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STUDY OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF LEAVES OF INDIGENOUS MEDICINAL PLANT ON LABORATORY ANIMALS.

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Abstract- *Convolvulus arvensis* belonging to family *Convolvulaceae* is used as an antioxidant and laxative herbal medicine. However review of literature does not show any scientific data regarding analgesic and anti-inflammatory activity on leaves of *Convolvulus arvensis.* Hence this study was carried out to scientifically investigate the possible analgesic and anti-inflammatory activity of leaves of *Convolvulus arvensis* in laboratory animals. Analgesic activity was evaluated with Eddy's hot plate method. Anti-inflammatory activity was evaluated with Carrageenan induced Arthritis inflammation in rats. In Eddy's hot plate method AECA showed significant analgesic effect at oral dose of 400 mg/kg ($p<0.001$). Vehicle group compared with pentazocine treated group, latency period of paw licking & jumping was found to increase. In the anti-inflammatory study, AECA reduced carrageenan induced paw edema in rats. Thus, it can be stated that, aqueous extract of leaves of *Convolvulus arvensis* possess analgesic and anti-inflammatory activity in lab animals.

Key words- *Convolvulus arvensis,* leaf extract, analgesic, anti-inflammatory

INTRODUCTION

Medicinal herbs have been used as a form of therapy for the relief of pain throughout history. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic efficacy. Taking into account the most important analgesic prototypes (e.g. salicylic acid and morphine) were originally derived from the plant sources. According to various medical literatures, several adverse reactions are known to be associated with the conventional nonsteroidal anti-inflammatory drugs, thereby limiting the widespread application of these agents. The study of plant species traditionally used as pain killers should still be seen as a fruitful research strategy in the search of new analgesic and antiinflammatory drugs.

Convolvulus arvensis belonging to family *Convolvulaceae* is used as an antioxidant and laxative herbal medicine. However review of literature does not show any scientific data regarding analgesic and anti-inflammatory activity on leaves of *Convolvulus arvensis.* Phytochemical studies on this plant showed the presence of Saponins, steroids, flavonoids and alkaloids, proteins and lipids (Manbir kaur, *et al*, 2012).

MATERIAL AND METHOD

Plant material

Convolvulus arvensis (Field bindweed) Fam. Convolvulaceae was collected in the month of August 2016 from Bhose (K), Pandharpur, Maharashtra.

Preparation of plant extract

The whole plant was shade dried and powdered. Dried powder (500 gm) was subjected to successive extractions by ultrasonication using distilled water. The extracts were filtered and

concentrated on a rotary evaporator (Medica Instrument, India) and stored in desiccator. The percentage yields of aqueous extract of *Convolvulus arvensis* (AECA) was 6.7 %, (Dhane NS, *& etal* 2016). The aqueous extract of *Convolvulus arvensis* (AECA) were stored in tightly closed amber color glass bottles in refrigerator.

Preparation of dosage form:

Dosage forms of aqueous extracts were prepared as per the following procedures.

Aqueous extracts:

10 mg aqueous extract of *Convolvulus arvensis* was dissolved in 10 ml distilled water to make up the required volume.

Drugs:

Accurately weighed quantities of 1.3 mg diclofenac sodium were suspended in 10 ml distilled water to make volume.

Accurately weighed quantities of 1.3 mg pentazocine were suspended in 10 ml distilled water to make volume.

Vehicles:

Distilled water was used as a Vehicle, without addition of extracts or drugs.

Experimental animals

Female Swiss albino mice (25-30 gm) and female Wistar rats (180-220 gm) were purchased from National Institute of Biosciences, Dhangawadi, Nigadewada road, off Pune Banglore highway, Tal. Bhor, dist: Pune pin code: 412205, Animals were housed in an air-conditioned room at a temperature of 25 ± 1 °C and relative humidity of 45 to 55% under 12-h light: 12-h dark cycle. The animals had free access to food pellets (Manufactured by Pranav Agro Industries Ltd., Sangli, India) and water.

Acute oral toxicity (AOT) study:

Healthy female Swiss albino mice of 25-30 g were used in acute toxicity studies as per OECD guidelines-425. The animals were fasted overnight and divided into 3 groups with 5 mice in each group. Extracts (AECA) were administered at dose of 2000 mg/kg, p.o. body weight. The mice were observed continuously for behavioral and autonomic profiles for 2 hrs and for any signs of toxicity or mortality up to 48 hrs (OECD-425, 2001).

Eddy's hot plate method in albino mice:

Female Swiss albino mice (25-30 g) were treated according to the method described by (Ezeja MI *et al*, 2011) (Danuta Malec., *et al.*2008) Selected swiss albino mice of either sex with a body weight between 25-30gm. Marked the animal with the picric acid. Kept the animal on fasting for about 3 hrs; provide free access to drinking water. Animals were divided into three groups (n=3) as standard, control & test. Standard & control group was composed of 6 animals in each while; test Group was have again made three group of six animals for three different doses level.

Group 1: control group- vehicle-saline solution

Group 2: standard group- standard drug-pentazocine (6 mg/kg)

Group 3: test group- extract (dose- AECA 200 mg/kg)

Group 4: test group- extract (dose- AECA 400 mg/kg)

Eddy's hot plate apparatus stabilized for 10 min. at temperature range of 55-56°C. Administered test compound & vehicle orally to the respective groups of animal. After one hour of dosing, placed the animal one by one on the hot plate & noted down. The time required for jumping or licking response whatever is first. Cut off time was 15 seconds

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Carrageenan induced Arthritis inflammation in rats:

Female Wistar rats (180-220 gm) were treated according to the method described by (Waleed K.G. *et al*, 2011). Wistar rats either male or female of body weight approx. 180-220 gm were selected. Picric acid was used for marking on paw region. Rats were divided into three groups (n=3) as standard, control & test. Standard & control group was composed of 6 rats in each while; test group was have again three groups of 6 rats for three different dose levels.

- **Group 1:** control group- vehicle-saline solution

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- **Group 2:** standard group- standard drug-

Observation	Control (Saline solution 2 ml/kg)	Standard (PZ 6 mg/kg)	Test 1 (CA 200 mg/kg)	Test 2 (CA 400 mg/kg)
Paw licking	5.000 ± 0.3651	12.67 ± 0.3333c	7.333 ± 0.4944bz	12.67 ± 0.4944

diclofenac sodium (5 mg/kg)

- **Group 3:** test group- extract (dose- AECA 200 mg/kg)
- **Group 4:** test group- extract (dose- AECA 400 mg/kg)

Initially noted the left hind paw volumes of all rats before the drug administration, with the help of the Vernier caliper. For this marked the paw with ink at level of the lateral malleolus (bony prominence on each side of the ankle) & immersed it in mercury/water up to this mark. The paw volume was displayed on the scale of Vernier caliper. Administered test compound (in three different dose levels) & vehicle orally to the respective groups of rats. After 30 minutes/1 hour, administered carrageenan by subcutaneous injection into the left hind paw. Recorded the paw volumes daily for 10 days using Vernier caliper.

RESULTS

Acute Toxicity Test (AOT)

Swiss albino mice were used in toxicity study. After dosing, each mouse was observed individually. Observations were made from the signs of toxicity, time of onset of signs of toxicity and length of recovery period. Experimental animals from both vehicle treated and AECA treated animals showed no signs of toxicity.

Effect of oral administration of *Convolvulus arvensis* Eddy's hot plate method in albino mice

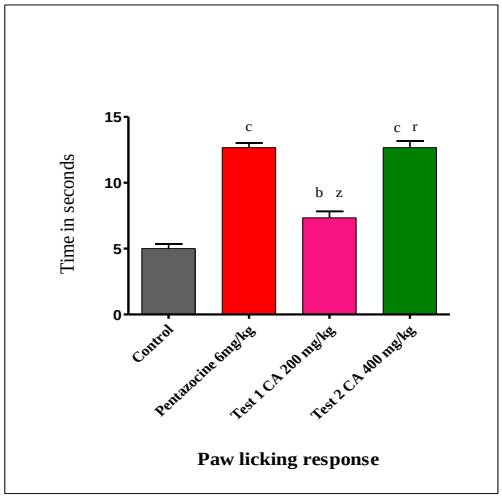
Paw Licking response:

Pentazocine (6 mg/kg,) p.o. treated group significantly ($p<0.001$) increased the paw licking latency at 60 and 90 minutes. Onset of action was observed at 60 minutes of administration of pentazocine (6 mg/kg,). However, aqueous extracts (*Convolvulus arvensis*) at doses of 200 and 400 mg/kg p.o. treated group showed minimum effect as compared with that of pentazocine (6 mg/kg,) p.o.

Effect of AECA at dose of 200 mg/kg, p.o. & 400 mg/kg, p.o. in albino Eddy's hot plate method (Paw licking response).

Values are expressed as mean ± S.E.M.; n = 6 mice per group. One way ANOVA followed by Dunnet test when normal compared with standard test1 and test 2 where p value are a<0.05, b<0.01 c<0.001, Standard compared with Test 1 and Test 2 p values are x<0.05, y<0.01, z<0.001.

Graph no 1: Effect of AECA at dose of 200mg/kg, p.o. & 400 mg/kg, p.o. in albino mice in Eddy's hot plate method (Paw licking response)



Values are expressed as mean ± S.E.M.; n=6 mice per group. One way ANOVA followed by Dunnet test when normal compared with standard, test1 and test2 p value are a<0.05, b<0.01 c<0.001, Standard compared with Test 1 and Test 2 p values are x<0.05, y<0.01, z<0.001.

Jumping response:

Pentazocine (6 mg/kg,) p.o. treated group significantly ($p<0.001$) increased the jumping latency at 60 and 90 minutes. Onset of action was observed at 60 minutes of administration of pentazocine (6 mg/kg,) p.o. However, aqueous extracts of (*Convolvulus arvensis*) 400 mg/kg have showed the same effect as compare to pentazocine (6 mg/kg,) p.o.

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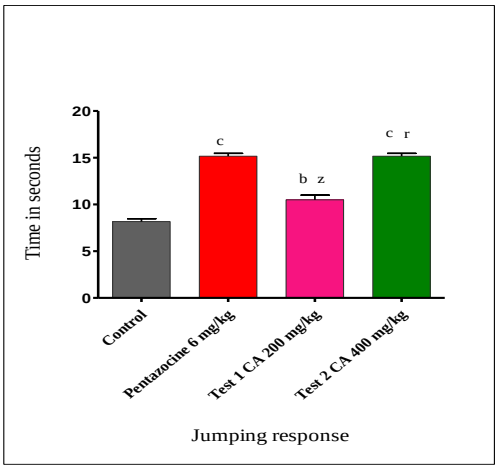
where 200 mg/kg of aqueous extract of (*Convolvulus arvensis*) showed minimum effect.

Effect of AECA at dose of 200 mg/kg, p.o. & 400 mg/kg, p.o. in albino mice using Eddy's hot plate method (jumping response).

Observation	Control Saline solution 2ml/kg	Standard (PZ 6 mg/kg)	Test 1 (CA 200 mg/kg)	Test 1 (CA 400 mg/kg)
Jumping response	8.167 ± 0.3073	15.17 ± 0.3073	10.50 ± 0.5000	15.17 ± 0.3073

Values are expressed as mean ± S.E.M.; n=6 mice per group. One way ANOVA followed by Dunnet test when normal compared with standard, test1 and test2 where p value a<0.05, b<0.01 c<0.001, Standard compared with Test 1 and Test 2.

Graph no 2: Effect of AECA at dose of 200 mg/kg, p.o. & 400 mg/kg, p.o. in albino mice against Eddy's hot plate method (jumping response)



Values are expressed as mean ± S.E.M.; n=6 mice per group. One way ANOVA followed by Dunnet test when normal compared with standard test1 and test2 where p value are a<0.05, b<0.01 c<0.001, Standard compared with Test 1 and Test 2 p values are x<0.05, y<0.01, z<0.001.

Carrageenan induced Arthritis inflammation in rats

There was a gradual increase in inflammation of paw of mice in the carrageenan control group. Arthritis induced by carrageenan is a model used for the evaluation of an agent with probable anti proliferative activity. 200 mg/kg p.o. of AECA & 400 mg/kg p.o. of AECA significantly (p<0.001) reduced the carrageenan induced rat paw edema. The maximum inhibition of rat paw edema by AECA (400 mg/kg p.o.) and Diclofenac sodium (5 mg/kg p.o.) was observed on the 8th day after carrageenan administration subcutaneously.

Effect of AECA on carrageenan induced arthritic inflammation in wistar rats.

Observation	Normal Control	Control Saline solution 2ml/kg	Standard (DS 5 mg/kg)	Test 1 (CA 200 mg/kg)	Test 2 (CA 400 mg/kg)
Anti Arthritis activity	5.500 ± 0.0000	7.288 ± 0.1941	5.675 ± 0.04532	6.388 ± 0.1329	5.913 ± 0.06105

Values are expressed as mean ± S.E.M.; n=6 mice per group. One way ANOVA followed by Dunnet test when normal compared with standard test1 and test2 where p value are a<0.05, b<0.01 c<0.001, Standard compared with Test 1 and Test 2 p values are x<0.05, y<0.01, z<0.001.

Graph no 3: Anti-inflammatory activity of AECA at dose of 200 mg/kg, p.o & 400 mg/kg, p.o. against carrageenan induced arthritic inflammation in wistar rats

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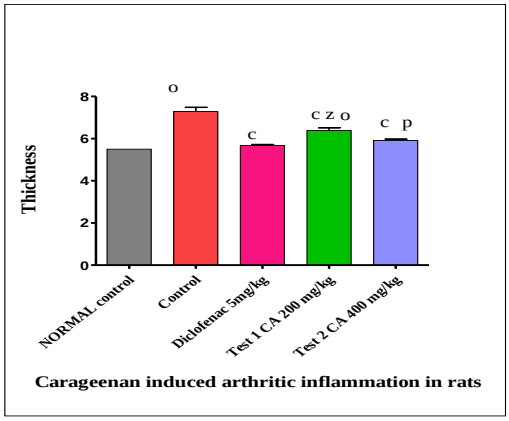

Values are expressed as mean ± S.E.M.; n=6 mice per group. One wayANOVA followed by Dunnet test when normal compared with standard test1 and test2 where p value are a<0.05, b<0.01 c<0.001, Standard compared with Test 1 and Test 2 p values are x<0.05, y<0.01, z<0.001

DISCUSSION AND CONCLUSION

Acute oral toxicity study was performed at the dose of 2000 mg/kg, p.o. revealed the non-toxic nature of aqueous extracts of *Convolvulus arvensis* (AECA). There were no toxic reactions or mortality with aqueous extracts of *Convolvulus arvensis* 200 mg/kg, p.o. The following doses were selected for the pharmacological studies i.e., 200mg/kg and 400 mg/kg, p.o. AECA.

In the present study of hot plate method thermal stress was employed for pain induction. An increase in basal reaction time is generally considered as an important parameter of analgesic activity. In this method, increase in stress tolerance capacity of the animals indicates the possible involvement of a higher centre (G. H. Vogel 2002).

The hot plate method involves spinal reflexes and is regarded as one of the most suitable methods for studying the involvement of centrally acting analgesics (T. R. Lavich & *et al* 2005). So it can be stated that AECA may have central analgesic effect, as it has shown significant increase in latency period. When control group was compared with test and standard group the test dose of AECA 400 mg/kg p.o. showed significantly increase in paw licking latency ($p<0.001$) and when compared with 200 mg/kg p.o. test its showed significance ($p<0.01$) analgesic activity, when standard was compared with 200 mg/kg p.o. test drug it showed significantly increase in paw licking ($p<0.001$) but when compared with test drug AECA 400 mg/kg p.o. it not showed any significance analgesic activity.

The jumping latency was also carried out, when control was compared with standard and test the test dose of AECA 400 mg/kg p.o. showed significantly increase in jumping latency ($p<0.001$) and when compared with test 200 mg/kg its showed significance activity ($p<0.01$). When standard was compared with test drug 200 mg/kg and 400 mg/kg p.o. its showed significantly increase in jumping latency ($p<0.001$).

Carrageenan is a family of linear sulphate polysaccharides extracted from the red seaweed marine alga *Chondrus crispus.* Lambda carrageenan is used in animal models of inflammation to test analgesics because dilute carrageenan solution (1-2%) injection causes swelling and pain (Costa et al., 2004). Inflammation induced by carrageenan is an acute and highly reproducible inflammatory model. Carrageenan has been widely used as an inflammagen capable of inducing experimental inflammation (William et al., 2010). This model has frequently been used to evaluate the anti-inflammatory agents (Panthong et al., 2007). The induction of edema by using carrageenan is believed to be biphasic in nature. The first phase involved within 1 h of carrageenan administration is associated with the release of histamine and serotonin from mast cells. The second phase starts after 1 h and is characterized by an increased release of prostaglandins (PGs) in the inflammatory area. During the second phase, the macrophages are known to release the large amounts of interleukin-1 (IL-1) which led to the increased accumulation of polymorphic nuclear cells (PMNs) to the site of inflammation. The activated PMNs then release the lysosomal enzymes and active oxygen species to destroy connective tissue and induce paw swelling (Marzouk et al., 2010). Statistical analysis revealed that, when normal control group was compared with control vehicle group, test group the test dose of AECA 200 mg/kg p.o. showed

significantly decrease in thickness of paw with ($p<0.001$). But when normal control group was compared with standard group & test group the test dose of AECA 400 mg/kg p.o. did not showed any significant. When control vehicle group was compared with test and standard group the test dose of AECA 200 mg/kg & 400 mg/kg p.o. showed significantly decrease in thickness of paw ($p<0.001$). when standard was compared with test drug (200 mg/kg p.o.) it also showed significantly decrease in thickness of paw ($p<0.001$). When standard group was compared with test drug (400 mg/kg) p.o. it did not showed any significant reduction in paw thickness.

Thus the experimental findings in the study demonstrated the analgesic, and anti-inflammatory activity of *Convolvulus arvensis* extracts. AECA showed weak analgesic and anti-inflammatory effect when compared with standard (Diclofenac sodium 5 mg/kg).

It was seen that AECA shows dose-dependent decrease in pain response of albino mice. So, it can be stated that, aqueous extract of *Convolvulus arvensis* may have analgesic activity. It was found to have moderate anti-inflammatory effect when compared to standard drug diclofenac sodium.

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