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EVALUATION OF ANTI-NOCICEPTIVE AND ANTI-PYRETIC ACTIVITY OF *AERVA PSEUDOTOMENTOSA* LEAVES AQUEOUS EXTRACT

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Keywords:

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ABSTRACT: *Aerva pseudotomentosa* Blatt. & Hallb. (Amaranthaceae) is arid region plant commonly known as Bui. It is used in ethno medicinal practices for the treatment of pain and inflammatory hyperalgesic disorders such as rheumatic pain, fever, inflammation, wounds and urinary disorders. This study was conducted to evaluate the anti-nociceptive, antipyretic effects of *A. pseudotomentosa* leaves aqueous extract. The aqueous extract of *A. pseudotomentosa* leaves (APAE) was prepared by maceration. Total phenolic and flavonoid contents were determined spectrophtometrically. Two dose levels (200 and 400 mg/kg) of the extract were administered by oral route to laboratory mice and rats. Peripheral nociception was induced in rodents using (acetic acid induced abdominal writhing and formalin), supra spinal (hot plate) and spinal (tail immersion) behavioral models of acute pain were used, while the fever was induced by using brewer's yeast. The total phenolic and flavonoid contents were estimated 359.3 mg tannic acid equivalents/g and 248.5 mg quercetin equivalents/g of extract, respectively in aqueous extract. The APAE at dose 400 mg/kg exhibited significant anti-nociceptive effect ($p < 0.001$) in all tests as well as pronounced antipyretic effect. In conclusion the leaves of *A. pseudotomentosa* exhibited significant peripheral, central anti-nociceptive effect and antipyretic potential, which substantiates its uses as folk remedies in treatment of inflammatory hyperalgesic disorders.

INTRODUCTION: *Aerva pseudotomentosa* Blatt. & Hallb. (*Aerva javanica* var. *bovei* Webb) is locally known as "Bui" in India. It is a perennial herbs of Amaranthaceae family that grows in Western arid zones of India and Nara desert of Pakistan. Different parts of the *Aerva pseudotomentosa* used as folk medicine for the treatment of various ailments. The *Thari people* of Nara desert of Pakistan traditionally use decoction of the whole plant as a remedy for toothache [1].

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Paste of inflorescence and seeds are applied topically to treat inflammation and face acne, additionally roots of the plant are used for the treatment of headache. *Aerva pseudotomentosa* is effective against lithiasis [2]. In Sudan whole plant is used in folklore medicine for its antipyretic property [3]. Recently some new folklore uses reported for this species. Paste of leaves and inflorescence applied topically for the treatment of wounds and painful inflammation of joints [4, 5].

Previous studies have demonstrated that ethanolic extract of whole plant is effective against human epidermoid carcinoma of nasopharynx and inflorescence has analgesic and anti-inflammatory activity [6, 7]. *Aerva pseudotomentosa* administered for gastric trouble and as anthelmintic in cattle.

In Somalia decoction of inflorescense given for the treatment of East coast fever, poultice of the inflorescence is applied externally to cure muscular injury of the animal [8, 9]. Various species of *Aerva* genus such as *Aerva lanata*, *Aerva sanguinolenta*, *Aerva javanica* var. *Javanica* have been reported for analgesic, anti-inflammatory, wound healing properties [10, 13]. Chemical studies carried out with these species demonstrated the presence of many phytoconstituents such as flavonoids (aervanone, isorhamnetin – 3 – O - β [4'''-p-coumaroyl -α rhamnosyl (16) galactoside]), ecdysteroids, ascorbic acid, β sitosterol, gallic acid, alkaloids, terpenoids and phenolic compounds [14, 15].

Despite the various *Aerva pseudotomentosa* folk medicinal uses in the management of pain, fever and inflammation. There is no systematic scientific study has been carried out to describe its anti-nociceptive and antipyretic potential. Hence present study evaluated the anti-nociceptive and anti-pyretic effect of *Aerva pseudotomentosa* leaves aqueous extract.

MATERIAL AND METHODS:

Chemicals and drugs: Tannic acid, quercetin were procured from the local market while, aspirin, pentazocin, indomethacin and paracetamol were obtained as gift sample from Ajanta pharmaceuticals Ltd., Mumbai, India.

Collection and authentication of plant material: The Plant was collected from Banad arid region of Jodhpur, Rajasthan, India in month of May, 2012, authenticated by Senior Scientist Dr. R.P. Pandey of Botanical Survey of India, Jodhpur. Specimen voucher no. BSI/AZC/I/2012/Tech/2012-13 (Pl. Id.) was deposited in the herbarium of the institute.

Preparation of extracts and phytochemical screening: The leaves of *A. pseudotomentosa* were shade dried and grinded to get a coarse powder and defatted with petroleum ether (60-80°C), then powdered drug was finally macerated with distilled water to obtain the aqueous extract. The extracts were filtered with Whatman filter paper no.1 and solvent was removed under reduced pressure at 50-60°C and stored in a desiccator with silica self indicating crystals for further use. The aqueous extract of dark brown solid consistency was obtained with percentage yield of 8% w/w. Preliminary phytochemical study of extract was carried out to determine the presence of flavonoids, alkaloids, terpenoids, saponins, fixed oils, steroids, glycosides in the extract [16].

Quantitative estimation of phytoconstituents:

Total phenolic and flavonoid content: The total phenolic and flavonoid content of the extract was determined spectrometrically. [17, 18]. A standard curve was obtained using various concentrations of tannic acid. Results were expressed as mg of tannic acid equivalents (TAE) per gram of extract. Total flavonoid content was measured by aluminum chloride colorimetric assay [19]. Total flavonoid content of the extracts was expressed as mg of quercetin equivalent per gram of extract.

Animals: Experiments were carried out using adult albino Wistar rats (180-200g) of either sex obtained from Animal house of the IPS College of pharmacy, registration no. 1039ac/07/CPCSEA Gwalior. The animals were kept in polypropylene cage at controlled room temperature 25° ± 2°C and 12 h light-dark cycle with standard pellet diet and water provided *ad libitum*. The animal experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and the protocols were approved by the Institutional Animal Ethics Committee (Approval Number IPS/COP/IAEC/01).

Grouping and treatments: The animals (n = 5) were divided into 4 groups. The APAE were dissolved in 0.9% saline for the purpose of administration.

Group I: Control groups received 0.9% normal saline (orally).

Group II: Standard groups received aspirin (100 mg/kg, orally) or (Indomethacin 10 mg/kg, orally) or (pentazocin 10 mg/kg, intra peritoneal) or (paracetamol 100 mg/kg, orally).

Group III & IV: APAE (200 and 400 mg/kg, orally) treated groups.

Pain was induced by 0.6% acetic acid (10 ml/kg i.p.) or 0.05 ml of 2.5 % formalin in dorsal surface of left hind paw or thermal nociception, while pyrexia was induced by Brewer's yeast in normal

saline 12.5% of 1 ml/100 gm body weight subcutaneously.

Acute toxicity studies: Acute toxicity study was carried out as per OECD (423) guidelines. The overnight fasted rats (*n* = 3) treated with 2000 mg/kg of extract and were observed for 24 hours for any lethality moribund stage [20].

Anti-nociceptive activity:

Acetic acid induced writhing method: The acetic acid (0.6% v/v, 10 ml/kg) was administered in intraperitoneal cavity of the mice, each animal was kept in a transparent cage and the nociceptive behavior was observed by counting the numbers of writhes between a period of 15 min, starting 5 min after the injection of acetic acid, mice were previously treated with oral administration of 0.9% normal saline, aspirin (100 mg/kg) or APAE (200 and 400 mg/kg). A half hour before administration of 0.6% acetic acid. The writhing response involve elongation of body, stretching of hind limbs and abdominal constriction [21].

Hot plate method: The hot plate test was used to measure analgesic activity with minor modification [22]. The temperature of the hot plate was set to a constant temperature of 55°C ± 1°C. The cut off reaction time for either paw licking or jumping was set 15 sec to avoid any physical injury or tissue damage. Each rat was separately placed on the hot plate in order to obtain the animal's response to thermal noxious stimuli. The time taken to a paw licking or jumping was recorded 0, 30, 60 and 90 min after oral administration of 0.9% normal saline, pentazocin (10 mg/kg, i.p.) and APAE (200 and 400 mg/kg, orally).

Tail immersion method: The lower portion of the animal tail was immersed in a beaker containing water, kept at constant temperature water bath at 55°C ± 1°C. Time taken by animal to withdraw the tail from water bath due to heat sensitivity induced pain was defined as reaction time. The reaction time was observed for 0, 30, 60 and 90 min after the administration of (0.9% normal saline, orally) pentazocin 10 mg/kg (i.p.). and APAE (200 and 400 mg/kg, orally). The cut off time was taken 10 sec to avoid any tissue injury [23].

Formalin test: A formalin solution (0.05 ml, 2.5 %) was injected in to the dorsal surface on the left hind paw of rats. Thirty minutes after injection of formalin solution, oral treatment with 0.9% normal saline, indomethacin and APAE (200 and 400 mg/kg, orally) were given to all groups. The number of licking and flinching of formalin injected paw during early phase (Phase1; 0-5 min) and late phase (Phase 2; 15-30 min) were recorded [24].

Antipyretic activity:

Brewer's yeast induced pyrexia: Antipyretic effect was evaluated as described [25]. Before inducing pyrexia animals were fasted for 18 h. The initial rectal temperature was recorded by inserting digital thermometer 3 c.m. into the rectum of the animal. Next fever is induced by Brewer's yeast in normal saline 12.5% of 1 ml/100 gm body weight subcutaneously. The rectal temperature was recorded after 18 h. Then those animals show 1°C rise in body temperature were administered with APAE (200 and 400 mg/kg) or paracetamol 100 mg/kg and 0.9% normal saline solution. The rectal temperature was recorded after 1, 2 and 3 h following the treatment.

Statistical analysis:

The data were analyzed with one-way ANOVA followed by Tukey's multiple comparisons post hoc test and two-way ANOVA Bonferroni post hoc tests using Prism pad statistics software ver. 4. A statistical difference of $P < 0.05$ was considered significant in all cases.

RESULTS:

Phytochemical screening: Preliminary phytochemical screening of APAE indicates the presence of flavonoids, alkaloids, terpenoids, saponins, fixed oils, steroids, glycosides.

Quantitative estimation of phytoconstitutents: Total flavonoid and total phenol content contents of APAE were estimated 248.5 mg quercetin equivalents/g of extract and 359.3 mg tannic acid equivalents/g of the extract respectively.

Acute toxicity study: Acute toxicity studies revealed no lethality or any toxic reactions or moribund state up to the end of the study period APAE was safe up to a dose level of 2000 mg/kg of body weight (limit test) and LD_{50} observed was more than 2500 mg/kg.

Analgesic activity:

Acetic acid induced writhing method: The APAE produce a dose-dependent effect on acetic acid induced writhing. Administration of APAE (200 and 400 mg/kg) significantly inhibited the number of writhing responses but the more pronounced effect was observed in APAE dose (400 mg/kg) with percentage inhibition (48.45%) but this effect was lower than aspirin (positive control) percentage inhibition (55.15%).The results are presented in **Table 1**.

TABLE 1: ANTI NOCICEPTIVE EFFECT OF APAE IN ACETIC ACID INDUCED WRITHING

Groups	Control	Aspirin 10 mg/kg	APAE 200 mg/kg	APAE 400 mg/kg
No. of Writhing	38.8 ± 1.82	17.4 ± 2.31***	32.8 ± 1.15 *	20 ± 1.09***
% Inhibtion	-	55.15	15.46	48.45

One way ANOVA Non repeated measure using Tukey Multi comparison test *p<0.05 **, p<0.01*** p<0.001

Hot plate and tail flick induced nociception in mice: APAE administered at doses (200 and 400 mg/kg) caused a significant increase in latency time as compared to control groups. In the hot plate method animals treated with APAE at dose 200 mg/kg exhibited significant anti-nociceptive effect only at 90 min ($p < 0.001$) while maximum latency time was observed in APAE dose of 400 mg/kg, which demonstrated significant analgesic effect ($p < 0.001$) at 60 min of administration which persists at 90 min. This effect is comparable to standard drug pentazocin. Similarly in tail flick method APAE at dose 400 mg/kg produced significant nociceptive effect at 60 min and 90 min while APAE dose of 200 mg/kg effective only at 90 min. Results are presented in **Table 2** and **3** respectively.

TABLE 2: ANTI NOCICEPTIVE EFFECT OF APAE IN HOT PLATE METHOD

Time (Min.)	Control group	Standard Pentazocin 10 mg/kg	APAE 200 mg/kg	APAE 400 mg/kg
0	4.01 ± 0.47	4.12 ± 0.19	3.71 ± 0.36	4.22 ± 0.39
30	3.99 ± 0.45	5.75 ± 0.34*	4.43 ± 0.34	4.87 ± 0.36
60	4.32 ± 0.41	8.12 ± 0.18***	5.77 ± 0. 46	7.25 ± 0.46***
90	4.11±0.36	11.26 ± 1.00***	7.34 ± 0.45***	9.31 ± 046***

Two way ANOVA Non repeated measure using Bon Ferroni posthoc test * p<0.05 ** p<0.01 ***p<0.001.

TABLE 3: ANTI NOCICEPTIVE EFFECT OF APAE IN TAIL IMMERSION METHOD

Time (Min.)	Control group	Standard Pentazocin 10 mg/kg	APAE 200 mg/kg	APAE 400 mg/kg
0	1.92 ± 0.28	4.0 ± 0.83	1.88 ± 0.38	2 ± 0.44
30	1.68 ± 0.20	5.9 ±0.95 ***	3.76 ±0.35	4.22± 0.31*
60	1.8 ± 0.33	7.5±1.30 ***	4.1±0.78 *	6.54±0.85 ***
90	1.78 ± 0.31	7.54±1.14***	6.42±0.34***	7.12±0.24***

Two way ANOVA Non repeated measure using Bon Ferroni posthoc test * p<0.05 ** p<0.01 *** p<0.001

Formalin test: APAE at doses of (200 and 400 mg/kg) exhibited a significant anti-nociceptive effect ($p < 0.001$) compared to the control group in both phases. APAE at dose 400 mg/kg exhibited significant analgesic activity in late phase (15-30 min.) which is comparable to reference drug indomethacin **Table 4**.

TABLE 4: ANALGESIC EFFECT OF APAE IN FORMALIN INDUCED PAIN MODEL

Groups	Licking time		% Inhibition	
	Early Phase 0-5 min	Late phase 15-30 min	Early Phase 0-5 min	Late phase 15-30 min
Control	75.22 ± 1.62	111 ± 3.11	-	-
Indomethacin100 mg/kg	61.2 ± 1.93***	20.4 ± 1.20***	18.61	81.62
APAE 200 mg/kg	45.4 ± 1.77 ***	79.2 ± 2.03***	39.62	28.64
APAE 400 mg/kg	32.6 ± 1.20***	26.4 ± 1***	56.64	76.21

Two way ANOVA Non repeated measure using Bon Ferroni post hoc test *p<0.05 ** p<0.01 *** p<0.001

Brewer's yeast induced pyrexia:

Antipyretic Activity: APAE in low dose 200 mg /kg, effective only at 2 and 3 hr ($p < 0.05$, $p < 0.001$ respectively) against pyrexia. While APAE dose of 400 mg/kg demonstrated significant antipyretic effect at 1, 2 and 3 hr ($p < 0.01$, $p < 0.01$, $p < 0.001$, respectively) maximum effect of extract was observed at 3 hr which is comparable to standard drug paracetamol 100 mg/kg, results are presented in **Table 5.**

TABLE 5: ANTIPYRETIC EFFECT OF APAE AGAINST BREWER'S YEAST INDUCED PYREXIA

Time (hrs)	Control	Standard Paracetamol 100 mg/kg	APAE 200 mg/kg	APAE 400 mg/kg
0	98.42 ± 0.18	98.14 ± 0.23	98.62 ± 0.58	98.71 ± 0.2
18 h	101.28 ± 0.31	101.50 ± 0.41	101.34 ± 0.42	101.38 ± 0.30
1	101.91 ± 0.41	99.84 ± 0.32**	100.56 ± 0.55	100.0 ± 0.43**
2	102.06 ± 0.22	97.29 ± 0.66***	100.24 ± 0.45*	99.88 ± 0.57**
3	101.46 ± 0.17	98.68 ± 0.49***	99.32 ± 0.29**	99.83 ± 0.37***

Two way ANOVA Non repeated measure using Bon Ferroni post hoc test * p<0.05 ** p<0.01*** p<0.001

DISCUSSION: The analgesic effect of APAE was assessed in different chemical and thermal pain models i.e. acetic acid induced writhing, hot plate test, formalin test, and tail immersion test. Nociception (Writhing) induced by acetic acid is one of the sensitive test to assess the analgesic effect of a drug. Acetic acid produces an acute peritoneal inflammation and induced nociceptive response may involve both direct stimulation of nociceptive afferent fibers due to pH reduction and synthesis of inflammatory mediators [26]. The results obtained from the study suggested that APAE at dose 400mg/kg exhibited pronounced anti-nociceptive effect (percentage inhibiton of 48.5%) could be by inhibiting cyclooxygenase pathway or lipooxygenase pathway. The APAE at dose (200 and 400mg/kg) demonstrated central anti-nociceptive effect when assessed by hot plate test.

In this test paw licking and jumping are two behavioral responses produced by thermal stimulus which is considered as supraspinal integrated components and increases in latency time in both responses confirms the significant ($p<0.001$) central analgesic effect of the drug. In tail immersion test increase in the reaction time against thermal stimuli pronounces the anti-nociceptive effect of the extract. The analgesic effect of APAE involves supraspinal as well as spinal components as demonstrated by use of the hotplate and tail immersion test [27, 29]. Formalin test was performed to assess the neurogenic and inflammatory pain [30]. Subcutaneous injection of formalin in the right hind paw of animals evoked a behavioral nociceptive response in biphasic pattern i.e. an initial acute period (phase 1) for about 3-5 min then after some time second phase characterized by the long duration of nociception up to 60 min begins from 15-20 min after formalin injection [31]. This appears to be depending on the combination of an inflammatory reaction, activation of N-methyl-asparate (NMDA receptors and the nitric oxide cascade in peripheral tissue and functional changes in the dorsal horn of the spinal cord [32, 34]. APAE at higher dose 400 mg/kg showed significant anti-nociceptive effect in both phases of formalin induced pain. Although it is difficult to understood the exact mechanism of peripheral and central analgesic effect of APAE. Brewer's yeast pyrexia is pathogenic fever which is induced by inflammatory mediators (IL-1, IL-2, TNFα, others) released by immune cells or peripheral mononuclear macrophage [35, 36]. These fever promoting cytokinins could interact with their receptors on brain endothelial cells and triggers release of PGE2 from COX- 2 pathwaay [37]. PGE2 is a key mediator of fever acting on thermo sensitive and thermo integrative hypothalamic integrative neurons [38].

The APAE produce antipyretic effects in higher dose 400 mg/kg comparable to the paracetamol treated group. Qualitative and quantitative phytochemical investigation (total phenolic & flavonoid content). Flavonoids, terpenoids, phenolic compounds are known to target prostaglandins which involve in pyrexia, pain perception and acute inflammation, so this could be logical to establish relationship that the anti-nociceptive and antipyretic potential of *Aerva pseudotomentosa* extract could be due to high phenolic content (359.3 mg tannic acid

equivalent/g of extract) which is shared by flavonoidal constituents (248.5 mg quercetin equivalents /g of extract) respectively. Previous chemical studies carried out with *Aerva javanica* a type variety of *Aerva pseudotomentosa* has revealed the prescense of kaempferol-3-O-β [4'''-E-p-coumaroyl-α-L-rhamnosyl (1-6) galactoside]) and its derivative, rutin, oleanolic acid, ecdysteroids, lupeol, gallic acid, caffeic acid, ferulic acid and β sitosterol [39, 42]. Probably these phytochemicals may attribute analgesic and antipyretic effect, suggesting a rational basis for traditional uses of this herb.

CONCLUSION: In conclusion, the leaves of *Aerva pseudotomentosa* possess significant anti-nociceptive and antipyretic potential which substantiates its use as folk remedies. This study validates central and peripheral analgesic potential of the plant in the treatment of pain along with useful traditional remedy for the treatment of fever. However, the isolation of compounds responsible for anti-nociceptive and antipyretic effect is subject to further investigation.

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