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Evaluation of Analgesic Activity of Crude Hydro-Alcoholic Extract of *Acacia Senegal* Pod

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ABSTRACT

The main objective of the present investigation is to evaluate the analgesic activity of hydro-alcoholic extract of *Acacia senegal* pods on mice. Analgesic activity of hydro-alcoholic extract of *Acacia senegal* pod at a dose of 200mg/kg, 400mg/kg and 800mg/kg were evaluated against drug Pentazocine at a dose of 17.5mg/kg. Adult Albino mice and rats of either sex of six numbers in each group were under taken for study and evaluated by Eddy's hot plate and tail immersion method. The all doses of *Acacia senegal* pod crude hydro-alcoholic extract were found to produce significant ($P<0.05$) analgesic activity. By Eddy's hot plate and tail immersion method, both showed significant activity ($P<0.05$) after 30 minutes. The results showed significant analgesic activity against stimuli in animals.

Keywords: *Acacia senegal* pod, Crude hydro-alcoholic extract, Eddy's hot plate method, Tail immersion method, Pentazocine.

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INTRODUCTION

The use of plant products is increasing in many segments of the population. According to an estimate, 80% of the world's population relies upon plants for their medication. Most of the synthetic drugs used to present for analgesic and anti-nociceptive effect cause many side and toxic effects. Plants still represent a large untapped source of structurally novel compounds that might serve as lead for development of novel drugs[1]. Many medicines from plant origin with analgesic had been used since long time without any adverse effect. North East India is considered as one of the "hotspots" for biodiversity in India, since out of the 1500 species of medicinal plants available in India, almost 350 species belong to Assam and many of these traditionally used plants have not yet been studied scientifically which can be used as a potential drug after scientific validation.

Acacia senegal Willd (*Leguminosae*) locally known as Arabic gum tree, locally it is called Kumbat in Sind[2], Rajasthan and Lasbella it is called Kher[3,4] distributed mainly in tropical and sub tropical region of southern parts of West Pakistan and India (Jaipur and Jodhpur), the species grows 2-15m tall with a flat or rounded crown[5]. *Acacia senegal* (*AS*) leaves are small, grey-green, alternate cream colored flowers occur with 2-12mm long spikes, pods are dehiscent (open by splitting at maturity) and seeds greenish-brown[6]. The tree is highly valued for centuries for Gum Arabic production, which is used in food, pharmaceuticals and other industries in the USA and Europe[7]. Gum Arabic is approved for use as food additives by the US Food and Drug Administration and enlisted substances that are generally recognized as safe[8]. Folk medicine, demulcent, emollient, the gum is used in inflammation of intestinal mucosa, and externally to cover inflamed surfaces, as burns and nodular leprosy. It is also said to be used for anti-tussive, astringent, cold coughs, diarrhoea, dysentery, expectorant gonorrhea, sore throat and urinary track[9]. Various chemical studies on *Acacia senegal*, gum contains nautral sugars (rhamnose, arabinose and galactose), acids (glucuronic acid and 4-methoxyglucuronic acid) calcium, magnesium, potassium and sodium[10]. The phyto-constituents reported in literature for plant *AS* (L.) Willd: flavones, catechin, tannins, polyphenols, chalcones, alkaloids and flavonoids[11]. Various pharmacological activities on *Acacia senegal* gum exudates offers protection against cyclophosphamide induced urinary bladder cytotoxicity[12]. Scavenging of nitric oxide by Gum Arabic has been reported to limit the acetaminophen-induced hepatotoxicity in mice[13]. Other studies have documented the antioxidant properties of Gum Arabic in a variety of animal model system[14]. Antioxidant potential and free radical scavenging activity by pod extracts of *Acacia senegal*[15] and Anti-diabetic activity of *Acacia senegal* pod extract in Streptozotocin-induced diabetic rats[16]. Bark of *AS* are made into a poultice

to treat bedsores and wounds. The investigation was initiated to identify wound-healing plants and lesser known edible plant in Oman that have high antioxidant capacity[11]. In present study we aimed to explore the analgesic activity of hydro-alcoholic extract of *Acacia senegal* pod.

MATERIALS AND METHOD

Chemicals detail and identification of plant material

All chemicals were of analytical grade. Pentazocine (Ranbaxy) was used for this study. The matured pod with seeds of *Acacia senegal* Willd was purchased from Central Arid Zone Research Institute, Jodhpur, Rajasthan. (India), the plant material was authenticated by Dr. Ashok Kumar Sharma, M.D. (Dravyaguna Vigyan), Prof. & Head of Department Shri Baba Mastnath Ayurvedic Degree College, Asthal Bohar, Rohtak.

Extraction process

Acacia senegal shed dried plant material (matured pods with seeds) were grinded and powdered material (100g) was used for extraction. The hydro-alcoholic and aqueous extracts were prepared by continuous hot percolation process, using Soxhlet apparatus. Both extracts were collected separately and dried in vacuum system. Hydro-alcoholic extract with 70% ethanol (Pod-HA) yields: 12.45% and aqueous extract with water (Pod-W) yields: 12.95%. Both extracts were condensed by re-distillation and dried in vacuum desiccators to obtain a final extract residue.

Experimental animals

Healthy adult Wister Albino rats and Albino mice were selected for the study. Animals were housed in polypropylene cages, maintained under standard conditions (12 hours light/dark cycle; 25±30^{o}C; 45-55% humidity). They were fed with standard pellet diet and water ad libitum. The Institutional Animal Ethical Committee of Janta College of Pharmacy Butana, (Sonepat) Haryana, India (CPCSEA-667/02/c/CPCSEA) approved the studies.

Determination of acute toxicity of the drug

Acute toxicity was determined in overnight fasting mice. Animals were divided into groups of 6 each and the extract Pod-HA of *Acacia senegal* was administrated orally with 1% CMC of 10, 30, 100, 300, 1000 and 1200mg/kg body weight. The mice were observed continuously for 2nd, 4th, 6th, 12th, 24th and up to 48 hours[17].

Analgesic activities

Eddy's hot plate method in mice

This study was carried out in Albino mice of either sex, weighing 20-25g. Animals were divided in different groups, including standard and control animals. Thirty minutes prior to hot plate

exposure, the mice were treated with test drug compound. The time of reaction to pain stimulus (interval between placing the mice on the Eddy's hot plate and the lick or jump response) of the mice placed on the hot plate heated at 55±0.5°C, was recorded every thirty minutes for duration of 3 hours after drug administration. To avoid the variance in temperature extent, animal were always kept in centre of hot plate. The efficacy of analgesic activity was determined by comparing the delay in pain stimulus in control and drug treated animals[18].

Tail immersion in hot water method

The present study was carried out in overnight fasted rats of either sex weighing from 200-250gm. Anti-nociceptive effect of the test substances was determined according to the initial (control) reaction time, noted in all the animals. Preliminary screening of animals were done to select animals having reflex stimuli lesser than five seconds at temperate 50^0C. The selected animal will be acclimatizing for experimental conditions prior to study. Rats were randomized in desired group and were treated with test compound (extracts), 30 minute prior to noxious stimuli. One to two cm tail of rat was immersed in warm water kept constant at 55°C. The reaction time taken by the animals to deflect their tails were accounted. The latent period of tail-flick response was taken as the index of anti-nociception and was determined before and at 30, 60, 90, 120 and 150 min. after the administration of drugs. The maximum reaction time was fixed: 15 seconds. Tail flick reaction time in control and drug treated groups were compared. The extant of analgesia was calculated by percentage reduction latent period of the tail-flick response[19].

Statistical analysis

The data were expressed as Standard Error Mean (SEM). The significance of difference among the groups was assessed by using one-way and multiple-way analysis of variance (ANOVA). The test was followed by Dunnet's test and p values were considered as significance.

RESULTS AND DISCUSSION

Acute toxicity studies

Acute toxicity studies revealed the nontoxic nature of the Pod-HA extract of *Acacia senegal*. There were no lethality or toxic reactions found at any of the doses selected until the end of the study period. All the animals were alive, healthy and active during the observation period.

Analgesic activity of *Acacia senegal* Pod-HA in mice by Eddy's hot plate method

In this model, the reaction time in Pod-HA treated group increased significantly ($P<0.05$) in comparison to the control group. The maximum effect was observed at the highest dose viz. 800mg/kg per oral at 90 min. which showed a reaction time of 9.08 sec., where as standard drug

Pentazocine (17.5mg/kg i.p.) showed a reaction time of 11.15 sec., at 60 min. The extract also showed dose and time dependent activity as given in (Table: 1).

Analgesic activity of *Acacia senegal* Pod-HA in rats by tail immersion method

In this tail immersion test, the increase in reaction time was significant ($P<0.05$) as compared to the control group of animals. Maximum effect was observed as 8.9 seconds at 90 minutes post treatment with 800mg/kg p.o. of Pod-HA, where as in the vehicle treated control group the reaction time was 3.83 seconds, at 90 minutes, it clearly indicating the analgesic property of the extract of interest as given in (Table: 2).

Table 1: Analgesic activity of Pod-HA of *Acacia senegal* in mice by Eddy's hot plate model.

Group	Drugs	Dose mg/kg	Reaction time in seconds					
			0 min	30 min	60 min	90 min	120 min	150 min
I	Control	-	3.74±0.23	3.87±0.31	4.04±0.20	4.06±0.26	3.77±0.29	3.77±0.28
II	Pentazocine	17.5	3.62±0.21	7.12±0.23*	11.15±0.18*	10.11±0.17*	7.22±0.17*	6.16±0.17*
III	Pod-HA	200	4.34±0.16	5.50±0.15*	6.36±0.15*	6.58±0.14*	6.72±0.14*	6.80±0.15*
IV	Pod-HA	400	3.86±0.21	5.46±0.22*	6.33±0.22*	07.14±0.23*	7.31±0.25*	7.22±0.26*
V	Pod-HA	800	3.80±0.11	5.94±0.12*	7.02±0.12*	09.08±0.13*	8.98±0.11*	8.84±0.13*

All values in terms of Mean ± SEM, n=6 in each group. *P <0.05 statistically highly significant as compared with control group.

Table 2: Analgesic activity of Pod-HA of *Acacia senegal* in rats by tail immersion method.

Group	Drugs	Dose mg/kg	Reaction time in seconds					
			0 min	30 min	60 min	90 min	120 min	150 min
I	Control	-	3.88±0.24	3.78±0.18	3.76±0.16	3.83±0.11	3.72±0.15	3.72±0.19
II	Pentazocine	17.5	3.59±0.15	8.53±0.17*	13.72±0.37*	13.19±0.12*	9.28±0.20*	7.23±0.11*
III	Pod-HA	200	3.86±0.11	5.10±0.09*	6.16±0.07*	7.33±0.12*	7.22±0.08*	6.93±0.08*
IV	Pod-HA	400	3.82±0.11	6.49±0.24*	7.90±0.22*	7.96±0.26*	7.54±0.34*	7.34±0.27*
V	Pod-HA	800	3.47±0.19	7.35±0.18*	8.07±0.15*	8.94±0.16*	8.55±0.13*	7.98±0.12*

All values in terms of Mean ± SEM, n=6 in each group. *P <0.05 statistically highly significant as compared with control group.

DISCUSSION

Preliminary phytochemical screening showed the presence of catechin, tannins, alkaloids and flavonoids in the *Acacia senegal* pod hydro-alcoholic extract, so the observed analgesic activity may be attributed due to these compounds. Moreover, recent studies suggest that the inflammatory tissue damage is due to the liberation of reactive oxygen species form phagocytes invading the inflammation sites[20]. There are also reports on the role of flavonoid, a powerful antioxidant[21, 22] in analgesic activity primarily by targeting prostaglandins[23, 24]. Again the Pod-HA extract of *Acacia senegal* demonstrated good antioxidant action in tested models. So, can be assumed that cyclo-oxygenase (COX) inhibitory activity, together with antioxidant activity may reduce the production of free arachidonic acid from phospholipids or may inhibit the enzyme system responsible for the synthesis of prostaglandins and ultimately relive pain-sensation.

Eddy's hot plate and tail immersion test are considered to be selective to examine compounds acting through OPIOID receptors, the extract increases mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while NSAIDs inhibit only peripheral pain[25, 26].

CONCLUSION

From the above investigation it is quite apparent that a crude hydro-alcoholic extract of *Acacia senegal* pod possesses the analgesic effect against different stimuli in small animals. This is evidenced by a significant increase in the reaction time by stimuli in different experimental models.

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