ANALGESIC ACTIVITY OF CINNAMALDEHYDE PER SE AND IT'S INTERACTION WITH DICLOFENAC SODIUM AND PENTAZOCINE IN SWISS ALBINO MICE.

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ABSTRACT: Cinnamon is one of the best-known spices used as an herbal medicine. Cinnamaldehyde is the most important constituents of cinnamon. The present study was aimed to evaluate the analgesic activity of Cinnamaldehyde per se and its interaction with diclofenac sodium and pentazocine in Swiss albino mice. Healthy mice of either sex weighing 20-30 grams were divided into 6 groups of 6 animals each. Peripheral analgesic activity was evaluated by acetic acid induced writhing test and central analgesic activity was studied using Eddy's hot plate method. Cinnamaldehyde (100 and 200 mg/kg) and its combination (Cinnamaldehyde 100 + 2.5 mg/kg standard drug), diclofenac sodium (2.5 and 5 mg/kg) and pentazocine (2.5 and 5 mg/kg) were given orally. Acetic acid induced writhing model showed that diclofenac sodium at doses of 2.5 and 5 mg/kg reduces writhing 44% and 66% respectively as compared to control when administered alone. Cinnamaldehyde at 100 and 200 mg/kg showed dose dependent decrease in writhes 54% and 81%, but when Cinnamaldehyde (100 mg/kg) was Co administered with diclofenac sodium (2.5mg/kg) showed significant decrease in writhes 84.43% with respect to control. While in Eddy's hot plate method Cinnamaldehyde not only showed hyperalgesia when given alone as compared to control, but also decreases the analgesic effect of pentazocine when combined with pentazocine in comparison with pentazocine alone and control group. The findings suggest that the Cinnamaldehyde significantly increases the analgesic activity of diclofenac sodium, but decreases the analgesic activity of pentazocine.

INTRODUCTION: Pain is one of the most common and frequent complaints of human being. It can be either defined to one specific area or it can be generalized to whole body. Perception of pain is a normal physiologic response of healthy nervous system and is mediated by various mediators like prostaglandins, serotonin, substance P etc. Currently there are various medications available for pain. Depending on the type of pain, either NSAIDs or opioids can be used. But there are various adverse effects related to these medications.

NSAIDs has high risk of gastric ulcers similarly opioids has wide range of CNS adverse effects like tolerance, dependence, CNS depression etc. As an alternative to NSAIDs and opiates newer analgesic drugs with lesser or negligible adverse effects along with high efficacy are being searched all over the world. According to WHO, about 80% of the world population still rely mainly on plant based drugs.

Cinnamon is one of the oldest and best-known spices in the world and is used as an herbal medicine. It belongs to family lauraece and is found in South India, Sri Lanka, Indonesia, Vietnam, Bangladesh and Nepal. Commonly known as dalchini, darchini or dhall cheene in Hindi. The active component of commercial cinnamon is the dried inner stem-bark of aromatic evergreen tree 10-15 meters tall. The most important constituents of...
Cinnamon are Cinnamaldehyde and eugenol, which are present in the essential oil of the bark thus contributing to the fragrance and to the various biological activities observed with cinnamon.\(^6\)

Cinnamon has been investigated for antioxidant property \(^7\), inhibition of tau aggregation \(^8\), anti-inflammatory activity \(^9\), anti-nociceptive activity \(^10\), peptic ulcer protection effects \(^11,12\) effect on cardiovascular system \(^11,13,14\) and hepatoprotective effects \(^15\), antihyperlipidemic activity \(^16\) and antidiabetic \(^17\). The present study was planned to investigate whether Cinnamaldehyde has any analgesic activity per se and how does it interact with diclofenac sodium and pentazocine?

**MATERIALS AND METHODS:**

Drugs and chemicals:
Cinnamaldehyde (CNM) was obtained from Science centre (Sunchem Pharma), Indore, Madhya Pradesh, India, having 98% purity. Tween twenty was purchased from the same and used as vehicle. Acetic acid - Ranbaxy lab limited, Mumbai, diclofenac sodium (DICLO) (Voveron injection, 25 mg/ml- 3 ml ampoule- Novartis Pharmaceuticals) and pentazocine (PTZ) (Fortwin injection, 30 mg/ml – 1ml ampoule, Ranbaxy pharmaceuticals Ltd.) were purchased from their authorized representatives.

Animals:
Swiss albino mice (20-30 gm) of either sex were used for the study. The animals were housed in polypropylene cages in central animal house. The rooms were maintained at the temperature of 25 ±5°C with 12 hour light/dark cycles. All the animal experiments were carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India guidelines. The animals were fasted overnight prior to the experiment and were given only water ad libitum. The study was approved by Institutional Animal Ethics Committee (IAEC), M.G.M. Medical College, Indore, India (IAEC-709/20/2010, dated 25/03/2015) and the work was conducted in the Department of Pharmacology.

Preparation of drugs for animal experiment:

CNM and other drugs, were dissolved in tween twenty 20% to maintain uniformity of the solvent.

**Experimental design:**
Animals were grouped in 6 groups comprising of 6 animals each. (N=6, n=6). All the drugs were administered orally as per the grouping done to all animals at the stipulated doses. The grouping done as follows:-
- Group I - Tween-20 20% (10ml/kg),
- Group II - Diclofenac sodium for peripheral activity and pentazocine for central activity (2.5mg/kg),
- Group III - diclofenac sodium or pentazocine 5mg/kg (as per the peripheral or central activity),
- Group IV- CNM (100mg/kg),
- Group V - CNM (200mg/kg), and
- GroupVI – CNM + diclofenac sodium or pentazocine (100+2.5mg/kg)

Procedure and Experiment:
Antinociceptive activity was assessed by two different models of nociception.

1. Acetic acid induced Writhing method to demonstrate peripheral analgesic activity:
**Principle:** Writhing method was used for the evaluation of peripheral analgesic activity. Painful reactions can be produced in experimental animals by applying noxious stimuli using chemical irritants such as acetic acid and bradykinin. Acetic acid induces writhing after intra peritoneal administration in mice.

**Procedure:**
Abdominal constrictions were induced, by 1 % v/v glacial acetic acid solution (10 ml/kg, i.p.) \(^18\), after 1 hour of group wise drug treatments. The number of abdominal writhes were measured over 10 min after the intra peritoneal injection of acetic acid. Results are expressed as percentage inhibition of abdominal constrictions with respect to control, total number of writhes and onset time of writhes were recorded. Abdominal constriction followed by extension of at least one hind limb is considered as one writh.

2. Eddy’s Hot plate model to demonstrate analgesic activity:
**Principle:** Painful reactions can be produced in experimental animals by applying noxious stimuli such as thermal – using radiant heat as a source of pain, chemical – using irritants such as acetic acid...
and bradykinin and physical pressure – using tail compression.

In the Eddy’s hot plate model, the animals are placed on the Eddy’s hot plate which consists of an electrically heated surface. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics (opioid analgesics), whereas peripheral analgesics do not generally affect these responses.\(^{19}\)

**Procedure:**

Animals were weighed, dosed according to grouping and placed on the hot plate maintained at temperature of 55±1 °C. Responses such as jumping, withdrawal and licking of the paws were seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid tissue damage of the animals 10 seconds was kept as a cut off time.\(^{20,21}\)

The time obtained in all the untreated groups of animals was considered as basal reaction time. Increase in the basal reaction time was the index of analgesia. All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. After selecting the animals, the drugs were administered to all animals as per grouping. The reaction times of the animals were then noted at 0.5, 1, and 2 hrs interval after drug administration.

**Statistical analysis:**

SPSS-20.00 statistical computer software was used to evaluate the results. Results are expressed as mean ± SEM. One way ANOVA followed by Tukey’s test was applied for multiple comparisons amongst different groups. \(p<0.05\) was regarded as statistically significant.

**RESULTS:**

**Analgesic activity of individual drugs:**

(a) Cinnamaldehyde: In acetic acid induced writhing method, CNM at the dose of 100 and 200 mg/kg showed significant decrease in number and delayed onset of writhes in the dose dependent manner \((p<0.05)\). Table 1

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Dose (p.o., mg/kg)</th>
<th>Number of writhes in 10 minutes</th>
<th>Percentage inhibition with respect to control</th>
<th>Onset of writhes in sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween-20 20%</td>
<td>10 ml/kg</td>
<td>32.67±1.74</td>
<td>–</td>
<td>97.67±5.85</td>
</tr>
<tr>
<td>DICLO 2.5</td>
<td></td>
<td>18.50±0.76*</td>
<td>43.38%</td>
<td>181.67±7.14*</td>
</tr>
<tr>
<td>DICLO 5</td>
<td></td>
<td>11.00±0.57&quot;</td>
<td>66.33%</td>
<td>276.67±4.75&quot;</td>
</tr>
<tr>
<td>CNM 100</td>
<td></td>
<td>15.33±0.95*</td>
<td>53.08%</td>
<td>416.50±4.51*</td>
</tr>
<tr>
<td>CNM 200</td>
<td></td>
<td>6.17±0.60*†</td>
<td>81.12%</td>
<td>402.33±5.51*†</td>
</tr>
<tr>
<td>CNM+DICLO 100+2.5</td>
<td></td>
<td>5.00±0.70*†</td>
<td>84.70%</td>
<td>402.33±5.51*†</td>
</tr>
</tbody>
</table>

One way ANOVA followed by multiple tukey’s comparison test. Values are mean ± SEM, \(n= 6\) in each group, \(df = 5, 30\)

\* \(p<0.05\), as compared to control.

\# \(p<0.05\) as compared to diclofenac 2.5 mg/kg.

\† \(p<0.05\) as compared to diclofenac 5 mg/kg.

Note:- Percent reduction = \([1 – (mean of writhes in test or standard group / mean of writhes in control group)] \times 100\)

While on Eddy’s hot plate it showed hyperalgesic effect at the dose of 200 mg/kg as compared control and pentazocine during all time points of study \((p<0.05)\). But with 100 mg/kg dose hyperalgesia was observed only at 0.5 hr as compared to control \((p<0.05)\) and at all time point when compared with pentazocine (5mg/kg). Table 2.

(b) Diclofenac sodium: Diclofenac sodium at a dose of 2.5 and 5 mg/kg produced significant decrease in number and delayed onset of writhes as compared to control \((p<0.05)\). Table 1

(c) Pentazocine: In Eddy’s hot plate method, with a dose of 5 mg/kg of pentazocine, significant antinociceptive effect was observed at 0.5 hr after
the treatment and persisted for the entire test period ($p<0.05$). With the subtherapeutic dose of pentazocine (2.5mg/kg) the antinociceptive effect was observed only at 2hr ($p<0.05$). **Table 2**

**Analgesic activity of combinations:**

(a) CNM and diclofenac sodium: Diclofenac sodium (2.5mg/kg) in combination with CNM (100mg/kg) produced significant decrease in number of writhes when compared to control value or either of the treatment alone ($p<0.05$). **Table 1**

(b) CNM and pentazocine: When CNM (100mg/kg) given in combination with pentazocine (2.5mg/kg) decreases the reaction time as compared to control and pentazocine 2.5 mg/kg significantly ($p<0.05$). **Table 2**.

No toxicity or mortality was observed during observation period of seven days after the completion of experiment.

### DISCUSSION:

Thus in the present study, Cinnamaldehyde (CNM) was studied for its analgesic potential in both peripheral (non-narcotic) and central (narcotic) type of pain models. Diclofenac sodium (2.5 and 5 mg/kg, p.o.) and pentazocine (2.5 and 5 mg/kg, p.o.) were used as standard drugs for comparing analgesic effects at peripheral and central levels, respectively.

The study on peripheral analgesic activity using the glacial acetic acid (1%) induced writhing method showed that CNM has peripheral analgesic potential. CNM increases pain threshold in Swiss albino mice in a dose dependent manner i.e. 100mg/kg and 200mg/kg. There was significant decrease in number of writhes ($p<0.05$) and also delay in the onset time of writhes ($p<0.05$) with CNM 200 mg/kg with respect to control and diclofenac 2.5 mg/kg and diclofenac 5 mg/kg.

The combination group (i.e. CNM 100mg/kg and diclofenac sodium 2.5mg/kg), showed significantly better analgesic activity as compared to control and standard drug groups (i.e. half and full dose of diclofenac sodium). ($p<0.05$)

CNM has hyperalgesic effect, at both doses i.e. 100mg/kg and 200mg/kg and it remained for whole study period i.e. 0hr, 1hr, 2hr, as compared to control and standard drug groups. ($p<0.05$) but there is no significant difference between hyperalgesic effect of both CNM doses ($p>0.05$).

On the contrary study for central analgesic activity reveals that CNM has hyperalgesic effect in a dose dependent manner and when it was given in combination with pentazocine it markedly decreased the analgesic effects of pentazocine. **Table 2**

When CNM (100mg/kg) was given in combination with pentazocine (2.5mg/kg), we found significant decrease in analgesic activity as compared to control and pentazocine (5 mg/kg) alone ($p<0.05$). The decreased analgesia, compared to the standard group pentazocine (5mg/kg), was evident as early as 0.5hr, and remained for the entire duration of study.
If we analyze the above findings we can say that the CNM showed hyperalgesic activity not only when given alone but also showed the same effects when it was combined with even subtherapeutic dose of standard drug. The lowering of analgesic effect response of pentazocine probably can’t be explained by peripheral pharmacokinetic interactions like lowered absorption of pentazocine by CNM or increased metabolism of pentazocine by microsomal enzyme system because hyperalgesic activity was seen with incremental dose of CNM per se also, which could be due to antagonism of endogenous narcotic endopeptides like endorphins, enkephalins and dynorphins which are acting on various narcotic receptors like mu, kappa and delta. This activity is similar to narcotic antagonist drugs like naloxone and naltrexone. The hyperalgesic activity of CNM could be due to stimulation of nociceptive receptors (NOP) or through agonistic action on Transient Receptor Potential Ankyrin 1 (TRPA1) which are involved in process of nociception and hyperalgesia.\(^\text{22, 23}\)

Pain is a complex process mediated by many physiological mediators e.g. prostaglandins, bradykinin, substance P etc. In the acetic acid induced writhing model the contractions induced by acetic acid in mice results from an acute inflammatory reaction with production of PGE2 and PGF2\(_{\alpha}\) in the peritoneal fluid.\(^\text{24, 25}\) Therefore, it is likely that CNM might suppress the formation of these substances or antagonize their action for exerting analgesic activity. Non steroid anti inflammatory drugs inhibits COX and thereby inhibits production of prostaglandins.\(^\text{26}\) The similar analgesic activity has been reported with CNM by Atta & Alkofahi in 1998\(^\text{10}\) and Annegowda HV in 2012.\(^\text{7}\) Against gastric ulceration\(^\text{12}\) If we consider this as a principle mechanism of CNM, then like prostaglandin synthesis inhibitors it must impair the mucosal defence of GIT by increasing gastric acid secretion, reducing mucus formation and increasing mucosal permeability. However the study of Alqasoumi S \textit{et al} showed that CNM is protective against gastric ulceration.\(^\text{11}\) Thus it appears that the analgesic activity of CNM is due to some other mechanism rather than inhibition of prostaglandin synthesis.

The pharmacokinetic reasons may be responsible for the enhancement of analgesia of low dose of diclofenac. This could be due to increased blood flow due to GIT irritation with consequent enhanced absorption. CNM may improving the absorption of drugs, perhaps by increasing GI blood flow by vasodialatation.\(^\text{11, 13}\)

Further studies are needed to reveal the exact mechanism of action responsible for the enhanced activity of diclofenac sodium and decreased activity of pentazocine. However the study adds to our concept that CNM can enhance the activity of diclofenac and inhibit the activity of pentazocine. So the addition of CNM may reduce the required dose of diclofenac that may help in reduction of toxicity. Contrastingly co administration with pentazocine may cause the requirement of higher dose lead to increased toxicity. Hence further studies are required to acknowledge these facts.

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**REFERENCES:**

8. George RC, Lew J, Graves DJ. Interaction of cinnamaldehyde and epicatechin with tau: implications of