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EVALUATION OF ANALGESIC AND ANTI INFLAMMATORY ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF *CROTALARIA PUSILLA* B. HEYNE EX. DC. IN EXPERIMENTAL ANIMALS

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ABSTRACT

The plant *Crotalaria pusilla* B. Heyne Ex. Dc. Crotalaria genus of family Fabaceae (alt. Leguminosae) Also placed in : Papilionaceae was extensively used in traditional system of medicine for various ailments like rheumatism, inflammation, anemia colic pain impetigo, menorrhagia, psoriasis., etc. In the present study hydro-alcoholic extract of stem was screened for different study like acute toxicity study, analgesic activity by thermal(hot plate) and chemical methods(Acetic acid induced writhing) and anti-inflammatory activity was evaluated on the basis of effect on formalin induced- arthritis (paw edema) in rats and measured by slide calipers method. In thermal method the maximum analgesic activity of the extract at dose 350mg/kg, 450mg/kg (p.o) increase pain threshold of rat towards thermal source and in chemical method the maximum analgesic activity of the extract at dose 350mg/kg, 500mg/kg (p.o) inhibits the abdominal constriction induced by acetic acid. The maximum anti-inflammatory activity of hydro-alcoholic extract at dose 300mg/kg, 400mg/kg reduce formalin induced arthritis (paw edema). From the result it was concluded that the hydro-alcoholic extract exhibit analgesic and anti-inflammatory activity by central and peripheral mechanism.

INTRODUCTION

Medicinal herbs have been used as a form of therapy for the relief of pain throughout history.The treatment of rheumatic disorder is an area in which the practitioners of traditional medicine enjoy patronage and success. Natural products in general and medicinal plants in particular, 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, 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 anti-inflammatory drugs[1,2]. Crotalaria genus of family Fabaceae (alt. Leguminosae) comprises of about more then 10 species that occurs in India. *Crotalaria pusilla* is very rare plant and are medium in size and native to W. Peninsula, native to tropical area of Asia. In India it is distributed at Andhra Pradesh, Bihar, Orissa, Goa, Gujarat, Karnataka, Kerala, Madhya Pradesh & Maharashtra. *Crotalaria pusilla* B. Heyne ex DC. is a annual, small herb belonging to family Fabaceae (Figure - 1) (alt. Leguminosae) Faboideae tribe: Crotalarieae.is described in ayurvedic literature. The whole plant, roots of the plant are extensively used in traditional system of medicine for various ailments like rheumatism, inflammation, anemia colic pain impetigo, menorrhagia, psoriasis etc. The stem contain alkaloids, glycosides, steroidal compound, protein & phytosterols. Plant are reported to contain cardinolide,14-16- dianhydrogitoxigenin, 3 –O- β- xylopyranoside. C-anagyroids It is charactarised by ester of tornefurcidine and 3- Hydroxyl 2,3,4- trimthyl glucoric acid , respectively.[3,4,5] However, no sufficient data were found regarding the pharmacological evaluation of the stem of *Crotalaria pusilla* B. Heyne ex DC. to substantiate their therapeutic claim. The aim of the present study is to investigate the analgesic and anti-inflammatory activities of the hydro-alcoholic stem extract of *Crotalaria pusilla* B. Heyne ex DC.

Figure No. 1 : Plant *Crotalaria pusilla*

MATERIALS AND METHODS

Formalin (Himedia Laboratories pvt ltd. Mumbai)

Sodium bicarbonate (Merck pvt.ltd)

Diclofenac sodium (Novartis, Thane)

Collection and authentication of Plant Materials

The plant '*Crotalaria pusilla'* were collected from nearest mountain area situated in Barpali, district Bargarh of Odisha in the month of November- December for the correct botanical identification. The plant material was authenticated from Botanical Survey of India (BSI), Howrah, Kolkata having reference no.CNH/I-I/49/2010/Tech.II/285. Few authentic samples was preserved in our department for future reference. The plant was washed with water & dried it in sunlight first hour & then it was dried in shade. The dried plant was coarsely powdered by means of mechanical grinder. The course powder was taken for studies.

Preparation of extracts

The stem were washed thoroughly, dried under a shade and pulverized. The coarse powder was extracted with hydroalcoholic (70:30) using soxhlet apparatus. The extract was dried using a rotary vacuum evaporator (BUCHI, GERMANY) and stored in a desiccators until further use. The percentage yield of hydroalcoholic extract from stem was 2.67% with reference to dry starting material[5, 6].

Animals

Wistar albino mice (20 ± 5 g) and Wistar albino rats 125-175g of either sex, were procured from institutional Animal House facility, and were used for study. The animals were housed in large polypropylene cages (38.23.10 cm) with not more than six animals per cage in a temperature controlled room temperature (25º ± 2°C) and provided with standardized pelleted feed and clean drinking water *ad libitum*. The protocol was approved from the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals)[7, 8].

ACUTE TOXICITY STUDY

The acute toxicity studies (LD50 determination) were carried out by the method described by Miller and Tainter[11]. Five groups of rat (125-175g of either sex) were taken and each group contains six rat which were deprived of food. Then the extract was injected intra peritoneally and animals were observed for general signs and symptoms of toxicity including mortality over a period of 24 hours.

ANALGESIC ACTIVITY

In this present study, for the evaluation of analgesic activity two models, i.e. hot plate reaction time (thermal methods) by using wistar albino rats and acetic acid induced writhing response (chemical method) using albino mice were employed according to the method of (Turner, 1965). The animals were divided into four groups of six animals for each model. Animals of each experiment, Group I served as control and received saline water(10 ml/kg/ p.o.) and acetic acid (10 mg/kg, p.o), respectively, group II served as reference group and received morphine hydrochloride and aspirin at the dose 7.5 mg/kg/p.o and 250 mg/kg/p.o respectively. groups III and group IV of each experiment served as treatment groups in which group III received extract at the doses of 350 mg/kg/p.o and 350 mg/kg/p.o and group IV received the extract at the dose of 450 mg/kg/p.o and 500 mg/kg/p.o, respectively[9,10,11].

Thermal method (Hot plate test)

The test was performed using Eddy's hot plate maintained at a temperature of 55 ± 1°C. The basal reaction time of all animals towards thermal heat was recorded. The animals which showed fore paw licking or jumping response within 6-8 secs were selected for the study. 60 min after the administration of test and reference compounds, the animals in all the six groups were individually exposed to the hot plate maintained at 55°C. The time taken in secs for fore paw licking or jumping was taken as reaction time. A cut off period of 15 secs is observed to avoid damage to the paws. The pain inhibition percentage (PIP) was calculated according to the following formula:

Pain inhibition percentage (PIP) = $((T_1-T_0)/T_0) \times 100$, T_1 is post-drug latency and T_0 is pre drug latency[9,10,11].

Acetic acid induced writhing test

Acetic acid was administered intra peritoneally to all the groups at the dose of 10 ml/kg body weight 60 min after the administration of test compounds. Analgesic activity was recorded by counting the number of writhes after the injection of acetic acid for a period of 10 min. A writhe is indicated by abdominal constriction and full extension of hind limb[8, 9].

ANTI INFLAMATORY ACTIVITY

The anti-inflammatory activity of the extracts was determined according to the method of Vogel *et.al*[6]. The rats were divided into four groups of six rats each. The group-1 (control group) received 0.9% (w/v) normal saline water, at a dose of (10 ml/kg/ p.o.). Group-II was treated with the hydro-alcoholic extracts at the dose 300 mg/kg, Group-III was treated with the hydro-alcoholic extracts at the dose 400 mg/kg and Group-IV was treated with the

standard drug, diclofenac sodium (10mg/kg) for ten days. On 1st and 3rd day formalin (0.1 ml of 2% solution) was injected in the sub planter tissue of the right hind paw for induction of arthritis. The degree of paw edema of all the groups was measured using a slide caliper at 60, 120, min after the administration of formalin to each group[8, 9].

RESULT AND DISCUSSION

Analgesic activity

The analgesic activity was evaluated using both thermal and chemical methods of analgesia in rat. These methods are used to detect central and peripheral analgesics. Hot plate tests are most sensitive to centrally acting analgesics whereas Acetic acid induced writhing test was used for detecting both central and peripheral analgesia, Thermal induced analgesia indicates narcotic involvement. Thermal analgesic tests are more sensitive to opioid μ receptors and non-thermal tests are to opioid κ receptors. Intra peritoneal administration of acetic acid releases prostaglandins and sympathomimetic system mediators like PGE2 and PGF2α and their levels were increased in the peritoneal fluid of the acetic acid induced rat. The centrally acting analgesics generally elevate the pain threshold of rat towards heat. Both the dose of hydro-alcoholic extract significantly increased the reaction time of animals towards the thermal source in a dose-dependent manner. Both dose of hydro-alcoholic extract significantly reduced the number of abdominal constrictions and stretching of hind limbs induced by the injection of acetic acid in a dose-dependent manner. Both the dose of hydro-alcoholic extract exhibited a writhing inhibition percentage of 19.8%, 33.49% respectively in a dose dependent manner. So the hydro-alcoholic extract of the rat at (350,450 mg/kg/p.o) exhibited greater activity (33.49%), (Table-2). which was comparable with the standard drug Aspirin (62.39%). The abdominal constrictions produced after administration of acetic acid is related to sensitization of analgesic receptors of prostaglandins. It is therefore possible that the extracts exert their analgesic effect probably by inhibiting the synthesis or action of prostaglandins[8,9,10].

Anti-inflammatory activity

Formalin-induced arthritis (paw edema- due to over growth of fibroblast) is a commonly used primary test for the screening of new anti-inflammatory agents and is believed to be biphasic. The first phase (1-2hr) is due to the release of histamine or serotonin and the second phase of edema is due to the release of prostaglandin. The results of this study indicate that the hydro-alcoholic extract of *Crotalaria pusilla* significantly reduced formalin induced paw edema in rats. Therefore, the mechanism of action may be by inhibition of histamine, serotonin or

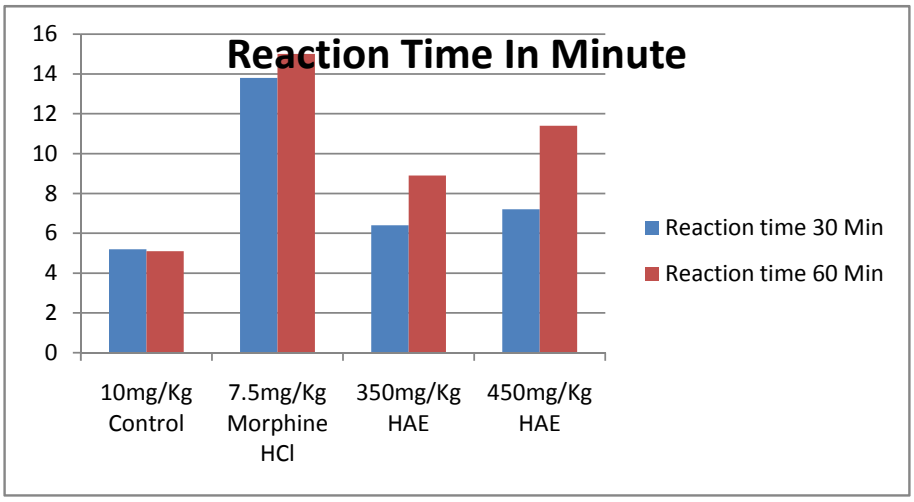
prostaglandin. In general, non-steroidal anti-inflammatory drugs produce their analgesic action through the inhibition of prostaglandin synthetase within the hypothalamus .Therefore, the analgesic activity of hydro-alcoholic extract of *Crotalaria pusilla* is probably by inhibition of prostaglandin synthesis in hypothalamus. The hydro-alcoholic extract of *Crotalaria pusilla* produced significant anti-inflammatory activity, the reduction in formalin induced arthritis (paw edema) decrease by hydro-alcoholic extracts after 2 hr was 9.6% and 12.9%, edema reduction by standard, diclofenac Na (10 mg/kg) was 17.6% (Table 3)[8,9,10].

CONCLUSION

From the above it was conclude that hydro-alcoholic extract of *Crotalaria pusilla* posses potent analgesic and anti inflammatory property. However, studies are required to establish its exact mode of action at a molecular level for its analgesic and anti inflammatory effect.

TABLE-1:ANALGESIC ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF STEM OF *CROTALARIA PUSILLA BY HOT PLATE METHOD*

Gr	Treatment	Dose	Basalreaction Time(sec)	Reaction time in min			
				15	30	60	120
I	Control (Normal Saline)	10mg/kg	5.6± 2.8	5.8± 2.8	5.2±1.9	5.1±2.1	5.6±2.4
II	Morphine hydrochloride	7.5mg/kg	6±2.2	9.3±2.9	13.8±2.4	15	10.2±2.1
III	HAE	350mg/kg	5.5±2.5	5.9±1.6	6.4±2.1	8.9±2.2	6.2±2.4
IV	HAE	450mg/kg	5.3±2.9	6.1±1.9	7.2±2.2	11.4±2.8	6.7±1.6

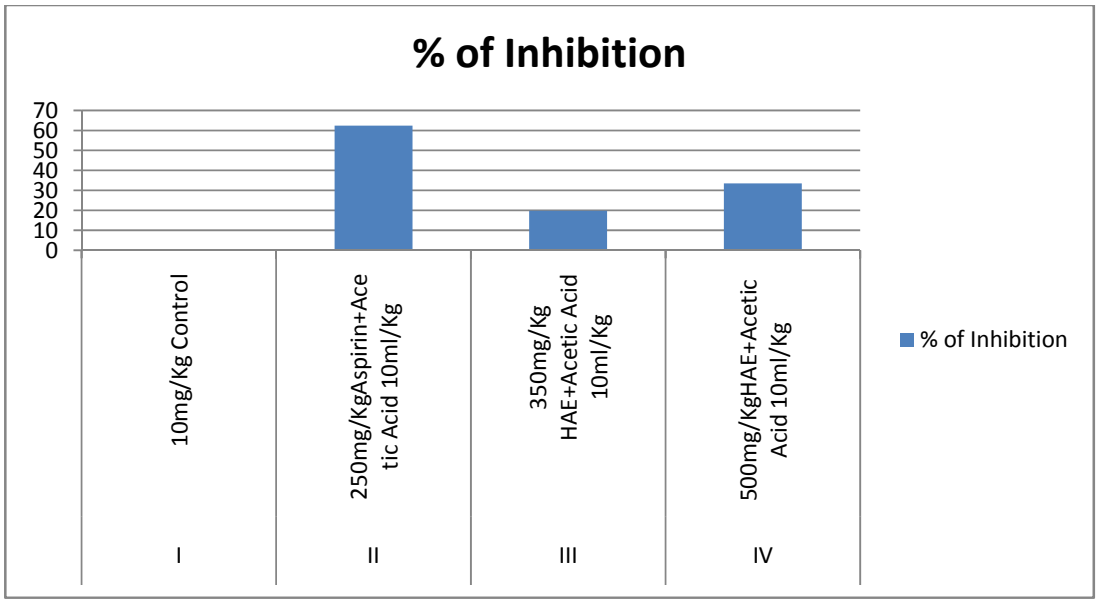


X-axis= Concentration Y-axis =Reaction time

Figure – 2: Effects of HAE on latency time of mice exposed to hot plate test. Data represent mean ± SEM of 6 animals. *p< 0.001 compared to control (One way ANOVA followed by student t test).HAE – Hydro-alcoholic extract of stem of *Crotalaria pusilla*

TABLE-2: ANALGESIC ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF STEM OF *CROTALARIA PUSILLA BY ACETIC ACID INDUCED WRITHING METHOD*

Groups	Treatment	Dose	Avg. no. of writhing	% Inhibition
I	Acetic Acid	10mg/kg	86.66, 1.36	……..
II	Aspirin + Acetic Acid	250mg/kg10 ml/kg	32.66, 0.79	62.39%
III	HAE+Acetic Acid	350 mg/kg	69.5, 1.4	19.8%
IV	HAE+Acetic Acid	500 mg/kg	58.5, 1.2	33.49%

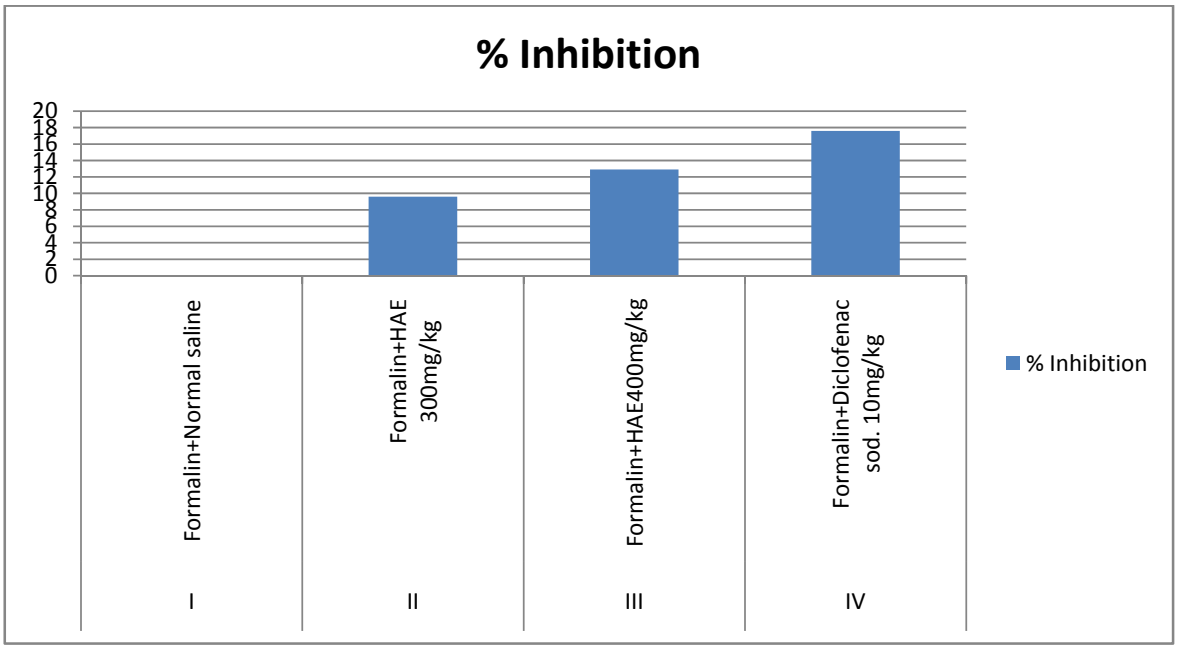


X-axis =Concentration, Y-axis = % Inhibition

Figure – 3: Effects of HAE on acetic acid induced writhing in mice. Data represent mean ± SEM of 6 animals. * $p < 0.001$ compared to control (One way ANOVA followed by student t test) HAE – Hydro-alcoholic extract of stem of *Crotalaria pusilla*

TABLE -3: ANTI INFLAMMATORY ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF STEM OF *CROTALARIA PUSILLA* BY USING SLIDE CALLIPER METHOD

Groups	Treatment	Paw diameter(mm) (After formalin)	Paw Diameter(mm) 1 hr	Paw Diameter(mm) 2 hr	% Inhibition
1	Formalin+ Normal saline	6.080±0.88	6.08±1.2	6.08±1.2	……..
2	Formalin + HAE 300mg/kg	6.04±0.86	07±1.12	5.5±1.08	9.6
3	Formalin + HAE 400mg/kg	6.7±0.95	5.6±1.06	5±1.115	12.9
4	Formalin + Diclofenac Sodium 10mg/kg	6.62±0.94	5.59±1.03	5.1±1.06	17.6



X-axis = Concentration, Y-axis = % Inhibition

Figure – 4: Effects of HAE on anti inflammatory activity. Data represent mean ± SEM of 6 animals. * P < 0.001 compared to control (One way ANOVA followed by student t test).

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