Research Article

Saponins isolated from roots of *Chlorophytum borivilianum* reduce acute and chronic inflammation and histone deacetylase

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ABSTRACT

**OBJECTIVE:** The roots of *Chlorophytum borivilianum* are used in traditional medicine for the treatment of arthritis and inflammation. The aim of the work was to evaluate the anti-inflammatory activity of isolated saponins from *Chlorophytum borivilianum* (ISCB).

**METHODS:** The ISCB was screened using the carrageenan-induced paw edema, histamine-induced paw edema, cotton pellet-induced granuloma, and Freund’s adjuvant-induced arthritis in rats at orally administered doses of 3, 10, and 30 mg/kg. Effect of ISCB on histone deacetylase (HDAC) level was measured by the HDAC assay at the highest dose (30 mg/kg).

**RESULTS:** The results showed that the ISCB significantly reduced carrageenan-induced inflammation, histamine-induced inflammation, cotton pellet-induced granuloma and Freund’s adjuvant-induced arthritis in rats. The ISCB at a dose of 30 mg/kg significantly inhibited HDAC level in rat paw tissue.

**CONCLUSION:** It is concluded that saponins isolated from roots of *C. borivilianum* possess anti-inflammatory and anti-arthritic properties. ISCB may act by inhibiting histamine, prostaglandin and HDAC. This suggests that ISCBs have potential for therapeutic use in the treatment of inflammation and arthritis.

**Keywords:** *Chlorophytum borivilianum*; plants, medicinal; saponin; carrageenan; inflammation; arthritis; histone deacetylase; rats


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1 Introduction

Inflammation is a defensive response of living tissue to any type of injury that leads to the local accumulation of plasmatic fluid and blood cells[1]. This process can be classified, by the duration of the condition, from acute to chronic. If the risk factor(s) that caused acute inflammation continue to operate, the condition will progress, becoming chronic inflammation[2]. Prevalence of inflammatory diseases has increased over the last 50 years[3]. Chronic inflammation is a fundamental contributor to diseases such as cancer, diabetes, rheumatoid arthritis, chronic obstructive pulmonary diseases and cardiovascular disease.

Globally, non-steroidal anti-inflammatory drugs (NSAIDs)
are the most frequently used medicines in the world today to treat mild to moderate inflammation and pain\textsuperscript{[9]}. Adverse effects of NSAIDs, including stomach ulcers, gastrointestinal bleeding, kidney failure, heart attacks and strokes\textsuperscript{[3]} are responsible for more than 100,000 hospitalizations and more than 16,000 deaths each year. These side effects occur in all age groups, however, patients over 75 years of age taking NSAIDs have a 1 in 110 chance of having life-threatening gastrointestinal bleeding, and 1 in 647 chance of death from these complications\textsuperscript{[9]}. Sales of NSAIDs have increased every year, despite their wide array of side effects. Thus, with the expanding use of NSAIDs, statistically rare side effects are being seen in greater numbers\textsuperscript{[7]}.

The severity of inflammation is essentially controlled by histone deacetylases (HDACs) through the modulation of cytokine expression. Inhibition of HDACs has been shown to regulate gene expression and cytokine production after stimulation with several stimuli. HDAC inhibitors reduce the production and/or activity of pro-inflammatory cytokines in vitro, and exert a potent effect in animal models of inflammatory diseases in vivo\textsuperscript{[8]}. Thus, there is a current need for research on inflammation. This need includes not only the study of immunological and cellular responses involved but also the development of anti-inflammatory drugs without side effects\textsuperscript{[7]}.

Plants used in traditional medicine currently provide a rich source of candidate drugs for the treatment of inflammation\textsuperscript{[9]}. Chlorophytum borivilianum (family Asparagaceae) is a potential herb traditionally used in India and China to treat arthritis, oligospermia, diabetes and dysuria. It has been shown to possess antiviral, anticancer, immunomodulatory, antistress, aphrodisiac, antimicrobial\textsuperscript{[10]}, improvement in male sex health\textsuperscript{[11]}, anthelmintic\textsuperscript{[12]} and hepatoprotective activity\textsuperscript{[13]}. Methanolic extracts of roots and leaves at 200 mg/kg p.o. have been shown to inhibit carrageenan-induced inflammation in rats\textsuperscript{[14]}. Similarly, methanolic extracts of root tubers at 200 and 400 mg/kg p.o. were able to inhibit carrageenan-induced inflammation and cotton pellet granuloma in rats\textsuperscript{[15]}. The roots are reported to contain between 2% and 4% saponins\textsuperscript{[16,17]}, which include borivilianosides A–D\textsuperscript{[18]}, borivilinoside E–H\textsuperscript{[19]}, chlorophytoside-I\textsuperscript{[20]}, furostanol and spirostanol saponins\textsuperscript{[21]}. However, there is paucity of scientific data describing the anti-inflammatory activity of saponins. The present study was conducted in order to evaluate the anti-inflammatory activity of isolated saponins from C. borivilianum (ISCB) on laboratory animals, and to quantify the effect of these saponins on the level of HDAC-1 expression.

2 Materials and methods

2.1 Plant materials

Roots of C. borivilianum Sant & Fern were procured from crude drug supplier M/s. Gokuldas Goverdhandas (237, Budhawar Peth Pune). The plant material was identified and authenticated by Dr. R. B. Bhagat, Head of Botany, Poona District Education Association’s Arts, Commerce and Science College, Pirangut, Pune (A.C.S.C.P./104/1/2012-2013).

2.2 Experimental animals

Wistar rats (200–250 g; National Institute of Biosciences, Manikbaug, Pune) of both sexes were used for this study. Rats in groups of 6 were housed in standard-size polypropylene cages under the following environmental conditions: temperature (25 ± 2 °C; relative humidity 45%–55%; 12:12 hour light and dark cycle; with food and water ad libitum.

All of the experimental procedures (Protocol No. JSCOPR/02/ IAEC/03/2012) used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) at our institute, constituted under the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Ethical guidelines were strictly followed during all the experiments.

2.3 Extraction of plant material

Roots of C. borivilianum Sant & Fern were cut into small pieces, shade-dried and coarsely powdered. Coarse powder (600 g) was subjected to successive extractions with petroleum ether, ethanol and water by cold maceration for 72 h at room temperature ((30 ± 2) °C). The extracts were dried on a water bath ((45 ± 5) °C). The yield of the water extract was 40 g (6.66%). The dried water extract (40 g; 6.66% of the powder) was again dissolved in water, and the slow addition of ethanol:acetone (1:5) caused precipitation of the saponins. This light yellow precipitate of crude saponins was separated, dried (yield 25 g; 4.1% from crude drug powder), confirmed with the foam test for saponins and used for pharmacological evaluation\textsuperscript{[22]}.

2.4 Acute toxicity study

Acute oral toxicity limit test at a dose of 2000 mg/kg was performed as per the OECD guideline AOT 425. Rats were observed signs of toxicity or mortality during the first 24 h (with special attention given during the first 4 h), and daily thereafter, for a total of 14 d. The 50% lethal dose (LD\textsubscript{50}) was calculated using OECD 425 software\textsuperscript{[23]}.

2.5 Carrageenan-induced paw edema in rats

Wistar rats of either sex (200–250 g) were fasted for 12 h and divided into five groups, with six animals in each group. Experimental groups were given the test drug dissolved in distilled water at doses of 3, 10, and 30 mg/kg p.o. The control group received the vehicle (distilled water; volume not exceeding 5 mL/kg). The reference group received diclofenac sodium 10 mg/kg p.o. for comparison. Edema was induced in all groups by injecting 0.1 mL of 1% carrageenan in the sub-plantar region of left paw 1 h after treatment. Edema volume was measured plethysmographically.
using plethysmometer (Orchid Scientific PLM02, Nashik, India), before administering carrageenan (V₀) and 0.5, 1, 2, 4, 6, 8 and 24 h after (Vₜ)¹⁴.  

2.6 Histamine-induced paw edema in rats  
In this method instead of carrageenan, rats were given an injection of 0.1 mL of 1% histamine in the sub-plantar region of left paw in order to induce edema. Edema volume was measured plethysmographically using plethysmometer (Orchid Scientific PLM02, Nashik, India), before administering histamine (V₀) and 0.5, 1 and 2 h after administration of histamine (Vₜ).¹⁴ The paw swelling was determined for each rat and the difference at Vₜ (0.5, 1, and 2 h) from V₀ was taken as the edema value. The percentages of inhibition were calculated according to the following formula²⁵.

\[
\text{Inhibition (\%)} = \left( \frac{V₀ - V₀\text{control} - (Vₜ - V₀)\text{treated\ group}}{V₀\text{control}} \right) \times 100
\]

2.7 Freund’s adjuvant-induced arthritis in rats  
Male Wistar rats (200–250 g) were divided into five groups, six animals in each group. On the first day, animals in the control group received the vehicle (distilled water), 3 test groups received three different doses of ISCB (3, 10 and 30 mg/kg p.o.), and the reference group received indomethacin 2 mg/kg p.o. for comparison. All animals received injections of 0.1 mL of complete Freund’s adjuvant in the sub-plantar region of the left hind paw 1 h after drug treatment on the first day only. The oral treatment continued for 12 d. From days 13 to 21, dosing was stopped. Edema volume was measured using plethysmometer (Orchid Scientific PLM02, Nashik, India) before administering Freund’s adjuvant (V₀) and on the 1st, 7th and 14th day after (Vₜ)¹⁴. The amount of paw edema was determined for each rat and the difference at Vₜ (days 1, 7, and 14) from V₀ was taken as the edema value. The percentages of inhibition were calculated according to the formula²⁵. Body weight of all animals was recorded on the 1st and 21st day. Hematological parameters including white blood cell count (WBC), hemoglobin (Hb), rheumatoid arthritis factor, and C-reactive protein (CRP) values were recorded on the 21st day.²⁴

2.8 Cotton pellet-induced granuloma in rats  
Wistar rats (200–250 g) of either sex were selected and divided into 5 groups, with 5 animals in each group. Food was with held 12 h before experiment, with free access to water. On day 1, test groups were treated with ISCB 3, 10, and 30 mg/kg p.o., dissolved in distilled water. The control group received the vehicle (distilled water). The reference group received diclofenac sodium 20 mg/kg p.o. for comparison. After 1 h of treatment animals were anesthetized. Sterile cotton pellets of 20 mg were implanted one in each scapula region of rats by making small subcutaneous incision under sterile conditions. The treatment with ISCB, reference drug or the vehicle was continued daily for a period of 7 d. The rats were sacrificed on the 8th day after an excess dose of anesthesia, and the pellets surrounded by granuloma tissues were dissected out, weighed, dried for 24 h at 60 °C and weighed again. The wet weight and dry weight of the pellet were recorded. The percent change between wet weight and dry weight was calculated²⁴. The level of inhibition of granuloma tissue development was calculated using the equation:

\[
\text{Inhibition (\%)} = \left( \frac{Tₜ - (Tₜ/Tₐ) \times 100}{Tₐ} \right)
\]

Where Tₜ = weight of granuloma tissue of treated group and Tₐ = weight of granuloma tissue of control group.

2.9 HDAC assay  
Wistar rats of both sexes (200–250 g) were selected, divided into two groups, with 5 rats in each group, and treated orally with vehicle (distilled water) and ISCB (30 mg/kg) respectively. All animals received an injection of 0.1 mL of 1% histamine in the sub-plantar region 1 h after drug treatment. Thirty minutes after administration of histamine, paw tissues were isolated, weighed and transferred to a homogenization tube containing 3 mL of cold phosphate buffer solution (0.01 mol/L, pH 7.0–7.2) and homogenized. The homogenates were centrifuged for 5 min at 3 113 × g, and the supernatant was collected and processed as per the procedure given in enzyme-linked immunosorbent assay kit (USCN Life Science, Inc., Wuhan, Hubei, China)²⁶. Absorbance was recorded at 450 nm using a plate reader.

2.10 Statistical analysis  
Data are presented as mean ± standard error of mean (SEM). Analysis of variance (ANOVA) followed by Dunnett’s t test or Student t test, was used to calculate statistical differences among treatment groups. Statistical analysis was conducted using InStat software (Version 3.06, GraphPad Software Inc., San Diego, CA, USA) and comparisons with a P value <0.05 were considered significant.

3 Results  
3.1 Acute oral toxicity study  
ISCB was administered in five healthy female rats at dose of 2 000 mg/kg p.o. All animals were observed for changes in general health or mortality during the first 24 h (with special attention given during the first 4 h), and daily thereafter, for 14 d. This dose did not produce any change in their general behavior or mortality. LD₅₀ was found to be more than 2 000 mg/kg calculated with AOT 425 Stat Pgm (Westat and USEPA, Version 1.0), an acute oral toxicity statistical program (OECD Test Guideline 425)²³. On the basis of the AOT 425 report, doses of 3, 10 and 30 mg/kg were selected for evaluating anti-inflammatory activity.

3.2 Carrageenan-induced paw edema in rats  
Subcutaneous injection of carrageenan, 0.1 mL of 1%
solution in the sub-plantar region, induced edema in the paws of experimental rats. At 6 h after induction, paw edema volume was significantly reduced by oral administration of ISCB at 3 and 30 mg/kg ($P<0.01$) compared to the control group.

Diclofenac sodium given at a dose of 10 mg/kg p.o. appeared to reduce paw edema volume at all measurement intervals. However, reduced paw edema volume was significant only at 4 h ($P<0.05$) and 6 h ($P<0.01$) (Figure 1).

## 3.3 Histamine-induced paw edema in rats

Subcutaneous injection of histamine, 0.1 mL of 1% solution, in the sub-plantar region induced edema in the paws of experimental rats. Oral administration of ISCB 3 mg/kg reduced mean paw edema volume at all intervals. This reduction was significant ($P<0.01$) at 1 h and 2 h, with 11% and 87% reduction in swelling (respectively) relative to the control group. ISCB given at 10 mg/kg p.o. decreased paw edema volume significantly at 2 h ($P<0.01$). This was an inhibition of 76% relative to the control. ISCB 30 mg/kg significantly reduced mean paw edema volume at 1 h ($P<0.05$) and had an inhibition of paw edema volume of 36% (Table 1).

### Figure 1
Effects of ISCB on edema induced by carrageenan in rat hind paw

Data are represented as mean ± standard error of mean ($n=6$). Data were analyzed by analysis of variance (df = 4, 25) followed by Dunnett’s $t$ test. Results were considered significant at $P<0.05$.

* $P<0.01$, vs control group.

ISCB: isolated saponins from *Chlorophytum borivilianum*; Diclo: Diclofenac sodium. Dose is represented by mg/kg.

### Table 1
Effects of ISCB on edema induced by histamine in rat hind paw

<table>
<thead>
<tr>
<th>Treatment (mg/kg p.o.)</th>
<th>$n$</th>
<th>Paw edema volume (mL) at different time intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0.49±0.06</td>
</tr>
<tr>
<td>ISCB (3)</td>
<td>6</td>
<td>0.39±0.05 (22%)</td>
</tr>
<tr>
<td>ISCB (10)</td>
<td>6</td>
<td>0.32±0.04 (34%)</td>
</tr>
<tr>
<td>ISCB (30)</td>
<td>6</td>
<td>0.36±0.03 (26%)</td>
</tr>
<tr>
<td>Diclofenac (10)</td>
<td>6</td>
<td>0.22±0.05&quot; (55%)</td>
</tr>
<tr>
<td><strong>$df$=4, 25</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statistical value</td>
<td></td>
<td>$F=4.383$</td>
</tr>
<tr>
<td>$P=0.008$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are represented as mean ± standard error of mean. In parenthesis: % inhibition ($n=6$). Data were analyzed by analysis of variance followed by Dunnett’s $t$ test. Results were considered significant at $P<0.05$. *$P<0.05$, **$P<0.01$, vs control group. ISCB: isolated saponins from *Chlorophytum borivilianum*. 

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Diclofenac sodium 10 mg/kg p.o. appeared to reduce mean paw edema volume at all intervals. However, reduced mean paw edema volume was only significant at 30 min (P<0.01) and 1 h, and percentage inhibition was 55% at 30 min and 80% at 1 h respectively, compared to the control group (Table 1).

3.4 Freund’s adjuvant-induced arthritis in rats

Administration of complete Freund’s adjuvant in the sub-planar region of rat paws induced inflammation of the paw in the control group. Oral administration of ISCB at 3 and 30 mg/kg p.o. significantly reduced paw edema volume on the 14th day (P<0.01) as compared to the control group (Table 2). Body weight loss measured on the 21st day was significantly reduced in all treatment groups relative to the control group (P<0.05 or P<0.01; Table 3). WBC count was found to be significantly reduced in ISCB at 3 mg/kg (P<0.01), 10 mg/kg (P<0.01) and 30 mg/kg (P<0.01) relative to the control group on day 21. Blood hemoglobin level was significantly increased in rats receiving ISCB at 3 mg/kg (P<0.01), 10 mg/kg (P<0.01) and 30 mg/kg (P<0.05) (Table 4). The CRP levels were also decreased (non-significantly) relative to the control arthritic rats on day 21 (Table 4).

Indomethacin at a dose 2 mg/kg p.o. significantly decreased paw edema volume (P<0.01; Table 2) on the 14th day of treatment. The reference drug also significantly reduced weight loss (P<0.01; Table 3), significantly decreased WBC count and non-significantly decreased CRP level, significantly reduced rheumatoid arthritis factor (P<0.05) and significantly increased blood haemoglobin level (P<0.01) as compared to the control group on the 21st day (Table 4).

3.5 Cotton pellet-induced granuloma in rats

Subcutaneous implantation of 20 mg sterile cotton pellets into the scapular region of rats resulted in an increase in both wet weight and dry weight of the cotton pellets when they were extracted on the 8th day. Administration of ISCB at doses of 3, 10, and 30 mg/kg p.o. significantly decreased the dry weight of cotton pellets extracted on day 8 relative to the control (P<0.01). The reduction in
pellet dry weight was of 60%, 48% and 52%, respectively, relative to the control. There was no significant difference in the wet weight of pellets.

Treatment with diclofenac sodium (20 mg/kg, p.o.) significantly decreased both wet weight and dry weight of cotton pellets \( (P<0.01) \). The relative reduction in weight was 45% and 57% (wet and dry respectively) relative to cotton pellets removed from the control rats (Table 5).

**Table 5** Effects of ISCB on granuloma induced by sterile cotton pellet (20 mg) in rats

<table>
<thead>
<tr>
<th>Treatment (mg/kg p.o.)</th>
<th>n</th>
<th>Wet weight (mg)</th>
<th>Inhibition (%)</th>
<th>Dry weight (mg)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>34±48</td>
<td>—</td>
<td>18±3±16</td>
<td>—</td>
</tr>
<tr>
<td>ISCB (3)</td>
<td>5</td>
<td>270±16</td>
<td>22</td>
<td>73±7±16</td>
<td>60</td>
</tr>
<tr>
<td>ISCB (10)</td>
<td>5</td>
<td>290±24</td>
<td>16</td>
<td>95±10±7±16</td>
<td>48</td>
</tr>
<tr>
<td>ISCB (30)</td>
<td>5</td>
<td>271±33</td>
<td>21</td>
<td>89±8±19±17</td>
<td>52</td>
</tr>
<tr>
<td>Diclofenac (20)</td>
<td>5</td>
<td>189±7±17</td>
<td>45</td>
<td>78±19±7±17</td>
<td>57</td>
</tr>
<tr>
<td>Statistical value</td>
<td>df = 4, 20</td>
<td>F = 5.628</td>
<td>df = 4, 20</td>
<td>F = 9.076</td>
<td>P = 0.004 6</td>
</tr>
</tbody>
</table>

Data are represented as mean ± standard error of mean. Data were analyzed by analysis of variance followed by Dunnett’s \( t \) test. \( P<0.05, \ P<0.01, \) vs control group. ISCB: isolated saponins from *Chlorophytum borivilianum*.

3.6 HDAC assay

Administration of ISCB (30 mg/kg p.o.) significantly decreased HDAC-1 level in paw tissue compared to the control group \( (P=0.006 7; \) Figure 2).

**Figure 2** Effects of ISCB on histone deacetylase-1 level in rat paw tissue

Data are represented as mean ± standard error of mean (\( n=5 \)). Data were analyzed by Student-\( t \) test. Results were considered significant at \( P<0.05 \). \( \ast P<0.01, \) vs control group. ISCB: isolated saponins from *Chlorophytum borivilianum*.

4 Discussion

In the present investigation, we evaluated the anti-inflammatory activity of *C. borivilianum* against induced inflammation and arthritis in rats.

Carrageenan-induced edema in the rat hind paw is the most widely used primary test for screening of anti-inflammatory agents. The process of carrageenan-induced edema is described in two phases. The first (early) phase results from histamine, serotonin, and bradykinin liberation, while the second (late) phase is associated with formation of prostaglandins. Suppression of the first phase may be attributed to inhibition of the release of early mediators, serotonin and histamine, and action in the second phase may be explained by an inhibition of cyclooxygenase.

In the present study ISCB (at doses of 3 and 30 mg/kg, p.o.) suppressed carrageenan-induced paw edema effectively at 6 h, indicating marked anti-inflammatory effect in late phase. However, administration of ISCB 10 mg/kg
p.o. had no significant effect on carrageenan-induced paw edema. The fact that the lowest dose exerted greater reduction in paw edema suggests that ISCB does not act in a dose-dependent manner. This can be explained by a possible alteration in the pharmacokinetics patterns of the saponins that are influenced by the dose\[^{33}\]. Diclofenac sodium (10 mg/kg p.o.) at 4 h and 6 h suppressed the paw edema produced by carrageenan, supporting its well-proven cyclo-oxygenase pathway inhibition\[^{32,33}\].

Histamine-induced paw edema is a well-established model used to study inflammation and neutrophil infiltration in paw tissue\[^{34}\]. Injection of histamine caused a rapid increase in edema, which peaked at 30 min. Histamine H1 receptors are involved in mediating the inflammation induced by various inflammatory agents. Thus, H1 receptor antagonist can act as anti-inflammatory agents\[^{33}\]. Inhibition of histamine-induced edema with treatment of ISCB at doses of 3, 10 and 30 mg/kg indicated anti-inflammatory activity by histamine inhibition. However, ISCB did not show a significant effect in the first phase of carrageenan-induced paw edema; this may be due to plasma extravasations, leakage of water and plasma protein from the affected tissue, along with release of histamine for the induction of edema in first phase\[^{35,36}\].

Freund’s adjuvant-induced arthritis in rats is the most common model used to evaluate arthritic activity. The development of arthritis is divided into four phases: the first phase extends from the 1st to 4th day with acute local inflammation and systemic effects; the second phase spans the 7th to 12th day with remission of acute inflammation and periarthritis; the third phase, 12th to 28th day, is characterized by chronic inflammation, periarthritis and osteogenic activity; the fourth phase extends from day 35th onwards with permanent articular deformity and minimal (burn-out) inflammation\[^{37}\].

Freund’s adjuvant develops chronic inflammation in multiple joints due to accumulation of inflammatory cells, accompanied by erosion of joint cartilage and bone destruction\[^{38}\]. Administration of ISCB at doses of 3 and 30 mg/kg p.o. significantly suppressed paw edema volume in the chronic phase (14th day) of Freund’s adjuvant-induced arthritis. This suggests that the inhibition of changes during the chronic phase is acted by the inhibition of inflammatory mediators.

Increase in the total leukocyte count in arthritis has been attributed to the stimulation of the immune system to act against the invading antigens\[^{39}\]. Acute phase proteins, like CRP, increase in response to stress or inflammation\[^{38}\]. Administrations of ISCB at doses of 3, 10, and 30 mg/kg p.o., significantly decreased leukocyte count and CRP level.

Body weight and hemoglobin level were considered as indicators of health status and recovery from disease\[^{37}\]. During the progress of arthritis in lab rats, weight loss has been observed in response to alterations in the metabolic activity of diseased rats\[^{40}\]. Anti-inflammatory drugs can correct the decreased absorption capacity of intestine during inflammation\[^{38,41}\]. ISCB administered at doses of 3, 10 and 300 mg/kg significantly reduced body weight loss; this was correlated by a general improvement of health status, as supported by a significant increase in blood hemoglobin level at day 21. This effect may be beneficial for joint preservation.

Cotton pellet implantation is a method for studying the efficacy of drugs against the proliferative phase of inflammation\[^{42,43}\]. The subcutaneous implantation of sterile cotton pellets in rats has been divided into three phases: transudative phase, exudative phase, and proliferative phase\[^{12,44}\]. NSAIDs decrease the size of the granuloma. This response operates through inhibiting granulocyte infiltration/inflammation, preventing formation of collagen fibers and suppressing mucopolysaccharides\[^{45}\].

Tissue water and plasma protein accumulation, due to plasma extravasation, significantly contribute to the induction of edema in the first phase of carrageenan-induced paw edema\[^{37}\]. ISCB administered at doses of 3 and 30 mg/kg p.o. had no significant effect in this phase. Administration of ISCB at doses of 3, 10 and 30 mg/kg p.o. significantly decreased dry weight but not wet weight of the cotton pellets, suggesting that ISCB could reduce proliferative events of granuloma formation. These results support earlier reports by Panda et al\[^{12}\], which demonstrated that methanolic extract of C. borivilianum inhibited the proliferative phase of granuloma formation. Diclofenac sodium significantly decreased both wet weight and dry weight of the cotton pellet granuloma by inhibiting proliferation of fibroblast and fluid accumulation in chronic inflammation.

HDAC-1 are broadly expressed throughout mammalian tissues\[^{46}\]. HDAC-1 has been shown to modulate gene expression and cytokine production after exposure to several stimuli. HDAC-1 inhibitor reduces the production and/or activity of pro-inflammatory cytokines, exerting a potent effect in animal models of inflammatory diseases\[^{48}\].

In the present study ISCB administered at a dose of 30 mg/kg p.o. significantly decreased the HDAC-1 level, suggesting that the drug may inhibit synthesis of inflammatory mediators by affecting inflammatory gene expression\[^{47}\] (Figure 2). Further detailed study of chronic inflammation is required to clarify the HDAC-1 inhibition mechanism.

5 Conclusion

It is concluded that isolated crude saponins from roots of C. borivilianum possess anti-inflammatory and anti-arthritis activity. The observed activity of the saponins used in these experiments may be due to the inhibition of inflammatory mediators such as histamine and prostaglandin.
and inhibition of fibroblast proliferation without affecting exudation. Inhibition of inflammatory mediators may be attributed to an alteration in gene expression, which itself may be due to ISCB’s inhibition of HDAC-1. Thus isolated saponins from *C. borivilianum* have demonstrated a potential for therapeutic use in the treatment of inflammation and arthritis, supporting some aspects of its use in traditional Chinese and Indian medicine.

### 6 Acknowledgements

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### 7 Conflict of interests

No conflict of interests to declare.

### REFERENCES


3. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and inhibition of fibroblast proliferation without affecting exudation. Inhibition of inflammatory mediators may be attributed to an alteration in gene expression, which itself may be due to ISCB’s inhibition of HDAC-1. Thus isolated saponins from *C. borivilianum* have demonstrated a potential for therapeutic use in the treatment of inflammation and arthritis, supporting some aspects of its use in traditional Chinese and Indian medicine.


