Analgesic and Antipyretic Activities of Gindarudine, a Morphine Alkaloid from *Stephania glabra*

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**Abstract:** Gindarudine (GN), a morphine alkaloid isolated from the tubers of *Stephania glabra* (Menispermaceae), was evaluated for analgesic and antipyretic activities because of closely resembling structure to that of thebaine. The analgesic activity of GN was evaluated on albino mice by hot plate and tail immersion methods whereas antipyretic activity was studied on Brewer’s yeast-induced pyrexia rats. Fever was induced by injecting 20 ml/kg (s.c.) of 20% aqueous suspension of Brewer’s yeast in normal saline and rectal temperature was recorded by clinical thermometer immediately before (-18 h) and 18 h after (0 h) yeast administration. GN at doses of 100 and 150 mg/kg, p.o. showed significant analgesic activity (p<0.05) by increasing the threshold potential of pain whereas doses of 200 and 300 mg/kg exhibited significant (p<0.05) antipyretic effect by decreasing the rectal temperature of rats in 1st, 3rd and 5th h after treatment. Aspirin (300 mg/kg, p.o.) and paracetamol (200 mg/kg, p.o.) were used as standard drugs for analgesic and antipyretic activities respectively. These findings demonstrate that GN have remarkable analgesic and anti-pyretic activities when compared with positive control and thus have great potential as a source for natural health products.

**Keywords:** Gindarudine, Menispermaceae, pyrexia rats, rectal temperature, *Stephania glabra*.

1. INTRODUCTION

Analgesics are primary need of patients to get rid of any kind of pain. Pain is one of the basic symptoms of all human ailments which is a sensorial modality and primarily protective. Analgesics only relieve pain in a particular complaint without affecting its cause [1]. The most eminent analgesics include opiates and NSAIDs, but they are not helpful in all cases due to their adverse effects. Consequentially, the new compounds having potent painkiller action without adverse effects are being required to investigate. Besides pain, the fever is another most common symptom of sickness which is caused by increase in the body temperature of an individual at a particular time [2]. In recent years, the natural analgesics and antipyretics of plant origin are being appreciated due to possible toxicity of synthetic drugs.

Gindarudine (GN), a morphine alkaloid has been isolated from the tubers of *Stephania glabra*. The structure including stereochemistry of the compound was described earlier [3]. The structure of GN was closely related to thebaine, a morphine derivative (Fig. 1). Since, the morphine alkaloids are well known for their analgesic, antipyretic and narcotic properties. Therefore, the new morphine alkaloid (GN) was evaluated for its analgesic and antipyretic potency in albino mice and Brewer’s yeast-induced pyrexia rats respectively.

2. MATERIALS AND METHODS

2.1. Drugs and Chemicals

Aspirin and paracetamol were supplied from Ind-Swift Ltd, Parwanoo, India. The doses were prepared with normal saline. The saline was prepared with 0.9% NaCl (Qualigens, Mumbai) in distilled water.

2.2. Isolation of Gindarudine

Air dried and finely powdered tubers of *S. glabra* were extracted with ethanol. The dried extract was subjected to silica gel column chromatography in chloroform by increasing polarity of methanol. The fraction from 10% methanol afforded brown crystals of GN (1).

2.3. Evaluation of Analgesic Activity

The analgesic activity of the GN was carried out by following the various researches [4,5]. Albino mice of either sex weighing about 40-50 g were selected and kept under a conventional light regimen with a dark night at room temperature (about 25 °C) and humidity. Animals were housed in plastic bottom cages and were allowed free access to standard laboratory feed and water. Experiments were performed according to the guidelines for the care and use of laboratory animals, from the CPCSEA, Ministry of Environment and Forest, Govt. of India. All the animals have been divided into five groups (I-V) and placed in separate cages, each

![Fig. (1). Chemical structure of gindarudine (1) and thebaine (2).](image-url)
consisting of 6 animals. The animals were acclimatized to the laboratory condition for one week before the onset of experiment. All the selected animals were kept fasted throughout the experimental period, but allowed free access to water ad libitum. The normal control group (I) only received normal saline. The test groups (II-IV) were treated with oral dose of 50, 100 and 150 mg/kg, p.o., of GN. The standard group (V) was treated with aspirin (300 mg/kg, p.o.). The analgesic activity of GN was determined by following two methods hot plate and tail immersion methods.

2.4. Tail Immersion Method

The tail immersion method [6] was applied in order to determine the analgesic activity of GN. The tail part (3 cm) of mice was immersed in a water bath (SISCO, India) maintained at a temperature of 55.00 ± 1.5 °C. A latency period of 20 s was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The time of just prior to reacting the pain was measured by a stop watch as the initial reaction time (Ti). After measuring the time (Ti), all the groups of animals (I-V) were administered orally by normal saline, GN (50, 100 and 150 mg/kg body weight) and aspirin (300 mg/kg body weight) respectively. After test and reference drugs administration, the time of response (Tr) between the onset of immersion and the withdrawal of the tail was measured at 0.5, 1, 1.5, 2, 2.5 and 3 h after a latency period of 30 min for each animal group. The analgesic activity was calculated in percent by following expression:

\[
\text{Analgesic activity (\%) } = \left( \frac{\text{Tr}}{\text{Ti}} \right) \times 100
\]

2.5. Hot Plate Method

The hot plate method was performed for all above groups of animals (I-V) according to described method of Toma et al. [7]. Each mouse was placed on Eddy’s hot plate (55±2 °C) with a cut off period of 20 s to avoid damage in the paw [8]. The reaction time for the mice to respond the thermal pain (licked fore or hind paws or jumped) was recorded at 0.5, 1, 2 and 3 h after oral administration of test and standard drugs. The reaction time for each treated group (Tr) was determined and compared with that obtained before treatment (To). Analgesic activity percentage was derived for each time by following expression:

\[
\text{Analgesic activity (\%) } = \left( \frac{\text{Tr}}{\text{To}} \right) \times 100
\]

2.6. Evaluation of Antipyretic Activity

The antipyretic activity of GN was evaluated using Brewer’s yeast-induced pyrexia rats by following the method described by Pendota et al. [9]. The animals were housed in the departmental animal house and exposed to normal light. Prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature were only selected for the study. The selected animals were deprived of food for 18 h but allowed free access to water ad libitum. Being deprived of food for 18 h, mice were divided into five groups of six animals each (I-V). Pyrexia was induced in all groups of the animals by injecting 20 ml/kg (s.c.) of 20% aqueous suspension of Brewer’s yeast in normal saline near the groin region of the animals. Rectal temperature was recorded by clinical thermometer (PC-Cheonan Choongnam, Korea) immediately before (-18 h) and 18 h after (0 h) Brewer’s yeast injection. The animals that showed an increase of at least 0.5 °C rise in temperature were only selected for the antipyretic activity by drug administration. The group of normal control (I) was not administered by any test drug and only received normal saline. The test groups (II-IV) were treated with oral dose of 100, 200 and 300 mg/kg, p.o. of test drug GN. The remaining group (positive control, V) was treated with oral dose of paracetamol (200 mg/kg, p.o.) as a standard drug. The rectal temperature of each groups were recorded at 1-5 h post-dosing.

2.7. LD50 Experiment

The animals were administered GN orally at doses of 100, 250 and 500 mg/kg, p.o., body weight and observed continuously for 3 days intermittently up to 12 days for any gross behavioral changes and deaths.

2.7. Statistical Analyses

Results are expressed as the mean ± S.E.M. of 6 independent experiments. The data were analyzed for statistical significance by one-way ANOVA test; P values < 0.05 were considered to be significant.

3. RESULTS AND DISCUSSION

For the determination of analgesic activity in albino mice, tail immersion and hot plate methods were used. The results from these methods were shown in Tables 1 and 2 respectively. GN at different doses (50, 100 and 150 mg/kg, p.o.) showed significant analgesic activity (p<0.05) by increasing the threshold potential of pain (increase reaction time) in comparison to normal control (saline) and aspirin. The dose at 150 mg/kg body weight was found more effective when compared with positive control (aspirin). The antipyretic activity of GN was performed using Brewer’s yeast-induced pyrexia rats and exhibited significant (p<0.05) results by decreasing in the rectal temperature at 1st, 2nd and 5th h after GN administration (Table 3). The dose of 300 mg/kg, GN showed remarkable antipyretic activity when compared with positive control (paracetamol).

The hot plate and tail immersion methods are considered as selective to examine compounds acting through opioid receptor. The doses of GN have been selected on the basis of LD50 experiments because no behavioral changes and lethality were observed upto 500 mg/kg, p.o. in the experimental animals after 12 days of experiments. GN increased “mean basal latency” which indicates that it acts via centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain whereas non-steroidal anti-inflammatory drugs inhibit only peripheral pain [10,11]. In present case the GN inhibited both mechanisms of pain, suggesting that the compound act as a narcotic analgesic. Traditionally, S. glabra used in fever complaints [3] which have been pharmacologically validated by positive response of GN against pyrexia. Pain is the most
Table 1. Analgesic Effects of GN by Tail Immersion Method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Analgesia TFLD or mean Increase in Latency After Drug Administration ± SEM (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+60</td>
</tr>
<tr>
<td>Saline</td>
<td>-</td>
<td>3.33±0.211</td>
</tr>
<tr>
<td>GN1</td>
<td>50</td>
<td>3.167±0.166*</td>
</tr>
<tr>
<td>GN2</td>
<td>100</td>
<td>3.00±0.365</td>
</tr>
<tr>
<td>GN3</td>
<td>150</td>
<td>3.167±0.307*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>300</td>
<td>2.83±0.307</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for six animals in each group; * = p<0.05 vs. normal control (saline)

Table 2. Analgesic Effects of GN by Hot Plate Method

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Reaction Time in Seconds at Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Saline</td>
<td>-</td>
<td>8.66±0.333</td>
</tr>
<tr>
<td>GN1</td>
<td>50</td>
<td>8.33±0.307</td>
</tr>
<tr>
<td>GN2</td>
<td>100</td>
<td>7.50±0.428</td>
</tr>
<tr>
<td>GN3</td>
<td>150</td>
<td>7.50±0.428</td>
</tr>
<tr>
<td>Aspirin</td>
<td>300</td>
<td>9.00±0.365</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for six animals in each group; * = p<0.05 vs. normal control (saline)

Table 3. Effect of GN on BREWER’S Yeast-induced Pyrexia Rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Rectal Temperature in °C at Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-18*</td>
</tr>
<tr>
<td>Saline</td>
<td>-</td>
<td>36.93±0.154</td>
</tr>
<tr>
<td>GN1</td>
<td>100</td>
<td>36.87±0.126</td>
</tr>
<tr>
<td>GN2</td>
<td>200</td>
<td>36.84±0.187</td>
</tr>
<tr>
<td>GN3</td>
<td>300</td>
<td>37.08±0.087</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>200</td>
<td>36.89±0.158</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for six animals in each group; *: temperature just before yeast injection; °: temperature just before drug administration; †: change in temperature following yeast injection; *: p<0.05 vs. normal control (saline)

common complaint of all kinds of injuries or tissue damages. An analgesic can act on peripheral or central nervous system of an individual. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptor site of pain, whereas CNS acting analgesic not only raises the threshold for pain, but after the physiological response to pain it also suppresses the patient’s anxiety and apprehension. Pain is an essential prelude to the repair process [12]. Aspirin offer relief from normal or inflammatory pain by suppressing the formation of pain substances in the peripheral tissues, where prostaglandins and bradykinin were suggested to play an important role in the pain process [13].

It is a well known fact that hypothalamus gland is responsible for rising or decreasing the normal body temperature (37 °C) of an individual which ensures a balance between heat production and heat loss. The disturbance of hy-
pothalamic thermostat leads to rising of body temperature which results in a complaint of fever [14]. The GN at different doses decreased the rectal temperature of Brewer’s yeast-induced pyrexia rats up to 1 °C, suggesting that the GN can act as a potential antipyretic drug. Paracetamol ensures a balance between heat production and heat loss of the body by acting on the hypothalamus gland and thus used as an effective antipyretic agent.

4. CONCLUSION

On the basis of present study, we may conclude that GN produces significant analgesic and antipyretic activities in dose-dependent manner on animal models. By the positive activity of GN against pyrexia, the traditional use of S. glabra has been pharmacologically validated. Since, GN showed remarkable activity when compared with standard drugs. Therefore, GN can be a substitute of synthetic analgesic or antipyretic drugs having adverse effects.

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REFERENCES