Anti-inflammatory, Analgesic and Antipyretic Properties of *Rubus niveus* Thunb. Root Acetone Extract

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ABSTRACT

**Objective:** Traditionally the fruits and other parts of *R. niveus* have been used by local people for the treatment of various ailments. Hence, the present investigation directed towards the anti-inflammatory, analgesic and antipyretic attributes of *Rubus niveus*. **Methods:** The root acetone extract (up to 2000 mg kg⁻¹ b.wt.) of *R. niveus* was used to observe acute toxicity in mice for 14 days. Anti-inflammatory activity was evaluated using carrageenan induced paw edema in rats and croton oil induced ear edema in mice. Acetic acid induced writhing response and Eddy’s hot plate mediated pain reaction in mice were used as the models of study to determine analgesic activity. Antipyretic activity was investigated by yeast induced pyrexia in rats. **Results:** The acute toxicity results demonstrated no mortality and signs of toxicity in the tested groups. The root acetone extract (200, 400 mg kg⁻¹) showed significant anti-inflammatory, analgesic and antipyretic activities. 200 (59.50%) and 400 mg kg⁻¹ (74.52%) doses significantly reduced the paw and ear edema (23.14, 54.33%) compared to the reference indomethacin in the anti-inflammatory testing, the doses also significantly reduced the writhing responses (p<0.01, p<0.001) and increased the latency period in analgesic activity compared to aspirin and morphine. The rectal temperatures of the rats were found to be decreased for both the doses when compared to the paracetamol. **Conclusion:** The present study suggests that *R. niveus* root acetone extract possess appreciable anti-inflammatory, analgesic and antipyretic properties and so it has immense scope as an effective source to develop drug for the treatment of inflammatory related diseases.

**Key words:** Anti-inflammatory, analgesic, antipyretic, *Rubus niveus*, toxicity


INTRODUCTION

Due to rich ethnomedical and pharmacological properties; *Rubus* sp. has been used in folk medicine. The ripen fruits of *R. niveus* are black in color and eaten by local people. The fresh root tips are used for curing excessive bleeding during menstrual cycle. The root tips are made into a paste with water and small pills are made. One pill per day, preferably with butter made from buffalo milk, is taken empty stomach in the morning for 7 days. Root is also used for the treatment of dysentery and diarrhoea.

Use of medicinal plants as a source of relief and cure from various illness is as old as humankind itself. Even today, medicinal plants provide a cheap source of drugs for majority of world’s population. Plants have provided and will continue to provide not only directly usable drugs but also a great variety of chemical compounds that can be used as a starting points for the synthesis of new drug with improved pharmacological properties. The genus *Rubus* is very diverse, includes over 750 species in 12 subgenera and is found on all continents except Antarctica.

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasma fluid and blood cells. The complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. However, many studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs are the major problem during their clinical uses. Therefore, nowadays the development of newer and more substantial anti-inflammatory drugs with lesser side effects is necessary.

The link between inflammation and cancer has been suggested by epidemiological and experimental data and confirmed by anti-inflammatory therapies that show efficacy in cancer prevention and treatment. Cancer-related inflammation leads to the hypothesis that anti-inflammatory agents may have potential as cancer preventative agents. In terms of Nonsteroidal Anti-inflammatory Agents (NSAIDs), there are several...
observational studies suggesting that aspirin reduces risk of certain cancers. Chronic inflammation represents a major pathological basis for tumour development. Although, inflammation acts as host defence mechanism against infection or injury and is primarily a self limiting process, inadequate resolution of inflammatory responses lead to various chronic disorders associated with cancers. In 1863, Rudolf Virchow proposed that chronic inflammation supports carcinogenesis. Since then, accumulating studies support this hypothesis and it is estimated that 20% of all cancers death are associated with chronic infection and inflammation.

On the basis of the previously found strong antioxidant activity we have selected this plant for the present study. Even though, this plant has immense ethnomedicinal value; a survey of literature revealed that the anti-inflammatory, analgesic and antipyretic properties of this plant using animal models have not yet been evaluated. Keeping this in view, the main objectives of the present study was to study the *in vivo* anti-inflammatory, analgesic and antipyretic activities of *R. niveus* root acetone extract to put forward a scope to develop an effective drug for the treatment of inflammatory related diseases.

**MATERIALS AND METHODS**

**Plant material collection and identification:** The fresh plant parts of *R. niveus* were collected from Shola forest of Marayoor, Kerala, India, during the month of September 2010. The collected plant material was identified and authenticated by (Voucher specimen No. BSI/SRC5/23/2010-11/Tech.1658) Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu.

**Processing and extraction:** Collected plants were cleaned properly, separately shade dried and powdered. The powdered root was extracted in Soxhlet apparatus using acetone. The extract was concentrated to dryness under reduced pressure in a rotary evaporator to yield dried root acetone extract.

**Animals and acute toxicity study:** Healthy wistar albino rats (150-180 g) and mice (25-30 g) of either sex and of approximately the same age were used for the study. They were fed with standard chow diet and water ad libitum and were housed in polypropylene cages in a well maintained and clean environment. The experimental protocol was subjected to scrutiny of institutional animal ethical committee for experimental clearance (KMCERT/Ph.D/03/2011).

The acute toxicity was performed as per organization for economic co-operation and development guidelines. Wistar albino rats and Swiss albino mice were used to assess the toxicity level. The root acetone extract at dose of 100, 500, 1000 and 2000 mg kg⁻¹ was administered to 3 rats and 3 mice in a single dose orally. The rats were fasted over-night and mice were fasted 3 h prior to the dosage. Animals are observed individually after drug administration at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days.

**Anti-inflammatory activity:** The acute inflammation is the initial response of the body to harmful stimuli. The self-defense reaction or inflammation can be induced in animals by carrageenan and croton oil to study the anti-inflammatory responses of *R. niveus* root acetone extract.

**Carrageenin induced paw edema:** The acute inflammation in rats was induced by carrageenan according to the modified method described by Galanti. Four groups of six animals each were used for the study. Increased paw thickness was induced by sub-plantar injection of 0.1 mL 1% carrageenan in normal saline into the right hind paw. The root acetone extract was administered at 200 and 400 mg kg⁻¹ orally 60 min before carrageenan induction. Indomethacin 20 mg kg⁻¹ p.o. was used as standard drug. The control group received the vehicle only. The mean increase in paw size was measured with vernier caliper at 0, 1, 2, 3, 4, 5, 6 and 7th hour after carrageenan injection in each group. The 0th hour reading was considered as the initial paw size of the animals. The data is shown as an increase in paw thickness and percentage inhibition of paw edema was produced by the treatment groups calculated in comparison with control animals.

**Croton oil induced ear edema:** To estimate the inhibitory activity of root acetone extract against acute inflammation, croton oil-induced mice ear edema was performed according to the method of Wang modified by Lin. Briefly, 10 μL acetone solution containing the 5% croton oil was applied topically to the right ear of mice. The left ear received an equal volume of acetone. Acetone extract were administrated orally at a dose of 200 and 400 mg kg⁻¹ about 60 min before the croton oil treatment. The left ear received the vehicle. As a reference, the Non-steroidal Anti-inflammatory Drug (NSAID), indomethacin (10 mg kg⁻¹) was used. Six hours later, the mice were sacrificed and both ears were removed uniformly by a sharp scissors and individually weighed on a sensitive balance. The edematous response was measured as the weight difference between the two plugs.
Analgesic activity: Peripheral and central analgesic activities of the tested extract were carried out in albino mice using acetic acid-induced writhing and hot plate methods, respectively.

Acetic acid induced writhing test: Male swiss albino mice were divided into four groups with six animals each. Group 1 served as control group 2 received standard drug Aspirin (100 mg kg\(^{-1}\)); group 3 and 4 received root acetone extract at doses of 200 and 400 mg kg\(^{-1}\). The acetic acid 0.6% v/v (10 mL kg\(^{-1}\), i.p.) was injected intraperitoneally 1 h after administration of the drugs. After administration of acetic acid, number of writhes (abdominal muscle contractions) were counted over a period of 15 min and immediately after acetic acid injection (0 time)\(^{10,11}\). Analgesic activity was expressed as the percentage protection against writhing produced by the tested extract compared with writhing at 0 time. Aspirin and root acetone extract were suspended in carboxy methyl cellulose (0.1%) before oral administration.

Eddy’s hot plate mediated pain reaction: The hot-plate test was performed to measure response latencies according to the method described by Eddy\(^{20}\). Male swiss albino mice were divided into four groups of six animals each. Group 1 served as control; group 2 served as standard which received morphine (10 mg kg\(^{-1}\)); group 3 and 4 served as plant extract at a dose of 200 and 400 mg kg\(^{-1}\), respectively. The animals were placed on the hot plate, maintained at 55±1°C. The pain threshold is considered to be reached when the animals lift and lick their paws or attempt to jump out of the hot plate. The time taken for the mice to react in this fashion was obtained using a stopwatch and noted as basal reaction time (0 min). A latency period of 15 sec (cut-off) was defined as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury\(^{21}\). The reaction time was re-investigated at 30, 60 and 120 min after the treatment and changes in the reaction time noted.

Antipyretic activity: Yeast induced hyperpyrexia model was used to evaluate the antipyretic activity of the root acetone extract of \(R. \) niveus.

Yeast induced pyrexia in rats: Hyperpyrexia was induced in rats by subcutaneous injection of 20 mL kg\(^{-1}\) b.wt. of a 15% aqueous suspension of Brewer’s yeast in the back below the nape of the neck\(^{22}\). Animals were divided into four groups of six each. The animals were then fasted approximately for 24 h, having free access to water. Control temperatures were taken 18 h after the yeast injection to determine the pyretic response to yeast. The rats which showed a rise in temperature of at least 0.6°C were taken for the study. The extract suspension (200 and 400 mg kg\(^{-1}\)) and the standard drug (paracetamol, 100 mg kg\(^{-1}\)) were given orally after 18 h of yeast injection. Before experimentation rectal temperatures of the rats were recorded (0 h). The temperatures were recorded at 1-6 h after the drug treatment\(^{23}\).

Statistical analysis: All the results were expressed as Mean±SEM. Statistical significance was determined by using the one way ANOVA followed by Dunnett’s multiple comparison tests. p<0.05 was considered statistically significant.

RESULTS
Acute toxicity: In the acute toxicity studies, four groups of rats and mice were administered with root acetone extract in graded doses of 100, 500, 1000 and 2000 mg kg\(^{-1}\) p.o., respectively. The animals were kept under observation for the change in behavior or death up to 14 days following the drug administration. The extract administration neither caused any significant change in the behaviors nor the death of animals in all the test groups. This indicates that the root acetone extract of \(R. \) niveus was safe up to a single dose of 2000 mg kg\(^{-1}\) b.wt. Hence we had selected 200 and 400 mg kg\(^{-1}\) oral doses to evaluate anti-inflammatory, analgesic and antipyretic activities.

Anti-inflammatory activity
Carrageenan induced paw edema: Carrageenan-induced inflammation in the rat paw represents a classical model for studying the acute inflammation; that was used for evaluation of anti-inflammatory activity of root acetone extract of \(R. \) niveus (Table 1). Pre-treatment with the standard drug indomethacin (20 mg kg\(^{-1}\)) (p<0.001) and root acetone extract at doses 200 and 400 mg kg\(^{-1}\) significantly (p<0.05 and p<0.01) prevented the increase in thickness of paw edema. It has been found that, acetone extract has good activity after 7 h, the standard drug produced a significant inhibitory effect (80.89%) followed by 400 and 200 mg kg\(^{-1}\) plant extract (74.52 and 59.50%), respectively.

Croton oil induced ear edema: The similar result was obtained from croton-oil induced ear inflammation in mice; the standard drug Indomethacin 10 mg kg\(^{-1}\) significantly (p<0.001) decreased the weight of inflamed ear. Table 2 shows percent reduction of the inflammatory
Table 1: Effect of R. nivus root acetone extract in carrageenan induced paw edema

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before carrageenan induction</th>
<th>After carrageenan induction (h)</th>
<th>Increase in paw thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>Control</td>
<td>4.67</td>
<td>6.46</td>
<td>6.69</td>
</tr>
<tr>
<td>Indomethacin (20 mg kg⁻¹)</td>
<td>4.63</td>
<td>5.72</td>
<td>5.46</td>
</tr>
<tr>
<td>RNRA (200 mg kg⁻¹)</td>
<td>4.86</td>
<td>5.79</td>
<td>6.08</td>
</tr>
<tr>
<td>RNRA (400 mg kg⁻¹)</td>
<td>4.94</td>
<td>5.49</td>
<td>5.73</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM (n = 6), significantly different at *p<0.05, **p<0.01, ***p<0.001 when compared to control. RNRA: R. nivus root acetone.

Table 2: Effect of R. nivus root acetone extract in croton oil induced ear edema after 6 h

<table>
<thead>
<tr>
<th>Groups</th>
<th>Difference in thickness (mm) between left and right ear</th>
<th>Difference in weight (mg) between left and right ear</th>
<th>Inhibition(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.48±0.08</td>
<td>118.40±2.90</td>
<td></td>
</tr>
<tr>
<td>Indomethacin (10 mg kg⁻¹)</td>
<td>0.11±0.17***</td>
<td>27.89±2.01***</td>
<td>66.52</td>
</tr>
<tr>
<td>RNRA (200 mg kg⁻¹)</td>
<td>0.27±0.07**</td>
<td>91.00±3.37*</td>
<td>23.14</td>
</tr>
<tr>
<td>RNRA (400 mg kg⁻¹)</td>
<td>0.27±0.05*</td>
<td>53.84±1.85***</td>
<td>54.52</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM (n = 6), significantly different at *p<0.05, **p<0.01, ***p<0.001 when compared to control. RNRA: R. nivus root acetone.

Table 3: Effect of R. nivus root acetone extract on acetic acid induced writhing

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of writhes (per 15 min)</th>
<th>Inhibition(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>67±8.281</td>
<td></td>
</tr>
<tr>
<td>Aspirin (100 mg kg⁻¹)</td>
<td>18.0±3.05***</td>
<td>73.13</td>
</tr>
<tr>
<td>RELM (200 mg kg⁻¹)</td>
<td>42.0±3.98**</td>
<td>37.31</td>
</tr>
<tr>
<td>RELM (400 mg kg⁻¹)</td>
<td>33.0±3.02***</td>
<td>50.75</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM (n = 6), significantly different at *p<0.05, **p<0.01, ***p<0.001 when compared to control. RNRA: R. nivus root acetone.

Response following topical application of croton oil. The topical application of croton oil-induced edema on the ears of mice caused a significant increase in the ear plug weight of the right ear compared with the vehicle-treated left ear after 6 h. A dose-dependent edema inhibition was observed in the root acetone extract treated groups in comparison with the non-steroidal anti-inflammatory drug indomethacin; a stronger and effective anti-inflammatory agent. The mouse ear plug weight was reduced by 76.52% after indomethacin treatment. However, R. nivus root acetone extract significantly reduced the edematous response by 23.14% for 200 mg kg⁻¹ (p<0.05) and by 54.53% for 400 mg kg⁻¹ (p<0.001), respectively.

Analgesic activity

Acetic acid induced writhing test: The results presented in Table 3 shows that the acetone extract at the doses 200 and 400 mg kg⁻¹ exhibited significant (p<0.01 and p<0.001) analgesic activity compared to the control at the rate of 37.31 and 50.75% inhibition, respectively. However, Aspirin (100 mg kg⁻¹) showed 73.13% inhibition. Significant protection against writhing was observed in animals treated with aspirin, 200 and 400 mg kg⁻¹ extract; where number of writhes after treatment were 18, 42 and 33, respectively compared to 67 in the control group.

Eddy's hot plate mediated pain reaction: As shown in Table 4, the root acetone extract produced significant analgesic activity. In this model, the higher dose (400 mg kg⁻¹) prolonged significantly the reaction time of animal with relatively extended duration of stimulation. At the higher dose level; the animals could withstand on the hot plate for 14, 13 and 13.8 sec at 30, 60 and 120 min reaction time which was the highest and comparable with that of the reference drug morphine 10 mg kg⁻¹ (7.8, 9.6 and 12.4 sec). The basal reaction time of the high dose and standard drug were 6 and 5.8 sec.

Antipyretic activity

Yeast induced pyrexia in rats: Effect of R. nivus root acetone extract on yeast induced hyperpyrexia is shown in Table 5. Subcutaneous injection of yeast suspension markedly elevated the rectal temperature of rats after 18 h of administration and the treatment with the root acetone extract at 200 and 400 mg kg⁻¹ significantly decreased the rectal temperature in a dose-dependent manner. The result obtained from both the standard (paracetamol) and plant extract treated rats were compared with that of control and a significant (p<0.001 and p<0.01) reduction in the yeast induced elevated rectal temperature was observed from 3rd to 7th h after the treatment.
Table 4: Effect of *R. nivens* root acetone extract on hot plate meditated pain reaction

<table>
<thead>
<tr>
<th>Groups</th>
<th>15 min</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.2 ± 0.23</td>
<td>9.4 ± 0.43</td>
<td>8.8 ± 0.53</td>
<td>9.8 ± 0.80</td>
<td></td>
</tr>
<tr>
<td>Morphine (10 mg kg⁻¹)</td>
<td>5.8 ± 0.87</td>
<td>7.8 ± 0.34***</td>
<td>9.6 ± 0.80***</td>
<td>12.4 ± 0.16***</td>
<td></td>
</tr>
<tr>
<td>RNRA (200 mg kg⁻¹)</td>
<td>5.8 ± 0.43</td>
<td>5.2 ± 0.87</td>
<td>11.0 ± 0.66***</td>
<td>8.4 ± 0.98</td>
<td></td>
</tr>
<tr>
<td>RNRA (400 mg kg⁻¹)</td>
<td>6.0 ± 0.90</td>
<td>14.0 ± 1.50**</td>
<td>13.0 ± 1.30**</td>
<td>13.8 ± 1.00***</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM (n = 6), significantly different at *p<0.05, **p<0.01, ***p<0.001 when compared to control, RNRA: *R. nivens* root acetone

Table 5: Antipyretic activity of *R. nivens* root acetone extract

<table>
<thead>
<tr>
<th>Groups</th>
<th>0th</th>
<th>18th</th>
<th>19th</th>
<th>20th</th>
<th>21st</th>
<th>22nd</th>
<th>23rd</th>
<th>24th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal temperature (°C)</td>
<td>37.10±0.45</td>
<td>39.02±0.34</td>
<td>39.96±0.21</td>
<td>38.94±0.36</td>
<td>39.08±0.29</td>
<td>39.22±0.39</td>
<td>38.86±0.52</td>
<td>38.84±0.48</td>
</tr>
<tr>
<td>Paracetamol (100 mg kg⁻¹)</td>
<td>38.96±0.46</td>
<td>38.58±0.18</td>
<td>38.16±0.79</td>
<td>37.40±0.35**</td>
<td>37.50±0.25***</td>
<td>37.40±0.19***</td>
<td>37.28±0.22***</td>
<td>37.06±0.18***</td>
</tr>
<tr>
<td>RNRA (200 mg kg⁻¹)</td>
<td>37.18±0.22</td>
<td>38.16±0.17</td>
<td>38.46±0.53</td>
<td>38.90±0.62</td>
<td>38.12±0.32*</td>
<td>38.30±0.41*</td>
<td>38.20±0.26</td>
<td>38.88±0.23</td>
</tr>
<tr>
<td>RNRA (400 mg kg⁻¹)</td>
<td>37.56±0.52</td>
<td>38.72±0.52</td>
<td>38.18±0.71</td>
<td>38.18±0.95**</td>
<td>38.66±0.72**</td>
<td>38.88±0.61**</td>
<td>38.30±0.98***</td>
<td>38.04±1.01***</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM (n = 6), significantly different at *p<0.05, **p<0.01, ***p<0.001 when compared to control, RNRA: *R. nivens* root acetone

**DISCUSSION**

The reported antioxidant activity of *R. nivens* as well as the traditional use encouraged us to extend our evaluation using *in vivo* models of anti-inflammatory, analgesic and antipyretic studies. It is well known that all the pharmaceutical companies are now interested in developing more effective drugs to treat inflammation, pain and fever with fewer side effects, plants being the best choice.

Carrageenan-induced hind paw edema has been widely used as an experimental model of acute inflammation which is used as primary test for the screening of new anti-inflammatory agents and is believed to be biphasic i.e. the early phase (up to 2 h) and late phase (1-6 h)²³. The early phase was associated with significantly severe inflammation, whereas the late phase was observed to have slow increase in volume of paw edema. The early phase has been attributed to the action of mediators such as histamine, serotonin and bradykinin on vascular permeability²⁴. The late phase edema has been shown to be a result of over production of prostaglandins²⁵. The data obtained revealed that the 400 mg kg⁻¹ root acetone extract of *R. nivens* possess potent anti-inflammatory effect in the carrageenan-induced acute model of inflammation.

The test of erythema in mouse ears induced by croton oil is commonly used for the evaluation of the effect of new anti-inflammatory drugs, topical steroids or nonsteroidal anti-inflammatory agents⁰.²⁷⁻²⁸. Dermatitis induced by croton oil represents a model of acute inflammatory response. The edema is mediated by cyclooxygenase metabolism of arachidonic acid²⁰.²¹.²². Regarding the croton-oil induced edema, groups treated with 200 and 400 mg kg⁻¹ doses of *R. nivens* root acetone extract showed statistically significant results when compared to control which were not statistically different from the effect of indomethacin (10 mg kg⁻¹), a nonsteroidal anti-inflammatory drug, obstructing the generation of these mediators.

Extensive research during the past 2 decades has revealed the mechanism by which continued oxidative stress can lead to chronic inflammation which in turn could mediate most chronic diseases including cancer, diabetes and cardiovascular, neurological and pulmonary diseases. Oxidative stress can activate a variety of transcription. Activation of these transcription factors can lead to the expression of over 500 different genes, including those for growth factors, inflammatory cytokines, chemokines, cell cycle regulatory molecules and anti-inflammatory molecules²⁹. A wide variety of inflammatory and immune cells present in persistently inflamed tissue generate Reactive Oxygen Species (ROS) and/or Reactive Nitrogen Species (RNS) which cause DNA damage and contribute to carcinogenesis³⁰. Barths ref reported that ROS are involved in a wide spectrum of diseases, including chronic inflammation and in a wide variety of cancers. Chronic inflammation is induced by biological, chemical and physical factors and is in turn associated with an increased risk of several human cancers. Hence the significant anti-inflammatory property of *R. nivens* root acetone extract will encourage the researches for the development of drugs in the treatment of cancer and other inflammatory related diseases since they are interconnected in many ways.

Acetie acid-induced writhing is a non-specific pain model and many compounds belonging to diverse pharmacological categories including opioids, non-steroidal anti-inflammatory drugs, calcium channel blockers, anticholinergics, antihistamines and corticosteroids show analgesic activity in this test³¹.
Acetic acid test is a visceral pain model produces a painful reaction and acute inflammation in the peritoneal area. Release of arachidonic acid and biosynthesis of prostaglandin via cyclooxygenase pathway plays a role in the nociceptive mechanism of this test. The analgesic effect of the tested compounds may be mediated through inhibition of cyclooxygenase and/or lipooxygenase (and other inflammatory mediators). Aspirin offers relief from inflammatory pain by suppressing the formation of pain mediators in the peripheral tissues, where prostaglandins and bradykinins were suggested to play an important role in the pain process. Prostaglandins elicit pain by the direct stimulation of sensory nerve endings. It is evident from the study that *R. niveus* exhibits significant peripheral analgesic effect in mice comparable with standard.

The classic hot plate model was followed to evaluate the analgesic activity of *R. niveus* root acetone extract. The hot plate model has been found suitable to investigate central antinociceptive activity because of several advantages, particularly the sensitivity to antinociceptives and limited tissue damage. Proinflammatory mediators like prostaglandins and bradykinins were suggested to play an important role in analgesia. The obtained results confirmed that root acetone extract at the dose 200 and 400 mg kg$^{-1}$ has a central analgesic effect which was compared with reference drug (aspirin 100 mg kg$^{-1}$). The analgesic effect of *R. niveus* might be attributable to the inhibition of the synthesis of some pro-inflammatory mediators, such as prostaglandins and cytokines.

Antipyretics are drugs which reduces the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point is elevated and a drug like paracetamol does not influence body temperature when it is elevated by factors such as exercise or increases in ambient temperature. Fever is thought to be produced by several endogenous substances including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), macrophage protein-1 (MIP-1) and prostaglandins. Brewer's yeast induces both TNF-α and prostaglandin synthesis. An antipyretic drug reduces fever by depressing inflammatory messages at both peripheral sites of tissue inflammation and within central nervous system thermoregulatory sites. These agents suppress peripheral production of pyrogenic cytokines such as TNF-α and interleukin-1β, while lowering the thermoregulatory set point by blocking central cyclooxygenase production of prostaglandin E2 (PGE2). The present results showed that *R. niveus* root acetone extract at two doses possessed a significant antipyretic effect in yeast-induced elevation of body temperature in rats and its effect is comparable to that of paracetamol (100 mg kg$^{-1}$).

The results of the present study revealed the acute anti-inflammatory, central and peripheral analgesic and antipyretic activity of the root acetone extract of *R. niveus*. The data reported in this study confirms the traditional use of *R. niveus* in the treatment of various inflammatory related disorders. There is a need for further studies to determine the mechanism behind these activities and the exploration of the exact compound or compounds responsible for the specific action.

REFERENCES


