“A STUDY OF ANALGESIC ACTIVITY OF AQUEOUS EXTRACTION OF POLYHERBAL FORMULATION IN RATS”

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ABSTRACT

This study was performed to investigate the analgesic activity of aqueous extraction of the seeds of *Coriandrum Sativum* and the seeds of *Nelumbo Nucifera* in rats using the Eddy’s hot plate method. The extract was administered orally in two different doses of 100mg/kg and 200mg/kg were able to decrease the analgesic activity in rats. This effect was comparable to that of the paracetamol (10mg/kg p.o.). These results indicate that polyherbal formulation is an effective analgesic agent.

KEYWORDS

Analgesic-like effect, *Coriandrum Sativum*, *Nelumbo Nucifera*, Eddy’s hot plate and Paracetamol.

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INTRODUCTON

Coriander (*Coriandrum Sativum*), also known as cilantro, Chinese parsley or dhania1 is an annual herb in the family Apiaceae. Coriander is native to regions spanning from southern Europe and North Africa to south western Asia. It is a soft plant growing to 50cm (20in) tall. The leaves are variable in shape, broadly lobed at the base of the plant, and slender and feathery higher on the flowering stems. The flowers are borne in small umbels, white or very pale pink, asymmetrical, with the petals pointing away from the centre of the umbel longer (5-6 mm or 0.20-0.24 in) than those pointing toward it (only 1-3 mm or 0.039-0.118 in...
The fruit is a globular, dry schizocarp 3-5 mm (0.12-0.20 in) in diameter. Although sometimes eaten alone, the seeds often are used as a spice or an added ingredient in other foods. The dry fruits are known as coriander seeds. In Indian cuisine they are called dhania. The word coriander in food preparation may refer solely to these seeds (as a spice), rather than to the plant. The seeds have a lemony citrus flavour when crushed, due to terpenes linalool and pinene.

AIM AND OBJECTIVE OF THE STUDY
Aim
The present study was taken up for evaluating the analgesic activity of aqueous extracts of the seeds of the Coriandrum Sativum and the seeds of the Nelumbo Nucifera.

Objective
The objective of present study are-
1. To prepare aqueous extract by successive extraction technique of the seeds of Coriandrum Sativum and the seeds of the Nelumbo Nucifera. Analyze them for the presence of phytoconstituents.
2. To establish pharmacological profile of the seeds of Coriandrum Sativum and the seeds of the Nelumbo Nucifera.

To assess analgesic activity in rats by following model:
Eddy’s hot plate model
Parameters to study: basal reaction time

Coriander

Plant kingdom
Kingdom - plantae
Order - Apiales
Family - apiaceae
Genus - coriandrum
Species - C.sativum
Synonym
Coriander-Coriander fruits.

Biological source

These are the fully dried ripe fruits of the plant known as Coriandrum Sativum linn (family-umbelliferae). The fruits contain not less than 0.3% of the volatile oil.

Geographical source

Plant is cultivated throught European countries, principally in Russia, Hungary and Holland. It is also cultivated in India, Egypt and morocco. In India, it is widely cultivated in Andhra Pradesh (Guntur, Anantpur), Maharashtra (jalgon and satara) west Bengal (Howrah and 24-paragana districts) uttarapradesh, Rajasthan and Jammu Kashmir.

Chemical constituents

Coriander containing 0.3% of 1% of volatile oil, fixed oil 13%, proteins 20%, 90% of D-linalool (coriandrol), coriandryl acetate, L-borneol, geraniol, flavonoids and pinene. Coriander leaves are rich in vitamin A. The fruit yields 5-7% ash. During ordinary storage of crude drug. The volatile oil composition alters considerable.

Nelumbo Nucifera

Scientific classification
Botanical name: Nelumbo Nucifera

Biological source

It is the fully dried seeds obtained from the plant of Nelumbo Nucifera belonging to the family nelumbonaceae.

Geographical source

Plant is cultivated throught European countries, principally in Russia, Hungary and Holland. It is also cultivated in India, Egypt and morocco. In India, it is widely cultivated in Andhra Pradesh (Guntur, Anantpur), Maharashtra (jalgon and satara) west Bengal (Howrah and 24-paragana districts) uttarapradesh, Rajasthan and Jammu Kashmir, China, Japan and North America.

Chemical constituents

Lotus containing the Flavonol - Miquelianin
Alkaloids-coclaurine and non coclaurine
The plant also containing Nuciferine and Aporphine.

PLAN OF WORK
MATERIAL AND METHODS

Plant materials

The seeds of the Coriandrum Sativum and the seeds of the Nelumbo Nucifera of were collected from A.M Reddy Memorial College of Pharmacy and local market petlurivaripalam, Narasaropet. The plant materials were identified and authenticated (prof. Dr. P. Satyanarayanaraju) at botany and...
microbiology department, Acharya Nagarjuna University, Guntur.

Preparation of extracts
The seeds of *Coriandrum Sativum* and *Nelumbo Nucifera* were washed, cutted into small pieces, and dried under shade. Coarse powder of the seeds was made and extracted by 70% aqueous (distilled water) for 72h at room temperature. The whole extract of individual plants was collected in conical flasks, filtered and the solvents were evaporated to dryness under reduced pressure. The poly-herbal formulation extract was then analyzed by qualitative tests and was found to contain carbohydrates, flavonoids, glycosides, alkaloids, saponins, tannins, triterpens and steroids.

Animals
Whister rats weighing 180-210 g of either sex were used for the study. The animals were housed in solid-bottomed polypropelene cages and acclimatized to animal house conditions. The rats were fed with commercial rats diet and water adlibitum. The experiments were designed and conducted in accordance with ethical norms approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPSCEA) and Institutional Animal Ethical Committee (IAEC).

Preparation of poly-herbal formulation
Poly-herbal formulation was prepared according to ED50 of individual herbs. ED50 of individual plants was found to be *Coriandrum Sativum* and *Nelumbo Nucifera* (low dose-100mg/kg, high dose-200mg/kg, p.o). The % contents of individual plant extract in poly-herbal formulation. The poly-herbal formulation was administered oral route at doses of 100mg/kg, 200mg/kg in the form of suspension prepared in double distilled water containing gum acacia (2% w/v) were purchased from local market.

The quantity of aqueous extracts of seeds required for formulating herbal drug (Group-3, 4, 5) are calculated on the basis of human dose of powder form and percentage practical yield of respective crude drugs. Two formulations are prepared using 2% w/v gum acacia as suspending agent and considered as Lower dose and higher dose formulation 100mg/kg, 200mg/kg.

Drug treatment
Whister rats were divided into five groups of 6 animals each.
Group I - Received control
Group II - Received standard drug 10mg/kg of paracetamol, i.p.
Group III - Received (test-A) low dose-100mg/kg and high dose-200mg/kg p.o.
Group IV - Received (test-B) low dose-100mg/kg and high dose-200mg/kg, p.o.
Group V - Received (test-A+B) low dose-100mg/kg and high dose-200mg/kg, p.o.

Acute toxicity study
Acute toxicity study was performed in accordance with OECD guidelines. No adverse effect or mortality was detected in whister rats up to 180-210gm/kg, p.o of poly-herbal formulation during the 24 to 72 hrs observation periods. For this period the rats were continuously observed for 5 hrs for any gross behavioral, neurological or autonomic toxic effect and lethally after 24 to 72 hrs. Acute toxicity studies were carried out on Wister rats according to method proposed by Ghosh. The prepared formulation were subjected to toxicity study and were found to be safe up to daily dose of 400 mg/kg of body wt. in rats of either sex with no toxic reaction being observed.

DETEMINATION OF ANALGESIC ACTIVITY
Eddy’s hot Plate Method
Instrument description
The hot-plate method performs rapid and precise screening of analgesic drug properties on small laboratory animals according to the ‘hot plate test’. The animal’s pain sensitivity alterations induced by a specific experimental context change and or genetic manipulations can be evaluated by this method. The hot-plate test initially described by N. B. Eddy and D. Leimbach (1953) evaluates thermal pain reflexes due to footpad contact with a heated surface. During the experiments, the animal is confined in a removable clear acrylic cylinder where the latency time to the first hind paw or jumping response is measured. In the hot plate, a thick aluminium plate (10mm) provides a high temperature stability and even
surface distribution. The plate temperature can be held at a set point between 45 and 62°C (+/-0.1°C) by multiple proportional feedback circuits that minimize overshoot. A built-in timer activated by an external foot switch allows precise measurement of reaction (0.1 sec precision).

A remote foot-switch controls the test start/stop allowing rapid hands-free experiments. The operator can read the animal reaction time from the display or from the PC computer using the SeDaCom software. Trail number, plate temperature and reaction time are sent to PC through a RS-232 port.

**DISCUSSION**

The medicinal plant is poly herbal formulations (*Coriander Sativum, Nelumbo Nucifera*) is collected from the medicinal plant garden of A.M Reddy Memorial College of Pharmacy, Petlurivaripalam, Narasaraopet, Guntur (d.t). This plant authentication was done in department of botany in Acharya Nagarjuna University.

In order to test the analgesic activity we have taken the reference or standard drug paracetamol. We selected the 30 rats for control, test and standard samples with regular intervals and taken the wash out period three days for each type of sample. Inject the sample to the rats through intraperitoneal route and oral dose in certain doses at regular intervals of time 0min, 15min, 30min, 45min, 60min and note the basal reaction response (jump response) in seconds by using Eddy’s hot plate method at 55°C.

<table>
<thead>
<tr>
<th>Kingdom: Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>(unranked): Angiosperms</td>
</tr>
<tr>
<td>(unranked): Eudicots</td>
</tr>
<tr>
<td>Order: Proteales</td>
</tr>
<tr>
<td>Family: Nelumbonaceae</td>
</tr>
<tr>
<td>Genus: Nelumbo</td>
</tr>
<tr>
<td>Species: <em>N. nucifera</em></td>
</tr>
</tbody>
</table>

- *Nelumbiumspeciosum* Willd.
- *Nymphaeanelumbo*
- *Nymphaeastellate*

Compare the response of control, standard drug and observe the responses by plotting the time (min) on X-axis and basal reaction response (jump response) in sec on Y-axis. So the standard response is increases compare to control, the standard response has more significant value.

Test - A when compare with aqueous extract low dose and high the aqueous high dose shown more significant value than control.

Test - B when compare with aqueous extract low dose and high the aqueous high dose shown more significant value than control.

Test - A+B (mixed) when compare with aqueous extract low dose and high the aqueous high dose shown more significant value than control (Table No.1 and Graph No-1-7).
### Table No.1

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Basal reaction (jump response) in sec (avg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>1</td>
<td>Group 1-Control</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Group II-Standard</td>
<td>100mg/kg</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Group III-Test-A</td>
<td>100mg/kg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(a)aqueous extract-low</td>
<td>200mg/kg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(b)aqueous extract-high</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Group IV-Test-B</td>
<td>100mg/kg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(a)aqueous extract-low</td>
<td>200mg/kg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(b)aqueous extract-high</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Group V-Test -A+B</td>
<td>100mg/kg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(a)aqueous extract-low</td>
<td>200mg/kg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(b)aqueous extract-high</td>
<td></td>
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</tr>
</tbody>
</table>

**Graph No.1: Control + Standard**

**Graph No.2: Control + standard + test (A) aqueous-low**
Graph No.3: Control + standard + test (A) aqueous-high

Graph No.4: Control + standard + test (B) aqueous-low

Graph No.5: Control + standard + test (B) aqueous-high
Figure No.1
CONCLUSION
In pharmacological screening method, the polyherbal formulations (coriander, lotus) seeds extraction when administered in rats shown less potent analgesic activity when compared to the standard drug, by using Eddy’s hot plate method maintained at 55°C temperature.

The phytochemical study it was proved that flavanoids, glycosides, steroids, alkaloids, tannins, saponins, reducing sugars, triterpenes and carbohydrates are present. From the study it was shown that the Aqueous extract low and high doses shown more significant response when compare with control. And it was proved that poly herbal formulation shows fewer side effects than individual. So the present study was in poly herbal formulation.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST
We declare that we have no conflict of interest.

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7. Wheat Beers.
15. Shen-Miller et al. "Long-living lotus: germination and soil gamma-irradiation of centuries-old fruits, and cultivation, growth, and phenotypic abnormalities of offspring", American Journal of Botany, Retrieved 2010-02-03, Sacred lotus (Nelumbo nucifera) has been cultivated as a crop in Asia for thousands of years, An ~1300-yr-old lotus fruit, recovered from an originally cultivated but now dry lakebed in northeastern China, is the oldest germinated and directly 14C-dated fruit known, In 1996, we traveled to the dry lake at Xipaozi Village, China, the source of the old viable fruits, 2002.