

Evaluation of *Acacia cyanophylla* for their analgesic, anti-pyretic and anti-inflammatory potentials

Arshad Iqbal¹, Siraj Ud Din², Jehan Bakht³ and Inam Ullah Khan⁴

¹Department of Botany, Islamia College Peshawar, Peshawar, Pakistan

²Department of Botany, University of Peshawar, Peshawar, Pakistan

³Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar, Pakistan

⁴Department of Pharmacy, University of Peshawar, Peshawar, Pakistan

Abstract: The present paper presents results of analgesic, antipyretic activity and anti-inflammatory potential of extract obtained from *Acacia cyanophylla* when tested at different doses. Analgesic potential of the crude methanolic extract tested by acetic acid assay was dose dependent and maximum activity of 61.60% was measured at 400 mg/kg. Analgesic activity by hot plate method revealed that maximum activity of 36.98% was noted when the mice were exposed to 90 minutes at higher dose of 400 mg/kg. Similar pattern for antipyretic activity was observed as noted for analgesic activity. Anti-inflammatory activity was dose and time dependent when evaluated by Carrageenan-induced paw edema and Xylene-induced ear edema model. Maximum anti-inflammatory activity (43.32%) was shown by crude methanolic extract of *Acacia cyanophylla* at 400mg/kg⁻¹ after 5 hours on Carrageenan-induced paw edema model. Similarly, maximum (68.80%) anti-inflammatory activity was noted when accessed by Xylene-induced ear edema model at the dose of 200mg/kg after 60 minutes. No *in vivo* toxicity of the extracts up to the dose of 2000mg/kg was observed using albino mice.

Keywords: Acetic acid, hot plate, carrageenan, xylene, plant extracts.

INTRODUCTION

Since time ago the use of plants as herbal remedies has been acknowledged by Chinese, Greeks, Romans, Egyptians, Syrians and Indians. About 25 to 45 percent of the current prescriptions contain a number of lead molecules derived from plants as a major source of ingredients (Subramanian *et al.*, 2009). Therapeutic agents including tannins, nitrogenous and phenolic compounds etc. obtained from medicinal plants are used as leading components in designing different drugs (Cox, 1994; Khan *et al.*, 2002; Khan and Khan, 2003). Different parts of a number of medicinally important plants possess antimicrobial activity and are employed as extract for raw drugs having number of curative characteristics (Bakht *et al.*, 2020; Mohammad *et al.*, 2020).

Acacia cyanophylla belongs to the family Fabaceae and genus *Acacia*. *Acacia cyanophylla* consists of variable shrubs or trees and is about 2-10m tall. The trunk is either single- or multi-stemmed, about 5-40cm and is straight to rather crooked. The root of this plant may grow to 16m deep in sand. *Acacia cyanophylla* is native to Australia and has been extensively planted for ornamental purposes (Martin, 1974; Akkari *et al.*, 2008). It is cultivated in Iran, Morocco, Algeria and Egypt and Pakistan, especially in Rawalpindi and Peshawar etc. Genus *acacia* possess anti-inflammatory, anti-pyretic as well as analgesic activity (Dongmo *et al.*, 2005). *Acacia cyanophylla* has an antinematode activity against gastrointestinal nematode

parasite in sheep (Akkari *et al.*, 2008; Asma *et al.*, 2013). *Acacia cyanophylla* possess sufficient amount of crude proteins and tannins (Maslin and McDonald, 2004). A compound iso-salipurposide has been isolated from its flowers (Ghouila *et al.*, 2012). The objectives of the present studies were to evaluate pharmaceutical potentials of *Acacia cyanophylla* using mouse model.

MATERIALS AND METHODS

Plant material and crude extraction

Plants of *Acacia cyanophylla* were harvested from Pakistan Forest Institute, University of Peshawar Khyber Pukhtunkhwa Pakistan. The plant specimen was identified and allotted voucher number of AI-003-ICP (Herbarium of Islamia College Peshawar). Stem bark was shade dried for three weeks, processed with electric grinder and soaked in analytical grade methanol (4.3 L) for 14 days at room temperature with occasional shaking. The soluble residue was concentrated with rotary evaporator after filtering methanol.

Experimental animals

The present research project was approved by the Institutional Ethical Committee. Mice of either sex were collected from the Department of Pharmacy, University of Peshawar. To keep these animals healthy, recommended guidelines were followed throughout the experiments (Muhammad *et al.*, 2008). Before screening, mice were provided with water only for 12 hours. The animals were fed according to the recommended guidelines

*Corresponding author: e-mail: jehanbakht@yahoo.co.uk

(Muhammad *et al.*, 2008). The tested animals were separated into 5 different groups (n=6). The animals of group I (negative control) were administered with normal saline at 10ml/kg (body weight). Group II (positive control) was treated as control. Groups III, IV and V were subjected with crude extracts at 100, 200 and 300mg/kg (body weight).

Antipyretic activity

Pyrexia induction with Brewer's yeast

Antipyretic effect of crude methanolic extract was screened by mice (30-35g body weight) of either sex. Group II (positive control) was treated with Paracetamol at 150 mg/kg (body weight). Normal body temperature of every animal was recorded with the help of digital thermometer. Aqueous suspension of Brewer's (15%) was subcutaneously injected at 10 ml/kg to mice to induce pyrexia. Digital thermometer was used to note the rise in body temperature 24 hours after the treatment. Only mice showing at least 0.5°C increase in body temperature were selected for further study (Khan *et al.*, 2008). Through i.p route all doses were injected to all groups. The temperature of rectum was noted regularly at 1st, 2nd, 3rd, 4th and 5th hour of each mouse in all treated groups. Reduction in body temperature (%) was measured by the following formula

$$\% \text{ Reduction in body temperature} = \frac{B - C_n}{B - A} \times 100$$

A is for normal body temperature

B indicates body temperature after 24 hrs

C is for temperature at 1st, 2nd, 3rd, 4th and 5th hr of treatment.

Analgesic activity

Acetic acid induced writhing

Group II (positive control) was subjected to Diclofenac sodium (standard drug) at 10 mg/kg (body weight). After half an hour, acetic acid (1%) was injected to all groups through intra-peritoneal route. The abdominal writhings (constrictions) started 5 minutes after acetic acid injection, which were counted for next 10 minutes (Khan *et al.*, 2008). The analgesic potential (%) was calculated with the following formula

$$\% \text{ Analgesic effect} = 100 - \frac{\text{No of writhing in tested animals}}{\text{No of writhing in control animals}} \times 100$$

Hot plate method

In this experiment, analgesic effect was measured by Eddy's Hot Plate method (Turner, 1965). The test animals were placed on hot plate individually maintained at 55±1°C. Every mouse was observed for reaction on hot plate in the form of paw licking or jumping. Group II was treated as positive control given Aspirin (100mg/kg). The animals were placed on Eddy's Hot Plate maintained at a temperature of 55±1°C. The time of 15 seconds was a cut off period to observe animals on hot plate. After the treatment, the reaction period in treated and control

animals was recorded at 0, 30, 60, 90 and 120 minutes (Janssen and Eddy, 1959).

Anti-inflammatory activity

Carrageenan Induced Paw Edema Model

Indomethacin (standard drug) was administered at 10 mg/kg (body weight) to group II (positive). Carrageenan of 1% was administered in sub planter tissue of the hind paw (right) of every animal (mouse) 30 minutes after the above mentioned treatments. Anti-inflammatory potential was recorded for 5 hrs using Plethysmometer (LE 7500 plan lab S.L) after the administration of Carrageenan (Khan *et al.*, 2009). The percent inhibition of edema was measured with the help of formula given below

$$\% \text{ Inhibition} = \frac{A - B}{B} \times 100$$

A is for edema volume in negative control

B for paw edema in tested groups.

Xylene-Induced Ear Edema

Group II (Positive control) was treated with Dexamethasone (1 mg/kg). After 30 min, the inner surface of the right ear was treated with xylene (0.03 ml) for the induction of edema (Nunez-Guillen *et al.*, 1997).

The percent inhibitory effect was measured as follow.

$$\% \text{ inhibition} = 100 (V_c - V_t / V_c)$$

V_c is for difference in weight of ear in control

V_t is for difference in weight of ear in group treated with standard and extract

STATISTICAL ANALYSIS

Computer software (MSTATC-version 5.5) was used for statistical analysis (Russel and Eisensmith, 1983). After ANOVA and upon obtaining significant difference at P<0.5, Least Significant Difference (LSD) test was applied at P<0.5 (Steel *et al.*, 1997).

RESULTS

Crude methanolic extract of the selected plant was observed safe at all the tested doses of 500, 1000 and 2000mg/kg i.p. During the evaluation period, all the tested animals were found normal. No considerable difference was found between saline and test groups in moving, eating, respiration and others behaviors. The crude extract (methanolic) of *Acacia cyanophylla* was applied to evaluate antipyretic activity effects at different doses (table 1). Paracetamol (standard) reduced the temperature at maximum of 88.18%. The data revealed that anti-pyretic activity showed dose dependent decrease in the temperature of mice exposed to plant extracts. At 1st hr of the treatment, none of the dose showed significant inhibitory effect. The antipyretic effect of 30.70, 43.98 and 45.49% was noted at 100, 200 and 300mg/kg dose of crude extract respectively when compared with positive and negative controls. Body temperature was reduced by

35.25, 54.88 and 61.56% at 100, 200 and 300mg/kg respectively at 2nd hr. All the subjected doses showed significant ($p<0.05$) antipyretic activity at 2nd hour. More significant ($p<0.01$) inhibition was measured at 200 and 300mg/kg doses. At 3rd hour, plant extract reduced the rectal temperature by 52.69, 68.04 and 72.54% using 100, 200 and 300mg/kg doses respectively. Antipyretic activity observed after 4th hr was 45.64, 67.29 and 69.80% for 100, 200 and 300mg/kg dose respectively when compared with positive and negative controls. At 5th hr, temperature reduction was 48.54, 66.16 and 70.58% for the test doses respectively.

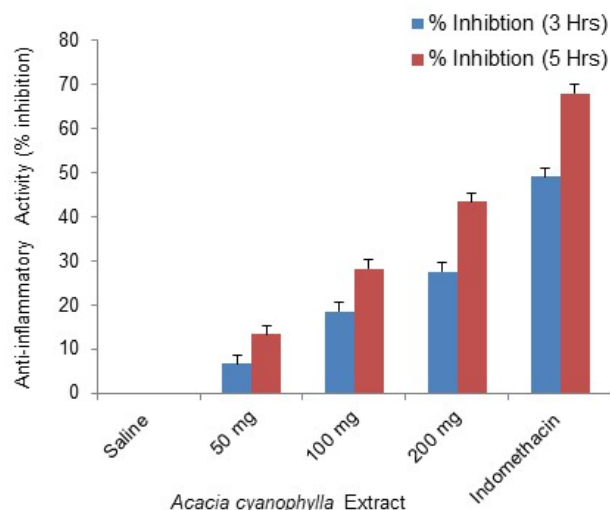


Fig. 1: Effect of different doses of crude methanolic extract of *Acacia cyanophylla* on anti-inflammatory activity (% inhibition) measured by Carrageenan Induced Paw Edema Model (Bar shows LSD at $P<0.05$).

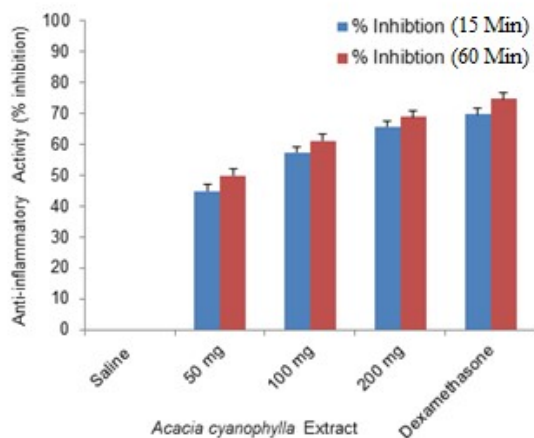


Fig. 2: Effect of different doses of crude methanolic extract of *Acacia cyanophylla* on anti-inflammatory activity (% inhibition) measured by Xylene-induced Ear Edema Model (Bar shows LSD at $P<0.05$).

Crude methanolic extract of *Acacia cyanophylla* showed decrease in number of writhings at various test doses using

Acetic Acid Induced Writhing method. Table 2 revealed that plant extract of 100, 200 and 400mg/kg produced significant ($p<0.05$) inhibitory effect when measured by acetic acid induced writhing method. The data suggested that the inhibitory effect was found to be dose dependent. Maximum inhibition of 61.60% was produced at the dose of 400mg/kg followed by 200mg/kg (49.09%) when compared with positive and negative controls. Similarly, inhibitory activity of 21.87% was measured for 100 mg/kg. The analgesic effect of plant extract from *Acacia cyanophylla* was significant ($p<0.05$) at the 400mg/kg only using Hot Plate method (table 3). The data indicated that analgesic activity of 11.32 and 30.07% was recorded at 200 and 400mg/kg respectively after 30 minutes of exposure. The inhibitory effect at 60 minutes was 20.09 and 33.68% respectively for the same doses. The nociceptive response observed at 90 minutes for 200 and 400mg/kg was 20.62 and 36.98%. After 2 hours, percent response demonstrated by 400mg/kg was 34.21.

Maximum anti-inflammatory effect of 27.59 and 43.32% was recorded for 200 mg/kg at 3 and 5 hours followed by 100 mg/kg which inhibited the inflammation by 18.45% at 3 hours and 28.27% at 5 hours using Carrageenan Induced Paw Edema Model (fig. 1). At 50mg/kg, the anti-inflammatory activity was 13.38 and 6.60% at 5 and 3 hours respectively. The *in-vivo* anti-inflammatory activity of *Acacia cyanophylla*, revealed that Carrageenan produced biphasic inflammatory events which was significantly ($p<0.05$) controlled by plant extracted samples.

Acacia cyanophylla plant extract showed profound anti-inflammatory effects when tested at different doses. The inhibitory effect of the tested plant extract was significant ($p<0.05$) at all doses using Xylene-Induced Ear Edema Model (fig. 2). Maximum activity was demonstrated by 200 mg/kg followed by 100mg/kg. The anti-inflammatory activity measured for 200mg/kg was 68.80% after 1 hour and 65.68% at 15 minutes. The inhibitory effect measured by 100mg/kg was 57.34% and 61.17% at 15 and 60 minutes respectively. The low dose of 50mg/kg reported activity of 44.84% at 15 minutes and 49.96% at 1 hour. The selected plant was found effective at all the subjected doses (50, 100 and 200mg/kg), however, was more effective after one hour (late phase). Standard drug (Dexamethasone) a steroid anti-inflammatory agent indicated significant reduction in the mean right ear weight of the tested animals (positive control).

DISCUSSION

All tested doses of crude methanolic extract of the selected plant were observed safe for the animals under study and adverse effects were noted. No considerable difference was found between saline and test groups in moving, eating, respiration and others behaviors. During

Table 1: Antipyretic activity of crude methanolic extract of *Acacia cyanophylla*

Treatments	Dose mg/kg	Rectal temperature (°C)						
		Normal	after 24h	After administration of drug				
				1h	2h	3h	4h	5h
Saline	10mL	36.69±0.52	39.71±0.26	38.67±0.31	38.62±0.44	38.61±0.21	38.71±0.33	38.77±0.33
Paracetamol	150mg	37.05±0.32	39.42±0.32	38.18**±0.26	37.78**±0.36	37.33**±0.39	37.41**±0.44	37.47**±0.47
Plant Extract	100	37.05±0.37	39.46±0.38	38.72±0.33	38.61*±0.39	38.19*±0.71	38.36*±0.32	38.29*±0.13
	200	37.07±0.52	39.73±0.41	38.56±0.52	38.27*±0.87	37.92**±0.47	37.94**±0.53	37.97**±0.31
	300	37.04±0.36	39.59±0.27	38.43±0.45	38.02*±0.62	37.74**±0.68	37.81**±0.71	37.79**±0.67

Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by ANOVA followed by Dunnett's test. * $P < 0.05$, ** $P < 0.01$ in comparison to control.

Table 2: Analgesic Activity of crude methanolic extract of *Acacia cyanophylla*

Treatments	Dose (mg/kg i.p.)	No. of writhing (10min)	% Analgesia
Saline	10ml/kg	63.38 ± 2.79	-
Plant Extract	100	49.52 ± 2.67*	21.87
	200	32.27 ± 2.03**	49.09
	400	24.34 ± 1.91**	61.60
Diclofenac Sod.	10	10.17 ± 1.27**	83.96

Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by following t- test. * $P < 0.05$, ** $P < 0.01$ in comparison to control.

Table 3: Analgesic activity of crude methanolic extract of *Acacia cyanophylla*

Treatments	Dose (mg/kg)	Latency of nociceptive response in min (mean ± SEM)				
		0	30	60	90	120
Vehicle	-	8.22±0.23	8.48±0.18	8.61±0.43	8.68±0.29	8.74±0.51
Plant Extract	200	8.31±0.27	9.44±0.51	10.34±0.42	10.47±0.78	9.81±0.29
Plant Extract	400	8.26±0.42	11.03±0.57*	11.51±0.39*	11.89±0.41*	11.73±0.38*
Tramadol	20	8.51±0.37	12.59±0.74**	15.89±0.52**	16.24±0.23**	15.62±0.23**

Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by ANOVA followed by Dunnett's test. * $P < 0.05$, ** $P < 0.01$ in comparison to control.

the course of action the toxic agents usually bind to the vital organs like kidneys and liver and produce harmful effects. Therefore it is very important to evaluate the toxic and harmful properties of an agent before further studies (Asante-Duah, 2002; Jothy *et al.*, 2011).

The anti-pyretic activity showed dose dependent decrease in the temperature of mice exposed to plant extracts. Initially none of the dose showed significant inhibitory effect. However, all the tested doses showed significant ($p < 0.05$) antipyretic activity at 2nd hour. More significant ($p < 0.01$) inhibition was measured after 3rd, 4th and 5th hours of treatment at 200 and 300mg/kg doses. These results showed plant extract acted both centrally and peripherally like aspirin (Ferreira *et al.*, 1978). Aspirin reduces the fever by decreasing prostaglandin E₂ brain concentration, especially through its action on COX-3 in the hypothalamus too (Vane, 1971; Ayaz *et al.*, 2017; 2018; Arshad *et al.*, 2019).

Crude methanolic extract of *Acacia cyanophylla* showed decrease in number of writhings at various test doses when measured by acetic acid induced writhing method. Plant extract of 100, 200 and 400mg/kg produced significant

($p < 0.05$) inhibitory effect. The data suggested that the inhibitory effect was found to be dose dependent and maximum inhibition was noted at the dose of 400mg/kg followed by 200mg/kg (Ayaz *et al.*, 2017; 2018; Arshad *et al.*, 2019). The analgesic effect of plant extract from *Acacia cyanophylla* was significant ($p < 0.05$) at the 400 mg/kg only. The data indicated that analgesic activity of was dose and time dependent and maximum analgesic activities were observed at the highest dose and more exposure time. This test is specifically applied to understand the analgesic effect of drugs and chemicals that act centrally such as morphine and its analogues. Anti-nociceptive drugs that act peripherally are found to be inactive on temperature induced hyper algesia (Coutaux *et al.*, 2005; Ayaz *et al.*, 2017; 2018 and Arshad *et al.*, 2019).

Using Carrageenan Induced Paw Edema Model, maximum anti-inflammatory effect was recorded for 200 mg/kg at 3 and 5 hours followed by 100mg/kg. The *in vivo* anti-inflammatory activity of *Acacia cyanophylla*, revealed that Carrageenan produced biphasic inflammatory events which was significantly ($p < 0.05$) controlled by plant extracted samples. Certain chemical

substances such as serotonin, histamines and some other related compounds in the first phase (90-180 minutes) of inflammation increased the volume of hind paw which characterized the second phase of inflammation (270-360 minutes). This increase in volume is due to the presence of certain inflammatory mediators (Khan *et al.*, 2011). Non-significant ($p>0.05$) differences in terms of morbidity and mortality was observed between the animals of treatment and negative controls. Similar results were also reported by Arshad *et al.* (2019).

Acacia cyanophylla plant extract showed profound anti-inflammatory effects when tested at different doses using Xylene-Induced Ear Edema Model. The inhibitory effect of the tested plant extract was significant ($p<0.05$) at all doses. Maximum activity was noted for 200mg/kg followed by 100mg/kg at 15 and 60 minutes. This might be due to phospholipase A₂ inhibition. Phospholipase A₂ play a key role in xylene induced inflammation (Lin *et al.*, 1992). All the selected plants were found effective at all the subjected doses (50, 100 and 200mg/kg), however, was more effective after one hour (late phase). Standard drug (Dexamethasone) a steroid anti-inflammatory agent indicated significant reduction in the mean right ear weight of the tested animals (positive control) due to inhibition of phospholipase A₂ (PL-A₂). These results showed that mechanism of action of the plant extracted samples resembles those of NSAID group of the anti-inflammatory drugs. These drugs have anti-inflammatory activities both in central and peripheral tissue (Okokon, 2011). These results also agree with Arshad *et al.* (2019).

CONCLUSION

From these results it can be concluded that extract of *Acacia cyanophylla* possess anti-inflammatory, anti-pyretic and analgesic activity when measured by different methods.

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