# Anti-diarrhea Effect of *Castanopsis sclerophylla* (Lindl) Schottk Fruit

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**Abstract** [**Objectives**] To explore the pharmacodynamic effect of the *Castanopsis sclerophylla* (Lindl) Schottk fruit on diarrhea model mice. [**Methods**] The anti-diarrheal efficacy of the *C. sclerophylla* fruit was evaluated using a castor oil-induced acute diarrhea model in mice and the small intestinal charcoal propulsion test, with assessment of onset time of diarrhea, frequency of loose stools, and small intestinal propulsion rate. [**Results**] Compared with the model group, the *C. sclerophylla* frui-treated group demonstrated significantly delayed onset of diarrhea, reduced frequency of loose stools, and decreased small intestinal charcoal propulsion rate in a dose-dependent manner, with the high-dose group achieving efficacy comparable to the loperamide-treated group. [**Conclusions**] The *C. sclerophylla* fruit has certain anti-diarrhea effect, and its anti-diarrhea mechanism may be related to the inhibition of small intestinal peristalsis.

**Key words** *Castanopsis sclerophylla* (Lindl) Schottk fruit, Diarrhea, Action mechanism

## 1 Introduction

*Castanopsis sclerophylla* (Lindl.) Schottk, an evergreen tree of the Fagaceae family, produces fruits with dual medicinal and edible values. These fruits can be consumed long-term and possess significant therapeutic potential. According to *Supplements to the Grand Compendium of Materia Medica*, they are described as having "bitter and astringent properties, effective in alleviating diarrhea and dysentery, suppressing hunger, enhancing vitality, resolving blood stasis, and quenching thirst". Traditionally, the fruit powder has been widely used in folk medicine across Jiangxi and Anhui provinces for diarrhea treatment with satisfactory efficacy. Literature reports further confirm its clinical effectiveness in pediatric autumn diarrhea and adult diarrheal disorders, demonstrating low adverse effects and good acceptability in children[1]. However, current research primarily focuses on food product development and bioactive component extraction, while systematic pharmacological investigations remain scarce. Its antidiarrheal application remains empirically based without scientific validation. Therefore, this study aims to evaluate its therapeutic effects on diarrheal animal models through pharmacodynamic evaluation, providing experimental evidence for clinical translation.

## 2 Materials and methods

**2.1 Experimental animals** SPF-grade KM mice (84 in total, 44 females and 40 males) with body weights of (20 ± 2) g were purchased from Guangdong Provincial Medical Laboratory Animal Center (License No.: SCXK (Yue) 2022-0002). The animals were housed in the barrier-level animal laboratory of Zhongshan Hospital of Traditional Chinese Medicine (Use License No.: SYXK (Yue) 2020-0109), with environmental temperature maintained at (22 ± 2) ℃, relative humidity at 50% - 70%, and a 12-h day/night cycle. SPF-grade feed and autoclaved water were provided ad libitum. All experimental procedures were approved by the Animal Ethics Committee of Zhongshan Hospital of Traditional Chinese Medicine.

**2.2 Medicines and reagents** The *C. sclerophylla* fruits were sourced from Zhangjia Village, Xinqiao, Wangdun Township, Duchang County, Jiujiang City, Jiangxi Province. They were identified by Chief Pharmacist Zeng Congyan from Zhongshan Hospital of Traditional Chinese Medicine as the fruits of *Castanopsis sclerophylla* (Lindl.) Schottky, a plant belonging to the Fagaceae family. The materials used included castor oil (Hubei Ketian Pharmaceutical Co., Ltd., Batch No.: 20231103) and Loperamide capsules (Xi'an Janssen Pharmaceutical Co., Ltd., Batch No.: 231018244).

**2.3 Preparation of *C. sclerophylla* fruit medicinal solution** Removed the outer shell of *C. sclerophylla* fruits, soaked in cold water with constant water changes until the leaching solution becomes colorless. Added water and grind into slurry, let stand, then sun-dried the sediment to obtain *C. sclerophylla* fruit powder, which was stored in a dry place for later use. Before use, prepared the medicinal solution at corresponding doses by mixing the *C. sclerophylla* fruit powder with an appropriate amount of distilled water.

**2.4 Castor oil-induced diarrhea experiment** Thirty SPF-grade KM mice (half male and half female) were acclimated for one week and randomly divided into model group, positive control group (loperamide group, 3.2 mg/kg), and low-, medium-, high-doses *C. sclerophylla* fruit groups (1.3, 2.6, 5.2 g/kg), with 6 mice per group. Mice in drug administration groups received corresponding drugs by gavage at 0.1 mL per 10 g body weight, while the model control group received equal volume of distilled water, once daily for 7 consecutive days. All mice were fasted with free access to water for 12 h before the last administra-

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tion. At 40 min after the final administration, each mouse was given 0.2 mL castor oil by gavage. Filter paper was placed under each cage and replaced hourly. Observations were continuously made for 4 h to record initial diarrhea time and count loose stools. Diarrhea inhibition rate (%) was calculated as: (Number of loose stools in model group − Number of loose stools in administration group)/Number of loose stools in model group × 100%. After 4 h, blood was collected via eyeball removal, followed by euthanasia and collection of organ and intestinal segment samples for preservation.

**2.5 Small intestine propulsion experiment** Animal grouping and drug administration followed Section **2.4**. Forty minutes after the last administration, mice in each group were administered 0.2 mL castor oil by gavage. Thirty minutes later, 0.2 mL ink was administered by gavage. At 20 min thereafter, mice were euthanized via cervical dislocation. The abdominal cavity was opened, and intestinal segments from the pylorus to the ileocecal junction were rapidly excised, placed on filter paper and gently straightened without tension. The length from the pylorus to the ileocecal junction was measured as the total small intestine length. The distance from the pylorus to the leading edge of charcoal powder was recorded as charcoal propulsion distance. Charcoal propulsion rate was calculated as: Charcoal propulsion rate (%) = Charcoal propulsion distance/Total small intestine length × 100%.

**2.6 Statistical methods** All experimental data were expressed as mean ± standard deviation ($\bar{x} \pm s$), and SPSS 27.0 statistical software was used for data analysis; one-way analysis of variance was used for comparison between groups, and *LSD* test was used for pairwise comparison; $P < 0.05$ was considered to be statistically significant.

## 3 Results and analysis

**3.1 Effects of *C. sclerophylla* fruit on castor oil-induced diarrhea in mice** Except for the blank control group, all groups of mice exhibited varying degrees of diarrhea after being administered castor oil by gavage for a period. Diarrheic mice manifested lethargy, rough fur, increased frequency of loose stools, yellowish-brown feces without fixed shape, increased fecal water content with viscous liquid and reduced solid content, indicating successful model establishment.

As shown in Table 1, compared with the model group, the positive control group and low-, medium-, high-dose *C. sclerophylla* fruit groups all showed significantly delayed initial diarrhea time and significantly reduced number of loose stools over the 4-h period ($P < 0.01$); compared with the positive control group, the high-dose *C. sclerophylla* fruit group demonstrated equivalent efficacy to loperamide, while low- and medium-dose groups showed no statistically significant difference in loose stool frequency ($P > 0.05$). The diarrhea inhibition rates of *C. sclerophylla* fruit groups were 41.30%, 41.30%, and 63.04%, respectively, indicating its potential effect against castor oil-induced diarrhea.

**Table 1 Effects of *Castanopsis sclerophylla* fruit on castor oil-induced diarrhea in mice** ($n = 6$, $\bar{x} \pm s$)

| Group | Time of initial diarrhea//min | Number of loose stools//times/4 h | Inhibition rate of loose stool//% |
|---|---|---|---|
| Model | 54.67 ± 8.385$^{\#\#}$ | 7.67 ± 0.333$^{\#\#}$ | – |
| Positive | 207.83 ± 19.791$^{**}$ | 2.83 ± 0.792$^{**}$ | 60.87 |
| Low dose | 136.50 ± 26.780$^{**\#\#}$ | 4.50 ± 0.620$^{**}$ | 41.30 |
| Medium dose | 138.67 ± 48.119$^{**\#\#}$ | 4.50 ± 0.719$^{**}$ | 41.30 |
| High dose | 189.67 ± 25.420$^{**}$ | 2.83 ± 0.477$^{**}$ | 63.04 |

**NOTE** Compared with the model group, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with the loperamide group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, the same below.

**3.2 Effects of *C. sclerophylla* fruit on small intestinal propulsion in mice with diarrhea** As shown in Table 2, compared with the model group, the positive control group and medium- and high-dose *C. sclerophylla* fruit groups significantly reduced small intestine propulsion rate; compared with the positive control group, there was no statistically significant difference in small intestine propulsion rate between medium- and high-dose *C. sclerophylla* fruit groups and the positive group, indicating equivalent efficacy to the positive drug loperamide with a dose-dependent relationship, demonstrating that *C. sclerophylla* fruit has inhibitory effects on castor oil-induced small intestine motility disorders.

**Table 2 Effect of *Castanopsis sclerophylla* fruit on small intestinal propulsion in mice with diarrhea** ($n = 6$, $\bar{x} \pm s$)

| Group | Length of small intestine//cm | Propulsion distance//cm | Propulsion rate//% |
|---|---|---|---|
| Model | 56.83 ± 1.352 | 35.00 ± 2.221$^{\#\#}$ | 61.36 ± 2.865$^{\#\#}$ |
| Positive | 58.67 ± 2.261 | 21.33 ± 2.486$^{**}$ | 36.33 ± 4.100$^{**}$ |
| Low dose | 58.00 ± 1.483 | 31.67 ± 4.271$^{\#}$ | 54.78 ± 7.306$^{\#}$ |
| Medium dose | 57.50 ± 0.428 | 25.00 ± 2.295$^{*}$ | 43.46 ± 3.957$^{*}$ |
| High dose | 58.83 ± 3.177 | 23.33 ± 1.994$^{**}$ | 42.54 ± 4.255$^{**}$ |

## 4 Discussion

Diarrhea is a common digestive tract disease caused by multiple pathogens and factors, which can lead to intestinal flora imbalance, absorption disorders, reduced nutritional status and immune function in humans and animals. As the second leading cause of childhood morbidity and mortality, it poses serious threats to human health. Traditional Chinese medicine offers advantages in treating diarrhea through multi-target mechanisms with fewer adverse reactions. The study subject *C. sclerophylla* fruit is a dual-purpose medicinal and edible material documented in historical materia medica. In regions such as Jiangxi and Anhui provinces, it has been traditionally used to treat diarrheal diseases with proven efficacy. Modern research on *C. sclerophylla* fruit remains limited, primarily focusing on food development and active component extraction: Liu Guangxian[2] isolated flavonoids, starch and pectin from the fruit; Hu Yonghu elaborated its culinary applications; Yang Wuying[3] determined its nutritional composition; Shen Mengjiao[4] developed a novel *C. sclerophylla* tofu product. However, systematic pharmacological studies are still lacking, and its

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inflammatory actions[12]. This multidimensional applicability highlights its significant industrial potential in pharmaceutical development (novel TCM formulations) and health-related industries (functional foods).

This study systematically analyzed *H. hedyotidea* samples from different geographical origins using HPLC fingerprinting technology and the *Traditional Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System* (2004A Edition). Through digital evaluation of parameters including retention times, relative peak areas of common characteristic peaks, and similarity indices, we conducted the first chemical component group-level quality control investigation. While most samples demonstrated high consistency, certain origin-specific samples exhibited similarity indices below 0.80, revealing substantial variations in chemical component composition that may influence the herb's quality.

The established HPLC fingerprint-based quality evaluation system effectively assesses both the consistency and intrinsic variability of *H. hedyotidea*, providing critical data support for quality control standard formulation and development of this medicinal material.

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anti-diarrheal application remains confined to few folk practices. Therefore, this study investigated the anti-diarrheal effects of *C. sclerophylla* fruit from a pharmacodynamic perspective.

This study evaluated the anti-diarrheal effects of *C. sclerophylla* fruit using a castor oil-induced diarrhea model. Castor oil, an irritant laxative, exerts its diarrheic mechanism by releasing ricinoleic acid in the gastrointestinal tract, which stimulates intestinal mucosa to produce prostaglandins and other endocrine substances that induce inflammation, enhance intestinal motility, and inhibit water-electrolyte absorption, ultimately leading to exudative diarrhea[5]. Experimental results demonstrated that *C. sclerophylla* fruit significantly delayed initial diarrhea onset time, reduced loose stool frequency, and alleviated diarrhea symptoms in a dose-dependent manner, with the high-dose group showing comparable efficacy to the positive control drug loperamide. The charcoal propulsion test revealed that *C. sclerophylla* fruit markedly inhibited intestinal propulsion in castor oil-induced diarrheic mice. Medium- and high-dose groups showed no significant difference in propulsion rate compared to the positive control group, indicating its inhibitory effect on small intestinal motility.

In conclusion, the anti-diarrheal mechanism of *C. sclerophylla* fruit may involve suppression of intestinal peristalsis. This study provides experimental evidence for further investigation into its anti-diarrheal mechanisms and offers references for its potential clinical application.

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