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EVALUATION OF ANALGESIC ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF ABUTILON INDICUM IN ALBINO MICE

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ABSTRACT

Pain as a sensory modality; represent a symptom for diagnosing various diseases and its associated conditions. Conventional synthetic drugs that are used to manage pain are not readily available and are associated with adverse effects. Thus, the use of herbal medicine from medicinal plants is an age old practice used by many communities to cure diseases. These medicinal plants are known to contain phytochemical compounds capable of relieving pain and healing diseases. The present study aims to investigate the analgesic activity of the Ethanolic and Aqueous extracts of *Abutilon indicum* in albino mice using, Acetic acid-induced abdominal writhing and hot plate analgesic models in albino Wistar mice were used for the study. The extracts were administered intraperitoneally at a dose of 50 mg/kg and 100mg/kg while Diclofenac sodium 10mg/kg and pentazocine 5mg/ served as standard in Acetate induced abdominal writhing and hot plate, method respectively.. The results are analysed by one way ANOVA followed by Dunnet's test The Ethanolic extract of 100mg /kg having significant analgesic activity in Acetic acid induced abdominal writhing response and percentage of inhibition (PI) ($p<0.01$)when compared to control.. The Ethanolic extract of 50mg/kg and 100mg/kg exhibited significant analgesic activity in the hot plate method ($p<0.001$) by increasing the pain reaction time(PAT) of the rats to sec 7.25±0.38 at 45 min after treatment in comparison to control (4.08±13)

KEYWORDS: *Abutilon indicum*, Diclofenac sodium, Acetic acid induced, Hot plate, PAT, PI.

INTRODUCTION

Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It is always a warning signal and primarily protective in nature but often causes a lot of discomfort and lead to many adverse effects.[1] Pain is a disabling accompaniment of many medical conditions and pain control is one of the most important therapeutic priorities[2] Analgesics are drugs used to treat or reduce pain and the classical analgesic drugs notably opiates and non-steroidal anti-inflammatory drugs have their origin in natural products but many synthetic compounds that act by the same mechanism have been developed and are associated with serious adverse effects such as ulceration, gastrointestinal bleeding, additive potential, respiratory distress, drowsiness, nausea etc.[3,4] Peripheral nerves transmit pain stimulus to the spinal cord which then links to the brain. Two types of nerve fibers are involved in this process; slow pain fibers and Fast pain fibers. Transmission of fast pain is through the Aδ fibers to the spinal cord while slow pain fibers is through the C-fibers. Fast pain nerve endings secrete neurotransmitter called glutamate, which transmits fast pain impulses to the brain in the cortex. Therefore localization of pain in certain part of the body becomes relatively precise.[5] Although pain is beneficial to the immune system however it causes a lot of suffering and discomfort to the victims, lowering the quality of life and therefore need to be managed. To suppress pain, NSAIDs are prescribed all over the world.[6,7] For severe or chronic malignant pain opioids analgesics are drugs of choice[8] However, prolonged use of these NSAIDs only provides asymptomatic relief and the greatest drawback lies in their toxicity to liver, kidney and gastrointestinal linings.[9] In this regard, herbal medicines from medicinal plants have been employed in complementary and alternative medicine (CAM) for treatment of pain as well as diseases related to these conditions.[10] Traditional medicinal herbs for over centuries have served as potential source for alternative medicine and the knowledge of herbal medicine has been passed on from generation to generation.[11] Considering that most of analgesic, anti-malarial and anti-pyretic synthetic drugs such as aspirin, morphine, artemisinin, atrophine and

chloroquine were derived from the plant products.[12] Many of these medicinal plants with analgesic activity had been used without any adverse effects. One of such medicinal plants is *Abutilon indicum*. The present study to investigate the Analgesic activity of Ethanolic and Aqueous extracts of stem of Abutilon *indicum.*

MATERIALS AND METHODS

Plant material

Abutilon indicum stem were collected from palakkad, Kerala, India and authenticated by Dr. P. Jayraman, Director of plant Anatomy Research Centre Chennai the *Abutilon indicum* was identified and deposited at the plant research centre, Chennai with the voucher number PRC/AI/2017. The fresh stem were separated and kept for shade drying. Dried stem material was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Extraction of Plant material

For preliminary phytochemical analysis, extract was prepared by weighing 600grams of the dried powdered stem were subjected to hot successive continuous extraction with different solvents as per the polarity, petroleum ether, benzene, chloroform, ethanol and finally with aqueous. The extracts were filtered in each step using Whatman filters paper. The filtrate was concentrated using a rotary evaporator at low temperature (40-45 ^{o}C) and pressure. The presence or absence of the primary and secondary phytoconstituents was detected by usual prescribed method.[13]

Chemicals and Drugs

Diclofenac sodium and Pentazocine, Tween 80 were purchased from Sigma Co. (Sigma St. Louis, MO). Absolute ethanol was of analytical grade and was purchased from Merck (German).The other reagents were of analytical grade.

Animals

Swiss albino mice 90-170gms maintained in the Animal house Facility of the Department of Pharmacology, Sanjo College of pharmaceutical studies, were used in these experiments. The animals were maintained on standard small animal feeds (Excel feed, Ilorin) and water *ad libitum*. This research was carried out in accordance with the rules governing the use of laboratory animals as accepted internationally. The experiment was conducted between the hours of 900 h and 1600 h. The experimental groups consisted of six animals. They were maintained at constant room temperature (22° ± 1 °C) and submitted to12 h light/dark cycle with free access to food and water.

Experimental procedure

Acute oral toxicity study

Acute oral toxicity was conducted as per OECD guidelines (Organisation of Economic Cooperation and Development) 423 (Acute toxic class method). The acute toxic class method is a step wise procedure of three animal of a single sex per step. Depending on the mortality and / or moribund status of animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number of animals while allowing for acceptable data based scientific conclusion. The method uses defined doses, (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for the classification of chemicals which causes acute toxicity. The method previously described by Lorke was adopted.[14]

Evaluation of analgesic effect[15-18]

Pain is not easily or satisfactorily defined and therefore is often interpreted as a suffering that results from the perception of painful stimuli. It's a common symptom and it indicates that something is wrong in the body and may give a clue to the nature of disease. Hence, "pain is a specific sensation with its own peripheral and central mechanisms independent of other five senses." Pain itself is not a disease; it is by far the most common medical complaint. It is usually perceived as an indication of ill health and most diseases have a component of pain. The control of pain is one of the most important uses to which drugs are put. Pain can be defined as the effect produced in consciousness by the arrival of nerve impulses generated by noxious stimuli in the brain. Drugs, which alter the pain sensitivity or remove pain, are called as painkiller or analgesics.

Acetic acid induced writhing response Method[19-20]

Acetic acid induced writhing method was adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (twist). Any writhing is considered as a positive response. Analgesic activity of Ethanolic and Aqueous extract extracts of *Abutilon indicum* was determined through acetic acid-induced pain in experimental animals.A total of thirty six Swiss albino mice were grouped into 6 groups of five animals each. Prior to pain induction and administration of the experimental doses, all the experimental animals were fasted for 12 hours but were allowed access to water *ad libitium*. Pain was induced by injecting 0.6% acetic acid solution at a dose of 10 mL/kg body weight into the left side of the abdomen intraperitoneally. Immediately, after injection with acetic acid abdominal muscle constriction in the abdomen and turning of body trunk of the laboratory animal was seen as an indication of pain. The different groups were treated as follows;

- Group I (normal control) was administered with Co solvent (Propylene glycol: Tween 80: Water-12:3:12).
- Group II (positive control) were induced with pain and administered with the standard drug. (Diclofenac sodium 10mg/kg body eight).

- Group III were induced with pain and administered with the 5omg/kg of AEAI.
- Group IV were induced with pain and administered with 100mg/kg of AEAI.
- Group V were induced with pain and administered with 50mg/kg of EEAI.
- Group VI were induced with pain and administered with 100mg/kg of EEAI.

Each mouse was then placed in a transparent observation box and the number of abdominal constrictions (writhes) for each mouse was counted for 15 minutes commencing 5 minutes after intraperitoneal injection of acetic acid. The percentage writhing inhibition was then calculated and tabulated. (Table 1)

$$\text{Percentage of writhing response} = \frac{C-T}{C} \times 100$$

Where, C- The vehicle-treated control group; T - Treated group value

Hot Plate Test[22]

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 hot plate, which is commercially available, consists of a electrically heated surface. The temperature is controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch. Evaluation of analgesic activity of the Ethanolic and aqueous extract of *Abutilon indicum* was also carried out using hot plate method. A total of thirty six Swiss albino mice were grouped into 6 groups of five animals each.

- Group I (normal control) was administered with Co solvent (Propylene glycol: Tween 80: Water-12:3:12)
- Group II (positive control) were induced with pain and administered with the standard drug (Pentazocine 5mg/kg body eight)
- Group III were induced with pain and administered with the 5omg/kg of AEAI.
- Group IV were induced with pain and administered with 100mg/kg of AEAI
- Group V were induced with pain and administered with 50mg/kg of EEAI
- Group VI were induced with pain and administered with 100mg/kg of EEAI

The rats were placed on a hot plate maintained at 55°C within the restrainer. The reaction time (in seconds) or latency period was determined as the time taken for the rats to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before (0 min) and at 15, 30, 45, 60 and 75 min after the administration of the treatments. The maximum reaction time was fixed at 60 sec to prevent any injury to the tissues of the paws. If the reading exceeds 15 sec, it would be considered as maximum analgesia Mean reaction time in seconds are recorded in (Table: 2).

Statistical analysis

The results are analysed by one way ANOVA followed by Dunnet's test and p value <0.01 was considered significant.

RESULTS

Acute toxicity

The results showed no clinical signs and mortality of the animal therefore an LD_{50} > 2000 mg/kg body weight may be assume

Acetic acid-induced writhing response

The effect of Ethanolic and Aqueous extracts of *Abutilon indicum* on the acetic acid- induced abdominal constrictions in mice is presented in the Table 1. The result shows that Ethanolic and Aqueous extracts (100 mg/kg), and the reference drug Diclofenac sodium (10 mg/kg) more significantly ($P < 0.01$) reduced abdominal writhing in mice when compared to the control group reducing the mean number of writhing from 12.5±2.7 in the control group to 5±0.37** at a dose of 100mg/kg of Ethanolic extract of *Abutilon indicum*. The reduction was in a dose dependent manner. Also the extract caused a dose dependent increase in inhibition of abdominal writhing, increasing it from 0% in control group to 60% at the dose 100 mg/kg of Ethanolic extract. Both the extracts at a dose of 50mg/kg are also significant (p<0.05) when compared to control group.100mg/kg of Ethanolic extracts was found more potent than all other extracts. The reference drug diclofenac sodium was found more potent than both the plant extracts at all of the dose level.

Table 1: Acetic acid induced writhing response of *Abutilon indicum* extracts.

Group no	Drug treatment	Dose mg/kg	Mean writhing response	Percentage inhibition
1	Control	27ml/kg	12.5±2.7	0
2	Diclofenac sodium	10	4±0.58**	68
3	AEAI	50	6.5±0.51*	48
4	AEAI	100	5.5±0.77**	56
5	EEAI	50	5.8±0.6*	53.60
6	EEAI	100	5±0.37**	60

One way ANOVA followed by dunnet's test. Values are mean ± SEM. n=6, in each group *p<0.05, **p<0.01

when compared to control. AEAI –Aqueous extract of *Abutilon indicum*, *EEAI*-Ethanolic extracts of *Abutilon indicum.*

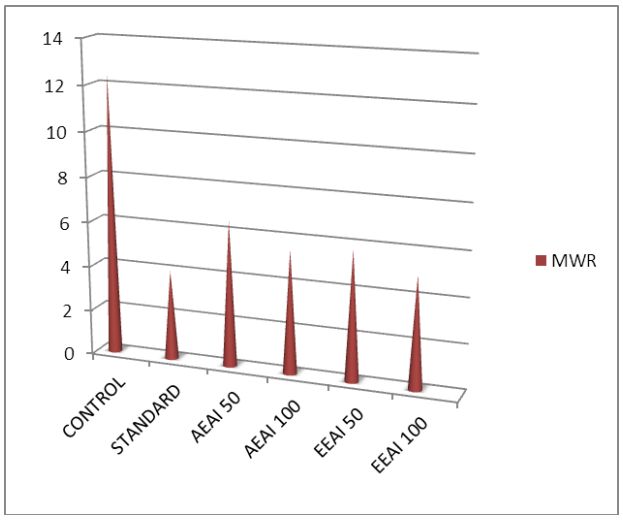


Figure 1: Mean Writhing response in Acetic Acid Induced Writhing in Mice, Standard – Diclofenac (10mg/kg), AEAI-Aqueous extract of *Abutilon indicum,* EEAI- Ethanolic extract of *Abutilon indicum.*

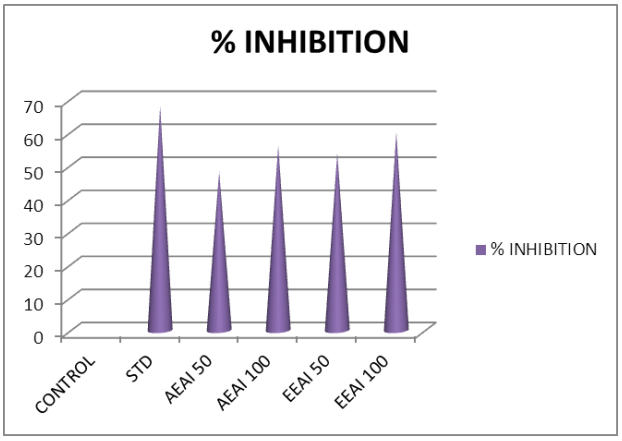


Figure 2: % of Inhibition in Acetic Acid Induced Writhing in Mice Standard – Diclofenac (10mg/kg), AEAI-Aqueous extract of *Abutilon indicum,* EEAI-Ethanolic extract of *Abutilon indicum.*

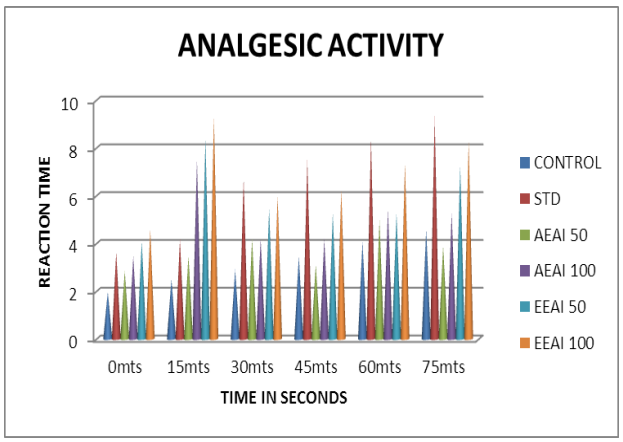
Hot Plate Method

The result of the effect of Ethanolic and Aqueous extracts of *Abutilon indicum* on the hot plate method is presented in Table 2. The result shows that Ethanolic extract 100mg/kg having more significant in mean reaction time when compared to control ($p<0.001$) .50mg/kg,100mg/kg of Aqueous extract of *Abutilon indicum* and 50 mg/kg of Ethanolic extracts shows significant in mean reaction time when compared to control .*($p<0.01$).*

Table 2: Analgesic activity of *Abutilon indicum* in hot plate method.

Group No,	Treatment	Dose (mg/kg)	Mean reaction time in seconds					
			0 Min	15 min	30 min	45 min	60 min	75 min
1	Control	Co-solvent	1.95±0.16	2.50±0.15	2.9±0.13	3.46±0.11	4.08±13***	4.60±0.1%
2	Pentazocine	5mg/kg	3.63±0.30	4.18±0.31	6.70±0.29**	7.5±0.29***	8.4±0.19***	9.3±0.12***
3	AEAI	50mg/kg	2.85±0.30	3.48±0.6	4.2±0.13**	4.18±0.18**	5.45±0.16**	5.98±0.22**
4	AEAI	100mg/kg	2.25±0.15	2.83±0.22	3.11±0.12**	4.18±0.06*	5.25±0.07**	6.25±0.11**
5	EEAI	50mg/kg	3.48±2.68	3.42±0.33	4.95±0.17**	5.43±0.11**	5.27±0.48**	7.36±0.11***
6	EEAI	100mg/kg	2.68±0.21	3.35±0.29	3.9±0.29*	5.28±0.19**	7.25±0.38***	8.28±0.3***

One way ANOVA followed by dunnet's test. Values are mean ± SEM. n=6, in each group *p<0.05, **p<0.01,***p <0.001 when compared to control.



DISCUSSION

The present study was designed to evaluate the analgesic potential of Ethanolic and Aqueous extracts of stem of *Abutilon indicum* in Swiss albino mice. To evaluate the analgesic activity of Ethanolic and Aqueous extracts of stem of *Abutilon indicum* the, acetic acid-induced pain test was used to induce abdominal writhing's in Swiss albino mice. Acetic acid-induced pain test has widely been used for screening new analgesic agents and it majorly involves cholinergic, histaminic peritoneal receptors, acetylcholine and histamine mediators. It is also used to asses peripherally acting analgesics.[23-24] According to[25] several chemicals can be used to induce writhing's in a laboratory animal for example acetic acid and phenylquinone When acetic acid is intraperitoneally injected into the experimental animal the following

characteristics are observed as indicators of pain; contraction of abdominal muscle, elongation of body part and extension of the hind limbs Therefore, such presentation is thought to be mediated by peritoneal receptors.[26] It has been proposed that acetic acid acts indirectly by releasing endogenous substances responsible for exciting the nerve endings and causing pain, but also excites neurons that are sensitive to drugs.[27] To suppress pain conventionally, nonsteroidal anti-inflammatory drugs are prescribed.[28] These drugs are used for treating various diseases such as arthritis, headache, pain and orthopaedic conditions. Analgesic drugs such as diclofenac relieve pain peripherally/centrally by inhibiting cyclooxygenase enzyme (COX-1 and COX-2). Inhibition of cyclooxygenase enzyme reduces the production of pain mediators such as prostaglandins, substance P, histamine, serotonin, and Bradykinin. Pain sensation is eventually reduced in the nociceptors.[29] These findings strongly suggest that Ethanolic and Aqueous extracts of stem of *Abutilon indicum* possess peripherally or centrally analgesic property Perhaps acting in a similar manner as conventionally used therapeutic drugs that reduce the pain perception in nociceptors by inhibiting production of prostaglandins. These results concur with other research studies on the evaluation analgesic activity of herbal plants extract using laboratory animals. Reduction in the number of abdominal writhing's in this study is in agreement with a study carried out by[30] on analgesic properties of acetone leaf extracts of *Carissa spinarum* in mice. The findings are also in line with studies by[31] on antinociceptive activity of *Toddalia asiatica* (L) Lam in models of central and peripheral pain.Studies conducted on herbal plants by many researchers have linked presence of secondary active metabolites such as flavonoids, saponins and alkaloids to analgesic activities among other properties.[32-33] Flavonoids have the ability to disrupt synthesis of eicosanoids.[34] Flavonoids also have the ability to reduce production of arachidonic acid through inhibition of neutrophils degranulation.[35] Besides flavonoids, alkaloids also have been associated with the ability to inhibit pain perception.[36]

CONCLUSION

The Ethanolic and Aqueous extracts of stem of *Abutilon indicum* in Swiss albino mice clearly demonstrated analgesic activities on acetic acid-induced pain in Swiss albino mice. The extract reduced the number of abdominal writhing's significantly when compared to the reference drug (diclofenac). This study therefore concludes that the medicinal plant posses analgesic properties. Suppression of pain in this study could be attributed to phytochemical constituents present in the extract. Therefore, it is possible to obtain analgesic agent from the plant and serve as an alternative bio-resource in managing pain. However, further research on the mechanism action of the extract should be carried out. The study thus, scientifically confirms the traditional use of the medicinal plant in management of pain.

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