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Pharmacological evaluation of *Acacia modesta* bark for antipyretic, anti-inflammatory, analgesic, antidepressant and anticoagulant activities in Sprague Dawley rats

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Abstract: In this study the bark of *Acacia modesta* was evaluated for anti-inflammatory, antipyretic, analgesic, antidepressant and anticoagulant activity by carrageenan, hot plat, forced swim and capillary tube method respectively in rats. Highest anti-inflammatory activity was exhibited by chloroform (AMC) extract (74.96% inhibition) while other two active fractions being n-hexane (AMH) and ethyl acetate (AME) exhibited 71.26% and 52.87% inhibition of edema respectively. On the other hand, the aqueous (AMA) fraction showed most effective response with 67.06% analgesic activity. Additionally, the significant ($p<0.05$) post-treatment antipyretic effect was found by all fractions in time dependent manner. The current findings showed that AMC, AME and AMA had significant reduction in immobility time in the antidepressant test, while AMH showed mild antidepressant activity. In anticoagulant assay, the coagulation time of crude extract *A. modesta* and its all fractions were comparable to that of positive control aspirin (208s). Moreover, neither mortality nor lethality was observed in the tested animals. Overall, the plant extracts showed potent anti-inflammatory, antipyretic, analgesic, antidepressant and anticoagulant activities which concludes that the bark of *A. modesta* have significant therapeutic potential.

Keywords: Analgesic, carrageenan, flavonoids, hyperthermia, pyrexia.

INTRODUCTION

Throughout the ages natural products have been used by humans for their basic requirements such as food, shelter and clothing. Particularly plants have provided medicines to combat numerous diseases such as leukemia, malaria, parasitic infections, diabetes, respiratory and cardiovascular diseases (Sarwar, 2016). The genus *Acacia* is a group of remarkable medicinal plants. Different members of genus *Acacia* are well known to have antioxidant, anti-inflammatory, antipyretic, analgesic and antidiabetic potentials. Like extract of *A. karroo*is is documented for its prominent anti-inflammatory potential in carrageenan assay and along with peripheral analgesic activity in experimental animals (Adedapo *et al.,* 2008). Similarly, ethanolic seed extract of *A. suma* was investigated for its anti-inflammatory and both central and peripheral analgesic activity (Mondal *et al.,* 2013). *A. nilotica* extract exhibited suppressive effect on yeast stimulated pyrexia in rats (Dafallah and Al-Mustafa, 1996) and *A. concina* leaves are considered as one of the best treatments of malaria (Bora *et al.,* 2007). *Acacia* gum can decrease the body mass index and body fat percentage among healthy adult females, which could be used in the treatment of obesity (Babiker *et al.,* 2012). *A. nilotica* has been reported for reducing cardiovascular disease risk in human, increasing level of low-density lipoproteins, decrease level of plasma total cholesterol and triglyceride (Lerman *et al.,* 2010). *A. bracteata* aqueous extract has shown reduced nephrotoxicity on albino rats, as it elevates urea, creatinine, aspartate transaminase and alanine transaminase levels along with reduction in the damage of the kidney tubules (Omer *et al.,* 2013).

A. modesta Wall is a deciduous plant from the family *Fabaceae* widely distributed in Pakistan, India and Afghanistan (Sarwar, 2016). It is commonly used as fuel, fodder, timber and the traditional folk remedies. The gum of the bark with butter, wheat flour, and almond is given to females after delivery as an energizer to alleviate body weakness (Selvam, 2008). Another preparation called *Zhuble sharbat* is prepared by dissolving one tea spoon of gum powder in one glass of water and is taken as a health booster (Ahmad *et al.,* 2012). Its ash made from branches

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is utilized as tranquilizer (Hussain *et al.,* 2010). Murad *et al.* (2011) reported that different preparations of this plant have been used for the cure of muscular problems and backache after delivery in the women since pre-historic times. The plant has commercial value, as a supply of medicinal gum, utilized for tooth cleaning in the form of "*Miswak*" and treatment of cough (Hussain *et al.,* 2010; Qureshi *et al.,* 2007).

In scientific literature; *A. modesta* leaves have been tested for anti-hyperglycemic properties in alloxan induced diabetic rats and the study reported the significant reduction in glycemic conditions (Jawla *et al.,* 2011). *A. modesta* bark was exhibited prominent antioxidant and hepatoprotective effects against paracetamol induced hepatotoxicity in mice (Rahaman and Chaudhry*,* 2015). Ahmad *et al*., screened the *A. modesta* crude extract and its fractions for antitermite, antifungal, nitric oxide scavenging and brine shrimp cytotoxic actions and reported significant activity (Ahmad *et al.,* 2011). Moreover, leaves of *A. modesta* has been reported for analgesic, anti-platelet and anti-inflammatory effects (Bukhari *et al.,* 2010). Seeds powder of *A. modesta* and other members of genus *Acacia* show a notable anti-hyperglycemic activity in alloxan induced diabetic symptoms (Singh *et al.,* 1975; Jawla *et al.,* 2011). Gum of this plant act as restorative (Qureshi *et al.,* 2007) and sex stimulant (Mahmood *et al.,* 2004). This plant is enriched with carbohydrates, flavonoids, glycosides, alkaloids, tannins and saponins (Jawla *et al.,* 2011) which are considered to be responsible for antimicrobial (Rashid and Hashmi*,* 1999), anti-hyperglycemic properties (Singh *et al.,* 1975), anti-inflammatory activity (Dafallah and Al-Mustafa*,* 1996). A very few reports are available on bark of *Acacia modesta* supporting folk remedies associated with this plant. Therefore, the present study was established to evaluate antipyretic, antidepressant, anti-inflammatory, analgesic and anticoagulant activity of bark of *Acacia modesta* plant.

MATERIALS AND METHODS

Plant collection

The bark of *A. modesta* was obtained from district Chakwal, Pakistan. The plant was recognized by Dr. M. Zafar (taxonomist), Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan and its voucher (number 175315) was submitted in the "Herbarium of medicinal Plants of Pakistan".

Preparation of extracts

The bark of the plant was collected, washed and air dried in shade for fifteen days. Then 2Kg of dry powder was soaked in crude methanol (4 liter). After 10 days, crude extract was filtered and dried in fume hood. About 60g of crude methanolic extract was progressed through solvent-solvent extraction having different polarity organic solvents including hexane, chloroform and ethyl acetate yielded 22g, 7g and 7g extract accordingly and were named as AMM (crude methanolic extract), AMH (n-hexane fraction), AMC (chloroform fraction) and AME (ethyl acetate fraction). Remaining soluble fraction was known as aqueous fraction (AMA) which yielded 24g of extract.

Animals and treatments

Sprague Dawley male healthy rats weighing about 180-210g, were obtained from Pakistan Agriculture Research Centre (PARC) and housed at animal facility, Quaid-i-Azam University Islamabad, Pakistan. Rats were randomly categorized in seven groups and each group contained seven animals. They were kept at standard healthy conditions, temperature 25±1°C, light/dark cycle (12/12) and having free contact to feed and water. Ethical committee of Quaid-i-Azam University Islamabad, Pakistan approved all the protocols used in the given study (Bch#0275). For the experiment, extracts were administered orally at 300mg/Kg (dissolved in 10% Dimethyl sulfoxide (DMSO)) of rat's body weight. Activity specific standard drug (Aspirin, Diclofenac potassium, Paracetamol, Fluoxetine HCl) were administered P.O. (*per os* (by mouth)) at 10mg/Kg of animal's body weight. No animal was sacrificed during study and the overall grouping was as followed.

Group-I: Negative control which contained 10% DMSO (10ml/Kg) in distilled water.
Group-II: Positive control which contained activity specific standard drug (10mg/Kg).
Group-III: Crude methanol extract of *A. modesta* (AMM) at 300mg/Kg.
Group-IV: n-hexane fraction of *A. modesta* (AMH) at 300mg/Kg.
Group-V: Chloroform fraction of *A. modesta* (AMC) at 300mg/Kg.
Group-VI: Ethyl acetate fraction of *A. modesta* (AME) at 300mg/Kg.
Group-VII: Aqueous fraction of *A. modesta* (AMA) at 300mg/Kg.

Acute toxicity study

Acute toxicity evaluation was carried out in accordance with the instructions of Organization for Economic Cooperation and Development (OECD). Rats (n=7) were treated with 500mg/Kg oral dose of each extract and all groups were keenly examined for any anomalous behavior or mortality up to 72 hours.

In-vitro anti-inflammatory assay

Acacia modesta was examined for its *in-vitro* anti-inflammatory potential by albumin denaturation protocol as reported earlier (Mizushima and Kobayashi*,* 1968; Sakat *et al.,* 2010). Briefly, test samples with final concentration of 500, 400, 300, 200 and 100µg/mL were dissolved with 1% aqueous suspension of bovine albumin. Aspirin was used as positive control. The reaction

mixtures were then incubated at 37ºC for 20min followed by 20min incubation in water bath set at 50ºC. Turbidity of the resulting mixture was recorded at 660nm using spectrophotometer. The test was performed in triplicate and data were compared with controls. Percentage of protein denaturation was evaluated by given formula and IC50 values were calculated with GraphPad Prism 5.

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

In-vivo anti-inflammatory activity

The *in-vivo* anti-inflammatory potential of extracts were examined by carrageen an induced method (Sajid *et al.,* 2017). Inflammation was induced after one hour of oral dosage by giving subcutaneous injection of carrageenan (100µl) into the left hind paw of each rat. By using Plethysmometer (Basile 7140), the paw volume reading was taken prior and subsequent to the carrageenan injection, that provide the control (0 hour) readings. Readings of the paw volume were taken at 1st, 2nd, 3rd and 4th hour. The results were then compared with the Diclofenac potassium and DMSO. The percentage of edema inhibition was calculated as follow:

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

Analgesic activity

Analgesic activity of *Acacia modesta* was determined by hot plate test (Ismail *et al.,* 2017a). Aspirin was served as positive control. Rats were enclosed in a cylinder mounted on analytical hot plate and the latency time was documented in seconds. Temperature of the hot plate was sustained at 55±2°C and the cut-off value of 20sec was set for whole experiment in order to avoid any kind of tissue damage. Before treatment, the reaction time (Ti) taken by each rat (for showing licking or jumping response) was recorded. The reaction time (Tf) and number of lickings was recorded at 0, 30, 60 and 120 minutes subsequent to the DMSO, drug and extract administration. Percentage activity was evaluated by using formula:

$$\% \text{ analgesia} = \frac{\text{Tf} - \text{Ti}}{\text{Ti}} \times 100$$

Antipyretic activity

A. modesta crude extract and fractions were scrutinized for antipyretic potential by the previously described method (Kang *et al.,* 2008). The regular body temperature (rectal) of each rat was determined with the help of thermometer (digital). Pyrexia was induced in all of the rats by giving subcutaneous injection of 20% aqueous solution of *Saccharomyces cerevisiae* (brewer's yeast). After injection, all of the rats were fasted for 24 hours and provided only with water. Following that, the rectal temperature of each animal was determined. Rats showing more than 1°C increase in temperature confirmed pyrexia whereas rats showing less than 1°C increase were excluded from the study. DMSO (10%) was given to the first group, paracetamol (standard drug) to the second group, while 300mg/Kg of crude methanol extract and its fractions were given to rest of the groups respectively. After 30 minutes of oral dose, rectal temperature of each group was noted for 4 hours, at interval of 1 hour.

Antidepressant activity

The anti-depressant potential of *A. modesta* was determined by the forced swimming test (Ismail *et al.,* 2016). In a training test, the rats were compelled to swim for 6 minutes in a water filled vertical cylinder (18 x 40 x 15cm). The temperature of water was maintained at 35°C. The ensuing anxiety (deprived of getting away) produced vigorous swimming action to escape. Provoked depression was assigned when rat stopped its vigorous limb movements. Next day, each group was administered with oral dose of extracts (300mg/Kg) according to the assigned groups. 10% DMSO and Fluoxetine HCl were utilized as controls. After half an hour, a camera (Nikon) was fixed on the top and animals were then released in cylinder filled with water and timer was started. Recording was stopped after six minutes, rats were taken out, dried and placed in their home cages. The total immobility time was calculated in the last four minutes of a total 6 minutes video with the help of XNote timer software.

Anticoagulant activity

Capillary tube method reported by Ismail and Mirza (2015) was followed to estimate the anticoagulant potential of plant extracts. DMSO (10%) and Aspirin were utilized as controls. After 1 hour of dose administration, the tail of each rat was disinfected with spirit and punctured with the help of sterile needle. To get a larger drop of blood, the tail was squeezed and the drop was then collected in capillary tubes. The tubes were maintained at 35°C and small pieces of capillary tube were busted at small intervals and the same procedure was repeated until thread (fibrin) appeared between the broken ends of capillary tube. The time period between the blood appearance and the thread formation was noted and regarded as the clotting time.

STATISTICAL ANALYSIS

In antidepressant and anticoagulant assays One-way while in rest of the assays Two-Way ANOVA followed by Tukey's multiple comparison was used to analyze data with the help of Statistix 8.1 software. Graphs were made by using Prism 5 (Graph Pad). Results were documented as mean ± S.D from three replicates for *in-vitro* and seven replicates in case of animal studies and $p<0.05$ was considered significant.

RESULTS

Acute toxicity test

Neither mortality was observed for any of the crude extract at 500mg/K (p.o) nor the extract produced any

kinds of prominent changes in the behavior during the time of observation.

In-vitro anti-inflammatory effect

In-vitro anti-inflammatory potential of *A. modesta* was measured by albumin degradation method. The extracts and its various fractions were proved to be active in preventing albumin denaturation as shown in table 1. The IC_{50} (µg/mL) values showed the inhibition of protein degradation in AMC >AMH >AME >AMM and AMA order. The percentage inhibition was found to be 30-45% for AMM, 60-70% for AMH, 68-76% for AMC, 42-54% for AME and 17-30% for AMA respectively at different concentration range (table 1). The results were compared with Aspirin (78-82%) at different concentration ranging from 100 to 500µg/mL (IC_{50}; 24.24µg/mL).

In-vivo anti-inflammatory effect

Carrageenan-induced rat paw edema test was performed on rats to evaluate anti-inflammatory activity and the results are documented in table 2. A time dependent action was observed which became maximum at 4th hour. At this time interval *A. modesta* extract and its fraction showed moderate but significant ($P<0.05$) activity with the percentage inhibition of 49.6%, 55.9%, 53.9%, 53.3% and 48.7% respectively for AMM, AMH, AMC, AME and AMA. Diclofenac potassium served as positive control which exhibited maximum activity at 4th hour interval (82.6%).

Analgesic effect

In hot plate test, thermal stimulation was determined to represent the analgesic effect. Results of *A. modesta* crude and fractions are tabulated in the form of latency time (table 3) and percentage analgesia (table 4). Overall, the extracts showed increase in the latency period with increasing time intervals (0, 30, 60 and 120min). However, AMM, AMH and AMC exhibited decreased in latency period at 120min (7.1±1.9, 7.6±1.0 and 7.6±2.7 respectively). Aqueous fraction (AMA) with latency time 16±2.4, 18.0±2.4, 19.4±1.1 and 19.7±0.8sec was almost close to the latency time of standard drug Aspirin (16.9±1.5, 19.0±1.5, 19.7±0.5 and 19.9±0.4) at 0, 30, 60 and 120 min respectively (table 3). AMA fraction showed percentage analgesia with 61.4%, 64.6% and 67.1% inhibition at 30, 60 and 120min which was approximately similar to Aspirin inhibition with 65.9%, 68.7% and 72.6% respectively (table 4).

Antipyretic effect

Antipyretic effect of various extracts of *A. modesta* stem bark was evaluated against yeast-induced pyrexia in rats. Body temperature (rectal) of animals was recorded before and after administration of subcutaneous injections of 20% aqueous solution of yeast (table 5). Among all fractions, chloroform fraction (AMC) was the most active fraction and significantly ($p<0.01$) reduced the body temperature after first hour of dose administration, and this effect was sustained up to four hours. The effect of AMC was very similar to that of standard drug paracetamol which exhibited significant decline in body temperature. Other active fractions were AMH, AME and AMA which exhibited significant antipyretic effect with $p<0.01$ while AMM showed significant effect with $p<0.05$. Overall, the activity was time dependent which was found more prominent with passage of time.

Antidepressant effect

Antidepressant activity of different extract/fractions of *A. modesta* bark was assessed by forced swim test. Negative control group was administered with DMSO (10%) which showed maximum immobility time 184±2sec expressing depression state while positive control group (Fluoxetine HCl) showed a significant reduction in the immobility time 28±1sec (fig. 1) signifying antidepressant state. In our test extracts a significant ($p<0.01$) decline in immobility time was recorded for AMC (68±2sec), AME (65±1sec) and AMA (75±3sec) representing good antidepressant activity. AMH also showed a decrease in immobility time (144±2sec) in a significant way ($p<0.01$), whereas the results of AMM were comparable with negative control (fig. 1) with lowest activity.

Anticoagulant effect

Anticoagulant activity of different fractions of *A. modesta* was evaluated by capillary tube method and results are presented in fig. 2 showing clotting time in seconds. DMSO showed coagulation time 50±2sec while positive control (Aspirin) exhibited prolonged clotting time 134±3sec which is indicative of good anticoagulant activity. The plant extract of *A. modesta* exhibited prominent and significant ($p<0.01$) anticoagulant effect with clotting time of 226±18sec, 216±2sec, 209±11sec, 196±8sec and 188±3sec for AME, AMA, AMH, AMC and AMM respectively. The plant extracts indicated remarkable anticoagulant activity as compared to the Aspirin (134±3sec).

DISCUSSION

This study was conducted to screen the anti-inflammatory, analgesic, antipyretic, antidepressant and anticoagulant effect of the crude extract and its fractions of the bark of *A. modesta*. The bark extracts of *A. modesta* showed a notable inhibitory effect on heat induced protein denaturation where AMC was the most active fraction. These results were in consistent with the results of *in-vivo* anti-inflammatory assay where the crude extract and fractions showed significant reduction in edema (Ismail *et al.,* 2017b). Carrageenan test having significant prognostic value is an efficient method for recognizing active anti-inflammatory agents (Kayani *et al.,* 2016; Kiran *et al.,* 2018) where formation of edema is considered to be a biphasic event (Rai *et al.,* 2018).

Table 1: Effect of *Acacia modesta* on heat induced protein denaturation

Treatment	Inhibition of protein denaturation					IC_{50} (µg/mL)
	500 µg/mL	400 µg/mL	300 µg/mL	200 µg/mL	100 µg/mL	
DMSO	0.07±0.005	0.07±0.001	0.08±0.002	0.08±0.002	0.08±0.002	N/A
Aspirin	82.91±1.48[a]	81.50±0.39[a]	80.43±0.39[a]	79.40±0.39[a]	78.21±0.50[a]	24.24[a]
AMM	45.89±0.26[e]	41.28±0.26[e]	37.44±0.25[e]	35.64±0.25[e]	30.26±0.25[e]	909.71[e]
AMH	70.60±2.33[c]	68.12±0.90[c]	66.15±0.51[c]	63.00±0.39[c]	60.25±0.25[c]	59.57[c]
AMC	76.07±3.92[b]	74.36±2.56[b]	71.11±0.53[b]	69.57±0.39[b]	67.95±0.26[b]	47.07[b]
AME	54.19±0.15[d]	52.22±0.40[d]	49.48±0.26[d]	47.01±3.92[d]	42.56±3.86[d]	105.01[d]
AMA	30.17±0.39[f]	28.21±2.56[f]	24.36±0.26[f]	20.00±0.25[f]	17.26±0.97[f]	2849.20[f]

Data values shown represent Mean±SD and percentage inhibition at different concentrations, (n=3). Different superscript ([a-f]) indicate significance at P<0.01 as compared with normal control (group DMSO). AMM; *A. modesta* crude methanol extract. AMH; *A. modesta* n-hexane fraction. AMC; *A. modesta* chloroform fraction. AME; *A. modesta* ethyl acetate fraction. AMA; *A. modesta* aqueous fraction.

Table 2: Effect of *Acacia modesta* on carrageenan-induced paw edema in rats

Treatment	Percentage inhibition to edema			
	1h	2h	3h	4h
DMSO	-	-	-	-
DIC	48.21±1.57[e]	60.14±1.48[a]	76.93±1.19[a]	82.6±1.16[a]
AMM	13.13±1.07[b]	18.05±2.57[e]	34.18±2.36[e]	49.61±1.45[e]
AMH	3.03±1.38[f]	35.61±1.51[b]	46.35±0.89[c]	55.91±0.611[c]
AMC	9.49±2.85[c]	30.70±4.32[c]	43.49±1.67[b]	53.94±1.80[b]
AME	19.25±2.44[a]	24.65±4.23[d]	40.48±2.45[d]	53.35±2.10[d]
AMA	11.36±1.22[d]	15.21±2.08[f]	31.80±1.81[f]	48.72±1.27[f]

Values shown represent Mean±SD (n=7). Means with different superscript ([a-f]) indicate significance at P<0.05 as compared with normal control (group DMSO). DIC; diclofenac potassium. AMM; *A. modesta* crude methanol extract. AMH; *A. modesta* n-hexane fraction. AMC; *A. modesta* chloroform fraction. AME; *A. modesta* ethyl acetate fraction. AMA; *A. modesta* aqueous fraction.

Table 3: Latency time of *Acacia modesta* in hot plate test

Treatment	Latency time in seconds			
	0	30 min	60 min	120 min
DMSO	2.29±0.49	4.29±0.49	6.57±0.79	6.71±0.76
Aspirin	16.86±1.46[a]	19.00±1.53[a]	19.71±0.49[a]	19.86±0.38[a]
AMM	5.29±1.70[d]	7.29±1.70[d]	7.71±3.04[e]	7.14±1.86[d]
AMH	4.86±1.57[de]	6.86±1.57[e]	8.00±2.52[d]	7.57±0.98[cd]
AMC	5.57±1.27[c]	7.57±1.27[cd]	7.86±2.85[cd]	7.57±2.70[e]
AME	3.00±1.4[f]	5.00±1.41[ef]	6.57±1.51[f]	7.14±0.90[f]
AMA	16.00±2.38[b]	18.00±2.38[b]	19.43±1.13[b]	19.71±0.76[b]

Values shown represent Mean±SD (n=7). Means with different superscript ([a-f]) indicate significance at P<0.05 as compared with normal control (group DMSO). AMM; *A. modesta* crude methanol extract. AMH; *A. modesta* n-Hexane fraction. AMC; *A. modesta* chloroform fraction. AME; *A. modesta* ethyl acetate fraction. AMA; *A. modesta* aqueous fraction.

Table 4: Percent analgesia of *Acacia modesta* in hot plate test

Treatment	Percent analgesia		
	30 min	60 min	120 min
DMSO	-	-	-
Aspirin	65.93±1.46[a]	68.72±0.62[a]	72.57±2.05[a]
AMM	14.53±1.17[d]	5.02±0.75[e]	4.92±0.75[d]
AMH	10.87±1.79[e]	6.17±0.83[d]	6.82±0.30[c]
AMC	15.30±1.37[c]	6.50±0.66[c]	4.91±1.30[e]
AME	2.57±0.89[f]	1.80±1.56[f]	2.17±1.87[f]
AMA	61.40±1.06[b]	64.63±1.40[b]	67.07±1.38[b]

Values shown represent Mean ± SD (n=7). Means with different superscript ([a-f]) indicate significance at P<0.05 as compared with normal control (group DMSO). AMM; *A. modesta* crude methanol extract. AMH; *A. modesta* n-hexane fraction. AMC; *A. modesta* chloroform fraction. AME; *A. modesta* ethyl acetate fraction. AMA; *A. modesta* aqueous fraction.

Table 5: Yeast-induced antipyretic activity of *Acacia modesta*

Treatment groups	Rectal temperature (^{0}C)		Mean rectal temperature (^{0}C) after oral dose ± SD			
	Before pyrexia	After pyrexia	1h	2h	3h	4h
Saline	33.20±0.27	34.90±0.24	34.90±0.24	34.70±0.40	34.50±0.45	34.50±0.45
Paracetamol	33.18±0.68	34.60±0.53	33.60±0.54^{b}	33.40±0.73^{bc}	33.20±0.87^{b}	33.20±0.87^{b}
AMM	32.26±0.37	35.28±0.49	34.36±0.24^{ab}	33.85±0.24^{ab}	33.37±0.45^{ab}	33.37±0.45^{ab}
AMH	32.24±0.54	34.42±0.53	33.80±0.68^{b}	33.30±0.34^{bc}	32.45±0.81^{b}	32.37±0.89^{b}
AMC	32.09±0.37	34.40±0.53	32.60±0.63^{cd}	32.40±0.38^{d}	32.20±0.39^{b}	32.21±0.39^{b}
AME	32.93±0.47	34.70±0.95	33.70±0.34^{bcd}	33.10±0.41^{bcd}	32.62±0.45^{b}	32.60±0.45^{b}
AMA	32.69±0.29	34.66±0.53	33.82±0.30^{a}	32.73±0.52^{cd}	32.40±0.52^{b}	32.41±0.52^{b}

Values are expressed as mean ± S.D (n=7). Different superscript letters (a-d) indicates significant difference ($p < 0.05$) as compared with normal control (group saline) AMM; *A. modesta* crude methanol extract of stem bark, AMH; n-hexane fraction of AMM, AMC; chloroform fraction of AMM, AME; ethyl acetate fraction of AMM, AMA; residual aqueous fraction of AMM).

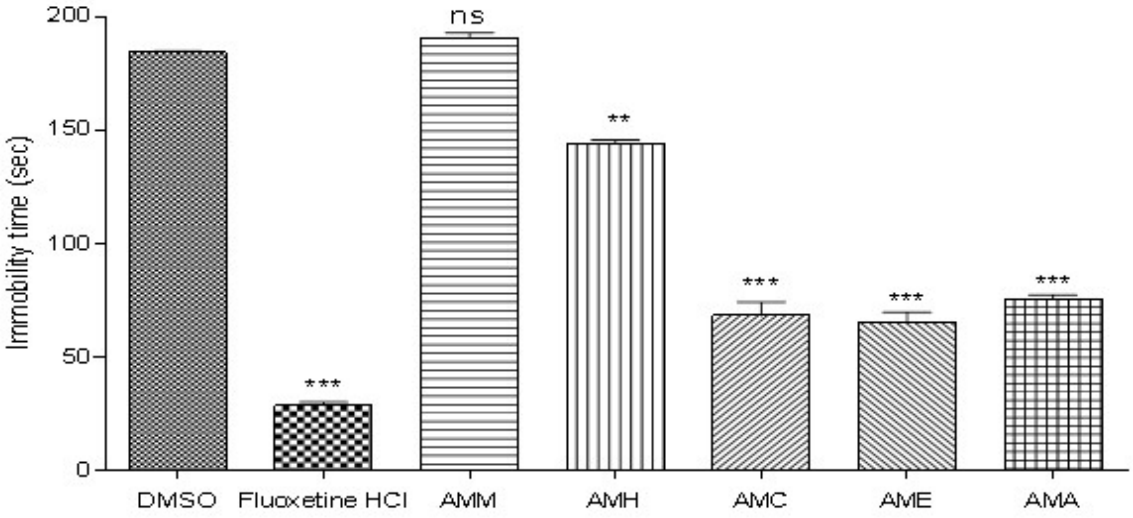

Fig. 1: Antidepressant activity of *Acacia modesta*

Values are expressed as mean ± S.D (n = 7) with statistical significance (**$p<0.01$ and ***$p<0.001$) as compared with normal control (group DMSO). AMM; *A. modesta* crude methanol extract of stem bark, AMH; n-hexane fraction of AMM, AMC; chloroform fraction of AMM, AME; ethyl acetate fraction of AMM, AMA; residual aqueous fraction of AMM).

The earlier phase is characterized by the serotonin and histamine discharge and the later phase is attributed to the discharge of lysosome, protease and prostaglandins (Rauf *et al.,* 2016). This later phase is susceptible to anti-inflammatory agents (Ismail *et al.,* 2017c). Anti-inflammatory effect of these extracts suggests that one of these mechanisms may be involved which needs to be further explored.

Hot plate method is used for the detection of centrally acting analgesics and found to be valid even in the absence of motor performance (McKune *et al.,* 2015). Overall, the bark extracts of *A. modesta* showed central analgesic effect being AMA the most active one.

In addition to this, bark extracts of *A. modesta* possessed a notable antipyretic effect in yeast induced pyrexia in rats. Fever may occur in several pathological conditions such as tissue injure, inflammation, graft rejection or infections. Temperature regulation in the body requires an insubstantial stability between generation and loss of heat (Lighton, 2018). During hyperthermia, the set point is raised up, the antipyretic agents like paracetamol affect the body normal temperature when it is raised by factors such as exercise or other physical activities (Devi *et al.,* 2003; Lundberg and Howatson, 2018). Natural antipyretics are agents, which decrease elevated body temperature with minimal side effects. These extracts of *A. modesta* could be potential source of herbal antipyretic agents.

Forced swim test is responsible for creating pathophysiology conditions comparable to human depression and is very precise and perceptive (Ismail *et al.,* 2018). A notable diminution in immobility period was observed for the methanolic extract and fractions in comparison to the controls. It is reported in the literature, that antidepressants act by reducing oxidative stress were responsible for depressive disorder (Ismail and Mirza, 2015; Dilshad *et al.,* 2016b). Plant are rich source of

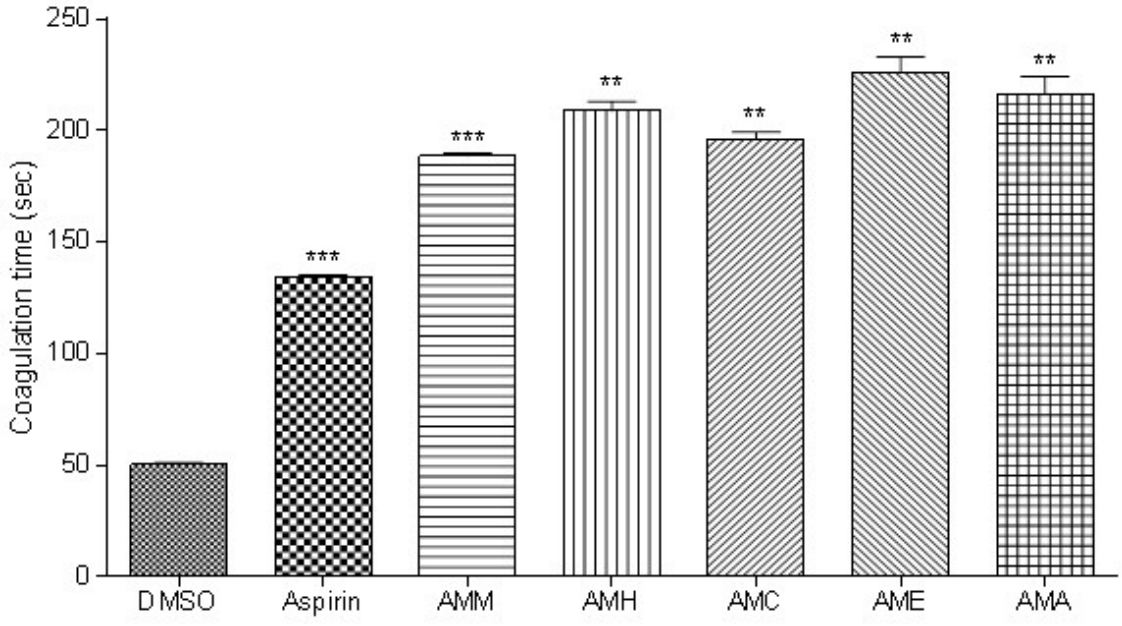

Fig. 2: Anticoagulant activity of *Acacia modesta*

Values are expressed as mean ± S.D (n = 7) with statistical significance (**p<0.01 and ***p<0.001) as compared with normal control (group DMSO). AMM; *A. modesta* crude methanol extract of stem bark, AMH; n-hexane fraction of AMM, AMC; chloroform fraction of AMM, AME; ethyl acetate fraction of AMM, AMA; residual aqueous fraction of AMM).

phytochemicals and flavonoids with several biological activities in central nerves system (Sajid *et al.,* 2016; Waheed *et al.,* 2015). Flavonoids present in the plants contain antioxidant property which was also responsible to enhance the antioxidant status on plasma (Heim *et al.,* 2002). Although, the role of flavonoids and phenolics in *A. modesta* have not been defined as antidepressant. However, these phytochemicals are well known for antidepressant activity (Velioglu and Mazza*,* 1991; Moallem *et al.,* 2007; Dilshad *et al.,* 2016a). Hence, it can be suggested that these compounds may be responsible for antidepressant effect.

The homeostatic pathways are responsible for the formation of clot or a plug by arresting the bleeding at the site of injury. In normal physiological conditions, there is equilibrium between these processes. The anticoagulant drugs inhibit blood clotting, which may attribute to heart attack and strokes. Since anticoagulants are utilized mainly by the heart patients, so instead of using synthetic blood thinners, physicians can rely on natural herbal products. Antioxidants are reported to counteract the hematological and blood coagulation turbulence (Ismail and Mirza*,* 2015; Kayani *et al.,* 2016). Hence our study confirms the strong anti-coagulant effect of *bark extract of A. modesta*. All fractions showed significant anticoagulant potential and the results are comparable to the standard drug aspirin (Ismail and Mirza*,* 2015; Ismail *et al.,* 2017b). The leaves extracts of the plants are reported to have antioxidant constituents like alkaloids and flavonoids (Jawla *et al.,* 2011), so it can be supposed that same chemical constituents in bark extract would also be responsible for anticoagulant activity of bark of *A. modesta* which require further studies to affirm.

CONCLUSION

From the above study, it can be summarized that bark of *Acacia modesta* is responsible for the significant anti-inflammatory, analgesic, antipyretic, antidepressant, and strong anticoagulant activities. These animal studies provide the base line to test these plant extracts on human cell lines to evaluate the validity and response. The given results suggested further studies to identify and isolate the compounds accountable for the examined activities of the plant.

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