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EVALUATION OF ANALGESIC ACTIVITY OF AQUEOUS EXTRACT OF *ALOE VERA* [AEAV] IN ALBINO WISTAR RATS

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ABSTRACT: *Aloe vera* has been used since ages to manage a number of diseases causing pain, inflammation and fever. However, its effectiveness has not been scientifically certified. NSAIDs & Opioid are used continuously for the pain management. Alternative analgesics are needed to escape from the serious adverse effects of NSAIDs. Our study demonstrated significant analgesic activity of *Aloe vera* at doses of 200 mg/kg and 500 mg/kg. **Materials and Methods:** The study was carried out using Wistar Albino rats (150-200gm). Soxhlets apparatus was used to prepare the aqueous extract of *Aloe vera* [AEAV]. The animals were divided into 4 groups (n=6) receiving different treatments. Hot plate method, acetic acid induced writhing method and tail flick tests were used. The results were subjected to statistical analysis by using one way ANOVA followed by Dunnett's test. **Results** Analgesic effect of high dose of AEAV at 500 mg/kg was similar with the standard drug indomethacin. The extract (200 & 500 mg/kg) exhibited a dose-dependent inhibition of writhing and also showed a significant ($P < 0.001$) inhibition in the hot plate method. The results indicate that the analgesic effect of AEAV is mediated both by central and peripheral mechanism.

INTRODUCTION: Pain is the subjective, unpleasant sensations that go along with damage or near damage to tissues. Chemicals released nearby as a result of cell injury either produces pain by direct stimulation or by stimulation of nerve endings responsible for the mediation of pain¹. Pharmacologic management of pain requires the use of analgesic drugs. For relief of pain and inflammation, nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used treatment in three areas: inflammatory rheumatism, osteoarthritis, and common pains, such as headache, trauma or minor tendonitis².

NSAIDs reduce pain and edema by suppressing the formation of prostaglandins (PG), by inhibiting the activity of the enzyme Cyclooxygenase 1 and 2 (COX-1 and COX-2). However, prostaglandins serve as key moderators of several components of gastro intestinal mucosal defense, so suppression of synthesis of PGs by NSAIDs greatly reduces the resistance of the mucosa to injury leading to faulty repair process³. Pain involves complicated pathophysiology. Peripheral stimulation of nociceptors by low pH, Substance P, histamine, bradykinins and most importantly PGs and leukotrienes plays a key role. COX enzyme is strategic enzyme producing an array of all these inflammatory cytokines that produce pain. COX is also seen in spinal cord where it produces prostaglandins and facilitates pain transmission. Along with pain pathway there are opioid receptors and monoaminergic pain modulating circuits that play role in pain modulation^{4,5}.

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Vernacular names of *Aloe vera*:

Assamese	–	Musabhar
Bengali	-	Ghrita kumari
Hindi	-	Kumari
Sanskrit	-	Ghritra Kumari
Urdu	-	Ailwa
Punjabi	-	Musabbar
Chinese	-	Lu Hui
French	-	Aloes
Japanese	-	Rokai
Russian	-	Aloe, Aloi, Sabur

The plant is a coarse looking perennial with short, thick somewhat stem, 30-60 cm high. The plant's complete life cycle is twelve years. It produces an average of twelve to thirty leaves. The odour is characteristic while the taste is nauseous and bitter⁶. Though, *Aloe* is native to North Africa and Spain, the plant is now also grown in the hot dry regions of Asia, Europe and America. Aloes is indigenous to Eastern and Southern Africa and grown in Cape colony, Zanzibar and islands of Socotra, also cultivated in Europe and many parts of India including northwest Himalayan region^{7,8}.

Aloe is made up of a vast range of compounds which can be divided into three large groups.

The first group, complex sugars (among which acemannan stands out), are inside the leaves gel and have an immune stimulating action. Next are the anthraquinones, contained in the outermost part of the skin, with a strong laxative action. Last of all are several substances with a wide array of actions such as minerals, vitamins, essential, non-essential and semi-essential amino acids, organic acids, phospholipids, enzymes, lignin and saponins^{7,8}.

Aloe vera gel exerts anti-inflammatory and analgesic properties. It has been utilized for reducing pain during dental treatments, mouth ulcers, sores, blisters, hemorrhoids and for wound healing in almost every parts of the World since ages^{7,8}. Thus we have undertaken this study to evaluate the analgesic property of the aqueous extract of *Aloe Vera* [AEAV].

MATERIALS AND METHODS: The study was conducted in Department of Pharmacology, Silchar Medical College & Hospital (SMCH), Assam (India). The experimental protocol was approved

by Institutional Animal Ethical Committee of the institution.

Toxicological evaluation of *Aloe vera* in Rats:

The study on the toxicological profile of *Aloe vera* was done by Saritha V and Anilakumar KR, 2010. The toxicity profile of the *Aloe* was studied in Wistar rats. A multiple oral administration of the extract at single dose of 4, 8, 16 g/kg body weights for 14 days did not produce signs of toxicity, behavioral appearances, changes on gross appearance. The sub-acute toxicity was established by administration of graded doses (1, 2, 4, 8 and 16g/kg orally) of the extract daily for 6 weeks and the effects on body weight, organ weight, histology as well as serum biochemical parameters were estimated. Body weight of dosed and control rats increased throughout the duration of treatment. They demonstrated significantly no difference in serum concentrations of aspartate amino transferase, alanine amino transferase, alkaline phosphatase, total protein, albumin, urea, creatinine, total and direct bilirubin. Histological findings on liver, kidney, small intestine, heart and brain revealed normal architecture and no obvious pathology was noted⁹.

Preparation of AEA V:

Collection and preservation of specimen:¹⁰ The herbal plant of *Aloe vera* was obtained from the campus of Silchar Medical College and Hospital, Assam. The plant sample was verified by Dr. Ashis Nath, Associate Professor, Department of Botany, Gurucharan College, Silchar. The collected leaves were washed in cold water. The lower 1 inch of the leaf base and the tapering 2-4 inch of the leaf top and the spines around the leaves were removed using a knife. The fresh leaves of *Aloe vera* were air dried at room temperature. The dried leaves were then powdered by electrical grinder and kept in a tight container. The dried powder obtained was 174 grams.

Extraction of plant leaves:¹⁰ The aqueous solvent was used in this study. The 174 grams of the dried powder was mixed with 1.7 liters of distilled water and was loaded in the thimble of Soxhlet apparatus (PIC I) at 70°C for 72 hr. The extracts obtained were then filtered using Whatmann No.1 paper.

The residue thus obtained was allowed to evaporate in glass petri dishes in room environment. When completely dry, the extract was scraped out, weighed and stored for future use. After

completion, the extracts were weighted and the percentage yield was calculated (initial weight of raw material / final weight of extract).



FIG. 1: PROCEDURE FOR EXTRACTION OF ALOE VERA



FIG. 2: AEA V

Percentage Yield Calculation:

Weight of the dried powdered leaves = 174 grams,
Weight of the extract (semi solid mass) obtained = 20 grams

$$\text{Percentage yield: } \frac{20}{174} \times 100 = 11.5\%$$

Period of study: September 2014 to August 2015.

Experimental animals used in the study: The Albino (Wistar) rats used for the study were obtained from Chakraborty Enterprise, Kolkata. They were housed in polypropylene cages and kept under controlled room temperature ($24 \pm 2^{\circ}\text{C}$) having relative humidity of 60 -70% in a 12 hr light-dark cycle. The rats were given free access to standard laboratory diet and water. Animals were deprived of food but not water for four hours before the experiment.

Criteria for experimental animals:

The experimental methods were carried out on:

Healthy Wistar Albino rats (*Rattus norvegicus*).

- i. Sex: of either sex.
- ii. Body weight: 150–200 grams.
- iii. Place used to keep the animals: Central Animal House, SMCH, Assam.

- iv. Diet: Standard animal diet with Bengal gram, wheat, maize, bread and sufficient amount of water.
- v. All the animals were taken care of under ethical consideration.

a) Inclusion Criteria:

- Animals weighing between 150-200 grams.
- Healthy animals with normal behavior and activity.
- Previously unused rats.

b) Exclusion criteria:

- Rats weighing less than 150 grams and more than 200 grams.
- Pregnant females and those that have delivered once.
- Previously used in other experiments.

Drugs used in the study:

- Indomethacin: Obtained from Jagsonpal Pharmaceuticals Ltd. Rudrapur, Uttarakhand.
- Acetic acid
- Normal saline
- Distilled water

Equipments and materials used in the study:

- Digital electronic weighing scale (GF400)
- Filter paper (Whatmann no. 1)
- Oral feeding gavage (Vishnu traders, Uttarakhand)
- Refrigerator (Electrolux)
- Single use syringe (DISPOVAN, Hindustan syringes and Medical devices, Ltd)
- Soxhlet apparatus
- Eddy's Hot Plate
- Sterile surgical gloves (MEDI-FIT, Shree Bhagwati Surgicals)
- Volumetric flask (BOROSIL)
- Glass petri dishes
- Mixer grinder

Experimental designs for screening of analgesic activity in experimental animals:

1. Hot Plate method: The wistar rats of either sex weighing 150-200 g were fasted overnight with *ad libitum* access to water. The animals were divided into four groups (n=6 in each group) viz.

- Group I: Normal saline 10 ml/kg per orally (p.o.) (control)
- Group II: Indomethacin 10 mg/kg p.o. (standard)
- Group III: AEA 200 mg/kg p.o. (test)
- Group IV: AEA 500 mg/kg p.o. (test)

Same grouping was followed for the below mentioned experimental tests. The hot plate was maintained at $55.0 \pm 10C$. Food was withdrawn 12 hours prior to drug administration till the finishing point of experiment. The animals were weighed and numbered appropriately. The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time taken to cause a discomfort reaction (licking paws or jumping) was recorded as response latency or reaction time. Before administration of the test compound or the standard, the normal reaction time was determined. The animals are submitted to the same testing procedure after 30, 60, 90, 120 and eventually 150 minutes after administration of the drug and test compound. For each individual animal the reaction time was recorded. Indomethacin 10mg/Kg was given as reference standard. A cut-off time of 30

seconds was followed to avoid any thermal injury to the paws¹¹.



FIG. 3: PROCEDURE FOR MEASURING THE WEIGHT OF EXPERIMENTAL ANIMALS



FIG. 4: PROCEDURE FOR ORAL FEEDING OF EXPERIMENTAL

2. Tail flick method:¹² Analgesic activity was assessed by Tail immersion method. A sensitivity test was performed by placing the tail of each rat on the radiant heat source. Animals which did not react by withdrawal of the tail in 30 s were considered insensitive and excluded from the study. The animals are allowed to adapt to the cages for 30 minutes before testing. The distal part of the tail

of each animal was marked (5 cm). This marked part of the tail was immersed in a beaker of freshly filled water of exactly 55°C. The extreme 3 cm of the rat's tail is immersed in a water bath containing water at a temperature of 55±0.5°C. Within a few minutes, we have to note the rat reaction of withdrawing the tail. The reaction time was recorded with a stop watch. Each animal served as its own control and two readings were obtained for the control at 0 to 10 min interval.

The average of the two values was the initial reaction time (Tb). The groups were given AEA V (200 and 500 mg/kg, p.o) indomethacin (10 mg/kg p.o.) and normal saline (p.o). The reaction time (Ta) for the test groups was taken at intervals of 0, 30, 60, 90, 120, 150 and after 180 minutes respectively after a latency period of 30 min following the administration of the extract and drugs. The cut-off time, i.e. time of no response was put at 120s. The reaction time was measured and calculated.

The following calculation was:

$$\text{Percentage analgesic activity} = \frac{T_a - T_b \times 100\%}{T_b}$$

3. Acetic acid induced writhing response in the rats: ¹³ The animals were divided into 4 groups, of 6 rats each. The groups were given AEA V (200 and 500 mg/kg, p.o) indomethacin (10 mg/kg p.o.) and normal saline (p.o.). Thirty minutes later, each rat was given i.p. injection of 0.6% Acetic Acid 1ml/kg. The writhing response per animal was recorded five minutes after Acetic Acid injection for duration of ten minutes. A writhe was indicated by abdominal contraction and stretching of the hind limbs.

TABLE 1: EFFECT OF AEA V ON HOT PLATE METHOD

Groups	Dose	Latency period (in minutes)						
		0	30	60	90	120	150	180
Normal saline	10 mg/kg	4.38 ± .07	3.7 ± .12	3.28 ± .07	2.82 ± .12	2.32 ± .09	1.85 ± .10	1.55 ± .08
Indomethacin	10mg/kg	4.87 ± .16	5.63 ± .17	6.13 ± .16	6.38 ± .20	6.72± .23	6.83 ± .21	6.95 ± .17
AEA V	200 mg/kg	5.38 ± .10	5.6 ± .07	5.92 ± .09	6.05 ± .07	6.1 ± .09	5.75 ± .04	5.43± .08
AEA V	500 mg/kg	4.32 ± .08	4.63 ± .14	4.82 ± .16	5.08 ± .8	5.32 ± .19	5.02 ± .18	4.65 ± .07

2. Acetic acid induced writhing in experimental animals: AEA V at 200 mg/kg and 500 mg/kg reduced significantly the number of abdominal constrictions induced by acetic acid in comparison with the control (**Table 2**). The standard drug Indomethacin at 10mg/kg produced 70.42%

The analgesic activity was expressed as percentage inhibition of abdominal contraction between control group and extract treated groups. A significant reduction of writhes in tested animals compared to those in the control group was considered as an antinociceptive response (reducing sensitivity to painful stimuli) and was calculated using the formula:

$$C-D/C \times 100$$

Where C is the average number of writhings for the control group of rats and D is the average writhings of the extract treated rats.

Statistical analysis: The values expressed as Mean ± SEM from 6 animals. The results were subjected to statistical analysis by using one way ANOVA followed by Dunnett's test to verify the significant difference if any among the groups. $p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$ were considered significant.

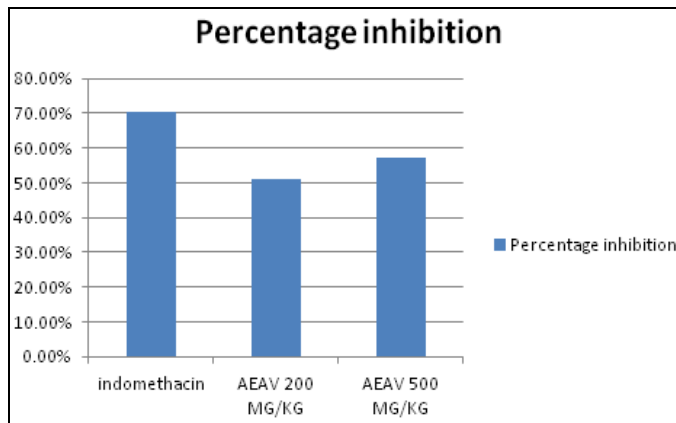
RESULTS:

1. Hot plate method: AEA V at doses of 200mg/kg & 500 mg/kg showed significant increase in the mean basal reaction time when compared with the control (**Table 1**). 500 mg/kg of AEA V showed significant increase in the basal reaction time ($p < 0.001$). At 120 minutes, 500 mg/kg of AEA V illustrated significant increase in basal reaction time which was almost matching with the standard drug Indomethacin. At the 180th minute, the effect of both 200 mg/kg and 500 mg/kg of AEA V showed a decline in comparison with the standard drug. Thus more studies will be required in future so that the duration of analgesic activity of *Aloe Vera* is to be established.

inhibition, AEA V at doses of 200 mg/kg and 500 mg/kg produced 50.94% & 57% inhibition respectively (**Fig. 5**).

TABLE 2: EFFECT OF AEA V ON ACETIC ACID INDUCED WRITHING IN EXPERIMENTAL ANIMALS

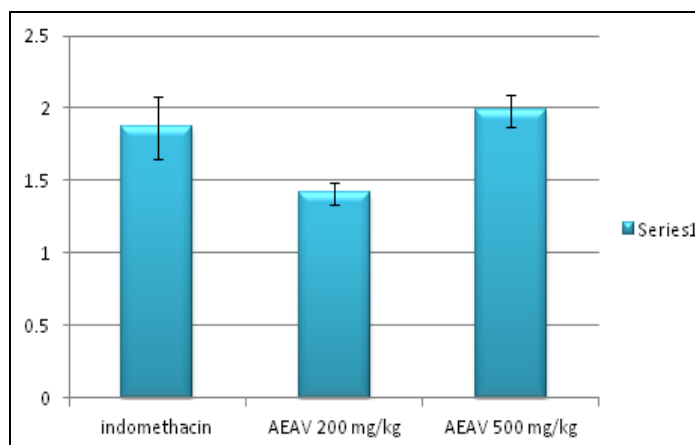
Groups	Dose	No of writhes	Percentage inhibition
Normal saline	10 mg/kg	6.93 ± 0.13	
Indomethacin	10mg/kg	2.05 ± 0.14	70.42%
AEAV	200 mg/kg	3.4 ± 0.14	50.94%
AEAV	500 mg/kg	2.98 ± 0.3	57%

**FIG 5: PERCENTAGE INHIBITION OF WRITHING BY INDOMETHACIN 10 mg/kg, AEA V 200 mg/kg & AEA V 500 mg/kg**

3. Tail flick method: The doses of both AEA V (200 mg/kg & 500 mg/kg) showed significant results ($p < 0.001$) after comparing the increase in latency of tail flick response with the control (Table 3). Highly significant results were observed during recordings made at the 60th minute. The difference in recordings made between the initial and the 180th minute (showing the increase in latency time) for AEA V at 500 mg/kg & the standard drug Indomethacin was almost parallel (Fig 6). It showed the efficacy of Aloe Vera at high doses was comparable to the standard drug Indomethacin.

TABLE 3: EFFECT OF AEA V ON TAIL FLICK METHOD

Groups	Dose	Reaction time in seconds at time minutes(ms) (Mean ± SEM)						
		0 ms	30 ms	60 ms	90 ms	120 ms	150 ms	180 ms
Normal saline	10 mg/kg	4.43 ± .11	4.18 ± .11	3.85 ± .12	3.28 ± .04	2.65 ± .08	2.32 ± .16	2.13 ± .23
Indomethacin	10mg/kg	4.67 ± .15	5.8 ± .14	6.32 ± .19	6.48 ± .21	6.67 ± .21	6.95 ± .23	7.13 ± .25
AEAV	200mg/kg	4.33 ± .16	5.08 ± .24	5.27 ± .20	5.42 ± .17	5.52 ± .15	5.65 ± .19	5.75 ± .28
AEAV	500 mg/kg	4.82 ± .16	5.82 ± .11	6.13 ± .21	6.40 ± .18	6.5 ± .25	6.72 ± .02	6.92 ± .26

**FIG 6: GRAPHICAL REPRESENTATION SHOWING THE DIFFERENCE IN RECORDINGS MADE BETWEEN THE INITIAL RECORDINGS AND THE RECORDINGS AT THE 180TH MINUTE**

DISCUSSION: Pain is centrally amended by a number of complex processes including opiate, dopaminergic descending noradrenergic and serotonergic systems³. The analgesic effect produced by these experiments possibly will be through central mechanisms involving the receptor systems or peripheral inhibition of PGs,

Leukotrienes, and other endogenous substances that are key players in pain. The results of this study displays that the AEA V possesses analgesic activity both in chemical and thermal pain models. Pain is distinguished as two types, peripheral or neurogenic pain.

It may involve the following pathological states: peripheral nociceptive afferent neurons which are activated by deadly stimuli and central mechanism which is activated by afferent inputs pain sensation. The hot plate was preferred to examine the central anti-nociceptive activity since it had several advantages particularly the sensitivity to strong antinociceptive and partial tissue damage¹⁴.

The study by Ghosh AK (2011) *et al.* showed *Aloe vera* to be a potent analgesic. They demonstrated that in pain induction by application of radiant heat on rat tail, the aqueous extract of *Aloe vera* gel at the dose of 200 and 300 mg/kg produced highly significant results in latency of tail flick at all time points. 300mg/kg of *Aloe Vera* showed significant increase in basal reaction time in hot plate method.

It was thus found that higher doses of *Aloe vera* are effective as an analgesic in the models functional for study of somatic pain¹⁵. This was similar to our study which clearly stated that *Aloe vera* (200 mg/kg & 500 mg/kg) produced significant results as analgesic in the hot plate method. The abdominal constriction reaction provoked by acetic acid is a sensitive method of evaluating peripherally acting analgesics. Acetic acid originates pain by liberating endogenous substances such as Serotonin, Histamine, PGs, Bradykinins and Substance P. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response. The method has also been associated with Prostanoids in general that is, increased levels of PGE2 and PGF2 α in peritoneal fluids as well as lipoxigenase products³. Egesic *et al.* (2011) confirmed that intraperitoneal administration of Acetic acid produced an abdominal writhing response by activating the chemo sensitive nociceptors in animals.

Aqueous extract of *Aloe barbadensis* at 25, 50 and 100 mg/kg significantly decreased the number of writhes induced by a 0.06% Acetic acid solution with an approximately 66.49%, 57.59% and 68.06% inhibition respectively, while the standard drug, piroxicam, revealed a protective effect of 63.35%. It is feasible that the analgesic effect of the extract could be as an outcome of its peripheral activity¹³. Safari VZ et al. (2016) stated that aqueous leaf extracts of *Aloe volkensii* reduced formalin induced pain in mice but not in a dose

dependent manner. At the dose level of 50 mg/kg body weight, the aqueous leaf extracts showed signs of significant analgesic effect in comparison with control and baseline groups ($p < 0.05$). Diclofenac as a reference drug produced marked reduction in pain¹⁶.

Our study also witnessed the same readings. Writhing test by Acetic acid done at our department illustrated Indomethacin at 10mg/kg produced 70.42% inhibition, AEAV at doses of 200 mg/kg and 500 mg/kg formed 50.94% & 57% inhibition respectively.

The study by Goutam P *et al.* (2015) on *Aloe vera* succulent (AVS) have shown analgesic property in tail flick latency, radiant heat method and writhing model (acetic acid induced writhing method). AVS (200 and 300 mg/kg) have shown significant analgesic activity. The analgesic effect of AVS with dose 300 mg/kg was equivalent to that of 10 mg/kg dose of Diclofenac sodium (standard drug). AVS showed significant reduction in writhes. Analgesic activity of AVS at 300mg/kg in writhing method was comparable with that of Diclofenac sodium (standard drug) at 10 mg/kg of dose¹⁷.

Our study further supports this result. In our study the tail flick method demonstrated that both the doses of AEAV at 200 mg/kg & 500 mg/kg produced significant analgesia.

CONCLUSION: AEAV was prepared by the Soxhlets apparatus. It produced significant results for analgesic efficacy at doses of 200 mg/kg & 500 mg/kg when compared with the standard. More studies are needed in the future to establish its duration of action and safety profile.

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CONFLICT OF INTEREST: All authors have no conflict of interest.

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