ANTI-ALLERGIC AND ANALGESIC ACTIVITY OF AERIAL PARTS OF HYBANTHUS ENNEA Spermus

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ABSTRACT

*Hybanthus enneaspermus* is commonly used plant in traditional medicine for the various condition such as urinary calculi, cardio protectivity, anti-arrhythmic, urinary tract infection, diarrhoea, dysuria, etc. In the present study are analgesic and anti allergic activity of petroleum ether and ethanolic extract of aerial part of the plant *Hybanthus enneaspermus*. The analgesic activity was screened by using hot plate method, tail immersion method, tail flick method. The anti allergic activity was screened by milk induced eosinophilia and leukocytes methods. The ethanolic extract have more significant activity of analgesic and anti allergic property than the petroleum ether extract *Hybanthus enneaspermus*. The extract possesses analgesic and anti-allergic activity which may be due to the presence of phytochemical constituents mainly flavonoids and polar constituents.

Keywords: *Hybanthus enneaspermus* analgesic activity, anti allergic activity, hot plate method, tail immersion method, tail flick method, milk induced eosinophilia, milk induced leukocytes method.

INTRODUCTION

Medical science during the last few decades, treatment of chronic pain still remains a challenge for medical community. All the available analgesic drugs such as NSAIDs have few adverse effects. As a result, the traditional system has gained importance in the field of medicine. Herbal plants are an important source of new chemical substances with potential therapeutic uses. Many chemical substances extracted from higher plants are used in medicine throughout the world. The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine. However, scientific evaluation is needed to provide evidences of their safety and efficacy. *Hybanthus enneaspermus* (Family: Violaceae) is well known for is different therapeutic uses. The whole plant is very acrid to relieve Strangury, painful dysentery, vomiting, burning sensation, wandering of the mind. Leaves of the plant are used as an external application for wound. It has been used as an antimalarial, antirheumatic, emmenagogue, sedative, antispasmodic, aphrodisiac and anti-asthmatic. The plant is also attributed to its antimicrobial and anti-plasmodial action, and it has been reported, in ancient ayurvedic literature, to cure conditions of urinary calculi. The roots of this plant have been indigenously used in epilepsy and hysteria. Early studies documented the presence of flavonoids, xanthine, terpenoids and glycosides in the *Hybanthus enneaspermus* plant. The present study has been designed to evaluate the analgesic activity and anti-allergic of ethanolic and petroleum ether extract of aerial parts of *Hybanthus enneaspermus*.

MATERIALS AND METHODS

Plant material

The aerial parts of fresh plant *Hybanthus enneaspermus* were collected from the Vandavasi Forest, Thiruvannamalai Dt, during December-2012. The plant was authenticated by Dr. J. Jayaraman, Ph.D., (PARC) Medicinal Plant Research Unit, Chennai, under the accession code PARC/2013/1997.

Preparation of extracts

Shade dried, cleaned from extraneous materials, mechanically grinded and coarsely powdered aerial parts of *Hybanthus enneaspermus* was subjected to successive solvent extraction in Soxhlet extractor using petroleum ether, ethanol as solvent. All the extracts were vaccum dried to produce petroleum ether (11.5%) and ethanol (12.6%) extracts respectively.

Phytochemical screening of active extracts

Various Phytochemical studies including test for carbohydrates, proteins, amino acids, steroid, alkaloids, glycosides and flavonoids compounds were carried out.

Animals

Albino mice (Swiss strain) weighing 25-30g either sex were obtained from the King’s institute Chennai. The animals were maintained at room temperature of (25 ± 2) °C with relative humidity of (75 ± 5) % under 12 hours dark and light cycle. The animals maintained under standard husbandry conditions and had free access to diet and water. The animals were allowed to acclimatize to the environment for 7 days prior to the experimental session. The animals were divided into different groups each consist of six animals and were fasted overnight prior to the experiments as per the instruction given in the Animal Ethical Committee.

Drugs and chemicals

Petroleum ether and ethanol were purchased from Chandanmal chemicals, Chennai. Diclofenac sodium were procured from Ranbaxy. All the chemicals used for the extraction and purification were all are of LR grade.

Acute toxicity study

Acute toxicity study was carried out as per the guidelines set by the Organization for Economic Co-operation Development (OECD guideline 425) received from the Committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA). Animals were divided into six groups (n=6). The animals were fasted for overnight with free access to food and water. The petroleum ether,
ethanol extracts was administered orally in doses of 500 mg/kg and 1000mg/kg of body weight to different groups of mice and observed over 14 days for mortality and physical of behavioural changes.

**Anti-allergic activity**

**Milk induced leucocytosis**

Mice were divided into three groups, six animals in each group. Animals belonging to group-I received distilled water 10 ml/kg, (p.o.). Animals belonging to group II, III, received boiled and cooled milk injection in dose of 4 ml/kg (s.c.). Animals belonging to groups II, III received petroleum ether and ethanol extract of *Hybanthus enneaspermus* plant (200 mg/kg, i.p) respectively 1 hour before milk injection. Blood samples were collected from each mouse from the tail vein. Total leucocyte count was done in each group before drug administration and 24 hour after milk injection difference in total leucocyte count before and 24 hour after drug administration was calculated.

**Milk induced eosinophilia**

Mice were divided into five groups, six animals in each group. Animals belonging to group-I received distilled water 10 ml/kg, (p.o.). Animals belonging to group II, III, received boiled and cooled milk injection in dose of 4 ml/kg (s.c.). Animals belonging to groups II, III to V petroleum ether, ethanol (200 mg/kg, i.p) respectively, 1 hr before milk injection. Blood samples were collected from each mouse from the retro orbital plexus, under light ether anaesthesia. Eosinophil count was done in each group before drug administration and 24 hr after milk injection. Difference in eosinophil count before and 24 hr. after drug administration was calculated.

**Analgesic activity**

Hot plate methods, Radiant heat tail flick and tail immersion method were employed to determine the analgesic activity.

**Hot plate method**

Mice were divided into four groups, six animals in each group. Group I: Distilled water 10 ml/kg, (p.o). Group II: Diclofenac sodium injection in dose of 25mg/kg (S.C). Group III: Petroleum ether Extract injection in dose of 200mg/kg (S.C). Group IV: Ethanol extract injection in dose of 100mg/kg (S.C). The analgesic activity of the extract was measured by hot-plate method. All drugs were given orally to the respective group mice as a suspension in gum acacia. The rats were placed on a hot plate maintained at 55±0.5°C. The reaction time was taken as the interval from the instant animal reached the hot plate until the moment animal licked its feet or jumped out. A cut off time of +10 sec was followed to avoid any thermal injury to the paws. The reaction time was recorded before and after +30, +60, +90, +120 and +180 min following administration of test or standard drug.

**Radiant heat tail flick method**

Mice were divided into four groups, six animals in each group. Group I: Distilled water (DW) 10 ml/kg(p.o).Group II: Diclofenac sodium injection in dose of 25mg/kg (S.C). Group III: Petroleum ether Extract injection in dose of 200mg/kg (S.C). Group IV: Ethanol extract injection in dose of 200mg/kg (S.C).The central analgesic activity was determined by radiant heat tail-flick model in mice. The analgesic activity of the plant extract was studied by measuring drug- induced changes in the sensitivity of the prescreened mice (the intensity of the light beam has been experimentally defined such that naive animals will withdraw their tails with into 4 s) to heat stress applied to their tails by using analgesiometer.

Tail-flick latency was assessed by the analgesiometer. All drugs were given orally to the respective group rats as a suspension in gum acacia. The strength of the current passing through the naked in chrome wire was kept constant at 5 ampere. The distance between heat source and the tail was 1.5 cm and the application site of the heat on the tail was maintained within 2 cm, measured from the root of the tail. Cut-off reaction time was +10 s to avoid any tissue injury during the process. Tail-flick latency was measured from +30 min after the drug administration.

**Tail immersion methods**

Mice were divided into four groups, six animals in each group. Group I: Distilled water (DW) 10 ml/kg,(p.o).Group II: Diclofenac sodium injection in dose of 25mg/kg (S.C).Group III:Petroleum ether Extract injection in dose of 200mg/kg (S.C).Group IV: Ethanol extract injection in dose of 200mg/kg (S.C).Mice divided in the groups of six each, were held in position in a suitable restrainer with the tail extending out. 2-3 cm area of the tail was marked and immersed in the water bath thermo-statistically maintained at 51°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The maximum cut off time for immersion was 180 seconds to avoid the injury of the tissues of tail. 0.2 ml of 0.9% NaCl solution was administered to control animals; plant extracts in doses of 300, 500 and 1000 mg/kg were given orally by intubation. The initial reading was taken immediately before administration of test and standard drugs and then 60, 90, 120,150 and 180 minutes after the administration. The criterion for analgesia was post drug latency which was greater than two times. Tail flick latency difference or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs.

**Statistical analysis**

The results are expressed as mean± SEM. The Dunnett’s test was used to make a statistical comparison between groups. Result with P<0.01 and P<0.05 were considered significant.

### RESULTS

**Table 1: Effect of *Hybanthus enneaspermus* plant extract on milk induced leukocytosis in mice**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Number of leukocytes (Cu.mm)</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td></td>
<td>6320 ± 15.47</td>
<td>1234.00 ± 60.05</td>
<td>5086 ± 44.99</td>
</tr>
<tr>
<td>II</td>
<td>Petroleum Ether</td>
<td></td>
<td>6172.00 ± 107.32</td>
<td>3300 ± 314.88</td>
<td>2872 ± 207.36</td>
</tr>
<tr>
<td>III</td>
<td>Ethanol</td>
<td></td>
<td>9632.00 ± 154.32</td>
<td>2932.00 ± 104.84</td>
<td>6700 ± 49.44</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM of a sample size of n=6; level of significance chosen was P=0.01. All treated groups were compared with control group. (One way ANOVA followed by Dunnett’s test).
Figure 1: Milk induced leukocytosis in mice

Figure 2: Milk induced eosinophilia in mice

Table 2: Effect of *Hybanthus enneaspermus* plant extracts on milk induced eosinophilia in mice

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Number of leukocytes (Cu.mm)</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>432.00 ± 3.40</td>
<td>543.00 ± 21.94</td>
<td>111 ± 18.54</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Petroleum Ether</td>
<td>82.65 ± 8.93</td>
<td>94.63 ± 8.96</td>
<td>11.98 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Ethanol</td>
<td>132.70 ± 3.20</td>
<td>63.33 ± 3.20</td>
<td>68.77 ± 0.6</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM of a sample size of n=6; level of significance chosen was *P*<0.01. All treated groups were compared with control group. (One way ANOVA followed by Dunnett’s test).

Table 3: Effect of *Hybanthus enneaspermus* plant extracts on differential Leukocytes count

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Differential leukocytes count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutrophils</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>6.37 ± 0.46</td>
</tr>
<tr>
<td>II</td>
<td>Petroleum Ether</td>
<td>4.32 ± 0.72</td>
</tr>
<tr>
<td>III</td>
<td>Ethanol</td>
<td>6.32 ± 0.72</td>
</tr>
</tbody>
</table>

Level of significance chosen was *P*<0.01. All treated groups were compared with control group.
Acute toxicity

From the acute toxicity study, the LD cut-off dose for extracts was found to be 1000 mg/kg body weight. Hence, the therapeutic doses were taken as 50 mg/kg and 100 mg/kg body weight.

Anti-allergic activity

Milk induced leukocytosis in mice

Subcutaneous injection of milk in dose of 4ml/kg, produced a significant (P<0.01) increase in the leukocyte count after 24 hour administration. Mice pre-treated with petroleum ether, Ethanol extract of *Hybanthus enneaspermus* have exhibited significant difference in total leukocyte before and after drug treatment. Ethanol extract of *Hybanthus enneaspermus* plant has highly inhibited the milk induced leukocytosis (P<0.01)
with dose of 200 mg/kg (i.p) as compare to petroleum ether extracts the result were listed in Table 1.

**Milk induced eosinophilia in mice**

Milk induced eosinophilia in mice subcutaneous injection of milk in dose of 4ml/kg, produced a significant (P<0.01) increase in the eosinophil count after 24 hour of its administration. Mice pre-treated with petroleum ether, and ethanol extract of *Hybanthus enneaspermus* plant have exhibited significant difference in eosinophil count before and after drug treatment. Ethanol extract of *Hybanthus enneaspermus* plant has highly inhibited the milk induced eosinophilia (P<0.01) with dose of 200mg/kg (i.p) as compare to petroleum ether extract and the result were listed in Table 2.

**Milk induced differential leukocyte count in mice**

Ethanol extract of *Hybanthus enneaspermus* plant showed highly significant inhibited the milk induced eosinophilia (P<0.01) with dose of 200mg/kg (i.p) as compare to petroleum ether extract, the result showed significant (P<0.01) i.e. up to 1.33% decrease in eosinophil count. But there is increased i.e. up to 19.42% of lymphocytes count. It indicates that increased leukocyte count in Table 3.

**Analgesic activity**

**Hot plate method**

The control drug increases the response latencies at various time intervals. The *Hybanthus enneaspermus* plant extract were dose as well as time dependent. Amongst all the doses used petroleum ether extract (200 mg/kg b.w) was most effective at 120 and 180 minutes as well as ethanol extract at 60 and 90 min comparable to the standard group which was highly significant (P<0.05, P<0.01) and the result are listed in Table 4

**Heat tail flick method**

In the tail flick method, the increases in latency period at different time points significantly differed (P<0.01) compared to baseline values within the same drug treated group. The extract and standard treated group significant increase (P<0.01) in reaction time in the dose of extract 200 mg/kg. The results are depreated in the Table 5.

**Tail immersion method**

In tail immersion test the result indicated a significant increase in reaction time at 120 and 180 min with both petroleum ether and ethanol (200 mg/kg). Whereas reference drug dclofenac sodium significantly increased the reaction time at 120/180 min Hence there groups are highly significant (P<0.01, P<0.05) and the results are gathered in the Table 6.

**DISCUSSION**

The pharmacognostical study is one of the major criteria for the identification of plant drug as well as drug standardization. The preliminary analysis of the plant extracts revealed the presence of constituents like flavonoids, alkaloids, and glycocides. The presence of these constituents may be responsible for their pharmacological activities. The acute toxicity studies revealed that there was no considerable change in body weight and after treatment of the experiment and no signs of same dose level for three days more and observed for 14 days, no change were observed from the first set of experiment. Bronchial asthma is characterized pathologically by an infiltration of eosinophils into the airway submucosa, phytochemical analysis of the ethanol extract shows the presence of carbohydrates, saponins. Thus we can speculate that these constituents might be responsible for anti-allergic activity. Literature shows that flavonoid is having anti-anaphylactic and anti-asthmatic activity. So anti-allergic activity showed by *Hybanthus enneaspermus* plant might be induced by these chemical moieties. After parenteral administration of milk there are increases in total leukocyte count (TLC) and this stressful condition can be normalized by administration of an anti-stress (or) adaptogenic drug. Eosinophilia is an abnormal increase in peripheral eosinophil count to more than 4% of total leukocytes. In the late phase, especially in the development of allergic asthma, eosinophils play role as an inflammatory cell. It was also demonstrated that parental administration of milk produces a marked and significant increase in leukocytes or eosinophils count after 24 hour of its administration. Thus, ethanol extract of *Hybanthus enneaspermus* plant shows more significant anti-allergic activity (P<0.01) as compare to petroleum ether extract in all three models of milk induced leukocytosis, milk induced eosinophilia and differential leukocytes count in mice. The hot plat method is considered to be selective for the drug acting centrally. The hot plat test measures the complex response to a non inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity. It is an establish fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally. Therefore the extract of *Hybanthus enneaspermus* plant must have a central activity.

In this tail flick, tail immersion and hot plate methods, both the extracts (petroleum ether + Ethanol (200 mg/kg) of plant increased the stress tolerance capacity of the animals and hence indicate the possible involvement of a higher centre. The time attained to reach the peak analgesic activity for standard drug (+30min) but the plant extract showed at (+90 min) and (120 Min) in hot plate, tail immersion and tail flick model respectively, which had no explanation and attributed as a limitation of our study. On the basis of their findings, if may be inferred that extract of *Hybanthus enneaspermus* has analgesic activities. These activities were related to the dose and there results corroborate the potential traditional use of the plat in folk medicine. At present, there are no reports on investigation to identity the active components present in extract of *Hybanthus enneaspermus*. Further investigations are anticipated to identity the active components and lead to their further clinical use.

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**REFERENCES**


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