One group was kept as a control, second group was given standard drug (Aspirin) and the animals of other groups were pretreated with test drugs

(300mg/kg and 500mg/kg doses) given orally 30 minutes before carrageenan injection. The crude extracts and standard drug Aspirin (300 mg/kg) were administered orally to the animals, respectively. Thirty minutes after the administration of test compounds and Aspirin, paw edema was induced in albino rats by injecting 0.1 ml of carrageenan suspension, into sub-plantar region of the left hind paw of each rat. The paw volume of all animals in all groups was measured at 0, 0.5, 1, 1.5, 2.5, 3.5, 4.5 and 24 hours intervals, after carrageenan administration. The differences in the paw volumes (i.e. edema volumes) of each animal of all the groups were calculated and compared with the changes in the edema volumes of control and the drug treated animals [6-8]. The results were expressed as percentage reduction in edema volume, which can be calculated by using the formula:

$$\% \text{ of inhibition} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

1.4. Statistical Analysis

All values were compared with the control and standard drug reading by taking out mean and standard error of mean. Level of significance was determined by ANOVA followed by Dunnett's test [9].

III. RESULTS

A.uva-ursi extract (300mg/kg) exhibited maximum inhibition of paw volume 14.28% at the end of 24 hours whereas *Uva-ursi* (500mg/kg) at 3.5 hours exhibited maximum paw volume inhibition 25.64%. *U. urens* (300mg/kg) exhibited maximum paw volume inhibition 13.79% after half hour. *U. urens* (500mg/kg) at 3.5 hours had 30.76% paw volume inhibition. *S. nigra* (300mg/kg) at 0.5 hour revealed 20.68% paw volume inhibition whereas *S. nigra* (500mg/kg) exhibited maximum paw volume inhibited 27.42% at 4.5 hours. *A. montana* (300mg/kg) exhibited maximum paw inhibition 18.75% at 1 hour. *A. montana* (500mg/kg) revealed 25.71% paw volume inhibition at 4.5 hours. *A. mellifica* (300mg/kg) at 1.5 hours exhibited maximum paw volume inhibition 11.76% while *A. mellifica* (500mg/kg) had 25.64% maximum paw volume inhibition at 3.5 hours. *D. purpureae* (300mg/kg) at 4.5 hours had maximum paw volume inhibition 28.57%. *D. purpureae* (500mg/kg) exhibited at 3.5 hours 25.64% paw volume inhibition. *T. occidentalis* (300mg/kg) at 1.5 hours had maximum paw inhibition volume 11.76%. *T. occidentalis* (500mg/kg) revealed 25.64% paw volume inhibition at 3.5 hours. The standard drug, Aspirin (300 mg/kg) had 22.22% maximum paw volume inhibition at 1.5 hours (Table 1 & Graph 1).

IV. DISCUSSION

The anti-inflammatory activity test of *A. uva-ursi* was performed on rats hind paw with the introduction of carrageenan. Significant results were obtained in comparison to Aspirin and control. This response may be attributed to arbutin, ursolic acid, α and β -amyrin, α -amyrin acetate, lupeol, salicylic acid, ferulic acid, caffeic acid and tannins [10]. Anti-inflammatory activity of *U. urens* might be due to chlorogenic acid, caffeic acid and butyric acid [11].

The *S. nigra* aqueous extract from its flowers inhibited the macrophage release of pro-inflammatory cytokines. The ability of aqueous extract to inhibit P13K has been suggested to be mediated at least partially through Quercetin [12]. Yesilada et al. (1997) also explored the *in vitro* inhibitory effects of *S. nigra* on interleukin-1 and tumor necrosis factor and found inhibitory activity [3]. *S. nigra* (300mg/kg) at 0.5 hour revealed 20.68% paw volume inhibition whereas *S. nigra* (500mg/kg) exhibited maximum paw volume inhibited 27.42% at 4.5 hours.

A. mellifica (300mg/kg) at 1.5 hours exhibited maximum paw volume inhibition 11.76% while *A. mellifica* (500mg/kg) had 25.64% maximum paw volume inhibition at 3.5 hours. Reported components of this insect drug are comprehensively studied by various researchers. The pharmacologically active constituents have been reported to be useful for the treatment of different diseases, for example Melittin is reported to produce immune-stimulatory and immune-suppressive effect; Apamine is anti-inflammatory, increases the defense capability, immune-suppressor and stimulates the CNS; MCD has anti-inflammatory effect; Adolapin inhibits the specific brain enzymes, cyclooxygenase and lipooxygenase; Protease inhibitor inhibits the activity of different proteases, thereby reducing inflammation [13-17].

Carrageenan induced anti-inflammatory activity was performed on *T. occidentalis* fresh plant by Jahan (2010) and in second and third hour at the dose of 300 mg/kg 31.53% and 30% inhibition was found whereas at 500 mg/kg 32.88% and 30% inhibition of paw edema was observed [18]. Our *T. occidentalis* mother tincture extract (300mg/kg) in 1.5 hours had maximum paw inhibition volume 11.76% and at the dose of 500mg/kg revealed 25.64% paw volume inhibition at 3.5 hours. Aspirin had 22.22% maximum paw volume inhibition at 1.5 hours. Further studies need to be carried out to validate the safety, efficacy as well as to determine the dose of these crude extracts required for treatment of inflammation associated with different pathologies.

V. CONCLUSION

The dose and time dependent anti-inflammatory activity was observed in the tested crude extract.

VI. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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Table 1: Shows the anti-inflammatory effect of crude extracts on 2% carrageenan induced paw edema in rats in comparison with Control and Aspirin

	Time (Hrs)							
	0	0.5	1.0	1.5	2.5	3.5	4.5	24
Control	6 ± 0.0083	7.73 ± 0.0061	8.23 ± 0.0065	9 ± 0.0083	9.24 ± 0.0046	9.75 ± 0.0063	8.74 ± 0.0065	7 ± 0.0096
<i>A. mellifica</i> (500mg/kg)	6.01 ± 0.0073	6.76 ± 0.0052	7.23 ± 0.0073	8.02 ± 0.0083	7.77 ± 0.0063*	7.73 ± 0.0061*	7 ± 0.0083	6.24 ± 0.0054
<i>A. mellifica</i> (300mg/kg)	6.01 ± 0.0073	6.76 ± 0.0052	7.23 ± 0.0073	8.02 ± 0.0083	7.77 ± 0.0063*	7.73 ± 0.0061*	7 ± 0.0083	6.24 ± 0.0054
<i>A. montana</i> (500mg/kg)	6 ± 0.0083	6.76 ± 0.0065	7.26 ± 0.0052	8 ± 0.0063	7.75 ± 0.0065*	7.73 ± 0.0073*	6.52 ± 0.0063*	6.48 ± 0.0052
<i>A. montana</i> (300mg/kg)	6 ± 0.0083	6.76 ± 0.0065	7.26 ± 0.0052	8 ± 0.0063	7.75 ± 0.0065*	7.73 ± 0.0073*	6.52 ± 0.0063*	6.48 ± 0.0052
<i>D. purpurea</i> (500mg/kg)	5.49 ± 0.0083	7.19 ± 0.0083	7.45 ± 0.0063	7.75 ± 0.0061*	8.1 ± 0.0063	7.75 ± 0.0063*	7.25 ± 0.0083	6.63 ± 0.0052
<i>D. purpurea</i> (300mg/kg)	5.49 ± 0.0083	7.19 ± 0.0083	7.45 ± 0.0063	7.75 ± 0.0061*	8.1 ± 0.0063	7.75 ± 0.0063*	7.25 ± 0.0083	6.63 ± 0.0052
<i>S. nigra</i> (500mg/kg)	6 ± 0.0063	6.76 ± 0.0052	7.26 ± 0.0054	7.51 ± 0.0065*	7.76 ± 0.0052*	7.73 ± 0.0065*	6.35 ± 0.0065*	6 ± 0.0096
<i>S. nigra</i> (300mg/kg)	6 ± 0.0063	6.76 ± 0.0052*	7.26 ± 0.0054	7.51 ± 0.0065*	7.76 ± 0.0052*	7.73 ± 0.0065*	6.35 ± 0.0065*	6 ± 0.0096
<i>T. occidentalis</i> (500mg/kg)	5.99 ± 0.0083	6.73 ± 0.0052*	7 ± 0.0083	7.48 ± 0.0073*	7.49 ± 0.0083*	7.75 ± 0.0046*	7.81 ± 0.0073	6.47 ± 0.0096
<i>T. occidentalis</i> (300mg/kg)	5.99 ± 0.0083	6.73 ± 0.0052	7 ± 0.0083	7.48 ± 0.0073*	7.49 ± 0.0083*	7.75 ± 0.0046*	7.81 ± 0.0073	6.47 ± 0.0096
<i>U. urens</i> (500mg/kg)	5.99 ± 0.0078	6.49 ± 0.0083	6.94 ± 0.0046*	7.51 ± 0.0054*	7 ± 0.0063*	6.77 ± 0.0074*	6.72 ± 0.0095*	6 ± 0.0083
<i>U. urens</i> (300mg/kg)	5.99 ± 0.0078	6.49 ± 0.0083	6.94 ± 0.0046*	7.51 ± 0.0054*	7 ± 0.0063*	6.77 ± 0.0074*	6.72 ± 0.0095*	6 ± 0.0083
<i>U. ursi</i> (500mg/kg)	6.01 ± 0.0052	6.73 ± 0.0052	7 ± 0.0083	7.76 ± 0.0052*	7.73 ± 0.0052*	7.23 ± 0.0052*	6.87 ± 0.0052*	6 ± 0.0083
<i>U. ursi</i> (300mg/kg)	5.58 ± 0.0046	6.5 ± 0.0063	7.50 ± 0.0077	8 ± 0.0063	8.15 ± 0.0063	7.75 ± 0.0063*	7.75 ± 0.0063	6 ± 0.0063
Aspirin (300 mg/kg)	6 ± 0.0063	6.75 ± 0.0063	6.85 ± 0.0063*	7 ± 0.0083	7.27 ± 0.004	8 ± 0.0077	7.74 ± 0.0036	6.75 ± 0.0063

Results were expressed along mean ± standard error mean. Significance of data were evaluated at *P < 0.05 compared with control group.

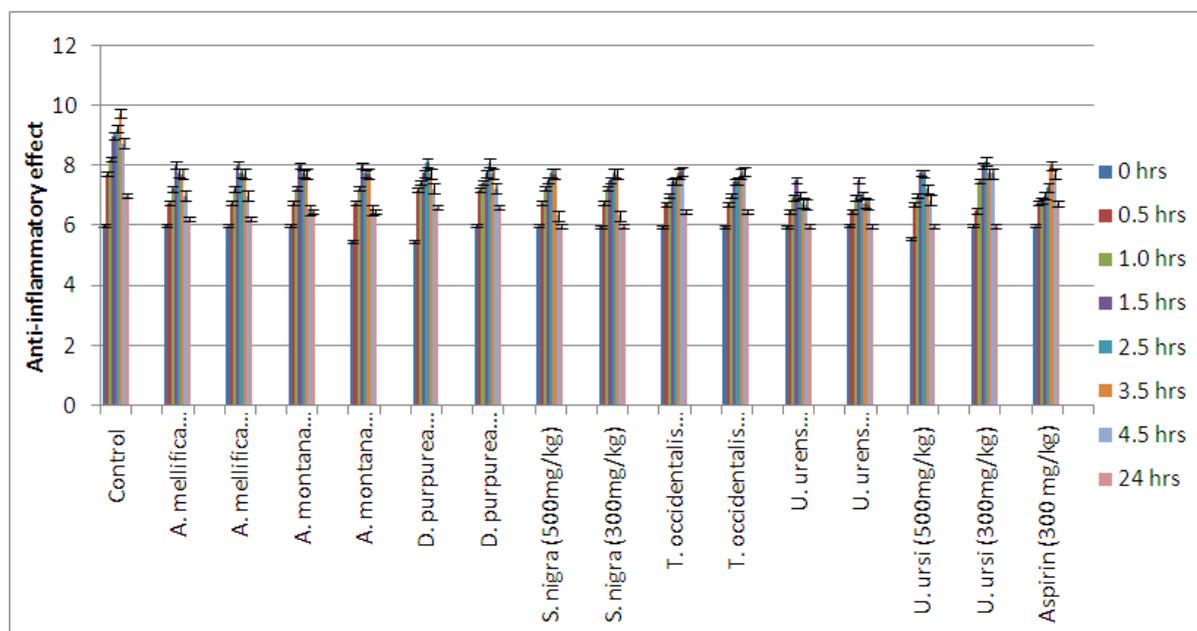


Figure 1: Shows the anti-inflammatory effect of crude extracts on 2% Carrageenan induced paw edema in rats in comparison to control and Aspirin