Evaluation of Anti-inflammatory, Analgesic and Antipyretic Potential of Parkinsonia aculeata. Linn Bark

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Abstract: The present study was aimed at evaluation of the analgesic, anti-inflammatory and antipyretic activity of total ethanolic and aqueous extract of bark of *P. aculeate* Linn in mice and rats. The ethanolic extract of *P. aculeate* L bark *at* a dose of 200 mg/kg body weight has shown significant analgesic, antipyretic and anti-inflammatory activity as compared to aqueous extract. The result of hot plate indicated that the total ethanolic extract shows a significant increase ($P<0.01$) in reaction time at 2 and 3 hours comparable to the reference drug Pentazocin but lesser ($P<0.05$) at 1 hr. The tail immersion and hot plate test reveal that this plant has high analgesic activity. This is because some form of error may be introduced with the animal handing while the test is being elicited. Both test show highest degree of analgesia in ethanolic extract compared to aqueous extract. The total ethanolic extract of *P. aculeate* L bark at the a dose of 200 mg/kg body weight has shown significant ($p<0.001$) antipyretic activity as compared to aqueous extract, it has shown significant fall in body temperature up to 4h following its admistration. The antipyretic activity stared as early as 1h and the effect was maintain for 4h.The response was comparable to that antipyretic activity of paracetamol a standard antipyretic drug. Total ethanolic extract significantly inhibited Carrageenin-induced paw oedemaas compared; it may be due to possible inhibition of lipooxygenase pathway.

Keywords: Analgesic, Anti-inflammatory, Antipyretic, Ethanolic extract, Aqueous extract, Tail immersion method, Eddy's Hot plate method. Paw edema method, Yeast induced pyrexia method.

INTRODUCTION:

The problem of uncontrolled pain led early human to seek remedies from any materials that they could lay their hand on. In recent times, focus on plant research has increase and non-steroidal anti-inflammatory drugs constitute one of the most widely used classes of drugs. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects. Herbal medicines are in line with nature, with less hazardous reaction [1].

Parkinsonia (Leguminosae) is a small genus containing three species which are common in tropical America and have been recently naturalized in hotter regions, e.g. Egypt and India[2]**.** *P.aculeata* is a tree from the family Fabaceae; common names include Mexican Palo Verde, *Parkinsonia*, Jerusalem thorn, or Jellybean tree[3]. Previous investigations showed that thebark from the plant contains orientin, iso-orientin, vitexin, isovitexin, lucenin-II, vicenin-II, diosmetin 6-C Bglucoside, apigenin, luteolin, kaempferol, chrysoeriol, epiorientin, parkinsonin-A, parkinsonin-B,and parkintin[2,4-6]. All the parts of the plant are known as antipyretic, diaphoretic, andabortifacient[7-8], hot water extract ofbark used orally as abortifacient in pregnant women. It cures boils and tumors if young twigs are crushed and applied. Leaf, bark and stem decoction are taken orally to treat fever, malaria. Reported phytoconstituents include β-amyrenone, β- amyrin, daucosterol, palmitic acid and β- sitosterol in dried aerial parts of *P. aculeata*, L-dopa isolated from dried seeds of *Parkinsoniaaculeata*, β- amyrin from dried stem bark of *P. aculeata*, prencence of alkaloids in bark, flower, leaf, stem of *P. aculeata*, Amino acid trytophan from dried seeds of *P. aculeata*, Glycerol β-butanoate α, α' 1- dipentanonate, β-Sitoeryl- β-D-glucoside, β-Sitosterol, glycerol α-heptanone kappa octanoate from stem of *P. aculeata*[9], reported to possess Antimicrobial activity[10], Antioxidant activity of the 70% hydroalcoholic extract ofbark of *P. aculeata*[11], Amoebicidal activity of different concentration of isolated rotenoids3. Many pharmacological activities viz. CNS depressant activity of ethanol-water(1:1) extract of dried aerial part, Smooth muscle stimulant activity of aqueous extract of dried aerial part of *Parkinsonia aculeata*, Antibacterial activity ethanol-water extract of dried leaf of *P. aculeata*, Antidiabetic effect taking water soluble fraction of aerial part of *Parkinsoniaaculeata*, Antidiabetic activity of the bark of *P. aculeata*[9], Hepatoprotective activity of *P. aculeatebark* extract posses potent against carbon tetrachloride(CCl4), Anti

spermatogenic activity of ethanolic extract of stem bark of *P. aculeata*, Anti malarial activity of crude extract of aerial parts (leaves) of *P. aculeata*[12], Analgesic, Anti-inflammatory and Antipyretic activity of total alcoholic and aqueous extract of bark of *P.aculeate*[13].

MATERIALS AND METHODS:

Plant Materials: Bark of *P. aculeate* L. was collected from local areas of Ajmer road, Jaipur, Rajasthan. The taxonomical identification of the plant was done by Dr. Gajendra Pal Singh, Department of Botany, University of Rajasthan, Jaipur, and voucher specimens were deposited at the herbarium, Department of Botany, University of Rajasthan, Jaipur. Bark was dried under shade, coarsely powdered and stored in airtight container for further use.

Preparation of Extract: The powdered Bark was Sox let-extracted with total ethanolic. The extract, on removal of solvent in vacuum, gave brown semisolid residue (yield: 9.8% w/w). The bark of *P. aculeata* was shade dried at room temperature, pulverized, and 100g of coarse powder was macerate exhaustively with water then being kept for 5 days in tightly sealed vessels at room temperature, protected from sunlight and shaken several times daily and add preservative(2% chloroform). Concentrate extract by distilling off the solvent and then evaporating to dryness on water –bath, gave brown semisolid residue (yield: 11.8% w/w) [14-15].

Phytochemical Screening: Preliminary Phytochemical investigation was carried out for extracts. Presence of alkaloids was determined by Mayer's, Dragendorf's, Wagner and Hager's test, Flavonoids by Shinoda, Ferric chloride and lead acetate tests, Saponins by Foam and haemolysis test and sterols by Salkowaski and Libermann and Burchards tests[16].

Animals Used: Wister rats of either sex weighing 180-200g and Swiss mice weighing 18-28g were maintained under standard nutritional and environmental conditions throughout the experiment. The animal was of food for 24 h before experimentation but allowed access to tap water throughout. Animal were divided into five (n=6) for each experimental model, control,

standard, two extract. Approval for the project was obtained by the institutional animal ethical committee, IAEC, Sri Balaji College of Pharmacy, Jaipur (Letter No. IAEC/2020/22)

Toxicity study [17]: *P. aculeate* was tested in single doses in each experimental model as per following the OECD guideline no. 420 fixed dose method procedure, the safest dose of total ethanolic extract and aqueous extract are 2000mg/kg body weight. The safe dose was found to be 2000mg/kg body weight; hence 1/10th of the dose was taken as effective dose which was found to be 200mg/kg body weight. Pentazocine 5mg/kg was used as the standard analgesic in hot-plate and Acetyl salicylic acid 640mg/kg p.o in tail immersion in mice. Paracetamol was used as standard drug (positive control) in anti-pyretic models in the dose of 200 mg/kg and required quantity was dissolved in normal saline. In the anti-inflammatory model aspirin was used as the standard drug in a dose of 200mg/kg.

Assessment of analgesic activity [18-19]

Hot Plate Method: In the hot plate method albino mice (18-28) were divided into four groups each consisting of six animals. One group served as a control (received vehicle), second group served as a standard (received Pentazocine 5mg/kg) while the third group received the ethanolic extract (As per b/w), and fourth group received the aqueous extract (As per b/w). The basal reaction time was noted before and 30, 60, 90 and 120 minutes after the administration of the drugs.

Tail Immersion Method: In the Tail immersion method albino rats (180-200g) were divided into four groups each consisting of six animals. One group served as a control (received vehicle), second group served as a standard (received Acetyl salicylic acid 640mg/kg p.o) while the third group received the ethanolicextract (As per b/w), and fourth group received the aqueous extract (As per b/w).The time in second to withdraw the tail clearly out the water was taken as the reaction time.

Assessment of anti- pyretic activity [20]

Induction of yeast-induced pyrexia: Rats were divided into four groups of six each for this experiment. The normal body temperature of each rat was measured rectally at predetermined

interval and recorded. The rats were trained to remain quiet in a restraint cage. A thermistor probe was inserted 3-4 cm deep into the rectum and fastened to the tail by adhesive tape. The temperature was measured on a thermometer. After measuring the basal rectal temperature, animals were given a subcutaneous injection of 10 ml/kg body wt. of 15% w/v yeast suspended in 0.5% w/v methyl cellulose solution. Rats were then returned to their housing cages. After 19 h of yeast injection, the animals were again restrained in individual cages for another recording of their rectal temperature as described above.

Drug administration: After 19 h of yeast injection, the total ethanolic and aqueous extracts were administered orally at doses of 200 mg/kg body wt. to two groups of animals, respectively. A similar volume (5ml/kg body wt.) of normal saline solution was administered orally to the control group. The fourth group of animals received the standard drug paracetamol (200 mg/kg body wt.) orally. Rats were restrained for recording of their rectal temperature at the nineteenth hour, immediate before total ethanolic and aqueous extract, saline or paracetamol administration, and again at one-hour intervals up to the twenty-third hour after yeast injection.

Assessment of anti- inflammatory activity[18,21-24]

Carrageenin-Induced Rat Paw Oedema: The rats were divided into four groups, each groups consisting of six animals. Oedema was induced by sub plantar injection of 0.1 ml of 1% freshly prepared suspension of Carrageenin into the right hind paw of each rat. The paw volume was measured before (O h) and 1 h after the injection of Carrageenin using a Plethysmometer. The total ethanolic and aqueous extractbark in 2% Tween 80 solution (200 mg/kg) was administered orally to two groups of rats, 30 min before the injection of Carrageenin. The third and fourth group of rats received 2% aqueous Tween 80 solution 10 ml/kg orally (control) and Aspirin 150 mg/kg as a reference drug.

Statistical Analysis: Values are expressed as mean ± S.E.M Statistical significance was analyzed using one way ANOVA.

RESULTS:

The OECD guideline 420 fixed dose methods study showed that extract was safe at a dose of 2000 mg /kg body weight. The ethanolic extract of *P. aculeate* Lbark at a dose of 200 mg/kg body weight has shown significant analgesic, antipyretic and anti-inflammatory activity as compared to aqueous extract. The analgesic activity ofbark of *P. aculeate* L was studied for its central activity. The result of hot plate indicated that the total ethanolic extract shows a significant increase ($P<0.001$) in reaction time at 2 and 3 hours comparable to the reference drug Pentazocin but lesser ($P<0.05$) at 1 hr. Aspirin leads to a relief from inflammatory pain by suppressing the formation of pain inducing substances in the peripheral tissues, prostaglandins and bradykinin were suggested to play an important role in the pain process [25]. Therefore it is likely that *P. aculeata*Lbark might suppress the formation of these substances. It has been widely accepted that Carrageenin-induced paw oedemamodel is applied for the evaluation of the antioedemal effect of drugs. Recent investigation demonstrated that Carrageenin oedema is effectively decreased by lipooxygenase inhibitors. In the present study, total alcoholic extract significantly inhibited Carrageenin-induced paw oedemaascompared; it may be due to possible inhibition of lipooxygenase pathway although such assumption obviously requires confirmation by further detailed experimentation [26]. The total ethanolic extract of *P. aculeata*Lbark at the a dose of 200 mg/kg body weight has shown significant ($p<0.001$) antipyretic activity as compared to aqueous extract, it has shown significant fall in body temperature up to 4h following its admistration. The antipyretic activity stared as early as 1h and the effect was maintain for 4h.The response was comparable to that antipyretic activity of paracetamol a standard antipyretic drug.
But aqueous extract did not showed significant activity as compared total ethanolic extract.

DISCUSSION:

A drug with anti-inflammatory activity usually exhibit antipyretic and analgesic properties. The best examples would be the non-steroidal anti-inflammatory drugs, which possess all three activities[22].. Inflammation is a defensive reaction of the local microcirculation to tissue injury arising from cell damages due to mechanical trauma, chemical, physical and thermal injury, antigen antibody reactions and infections. The signs and symptoms of inflammation include redness, swelling, heat,pain and loss of function of the affected area. Pain is an unpleasant sensation that is a consequence of complex neurochemical processes in the central and peripheral nervous systems. Non-steroidal anti-inflammatory drugs (NSAIDS) and opioids are used in

management of mild to moderate and severe pains respectively. These drugs have serious limitations due to their side effects. Most of the drugs used presently for the management of pain and inflammation possess some side and toxic effects. It is therefore, inevitable to search for new, less toxic and more effective anti-inflammatory and analgesic agents [27]. Fever may due to infection or one of the sequels of tissue damage, inflammation, graft rejection, or other disease states. Antipyretic are agent which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature. Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins which set the thermoregulation center at a lower temperature [28]. In the present study, total ethanolic extract significantly inhibited Carrageenin-induced paw oedemaas compared to aqueous extract. The tail immersion and hot plate test reveal that this plant has high analgesic activity. This is because some form of error may be introduced with the animal handing while the test is being elicited. Both test show highest degree of analgesia in ethanolic extract compared to aqueous extract. ethanolic extract ofbark possesses a significant antipyretic effect in yeast provoked elevation of body temperature in rats as compared to aqueous extract but its effect less than that of paracetamol (standard drug). The result clearly indicate that the total ethanolic extract of *P. aculeata*. Lbark in context of analgesic, antipyretic and anti-inflammatory activity. The detailed study is required in order to identify the actual active constituent from this drug.

Table no. 01: Effect of Pentazocine, ethanolic extract, and Aqueous extract of bark of *P. Aculeate* on Eddy's Hot plate test in mice.

S. No.	Treatment	Reaction time in seconds				
		0 min.	30 min.	6o min.	90 min.	120 min.
1.	Control	2.80±0.3070	3.64±0.3330	3.64±0.3330	2.88±0.2482	2.88±0.2482
2.	Pentazocine (5 mg/kg.S.C)	2.80±0.3070	3.63±0.2105	5.48±0.2234**	6.64±0.3330**	7.48±0.4280**
3.	PAFML (200mg/kg, b.w)	2.80±0.1665	3.30±0.2105	4.56±0.2008**	5.73±0.3070**	6.64±0.2105**
4.	PAFAQ (200mg/kg, b.w)	2.98±0.2580	3.48±0.2234	3.64±0.2105	4.4±0.2234*	5.23±0.3330**

The results were analyzed by ANOVA followed by Dunnet's test (p-value $\leq$0.05 was taken as significant).

Table no: 02, Effect of Acetyl salicylic acid, ethanolic extract, and aqueous extract of bark of *P.aculeata* on Tail immersion test in rats.

S. No.	Treatment	Reaction time in seconds						
		0 min.	30 min.	1st hr.	2nd hr.	3rdhr	4thhr	6thhr
1.	Control	2.80±0.1465	2.85±0.1665	2.96±0.2582	2.96±0.2582	2.96±0.2582	3.15±0.3071	3.15±0.1665
2.	Acetyl salicylic acid (640 mg/kg.)	3.11±0.3070	3.30±0.2105	3.80±0.3070	5.64±0.3329**	6.80±0.3070**	7.65±0.2105**	7.98±0.2580**
3.	PAFML (200mg/kg, b.w)	3.11±0.1657	3.13±0.1665	3.81±0.1665	4.15±0.1665	6.00±0.3648**	6.48±0.2232**	6.65±0.2104**
4.	PAFAQ (200mg/kg, b.w)	3.15±0.3053	2.60±0.2100	3.30±0.2105	3.97±0.3648	4.48±0.2234*	4.98±0.3648**	4.97±0.3645**

The results were analyzed by ANOVA followed by Dunnet's test (p-value £0.05 was taken as significant).

Table no. 03: Effect of Paracetamol, Ethanolic and Aqueous extracts of bark of *P. aculeate* on yeast-induced pyrexia.

S. No.	Treatment	Initial temp (°C)	Temp. after 19 hr yeast admn.(°C)	Temp. at different hr after treatment (°C)			
				20 hr	21 hr	22 hr	23 hr
1.	Control	37.15±0.0943	39.14±0.0665	37.28±0.5571	39.43±0.6705	39.48±0.0652	39.54±0.0840
2.	Paracetamol (150 mg/b.w)	37.31±0.0892	39.36±0.598	38082±0.7186	38.16±0.1885	37.48±0.07947**	37.49±0.598**
3.	PAFML (200mg/b.w)	37.26±0.0598	39.44±0.1172	38.54±0.0985	38.20±0.0491	38±0.0598**	38.04±0.0136**
4.	PAFAQ 200mg/,b.w	37.53±0.0761	39.51±0.114	38.6±0.1123	38.44±0.798	38.46±0.0802	38.19±0.0790*

The results were analyzed by ANOVA followed by Dunnet's test (p-value £0.05 was taken as significant).

Table no. 04: Effect of Aspirin, ethanolic extract, and Aqueous extract of bark of *P. aculeate* on paw edema in Carrageenin paw edema model in rat.

S. No.	Treatment	(Paw size) Change in volume (ml) at h				
		0 Hr.	1 Hr.	2 Hr.	3 Hr.	4 Hr.
1.	Control	0.82±0.0158	1.00±0.0273	1.14±0.0515	1.17±0.0391	1.19±0.0337
2.	Aspirin 150 mg/ b.w	0.75±0.0313	0.72±0.0047	0.68±0.0077**	0.67±0.0058**	0.62±0.0083**
3.	PAFML (200mg/kg, b.w)	0.72±0.0058	0.75±0.0075	0.81±0.0110	0.80±0.0101**	0.76±0.0050**
4.	PAFAQ (200mg/kg,	0.77±0.0120	0.80±0.0138	0.81±0.0130	0.83±0.0120	0.80±0.0060**

	b.w)					

The results were analyzed by ANOVA followed by Dunnet's test (p-value £0.05 was taken as significant).

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