Evaluation of Anti-inflammatory activity of Verbena officinalis leaf
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Abstract
Verbena officinalis Linn, the common vervain or common verbena, is a perennial herb native to Europe. It grows up to a meter high, with an upright habitus. The lobed leaves are toothed; the delicate spikes hold mauve flowers. This plant prefers limey soils. The aim of this study was to overview its therapeutic effects than its nutritive and industrial effects. This review article was carried out by searching studies in PubMed, Medline, Web of Science, and Iran Medex databases. In this study, studies was accepted for further screening and met all our inclusion criteria [in English, full text, therapeutic effects of Verbena officinalis Linn and dated mainly from the year 1964 to 2015. The search terms were “Verbena officinalis Linn,” lemon balm, “therapeutic properties”, “pharmacological effects”. It is commonly used for anti-inflammatory effect, ameliorative effect, and antitumor, pharmacokinetic effect, antioxidant and antifungal activity, antiradical efficacy, analgesic activity, neuroprotective effects, anti-inflammatory activity, inflammatory, wound healing, anti-trypanosomacruzi activity, antinociceptive and antioxidant activities, antioxidant/anti-inflammatory, antirhinosinusitis, biological activity, anti-skin infection. Verbena officinalis Linn. Possess very therapeutic effects. Nevertheless, it is still required to carry out more studies on this plant to identify its other properties.

Key words: Inflammation, Analgesic

Introduction
The inflammation response can be provoked by physical, chemical, and biologic agents, including mechanical trauma, exposure to excessive amounts of sunlight, x-rays and radioactive materials, corrosive chemicals, extremes of heat and cold, or by infectious agents such as bacteria, viruses, and other pathogenic microorganisms. Although these infectious agents can produce inflammation, (Harvard, 2008) The classic signs of inflammation are heat, redness, swelling, pain, and loss of function. These are manifestations of the physiologic changes that occur during the inflammatory process.
The three major components of this process are. (Krig.T, 2007)
1. Changes in the caliber of blood vessels and the rate of blood flow through them
2. Increased capillary permeability;
3. Exudation.

Material and Method
Procurement of the plant parts
The seed of Verbena officinalis collect from the market, Indore (M.P)
Preparation of extracts-
The plant material whole plant of Verbena officinalis dried under leaf. Ethenolic extracts whole plant of Verbena officinalis prepared.
Phytochemical study-
the phytochemical evaluation of ethenolic seed extract of Verbena officinalis will carried out as per standard methods .The presence of flavonoids will determined by lead acetate test, tannins by acetic acid test, saponins by foam test and steroids will determine.
Animals
Westar albino rats, weighing 150-250g procured from CPCSEA registered source. They will house in polypropylene cages under standard light/dark cycle, with food and water provided ad libitum. The experimental protocols will approve by the Institutional Animal Ethics Committee and conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.
Chemical
Carrageenan, indomethacin,
Experimental protocol
Overnight fasted animals were selected randomly on the day of experiment for administration of vehicle, standard drug and study drug. The animals were acclimatized one hour before for behavioral tests. Thirty minutes and 1 hour time interval between drug administration and behavioral tests were maintained in case of intraperitoneally and oral administration respectively. The animals were divided into five groups of five animals each as follows:

Group I (n=6) control, resive normal saline, i.p.
Group II (n=6) standard andomethacin 10 mg/kg
Group III (n=6) El i.p
Group IV (n=6) El i.p
Group (n=6) El i.p

Behavioral tests
Carragenan induce paw edema test
Male albino Wister rats/mice with a body weight between 150 and 250g are used. The animals are divided into 5 groups have 5 animals. The animals are starved overnight. one hour after oral administration of the ethanol extract of Verbena officinalis’s reference drug and vehicle (control), an injection of 0.1 ml of carrageenan was made into the right hind paw limb of each rat under the sub plantar of the left hind paw. Measurement of paw volume was done by mean of volume displacement after carrageenan injection and after 1, 2, 3 and 4 hr. Inhibition were obtained using the following ration: is the average volume for each after treatment, and is the average volume for each group before any treatment. (book S.K kulkarni 1998)

Cotton pallet granuloma test
The male rat was anesthetized with diethyl ether and sterilized cotton pellet weighing approximately 50mg was inserted in the subcutaneous layer of groin. The incised skin was properly sutured and a disinfectant was applied to prevent infection. The animals were treated with vehicle or alcoholic extraction once a day for 7 days. On the 8th day animals were sacrificed with ether overdose and the pellets along with granuloma mass were removed, washed and dried at 60-70° c till constant weight was obtained. The granuloma weights were obtained from control and treatment group and compared

Statistical analysis
All the data represent mean±S.E.M. values. The data were analyzed by means of analysis of variance (ANOVA). Whenever ANOVA was significant, further multiple comparisons were made using Turkey’s test as the post hoc test. All analyses were performed using the SPSS statistical software. The levels of statistical significance ranged from p<0.05 to p<0.01.

Results and Conclusion
The results of the present study have showed that the crude extract of the investigated plant exhibited very high anti-inflammatory activities. This activity may be linked with presence of polyphenolic compounds present in the extract. A main constituent of Verbena officinalis’s which are reported to be anti-inflammatory, antiasthma tic, analgesic, anti-inflammatory and antioxidant and these findings are in concordance with our results.

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Phytochemical constituents</th>
<th>Ethenolic extract of Verbena officinalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Flavanoids</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Phenols</td>
<td>–</td>
</tr>
<tr>
<td>4.</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Carbohydrates</td>
<td>–</td>
</tr>
<tr>
<td>8.</td>
<td>Terpinoids</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Proteins And Amino Acids</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 1: Phytochemical constituents of ethenolic extract of Verbena officinalis:
Table 2: effect of *Verbena officinalis* on immobility period of rats using carragenan method:

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug (Dose), Route</th>
<th>Change In Paw Volume (Ml) Mean ± Sem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Tween 80 (5%) i.p</td>
<td>1.8±0.02</td>
</tr>
<tr>
<td>Standard</td>
<td>Indomethacin (10Mg/Kg), i.p</td>
<td>81.56 (0.30±0.09)</td>
</tr>
<tr>
<td>EI</td>
<td>100 Mg/Kg, i.p</td>
<td>43.68 (1.06±0.14)</td>
</tr>
<tr>
<td>EI</td>
<td>300Mg/Kg, i.p</td>
<td>1.57 (1.77±0.14)</td>
</tr>
<tr>
<td>EI</td>
<td>500Mg/Kh, i.p</td>
<td>64.21(0.58±0.11)</td>
</tr>
</tbody>
</table>

N=5. Results are expressed as mean ± sem. *p>0.05, **p<0.01 as compared to control, ab= ethanol seed extract.

Table 3: effect of *Verbena officinalis* on immobility period of rats using cotton granuloma pallet method:

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug (dose), route</th>
<th>Weight of dry cotton pellet granuloma (mg)</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Tween 80 (5%) i.p</td>
<td>200±0.23</td>
<td>0</td>
</tr>
<tr>
<td>Standard</td>
<td>Indomethacin (10mg/kg), i.p</td>
<td>14.4±3.3**</td>
<td>92.87</td>
</tr>
<tr>
<td>EI</td>
<td>100 mg/kg, i.p</td>
<td>138±3.2</td>
<td>31.11</td>
</tr>
<tr>
<td>EI</td>
<td>300 mg/kg, i.p</td>
<td>127±1.1</td>
<td>36.13</td>
</tr>
<tr>
<td>EI</td>
<td>500 mg/kg, i.p</td>
<td>85±9.1**</td>
<td>56.93</td>
</tr>
</tbody>
</table>

N=Result Are Expresses as Mean ± Sem. *P>, **< As Compared to control, Ab= Ethanolic seed extract
Conclusion
Verbena preparation have a long history of human use for their anti-inflammatory properties, however, only recently have some of the compounds responsible for this activity and their mechanisms of action been identified.

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References

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