



Pharmacological Evaluation of Analgesic and Anti-inflammatory Activities of Ethanolic root Extract of *Saurauia roxburghii* Wall. in Wistar Rats

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Account Information	ABSTRACT
Original Research article	Pain and inflammation are common biological responses regulated by multiple mediators and remain important targets for pharmacological research. <i>Saurauia roxburghii</i> Wall. is a medicinal plant reported to possess phytochemical and pharmacological potential. The present study investigated the analgesic and anti-inflammatory activities of an ethanolic root extract of <i>S. roxburghii</i> in Wistar rats. An ethanolic root extract was prepared by Soxhlet extraction and subjected to preliminary phytochemical screening. Analgesic activity was evaluated using the Eddy hot-plate test at 200 and 400 mg/kg, with diclofenac sodium (50 mg/kg) as the reference treatment. Anti-inflammatory activity was assessed using carrageenan-induced paw edema at 200 and 400 mg/kg, with indomethacin (10 mg/kg) as the reference treatment. Reaction latency and paw edema were recorded at predefined time points. The source manuscript reports an extract yield of 27.32%. Preliminary phytochemical screening indicated alkaloids, flavonoids, glycosides, tannins/phenolic compounds, terpenoids and saponins. Recalculation of the supplied hot-plate observations showed a 60-min reaction latency of 33.40 s for 200 mg/kg, 39.00 s for 400 mg/kg and 40.80 s for diclofenac sodium. Using the stated 45-s cut-off and the recalculated vehicle-control baseline, the corresponding maximum possible analgesia values were 58.07%, 78.31% and 84.82%, respectively. In the carrageenan model, recalculation from the reported mean paw volumes gave 6.74–31.79% inhibition for 200 mg/kg and 9.55–37.95% for 400 mg/kg across 1–5 h, compared with 9.55–39.49% for indomethacin. The ethanolic root extract of <i>S. roxburghii</i> showed measurable analgesic and anti-inflammatory effects in the experimental models used, with greater activity at 400 mg/kg than at 200 mg/kg. The findings support further investigation but do not establish clinical efficacy or a specific molecular mechanism. Ethical, numerical and statistical inconsistencies identified in the source records should be resolved before final submission.
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INTRODUCTION

Pain is a multidimensional sensory and affective experience associated with actual or potential tissue injury. Inflammation is a coordinated biological response involving vascular changes, inflammatory mediators and recruitment of immune cells. Excessive or persistent inflammatory responses can contribute to tissue dysfunction and disease. Medicinal plants remain an important source of chemically diverse compounds with analgesic, anti-inflammatory and antioxidant properties. *Saurauia roxburghii* Wall. (family Actinidiaceae) has been described in ethnomedicinal literature, and phytochemical and pharmacological investigations have suggested potential therapeutic activities. A review of *S. roxburghii* has summarized its traditional uses, phytochemistry and reported biological activities, while earlier experimental work has described antioxidant and antidiabetic effects of its extracts.[1,2] The Eddy hot-plate test is commonly used in preclinical analgesic screening, whereas carrageenan-induced paw edema is a widely used model of acute inflammation. [3-6] The

present study was designed to evaluate the analgesic and anti-inflammatory effects of an ethanolic root extract of *S. roxburghii* using these two experimental models.

Materials and Methods

Root of *S. roxburghii* were air-dried, powdered and extracted with ethanol using a Soxhlet apparatus. The source manuscript states that 100 g of powdered root were extracted continuously for 24 h and that the concentrated extract was obtained by evaporation on a water bath. The dried extract was described as a dark reddish-brown, semisolid material. The source manuscript reports a recovered dried residue of 24.82 g but separately reports an extraction yield of 27.32%. For 100 g starting material, 24.82 g corresponds to 24.82% yield. The final yield must therefore be verified from the original laboratory record. The source document also states that the Soxhlet flask was heated to approximately 600°C. This value is not plausible for conventional ethanolic Soxhlet extraction and should be treated as a transcription error until verified. The actual heating conditions should be inserted from the laboratory record before publication. The ethanolic extract was screened qualitatively for major phytochemical groups using colour and precipitation reactions. The reported findings are summarized in Table These tests are qualitative and do not establish the identity, concentration or biological activity of individual constituents.

Phytochemical group	Reported tests	Reported observation	Interpretation
Alkaloids	Wagner, Mayer, Dragendorff, Hager	Characteristic precipitate/colour reactions	Positive
Flavonoids	Lead acetate, sulfuric acid, zinc	Characteristic colour/precipitate reactions	Positive
Glycosides	Baljet, Legal	Yellow-orange/pink colouration	Positive
Tannins/phenolics	Ferric chloride, dilute nitric acid	Blue/reddish-yellow colouration	Positive
Terpenoids	Salkowski	Observation in source is internally inconsistent	Verify
Saponins	Froth/foam tests	One test positive and one negative	Variable/verify
Volatile oils	Solubility test	Reported soluble in 90% alcohol	Verify

Healthy Wistar rats weighing approximately 150–200 g was reported to have been used. Animals were housed under controlled laboratory conditions with a 12-h light/dark cycle, temperature approximately 22±3°C and relative humidity of 50–70%, with free access to water and standard laboratory feed. Animals were acclimatized for 7 days before experimentation. The supplied manuscript does not provide the Institutional Animal Ethics Committee (IAEC) approval number, CPCSEA registration number, protocol number or approval date. These details are essential for publication of an animal experiment and must be inserted from the original institutional records. No approval number has been fabricated in this revision. [7] The extract was administered orally at 200 and 400 mg/kg. The source manuscript states that dose selection was based on previously reported safety information. A literature-reported LD50 should not be presented as an LD50 determined in the present study unless an acute toxicity experiment was actually performed and documented. Where an acute oral toxicity study was conducted, its protocol and results should be reported according to the applicable OECD guideline.[8] The source manuscript describes preparation of extract solutions in normal saline and administration by oral gavage. The exact formulation concentration, dose volume and administration schedule should be checked against the laboratory records. Diclofenac sodium (50 mg/kg) was used as the reference treatment in the hot-plate experiment and indomethacin (10 mg/kg) in the carrageenan model. Analgesic activity was assessed using the Eddy hot-plate method. The source manuscript reports a

plate temperature of approximately 55°C and a 45-s cut-off to minimize thermal injury. Reaction latency was recorded before treatment and at 15, 30, 45 and 60 min after treatment. The supplied group-design table states 2 animals in the vehicle-control group and 5 animals in each treatment group. However, the individual raw-data table contains observations for 3 vehicle-control animals and 5 animals in each of the other groups. This is an internal inconsistency. For descriptive recalculation in this revision, the individual observations in the supplied table were used; the final animal number must be reconciled with the original animal log.

Maximum possible analgesia (MPA) was calculated as:

MPA (%) = [(post-treatment latency – vehicle-control baseline latency)/(cut-off latency – vehicle-control baseline latency)] × 100.

Carrageenan-induced paw edema

Anti-inflammatory activity was evaluated using carrageenan-induced paw edema. The source manuscript reports subplantar injection of 0.1 mL of 1% carrageenan in normal saline into the right hind paw. Test extract or indomethacin was administered orally 1 h before carrageenan challenge. Paw volume was recorded at 1, 2, 3, 4 and 5 h after carrageenan injection using a plethysmometer. The reported groups were normal control, carrageenan disease control, *S. roxburghii* extract 200 mg/kg, *S. roxburghii* extract 400 mg/kg and indomethacin 10 mg/kg. The stated group sizes (n=2, n=3, n=5, n=5 and n=5, respectively) total 20 animals.

Percentage inhibition was calculated as: % inhibition = [(Vc – Vt)/Vc] × 100, where Vc is the mean paw volume of the carrageenan disease-control group and Vt is the mean paw volume of the treated group at the same time point.

RESULT

Extract characteristics

Parameter	Reported observation
Extraction solvent	Ethanol
Extraction method	Soxhlet extraction
Starting plant material	100 g powdered root
Extraction duration	24 h (as reported)
Appearance	Dark reddish-brown, semisolid
Reported yield	27.32%; requires verification against the reported 24.82 g residue

The extract was described as dark reddish-brown and semisolid. The reported yield requires verification because the reported recovered mass and percentage are inconsistent.

Analgesic activity

Group	n	0 min	15 min	30 min (first)	30 min (duplicate source column)	45 min	60 min
Vehicle control	3	17.33±0.33	17.00±0.58	16.67±0.33	16.67±0.33	17.33±0.67	17.67±0.33
<i>S. roxburghii</i> extract 200 mg/kg	5	22.80±0.37	26.60±0.40	28.80±0.37	28.80±0.37	30.80±0.37	33.40±0.60
<i>S. roxburghii</i> extract 400 mg/kg	5	23.60±0.24	30.00±0.32	32.60±0.24	32.60±0.24	35.80±0.37	39.00±0.32
Diclofenac sodium 50 mg/kg	5	24.40±0.40	30.80±0.37	30.80±0.37	34.20±0.37	37.40±0.24	40.80±0.37

The raw observations show a time-related increase in reaction latency after both extract doses. At 60 min, mean latency was 33.40 s at 200 mg/kg, 39.00 s at 400 mg/kg and 40.80 s with diclofenac sodium.

Treatment	0 min	15 min	30 min (first)	30 min (duplicate source column)	45 min	60 min
S. roxburghii extract 200 mg/kg	19.76	33.49	41.45	41.45	48.67	58.07
S. roxburghii extract 400 mg/kg	22.65	45.78	55.18	55.18	66.75	78.31
Diclofenac sodium 50 mg/kg	25.54	48.67	48.67	60.96	72.53	84.82

3.3 Anti-inflammatory activity

The supplied raw table contains a duplicated 30-min column with identical values. For analysis, the duplicated column represents the same 30-min observation and should not be treated as an additional time point. Using the stated 45-s cut-off and the recalculated vehicle-control baseline of 17.33 s, the calculated MPA at 60 min was 58.07% for 200 mg/kg, 78.31% for 400 mg/kg and 84.82% for diclofenac sodium. These are descriptive recalculations from the supplied raw observations and should be checked against the original statistical worksheet.

Group	Treatment	Dose	1 h	2 h	3 h	4 h	5 h
Normal control	0.9% NaCl	1 mL/kg	1.07±0.01	1.09±0.01	1.10±0.01	1.11±0.01	1.10±0.01
Disease control	1% carrageenan	0.1 mL	1.78±0.01	1.81±0.015	1.86±0.015	1.91±0.01	1.95±0.01
Extract	S. roxburghii	200 mg/kg	1.66±0.02	1.57±0.04	1.54±0.02	1.46±0.035	1.33±0.035
Extract	S. roxburghii	400 mg/kg	1.61±0.035	1.53±0.06	1.41±0.03	1.32±0.02	1.21±0.03
Reference	Indomethacin	10 mg/kg	1.61±0.04	1.51±0.055	1.39±0.035	1.27±0.03	1.18±0.03
Treatment	Dose	1 h	2 h	3 h	4 h	5 h	
S. roxburghii extract	200 mg/kg	6.74	13.26	17.20	23.56	31.79	
S. roxburghii extract	400 mg/kg	9.55	15.47	24.19	30.89	37.95	
Indomethacin	10 mg/kg	9.55	16.57	25.27	33.51	39.49	

The extract reduced carrageenan-induced paw edema at both doses. At 5 h, inhibition was 31.79% at 200 mg/kg and 37.95% at 400 mg/kg, compared with 39.49% for indomethacin.

Discussion

The previously reported value of 89.55% inhibition at 1 h for the 400 mg/kg group was mathematically incorrect. Recalculation from the reported mean paw volumes gives 9.55%. The values in the inhibition table above have been recalculated using the stated formula. The hot-plate test measures behavioural responses to a noxious thermal stimulus and is commonly used in analgesic screening. The increase in latency observed after extract administration is compatible with an analgesic effect in this model. However, the experiment does not establish a specific receptor or molecular pathway. The mechanistic statements in the source manuscript concerning prostaglandin synthesis and the arachidonic-acid pathway were not directly tested and have

therefore been removed. Carrageenan-induced paw edema is an established model of acute inflammation. The reduction in paw volume after extract pretreatment indicates anti-inflammatory activity under the conditions tested. The 400 mg/kg extract approached the response of indomethacin at later time points, but the reported data do not justify describing the two treatments as equivalent. Preliminary phytochemical screening suggested several classes of secondary metabolites. Because the screening was qualitative, it cannot identify individual active molecules or establish which constituents produced the observed pharmacological effects. Further studies should include chromatographic fingerprinting, quantitative phytochemical analysis, isolation or characterization of active constituents, mechanistic assays and adequately powered confirmatory experiments. The study also has methodological limitations that affect interpretation. The source manuscript contains inconsistencies in animal numbers, extraction yield, extraction temperature and statistical reporting. In the hot-plate raw table, the vehicle-control group contains three animals although the group-design table states two. The small and unequal group sizes further limit statistical inference. These issues should be reconciled with the original laboratory records before peer-review submission.

CONCLUSION

Under the experimental conditions reported, the ethanolic root extract of *Saurauia roxburghii* Wall. demonstrated analgesic activity in the hot-plate model and anti-inflammatory activity in carrageenan-induced paw edema in Wistar rats. The 400 mg/kg dose produced greater responses than the 200 mg/kg dose within the tested range, while the reference drugs produced the highest responses at the final observation point. The findings support further pharmacological investigation but do not establish clinical efficacy, human safety or a definitive mechanism of action.

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