ANTINOCICEPTIVE EFFECTS
OF POLY HERBAL OIL EXTRACT (PHOE)

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ABSTRACT
A number of drugs are used widely in the traditional system of medicine or Tibb-e-Unani
(Unani medicine) in the management of many diseases but these drug mostly, have not
been investigated for their described effects. This study aimed to investigate
antinociceptive and anti-inflammatory effects of Herbal Oil (1, 5 and 10 ml/kg)
administered orally in mice and rats, using standard behavioral paradigms.
It is a poly herbal preparation used in traditional medicine as a remedy for pains and other
ailments. The data show that orally administered herbal oil promotes antinociceptive
activity against Acetic Acid induced writhing and Formalin induced pain models. The oil
also produced a dose dependent inhibition of carrageenin-induced rat paw edema. These
effects were significant when compared with saline control group. The results indicate
potent antinociceptive component in oil, which justify at least partially the folkloric use
of oil in relieving pains.

INTRODUCTION
Pain is “an unpleasant sensory and
emotional experience associated with actual or
potential tissue damage, or described in terms
of such damage” (Merskey, 1986) and a very
common phenomenon. There is no doubt that
pain acts as a warning signal against
disturbances either in the body or in the
external environment of an individual. The
principal objective of the treatment of pain is
to remove or abolish the cause of pain. But it
is not always possible to do so; hence,
algesics are used for the symptomatic
treatment of pain like NSAID (Abbott et al.,
1996). Opioids and antidepressant (lung et al.,
1997). Almost all pharmacological treatments
may produce side effects (Goldberg, 1984).
Although alternative treatments are
increasingly being used to alleviate affective
disorders (Wiklund et al., 1999). Alternative
medicine claims to posses many save and
effective drugs useful in various disorders.
Only few have been investigated for their
effects. Antinociceptive herbal Oil is a poly
herbal oily preparation containing 7 different
ingredients (Table 1). It is standardized
preparation used as analgesic oil traditionally.
Although some of its ingredients have been
studied for various pharmacological effects
and their reported activities are as follows:

Linum usitatissimum L. belongs to family
Linaceae. In traditional medicine Linum
usitatissimum L. used as a mild laxative and its
topical application for local inflammation
(Carson, 1998). It is still official in the
Chinese pharmacopeia for constipation and
dry itching skin (Tu, 1992). They are used for
 inflammations, abscesses and relief of pain in
American conventional medicine (Taber,
1962).

Trachyspermum ammi (L.) Sprague belongs to family Umbelliferae/Apicideae. According to Azerbaijani folk medicine, decoctions of ajowan seeds have analgesic properties (Irvani, 1834; Mu'min, 1713 and Ansari, 1818).
Antinociceptive Effects of Poly Herbal oil extract

*Mvristica fragrans* Houtt. belongs to family Myristaceae, has been found to exhibit anti-inflammatory activity (Ozaki *et al*., 1989). Hussain and Rao (1991) reported the chemopreventive action of *Myristica fragrans* on 3-methylcholanthrene (MCA)-induced carcinogenesis in the uterine cervix of virgin mice.

*Syzzygium aromaticum* (Linn.) belongs to family Myrtaceae. Antioxidant potential of the *syzzygium aromaticum* was reported by Shymala *et al*. (2003), suggest that it possesses the hypolipidemic effect in rats may be due to its ability to combat oxidative stress by quenching free radicals generated in the body.

*Colchicum luteum* Baker belongs to family Liliaceae is antirhyrmatic and to allay pain in arthritis and gout (Khan *et al*., 1997).

*Celastnis paniculata* (Willd.) belongs to family Liliaceae. The extract showed marked CNS depressing activity (Singh *et al*., 1974). It is also considered to be analgesic (Duke *et al*., 1985) and used in the treatment of rheumatism, leprosy, gout, fevers and paralysis (Chopra, 1986).

*Pinus roxburghii* Sargent belongs to family Pinaceae. Owing to counter-irritant and anti-inflammatory properties of the pine needle baths, they may relieve allergic itch (Damirov, 1988). Pine oil has strong Anti-inflammatory, antiseptic and diuretic properties, it also promotes granulation of wounds and is used as disinfectant and deodorant (Goryachev, 1952).

Keeping this view in mind about the pharmacological effects of different plants a herbal oil is prepared and the present study was conducted to determine the medicinal properties of herbal oil, we investigated the analgesic and anti-inflammatory activities using different animals models.

### MATERIALS AND METHODS

#### Preparation of oil:

Fresh *Ajwain Desi* (*Trachyspermum ammi* (L.) Sprague), *Jawatri* (*Myristica fragrans* Houtt.), *Long* (*Syzygium aromaticum* Linn.) *Saranjan Talkh* (*Colchicum luteum* Baker), *Mal Kangni* (*Celastrus paniculata* Willd.) and *Behroza* (*Pirius roxburhii* Sarg.) were purchased from local market, crushed and soaked in 1 litre water for 24h. Next day *Alsi* (*Linum usitatissimum* Linn) is added in this and boil it until all water is evaporated and all the ingredients have burnt than filter it and add pine oil (*Pinus roxburghii* Sargent.) in it. Keep it in sunrays for 10-15 days. At the end yellowish brown color oil was obtained and used during the whole study (Table 1).

#### Animals:

Adult male NMRI mice (20-25g) and Sprague Dawley rats (200-225g) were obtained from Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences.

### Table 1: Ingredients of Herbal oil

<table>
<thead>
<tr>
<th>Unani Name</th>
<th>Scientific Name</th>
<th>Parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alsi, Tukme Katan</td>
<td><em>Linum usitatissimum</em> Linn.</td>
<td>Seeds oil</td>
</tr>
<tr>
<td>Ajwain Desi</td>
<td><em>Trachyspermium ammi</em> (L) Sprague</td>
<td>Seeds oil</td>
</tr>
<tr>
<td>Jawatri</td>
<td><em>Myristica fragrans</em> Houtt.</td>
<td>Seed</td>
</tr>
<tr>
<td>Long</td>
<td><em>Syzygium aromaticum</em> (Linn.)</td>
<td>Flower buds</td>
</tr>
<tr>
<td>Suranjan Talkh</td>
<td><em>Colchicum luteum</em> Baker</td>
<td>Roots or Tuber</td>
</tr>
<tr>
<td>Mal Kangni</td>
<td><em>Celastrus paniculata</em> Willd.</td>
<td>Seeds</td>
</tr>
<tr>
<td>Behroza</td>
<td><em>Pirius roxburhii</em> Sarg.</td>
<td>Oleo-resin</td>
</tr>
</tbody>
</table>
Aisha Azmat et al.

Effect of PHOE on acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
<th>25 min</th>
<th>30 min</th>
<th>Total (min)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.55 ± 1.96</td>
<td>25.11 ± 3.43</td>
<td>20.11 ± 1.86</td>
<td>16.75 ± 3.06</td>
<td>12.14 ± 2.82</td>
<td>9.85 ± 2.11</td>
<td>89.77 ± 11.1</td>
<td>61.2</td>
</tr>
<tr>
<td>1 ml/kg</td>
<td>6.16 ± 1.03</td>
<td>9.5 ± 1.38</td>
<td>7.18 ± 0.74</td>
<td>5.5 ± 0.7</td>
<td>4.5 ± 1.02</td>
<td>2 ± 0.71</td>
<td>34.83 ± 4.49*</td>
<td>55.4</td>
</tr>
<tr>
<td>5 ml/kg</td>
<td>3.83 ± 0.74</td>
<td>11.83 ± 0.64</td>
<td>8.33 ± 0.49</td>
<td>7.16 ± 0.58</td>
<td>4.33 ± 0.52</td>
<td>2.07 ± 0.82</td>
<td>40 ± 2.48*</td>
<td>43.8</td>
</tr>
<tr>
<td>10 ml/kg</td>
<td>6.75 ± 1.34</td>
<td>15.16 ± 1.22</td>
<td>11.91 ± 1.59</td>
<td>10.77 ± 0.88</td>
<td>7.88 ± 1.56</td>
<td>4.62 ± 1.05</td>
<td>50.61 ± 6.18*</td>
<td>43.8</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. (n=10), *p<0.05 significantly different from control (Student’s t-test).

Effect of oil on the Writhing Response Induced by Acetic Acid

The writhing test described by Koster et al. (1959) was adopted. A total of 24 Mice divided into four groups (n=6) were used and treated as follows; group I received normal saline (10ml/kg) p.o., group 2-4 received 1, 5, and 10ml/kg p.o. of the herbal oil respectively. 30 min later, 10ml/kg of 0.7% aqueous solution of acetic acid were given to all mice. Each mouse was placed in a transparent observational cage and the abdominal constrictions resulting from intraperitoneal injection of Acetic Acid consisting of the contraction of abdominal muscle together with a stretching of hind limbs, were counted according to procedure described by Santos et al. (1994), Correa et al. (1996), Bersa et al (1996). Result were presented as percent inhibition of analgesia, calculated as the reduction in the number of writhes between control animals and those pretreated with either dose of oil.

Formalin induced nociception:

This test was carried out in four group of mice (n=6) pretreated with either saline or the oil (1, 5 and 10ml/kg) p.o. Twenty microliters of 1% Formaldehyde was injected into the sub-plantar surface of right hand paw of mice (Hunskaar et al., 1985; Tjolsen et al., 1992). The time in seconds spent in licking and biting responses of the injected paw was taken as an indicator of pain response in the mice. Responses were measured for 0-5 min (first phase) and 15-30 min (second phase) after formalin injection. Oil (1, 5 and 10ml/kg) was administered 30 min before formalin injection. Control group was treated with Saline (10 ml/kg).

Carrageenin-induced paw edema in rats:

The paw edema/Inflammation was induced by carrageenin (0.1 ml, 1% w/v in normal saline) into sub-plantar tissue of right hind paw (Winter et al., 1962). The linear paw circumference was measured at hourly interval for 6 h (Bangbose and Noamesi, 1981).

Herbal oil (1 and 10 ml/kg) and saline (10 ml/kg) were administered orally 1h before induction of inflammation.

Acute toxicity:

Mice were administered orally with the herbal oil (1, 5 and 10 ml/kg) and the incidence of Mortality was noted up to 24 h in each group.

(Dr. HMIIPHS) and were housed in groups of 6 per cage for seven days prior to experimentation with free access to food and tap water ad libitum and kept on a 12h light/dark cycle. Each experimental group consisted of six animals. All animals were housed in an air-conditioned room at 23±1 oC during the quarantine period.
Antinociceptive Effects of Poly Herbal oil extract

STATISTICAL ANALYSIS

Results are presented as means ± S.E.M. Student’s t-test was applied to determine a significant between the control group and experimental groups in all the experiments. P<0.05 was considered significant.

RESULT

Acetic Acid – induced writhing in mice:

The intraperitoneal injection of 0.6% (v/v) solution of acetic acid in mice induced a writhing response. The PHOE exhibited marked reduction in the number of abdominal constriction between 0 and 30 min (Table 2). This reduction is more pronounced at low dose than higher dose. At 10, 5 and 1 ml/kg it cause 43, 55, 61% inhibition respectively (Table 2).

Formalin-induced paw licking in mice:

PHOE evaluated against formalin induced pain in mice at 1, 5 and 10 ml/kg and the effects in early and late phases of formalin test are shown in Table 3. PHOE was active in both early and late phases of the formalin test showing a pain inhibition of 47, 43 and 32% of the early phase (0-5 min) and 81, 80, 72% of the late phase for 10, 5 and 1 ml/kg of the oil, respectively (Table 3). Higher pain inhibition was, however, observed in the late phase.

Carrageenin-induced edema:

The results of animal experiments are shown in Table 4. In the acute inflammatory model, the oil in the dose of 10 and 1 ml/kg p.o. showed pronounced dose dependent inhibition of paw edema that was 63 and 35% respectively (Table 4).

Toxicity Studies:

PHOE was well tolerated up to a dose of 10ml/kg, through oral route. No mortality was observed during 24h period. In the initial pharmacological screening, it was observed that the dose produced palpebral ptosis a reduction in locomotion (non quantified observation).

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### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Score of Pain</th>
<th>Score of Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-5 min % inhibition</td>
<td>15-30 min % inhibition</td>
</tr>
<tr>
<td>Control</td>
<td>1.67 ± 0.19</td>
<td>4.002 ± 1.28</td>
</tr>
<tr>
<td>1 ml/kg</td>
<td>1.12 ± 0.03*</td>
<td>32.9</td>
</tr>
<tr>
<td>5 ml/kg</td>
<td>0.94 ± 0.18*</td>
<td>43.7</td>
</tr>
<tr>
<td>10 ml/kg</td>
<td>0.87 ± 0.19*</td>
<td>47.9</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. (n=6). *P<0.05 significantly different from control (Student’s t-test).

### Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Carrageenin-induced rat paw edema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increase in paw diameter</td>
</tr>
<tr>
<td>Control</td>
<td>0.54 ± 0.08</td>
</tr>
<tr>
<td>1 ml/kg</td>
<td>0.35 ± 0.005</td>
</tr>
<tr>
<td>10 ml/kg</td>
<td>0.2 ± 0.07</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. (n=6). *P<0.05 significantly different from control (Student’s t-test).
DISCUSSION

This study is aimed to evaluate the scientific basis and to clarify the traditional use of PHOE against pain and inflammation as a painkiller in folk medicine. Acetic acid induced abdominal constriction is used method for evaluation of peripheral analgesic effect (Gene et al., 1998). The results showed that PHOE exhibited antinociceptive potency against chemical induced nociception (Pain) in mice. It significantly inhibit the abdominal constriction induced by acetic acid in mice. Acetic acid cause inflammatory pain by inducing capillary permeability (Amico--Roxas, 1984) and cause an increase in peritoneal fluids of PGE2 and PGF2α which increases the sensitivity of nociceptors and perception of pain (Daeaedt et al., 1980; Bentley et al., 1983). Therefore, the observed effects in this study suggest that at all doses, PHOE analgesic effect could be peripherally mediated, might have occurred via prostaglandin synthesis inhibition.

Injection of formalin in sub-planter surface of right hand paw of mice produces a distinct biphasic pain, termed early and late phase. Shibata et al. (1989) reported that drugs that act primarily on the central nervous system inhibited both phases equally while peripherally acting drugs inhibit the late phase. In the present study PHOE inhibit both phases of formalin test indicated that central and peripheral both mechanisms are involved. However, higher inhibition was observed in the late phase strengthening the peripheral analgesic action of PHOE in addition to central mechanism of analgesia.

Earlier studies had indicated that carrageenin induced inflammation is the standard experimental model for acute inflammation, is known to be a peripheral process (Ahmadiani et al., 2000). Effect of the PHOE on carrageenin-induced paw edema was most pronounced and results showed that suppress the exudates inflammation, therefore, suggest that it possesses anti-inflammatory activity.

In our opinion, all the constituents of PHOE are used in Azerbaijani traditional medicine, Chinese pharmacopoeia and American conventional medicine in relieving pain and inflammation. All the effects independently produced by various ingredients in PHOE are producing a total beneficial effect.

The present results indicate that PHOE used traditionally for the treatment of pain and inflammation have ability to reduce the number of writhes, inhibit both phase of formalin-induced pain and suppression of carrageenin induce edema. Apparently analgesic effect of PHOE is probably mediated via inhibition of prostaglandin synthesis, suggesting more of a peripheral activity and confirms its analgesic and anti-inflammatory properties that validates the claim and folkloric use of PHOE in Unani system of medicine for the management of pain.

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REFERENCES


Antinociceptive Effects of Poly Herbal oil extract


