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Pharmacological evaluation of analgesic, anti-inflammatory and antidiarrheal activities of methanolic extract of *Neolitsea sericea* (Blume) Koidz

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Abstract
Neolitsea sericea, commonly known as Japanese silver tree, is used as herbal medicine for the treatment of inflammation, fever, body pain and diarrhoea. This plant is distributed in the South and Southeast Asia. The present study was aimed at the evaluation of analgesic, anti-inflammatory and antidiarrheal activities of the methanolic crude extract of *Neolitsea sericea* to authenticate its use in traditional medicine. Methanolic extract of leaves was prepared, and preliminary phytochemical analysis was carried out. *In vivo* assays were performed in Wistar albino rats to assess the analgesic activity by tail immersion method. Anti-inflammatory activity was evaluated by carrageenan-induced paw edema in rat model while the antidiarrheal activity was determined by charcoal meal test. The results of *in vivo* experiments revealed that the extract possesses significant analgesic activity at $p<0.001$ level in a dose dependent manner. Carrageenan-induced paw edema test in rats exhibited 26.95% and 28.95% inhibition of inflammation, respectively, at 200 and 400 mg/ kg b.w. dosage level after 4 h of drug administration. Statistically significant reduction in gastrointestinal transit was observed at 400 mg/ kg b.w. in charcoal meal test. The results of the present investigation proved that *Neolitsea sericea* is endowed with analgesic, anti-inflammatory and antidiarrheal properties in rats.

Keywords: analgesic, anti-inflammatory, antidiarrheal, medicinal plant, *Neolitsea sericea*, tail immersion test, carrageenan induced, charcoal meal

Introduction
The use of plant products for their healing effects has been traced back to several centuries in the history of human civilization. The plant-based medicine system originated initially within the local areas and has flourished into a reputed traditional medicine system. The major advantages of plant-based medicine are their affordable cost, low incidence of adverse effects, and easy access. World Health Organization has reported that around 80% of human population depends on herbal medicines around the globe. Approximately 40,000 to 70,000 plants are being utilized for various ailments [1]. Western Ghats is one of the global biodiversity hotspots of India which is popularly known for its high level of endemism in plants and animals. Several plants are endowed with medicinal properties and as many as 500 plants from Western Ghats are being used by the people to treat various kinds of ailments [2].
Neolitsea is one of the major genera in the family Lauraceae which grows as shrub or evergreen tree. There are about 100 species in this genera which are widely distributed in tropical countries of Asia, including India. A few of the *Neolitsea* species have been reported to possess medicinal properties as they are used in Chinese folk medicines. Some parts of these plants have been traditionally used for certain ailments such as abdominal distension, pain and edema [3]. However, reports are scarce on the *in vivo* biological activities of *Neolitsea sericea*. Hence an attempt was made to determine the analgesic, anti-inflammatory and antidiarrheal effect of this plant collected from the southern Western Ghats, Tamil Nadu, India using rat as animal model.

Materials and methods
Plant materials
The leaves of *Neolitsea sericea* was collected from Ayyanar Kovil forest area, Southern Western Ghats, Tamil Nadu, India, during winter. The plant was taxonomically identified by Dr. R. Ramasubbu, Professor, Department of Biology, Gandhigram Rural Institute, Dindigul, Tamil Nadu, India. A voucher specimen of this plant material has been deposited at the herbarium of the Department of Botany, V.H.N. Senthikumara Nadar College, Virudhunagar, India, for future reference. The leaves were washed in fresh water to remove extraneous matter and shade dried at the room temperature.

Preparation of the plant extract
The shade dried leaves were ground to fine powder using a mixer grinder. Thirty gram of this powder was packed inside the thimble of the Soxhlet extraction unit. It was extracted with methanol at 55°C for 48 h. The

viscous paste of the methanolic extract was dried in a hot air oven at 50℃ which resulted in a yield of 6.8%. It was labelled and stored in a screw-cap tube at 4℃ for further analysis.

Chemicals

All the chemicals used in the present investigation were of analytical grade. They were purchased from different sources: i.e., methanol from SD fine chemicals, activated charcoal from HiMedia, paracetamol, diclofenac sodium and atropine sulfate from Cipla Ltd., Mumbai, and all other reagents from Merck (India).

Phytochemical screening

Plants possess a vast array of secondary metabolites that are attributed to several biological activities. The crude methanolic extract of *Neolitsea sericea* was screened for the presence of various phytoconstituents such as total flavonoids, alkaloids, saponins, tannins, terpenes, proteins, and carbohydrates.

Experimental animals

Male Wister albino rats, weighing 150 – 180 g were used for the screening of pharmacological activities. The animals were procured from registered animal breeder and housed in the animal house, where the temperature was maintained at 25±2℃, relative humidity at 45 – 55%, and light and dark cycle of 12 and 12 h, respectively. They were kept in polycarbonate cages measuring 45 cm × 30 cm× 15 cm with a density of five animals per cage. The animals were provided with standard pellet diet procured from Poultry Research Station, Chennai and had free access to water. They were acclimatized to the laboratory conditions for 10 days prior to the experiment. All the studies were carried out in accordance with the guidelines prescribed by CPCSEA, and the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC Reg. No: 509/02/C/CPCSEA/2002-10/07/2002).

Experimental design

Analgesic activity by Tail immersion method

The analgesic activity of leaf extract of *Neolitsea sericea* was evaluated by tail immersion method [4]. Sixteen male Wister rats were divided into 4 groups, each comprising of 4 animals. The rats of control group were orally administered with distilled water (5 ml/ kg body weight). The animals of standard group were intraperitoneally injected with diclofenac sodium (10 mg/ kg). Group III and IV animals received 200 and 400 mg/ kg doses of methanolic extract of *Neolitsea sericea*. The tail of each rat was marked at 3 cm from the tip and was immersed in hot water maintained at 55±0.5°C. The rats responded to the heat stimuli by withdrawing the tail from the water bath. The withdrawal time (in sec) was recorded to determine the basal latency time. The cut-off time was kept at 15 s to prevent tissue injury to the tail. After the assessment of basal latency, the animals were administered with respective doses of standard drug and plant extract. The reaction time was recorded at 30, 60, 90 and 120 min after drug treatment.

Anti-inflammatory activity in Carrageenan-induced paw edema model

The anti-inflammatory effect was assessed by the carrageenan-induced paw edema model in rat [5]. Sixteen male rats were randomly divided into four groups each consisting of four animals. The rats in Group I served as control and received 1 ml of distilled water. Group II animals were treated with 30 mg/ kg of Diclofenac sodium. The rats in Group III and IV were orally administered with 200 and 400 mg/ kg of *Neolitsea sericea* extract, respectively. After 30 minutes of extract administration, the left hind paw of each rat was subcutaneously injected with 0.1 mL of 1% (w/ v) carrageenan into the sub-plantar region. Immediately after injection, the volume of injected paw was measured by a digital Plethysmometer (Orchid Scientific & Innovative India Pvt. Ltd., India). The paw thickness of experimental rats was recorded after one hour of carrageenan injection at 1, 2, 3 and 4 h. The anti-inflammatory activity was determined by the difference in volume of paw edema before and after carrageenan injection. The percentage (%) inhibition of inflammation in each group was determined by the following formula:

$$Inhibition\ (\%) = \frac{(V_c - V_t)}{V_c} \times 100$$

Where, VC = Mean change in paw thickness for the control group
Vt = Mean change in paw thickness for drug treated groups

Antidiarrheal activity by gastrointestinal transit test

The method employed in this test was described by Tagne Michel *et al*. [6] with minor modifications. Another set of 16 rats were randomly selected and divided into 4 groups of 4 animals each. They were fasted for 18 h but had free access to water. After the fasting period, the animals in the normal control group were orally administered with distilled water (10 mL/ kg). Group II animals received Atropine sulphate at 5 mg/ kg. The methanolic extract of *Neolitsea sericea* at the dosages of 200 and 400 mg/ kg was orally given to the animals of Group III and VI respectively. After 30 minutes of drug treatment, each animal was orally administered with 10 mL/ kg of 5% charcoal meal as peristaltic marker. Thirty minutes later, the animals were euthanized by ether anaesthesia

and the small intestine was removed by dissection. The total length of small intestine and the distance covered by charcoal meal through the intestine were measured. From these values, the peristaltic index (PI) was calculated as follows:

$$Peristaltic\ Index\ (PI) = \frac{Distance\ covered\ by\ charcoal\ meal\ in\ small\ intestine\ (cm)}{Total\ length\ of\ the\ small\ intestine\ (cm)} \times 100$$

The inhibition of diarrhoea (%) was calculated by the following formula

$$Inhibition\ (\%) = \left(\frac{PI_c - PI_t}{PI_c}\right) \times 100$$

Where, PI_c = Peristaltic index of control animals
PI_t = Peristaltic index of the drug treated animals

Statistical analysis
The experimental data were expressed as Mean ± SEM. Statistical significance of the data was determined by one way ANOVA which was followed by Scheffe's post hoc test using the SPSS 20.0 software. Statistical differences were considered significant at $p<0.05$.

Results

Preliminary phytochemical analysis
The qualitative analysis of the methanol leaf extract of *Neolitsea sericea* revealed the presence of various secondary metabolites such as flavonoids, saponins, alkaloids, tannins, etc.

Tail immersion test
The results of analgesic activity of *Neolitsea sericea* extract evaluated by tail immersion test are given in Table 1. It showed that the 200 and 400 mg/ kg doses of extract had significant effect on the inhibition of pain when compared to control. The activity was maximum for the 400 mg/ kg dose which was comparable to the standard drug diclofenac sodium. The analgesic effect was dose dependent at the tested doses of *Neolitsea sericea*. The reaction time to heat stimulus had increased significantly ($p<0.001$) in the rats treated with the standard drug diclofenac sodium from 30 min onwards.

Carrageenan-induced paw edema
The methanolic extract of leaves of *Neolitsea sericea* exhibited a dose dependent inhibition of paw edema from 2 h to 4 h after the administration of extract (Table 2). The results show that the standard drug diclofenac sodium significantly ($p<001$) inhibited the inflammation activity (9.74%, 13.80%, 20.74% and 32.67%, respectively, at 1 h interval for 4 h). The *Neolitsea sericea* extract treated rats at the dosage level of 400 mg/ kg b.w. exhibited 28.95% inhibition of inflammation after 4 h of drug treatment (Table 3).

Gastrointestinal transit test
The outcomes of antidiarrheal activity of *Neolitsea sericea* methanolic extract are given in Table 4. The relative mobility of charcoal in control animals was higher than the drug treated groups. The antidiarrheal activity was highly significant ($p<0.001$) at the dosage level of 400 mg/ kg which is comparable to Atropine sulphate (5 mg/ kg). It has reduced the passage of charcoal meal through alimentary canal and caused the most significant inhibition (42%) of intestinal transit when compared to control. The percentage inhibition of diarrhoea in the 200 mg/ kg treated group was not statistically significant.

Discussion

In the present study, the pharmacological effects of leaves of *Neolitsea sericea* for its analgesic, anti-inflammatory and antidiarrheal activities. Tail immersion test was employed to assess the analgesic activity of the methanolic extract. As evident from Table 1, the extract significantly inhibited the pain by centrally acting mechanism. This model can be considered as an essential index to determine the for central analgesic effects of agents that prolong the reaction time of rodents [7]. This kind of effect was observed in the *Neolitsea sericea* treated animals. Analgesic drugs are acting by increasing the pain threshold of animals caused by heat and pressure [8]. The thermal stimulus influences the μ2/δ and μ1/ μ2 opioid receptors that are playing a vital role in the spinal and supraspinal reflex mechanisms [9]. The analgesic effect can be attributed to the suppression of synthesis of cyclooxygenase [10].
As another phase of the study, the anti-inflammatory activity of *Neolitsea sericea* extract was evaluated in carrageenan-induced paw odema in rats. The methanol extract has showed significant inhibitory effect on the inflammation. Generally, this model has been used to evaluate the anti-inflammatory effect of new drug molecules. The inflammation caused by carrageenan injection is biphasic in nature. During the initial phase, an array of molecules such as histamine, kinins, h-hydroxy tryptamine, cyclooxygenase and leukotriens are released. Production of prostaglandins, bradykinin, neutrophil infiltration, etc. are associated with the delayed

phase of inflammation [11]. In the present investigation, *Neolitsea sericea* had significantly ameliorated the inflammation caused by carrageenan after 2 h. This result may be attributed to the inhibition of cyclooxygenase synthesis. Similar kind of results was also observed in the animals treated with the standard drug diclofenac sodium. Several cytokines such as Tumour necrosis factor (TNF-α), Interleukin-1β (IL-1β), IL-2, and PGE_2 are playing a vital role in the inflammatory response. This corroborates with the earlier report on the immunomodulatory effects of leaf extracts of *Neolitsea sericea* [12]. It has been reported that flavonoids also possess anti-inflammatory property [13]. Further studies on the purification and isolation of phytocompounds are required to understand the exact mechanism of inhibitory action of the *Neolitsea sericea* extract.

From the Table 4, it is evident that the methanolic extract of *Neolitsea sericea* exhibited antidiarrheal in the gastrointestinal transit test. The 400 mg/ kg dose of extract had influenced the movement of charcoal in the small intestine. It is also evident that there was a dose-dependent effect of the plant extract. The standard drug atropine sulphate appeared to cause profound effect on the inhibition of diarrhoea. There are several mechanisms to explain the antidiarrheal effect in intestinal transit of charcoal meal. The inhibition of muscle contraction and motility may also be responsible for the reduction in the intestinal propulsion. This reduction causes the gut contents to remain in the intestine for longer period which may promote the absorption of intestinal water and electrolytes [14].

A few plants of Neolitsea genera have been reported to possess antioxidant, anti-inflammatory and antiapoptotic responses in guinea pig [15]. These activities have been attributed to thaliporphine, an alkaloid, isolated from *Neolitsea konishii.* The phytochemical studies of leaf extract of *Neolitsea sericea* revealed the presence of alkaloids and terpenoids [16, 17]. The alkaloids are having extensive biological activities including analgesic, anti-inflammatory and anticholinergic. Terpenoids are also found to have anti-inflammatory, antitumour, antimicrobial and hypoglycemic effects [18, 19]. Therefore, the pharmacological effects on the experimental animas might have been produced due to the presence of these phytoconstituents in the methanolic leaf extract of *Neolitsea sericea*.

Tables

Table 1: Analgesic effect of methanolic extract of *Neolitsea sericea* on withdrawal reflexes in tail immersion test in Wistar rats

Groups	Dose	Reaction time (in sec) after administration of drugs at different time intervals				
		0 min	30 min	60 min	90 min	120 min
Control (Distilled water)	5 ml/ kg	4.15 ± 0.24	4.15 ± 0.26	3.90 ± 0.42	4.15 ± 0.46	4.15 ± 0.42
Diclofenac Sodium	100 mg/ kg	4.40 ± 0.64	9.65 ± 0.84**	11.15 ± 0.86**	11.65 ± 0.62**	11.15 ± 0.47**
Neolitsea sericea	200 mg/ kg	4.15 ± 0.24	6.65 ± 0.48	7.15 ± 0.27	7.40 ± 0.64*	6.85 ± 0.47
Neolitsea sericea	400 mg/ kg	4.15 ± 0.47	8.15 ± 0.46**	11.40 ± 0.95**	11.70 ± 0.63**	11.10 ± 0.72**

The values are expressed as Mean ± SEM (n = 4). The symbols * and ** represent highly significant levels at $p<0.05$ and $p<0.001$ respectively when compared with control

Table 2: Effect of methanolic extract of the leaves of *Neolitsea sericea* with carrageenan-induced paw edema in rats

Groups	Dose	Normal paw thickness (0 h)	Change in paw thickness after drug administration (mm)			
			1 h	2 h	3 h	4 h
Control	-	7.26 ± 0.14	9.14 ± 0.13	9.42 ± 0.12	10.03 ± 0.09	11.02 ± 0.13
Standard (Diclofenac sodium)	30 mg/ kg	7.25 ± 0.15	8.25 ± 0.14**	8.12 ± 0.13**	7.95 ± 0.15**	7.42 ± 0.18**
Neolitsea sericea	200 mg/ kg	7.27 ± 0.17	8.62 ± 0.18	8.41 ± 0.15**	8.44 ± 0.19**	8.05 ± 0.17**
Neolitsea sericea	400 mg/ kg	7.24 ± 0.15	8.35 ± 0.19	8.22 ± 0.12**	8.09 ± 0.18**	7.83 ± 0.16**

The values are expressed as Mean ± SEM (n = 4). The symbols * and ** represent highly significant levels at $p<0.01$ and $p<0.001$ respectively when compared with control

Table 3: Percentage inhibition of inflammation exerted by *Neolitsea sericea* extract

Groups	Dose	Inhibition of inflammation induced by carrageenan after drug administration (%)			
		1 hr	2 hr	3 hr	4 hr
Standard (Diclofenac sodium)	30 mg/ kg	9.74	13.80	20.74	32.67
Neolitsea sericea	200 mg/ kg	5.69	10.72	15.85	26.95
Neolitsea sericea	400 mg/ kg	8.64	12.74	19.34	28.95

Table 4: The effect of methanolic extract of *Neolitsea sericea* on intestinal transit of charcoal meal in Wister rats

Groups	Dose	Mean length of Small Intestine (cm)	Mean distance travelled by Charcoal (cm)	Peristaltic Index (%)	Inhibition of Diarrhoea (%)
Control (Distilled water)	10 mL/ kg	84.55 ± 6.23	71.15 ± 4.72	84.15	-
Standard (Atropine sulphate)	5 mg/ kg	105.30 ± 4.75**	47.65 ± 1.54**	45.25	46.23
Neolitsea sericea	200 mg/ kg	89.35 ± 5.82	60.40 ± 2.36	67.60	19.67
Neolitsea sericea	400 mg/ kg	98.65 ± 3.34*	48.15 ± 2.49**	48.81	42.00

The values are expressed as Mean ± SEM (n = 4). The symbols * and ** represent highly significant levels at $p<0.01$ and $p<0.001$ respectively when compared with control

Conclusion

In conclusion, the present study has revealed that the methanolic extract of leaves of *Neolitsea sericea* possess potential analgesic, anti-inflammatory and antidiarrheal activity in rats. This knowledge could be utilised to formulate new drug molecules to treat the ailments related to pain, inflammation, and diarrhoea.

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