Journal for ReAttach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 August; Vol 6 (8s): 755-760

Antidiarrheal And Acute Toxicity Study of Ethanolic Extract of *Quercus Leucotrichophora* in Swiss Albino Mice

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ABSTRACT
Quercus leucotrichophora, historically used to treat various ailments, including diarrheal, was the focus of a study evaluating its antidiarrheal efficacy against castor oil-induced diarrheal in mice. The study investigated three different doses (100, 200, and 400 mg/kg) of an 80% ethanolic leaf extract of *Q. leucotrichophora* in Swiss albino mice, employing models of gastrointestinal transit, enteropooling, and diarrheal induced by castor oil. The results showed that, compared to the control group, the plant extract significantly reduced the frequency of both moist and total fecal output at all tested doses. The study demonstrated a significant reduction in intraluminal fluid accumulation in the group treated with the plant extract at all dose levels. Moreover, the plant extract exhibited substantial antimotility activity at higher doses when compared to the control group. In conclusion, the findings suggest that the 80% ethanolic leaf extract of *Q. leucotrichophora* supports the traditional claim of the plant's antidiarrheal efficacy, although further research is warranted for a more comprehensive understanding.

Keywords: Antidiarrheal, castor oil, enteropooling, gastrointestinal motility, *Quercus leucotrichophora*

1. INTRODUCTION

Diarrheal infections pose a significant public health concern, causing numerous deaths each year and contributing to substantial morbidity and mortality, particularly in underdeveloped countries.[1] As of 2015, diarrheal stands as one of the primary causes of death among children, contributing to approximately 9% of all pediatric fatalities worldwide.[2] The regions with the highest rates of child deaths related to diarrheal were reported to be sub-Saharan Africa and southern Asia.[3] In Ethiopia, a country among the top 15 nations where diarrheal disease contributes to about three-fourths of child fatalities, the illness poses a significant public health concern.[4] Overall, despite the best efforts of various governments and international organizations to reduce it, the prevalence of diarrheal disease remains high.[5]

Despite significant technological advancements in contemporary medicine, eighty percent of people in developing nations still rely on traditional healing methods and medicinal plants for their everyday medical requirements.[6] In a similar vein, Ethiopia has long used plants as a source of medications to treat a variety of ailments that affect both people and their animals.[7] Because of its natural origin and fewer side effects, the use of herbal therapy is becoming more and more common in both developed and developing countries. Additionally, natural items have proven successful in the production of pharmaceuticals; currently, over 50% of the most popular prescription medications come from herbal products.[8,9] As a result, relying on conventional medical practise, the World Health Organization (WHO) promoted research for the prevention and treatment of diarrheal infections. Through its Diarrheal Control Program, the World Health Organization has used traditional medicine to combat the effects of diarrhoea.[10,11] The usage of opioid-like antimotility medications is being limited due to side effects, which is encouraging researchers to look for new antidiarrheal substances with unique chemical structures and modes of action. As a result, scientists are focusing more and more on traditional medicine in an effort to expand the range of medications available for treating diarrheal illnesses.[12,13]

Q. Leucotrichophora is an evergreen tree in the Fagaceae family. It is typically found in the Himalayan region, which encompasses Kashmir, Himachal Pradesh, Uttarakhand, Nepal, and the northeastern regions of India. Its height is around 40 metres, and its latitudinal range is primarily between 800 and 2300 metres. It is the primary fuel source and the primary fodder tree in these areas.[14,15] To enhance the quality of the human healthcare system, traditional medicine has made use of the leaves, seeds, and bark of *Q. leucotrichophora*. The bark, leaves, and roots of the tree are used to cure stomach pain, haemorrhages, diarrhoea, and gonorrhoea.[16]

2. METHODS

2.1 Preparation of extracts

Quercus leucotrichophora leaves were separately shade dried. Grinder is use for the pulverization of powder. In Soxhlet apparatus, the roots powder was extracted by ethanol and distilled water. After that the extract was hot filtered. By using distillation process solvents is removed. By reducing the pressure, the solvent is fully removed.

2.2 Experimental Animals

This research will involve mice with a weight ranging from 20 to 50 grams, obtained from a reputable supplier in Switzerland. The mice will reside in plastic enclosures with unrestricted access to pellet food and water. The enclosures will be maintained at a temperature of 22±3 ºC and will follow a 12-hour light and dark cycle. To ensure hygiene, cages will be thoroughly cleaned and excrement removed three times a week.

Before the commencement of the trials, the mice will spend a week in laboratory conditions. Food will be withheld for eighteen hours before the start of each trial. Water will only be accessible in designated entry pools, where food and water are provided. International guidelines for the use and care of experimental animals will be strictly adhered to in terms of handling and care.

2.3 Grouping and Dosing of Animals

Five groups of three mice each, treated with the extract, and two control groups with five mice each were randomly assigned. The appropriate treatments were administered to each group through oral gavage. The first group served as the negative control and received 10 milliliters per kilogram of DMSO, while the fifth group, the positive control, received 3 milligrams per kilogram of loperamide. The second, third, and fourth groups were given 100, 200, and 400 mg/kg of the extract, respectively. Dosages for the 80% ethanol were determined as 100, 200, and 400 mg/kg using an acute toxicity test. In other words, the middle dose was established by taking one-tenth of the 2000 mg/kg dose used in the acute toxicity test, and the lowest and highest doses were determined by taking half of and twice the middle dose, respectively.

On the day of the experiments, the animals received a freshly prepared solution of the extract, which had been reconstituted with the appropriate quantities of DMSO. Similarly, the animals were administered loperamide, the positive control, after it had been reconstituted in DMSO. The volume administered extract- and loperamide-treated groups was determined by first establishing the mg/kg needed for each animal and then calculating the equivalent volume containing the required mg of extract or loperamide from the reconstituted solution for each animal.

2.4 Acute Oral Toxicity Test

In compliance with the Organization for Economic Cooperation and Development (OECD) Guidelines 2008: 425, a single female mouse underwent a three-hour fast and received a sole oral gavage dosage of 2000 mg/kg of the 80% ethanol. Subsequently, four additional female mice, also subjected to a three-hour fast, were administered the same dosages based on the outcomes of the initial mouse.

Each animal underwent individual examination for potential signs of toxicity, including alterations in eating habits, water intake, locomotor activity, lethargy, grooming, any indications of weakness or distress, or fatality during the initial thirty minutes. Thereafter, daily observations were conducted for a total of fourteen days, with specific focus on the initial four hours.

2.5 Determination of Antidiarrheal Activity

2.5.1 Castor Oil–Induced Diarrhea - The mouse model for inducing diarrhea in this study involved the use of castor oil. Swiss albino mice of both sexes, after an eighteen-hour fast, were grouped into five sets, each comprising five animals. Following the administration of appropriate quantities of loperamide and extract to each animal, as detailed in the grouping and dosing section, 0.5 mL of castor oil was administered to each mouse. Subsequently, each individual mouse was placed in a plastic cage with a white paper floor. The mice were continuously observed for four hours, during which the frequency of feces, onset of diarrhea, and the weight of each mouse's feces—both wet and total—were recorded. The following formulas were employed to calculate fecal output weight and the percentages of diarrhea inhibition:

Percent of wet feces Inhibition:

$$\frac{\text{Average no. of wet feces of control} - \text{Average No. of wet feces of Drug Treated Group}}{\text{Average no. of wet feces of control}} \times 100$$

Percentage of Wet Fecal Out Put:

$$\frac{\text{Average weight of wet feces of control} - \text{Average weight of wet feces of Drug Treated Group}}{\text{Average weight wet feces of control}} \times 100$$

Percentage of total Fecal Out Put:

$$\frac{\text{Average weight of total feces of control} - \text{Average weight of total feces of Drug Treated Group}}{\textit{Average weight of total feces of control}} \times 100$$

2.5.2 Castor Oil–Induced Gastrointestinal Motility

After an eighteen-hour fast, thirty mice were randomly divided into five groups, each comprising five animals, and were treated according to the guidelines outlined in the animal grouping and dosage section. Following the administration of loperamide and extracts for an hour, each mouse received 0.5 mL of castor oil orally. Additionally, one hour after castor oil administration, each mouse was orally given 1 mL of a marker (5 percent activated charcoal suspension in distilled water). After an hour of activated charcoal administration, all mice were euthanized, and their small intestines, from the pylorus to the cecum, were removed and placed on a sterile surface. The length of the intestine from the pylorus to the cecum was measured after a thorough examination, and the distance covered by the charcoal meal was expressed as a percentage of that total length (peristaltic index). The percentage of inhibition was then expressed using a formula.

Peristalsis Index:

Distance Travelled by Charcoal × 100 Length of Intestine

Percent Inhibition:

Peristalsis Index of Control Group - Peristalsis Index of Drug Treated Group Peristalsis Index of Control Group

2.5.3 Castor Oil Induced Enterpooling

Thirty mice of both sexes will be randomly divided into five groups, with six mice per group, and will be subjected to an eighteen-hour fast, during which they will be deprived of food and water. One hour after the administration of extract and loperamide, as outlined in the grouping and dosage section, each animal will be orally given 0.5 mL of castor oil. Following the delivery of castor oil for one hour, all mice will be euthanized by cervical dislocation. After ligating the pyloric end and ileocecal junction, the abdomen of each mouse will be opened, and the small intestine will be excised and weighed. A graduated tube containing the intestinal contents will be pressed, and the volume will be measured. The difference between the small and empty intestines will be computed once the intestine is weighed again. The subsequent observations will be noted. Percent of Inhibition using Intestinal Weight with content:

Percent of Inhibition using Intestinal Weight with content:

Mean Weight of intestine with content for control- Mean Weight of intestine with content for Drug Treated ×100
Mean Weight of intestine with content for control

Percent of Inhibition using Volume of Intestinal Content:

Mean Volume of Intestinal Content for control- Mean Volume of Intestinal Content for drug treated×*100*
Mean Weight of intestine with content for control

2.6 In vivo Anti-Diarrheal Index

The in vivo antidiarrheal index (ADI) for the extract-treated group and the positive control group was calculated using various data from the aforementioned tests with the following formula:

$$\text{ADI} = \sqrt[3]{\text{D freq} \times \text{G meq} \times \text{P freq}}$$

Where:

D freq: Diarrheal Onset/ Delay in Defecation from Castor oil induced diarrhea.

G meq: Gut meal travel reduction, obtained by Percent Inhibition from Gastro Intestinal Motility Test

P freq: Purging frequency or reduction in the number of wet stools obtained from castor oil diarrheal model

3. RESULT

3.1 Acute toxicity study

Administration of 2000 mg/kg QLME did not result in noticeable changes in physiological and behavioural markers. No mortality was observed during the 14 days of extract treatment, indicating that the QLME LD50 was greater than 2000 mg/kg. On the fourteenth day, the body weight of the treated group was 140.3 ± 1.53 g, while that of the NC group was 139.3 ± 2.52 g. These differences were not statistically significant.

3.2 Effects of *Q. leucotrichophora* Leaf Extract on Castor Oil–Induced Diarrheal Model

The 80% ethanol extract of *Q. leucotrichophora* leaves significantly delayed the onset of diarrheal at all test doses administered to the animals. In comparison to the control group, all extract doses significantly reduced the frequency of defecation. Additionally, both the average weight of moist feces and the average weight of total fecal production were significantly lower in the plant extract group at all test levels compared to the control group. The highest percentage of defecation inhibition, at 89%, was observed at the dose of 400 mg/kg of the plant 80% ethanol leaf extract, which is comparable to the common medication loperamide.

Dose (mg/kg)	Onset of Diarrhea (min)	Number of Wet Feces	Total Number of Feces	Average Weight of Wet Feces (g)	Average Weight of Total Feces (g)	% of Inhibition of Defecation	%WWFO	%WTFO
Control	83.5±9.48	5.6±0.98	6.9±0.85	1.24±0.15	1.38±0.19	0	0	0
80% ethanol extract (100mg/kg)	152.2±19.45	1.8±0.25	3.3±0.81	0.19±0.06	0.23±0.05	68.25	14.63	15.42
80% ethanol extract (200mg/kg)	190.9±11.37	1.5±0.25	2.3±0.25	0.15±0.05	0.22±0.06	75.2	11.25	15.33
80% ethanol extract (400mg/kg)	201.2±4.32	13. ±0.3	1.9±0.38	0.12±0.02	0.021±0.02	79.25	9.64	15.36
Loperamide 3mg/kg	192.3±13.65	1.1±0.02	1.5±0.22	0.25±0.03	0.56±0.06	89.15	7.35	9.65

Table 1. Antidiarrheal Effect of 80% ethanol Extract of the Leaves of *Q. leucotrichophora* on Castor Oil–Induced Diarrhea.

3.3 Effects of *Q. leucotrichophora* Leaf Extract on Castor Oil–Induced Gastrointestinal Transit

In the negative control group, the charcoal meal had a peristaltic index of 78.25±5.36. The plant extract significantly reduced the distance travelled by the charcoal meal at doses of 200 mg/kg (13.26±5.36) and 400 mg/kg (9.6±4.36). However, at a dose of 100 mg/kg, the plant extract did not exhibit a statistically significant reduction in the propulsion of the charcoal marker compared to the negative control group. In comparison, the standard medicine loperamide showed a substantial reduction in the distance travelled by the charcoal meal, achieving the highest percentage of inhibition compared to the negative control group.

Dose (mg/kg)	Length of small intestine (cm)	Distance travelled by charcoal meal (cm)	Peristaltic index (%)	% inhibition
Control	51.25±3.07	39.65±3.48	78.25±5.36	0
80% ethanol extract (100mg/kg)	54.36±3.65	24.25±6.65	44.25±11.25	44.25
80% ethanol extract (200mg/kg)	60.35±3.22	13.26±5.36	21.36±9.65	73.65
80% ethanol extract (400mg/kg)	57.35±2.36	9.6±4.36	17.21±6.35	80.35
Loperamide 3mg/kg	59.65±3.25	9.65±6.65	15.36±7.65	82.36

Table 2. Effect of 80% ethanol Extract of the Leaves of *Q. leucotrichophora* on Gastrointestinal Transit in Mice.

3.4 Effects of *Q. leucotrichophora* Leaf Extract on Gastrointestinal Fluid Accumulation

The negative control group exhibited an intestinal contents volume of 0.86 ± 0.13 and a weight of 1.19 ± 0.08, respectively. The accumulation of gastrointestinal fluid induced by castor oil was significantly reduced by all dosages of the plant extract. Consequently, the intestinal contents volume for the groups treated with extract at doses of 100, 200, and 400 mg/kg were, respectively, 0.36 ± 0.05, 0.34 ± 0.04, and 0.30 ± 0.03. Additionally, the weight of intestinal contents at 100 mg/kg (0.58 ± 0.07), 200 mg/kg (0.52 ± 0.11), and 400 mg/kg (0.51 ± 0.14) was significantly reduced by the plant extract compared to the negative control group.

Dose (mg/kg)	Volume of intestinal contents (mL)	% inhibition	Weight of intestinal contents (g)	% inhibition
Control	0.89±0.13	0	1.19±0.08	0
80% ethanol extract (100mg/kg)	0.36±0.05	59.15	0.58±0.08	52.36

80% ethanol extract (200mg/kg)	0.34±0.04	60.48	0.52±0.11	56.30
80% ethanol extract (400mg/kg)	0.30±0.03	65.16	0.51±0.14	57.14
Loperamide 3mg/kg	0.26±0.04	69.78	0.47±0.11	60.54

Table 3. Effects of 80% ethanol Extract of the Leaves of *Q. leucotrichophora* on Gastrointestinal Fluid Accumulation in Mice

3.5 In Vivo Antidiarrheal Index

The cumulative impact of the plant extract on the frequency of defecation, the onset of diarrheal stool, and the accumulation of intestinal fluid is quantified by the ADI. Plant extracts at dosages of 100, 200, and 400 mg/kg had ADI values of 63.56, 89.64, and 96.72, respectively. These results indicate that the plant extract exhibited a dose-dependent antidiarrheal index, reaching its peak at 400 mg/kg.

Dose (mg/kg)	**Delay in defecation (Time of onset in min, Dfreq) (%)**	**Gut meal travel distance (Gfreq) (%)**	**Purging frequency in number of wet stool (%)**	**Antidiarrheal index**
Control	0	0	0	0
80% ethanol (100mg/kg)	82.73	45.25	67.86	63.56
80% ethanol (200mg/kg)	128.36	72.36	76	89.64
80% ethanol (400mg/kg)	141.25	80.36	79.65	96.72
Loperamide 3mg/kg	130.6	82.65	83.19	96.65

Table 4. In Vivo Antidiarrheal Indices of 80% ethanol Extract of the Leaves of *Q. leucotrichophora*

4. CONCLUSION

The antidiarrheal properties of the *Q. leucotrichophora* leaf extract (80% ethanol) was evaluated using Swiss albino mice as animal models. The plant extract exhibited a significant delay in the onset of diarrhea, reduced the frequency of wet feces, and demonstrated strong antisecretory effects at all experimentally examined doses. Additionally, the plant extracts showed antimotility action at higher concentrations. The study results supported the traditional belief in the plant's antidiarrheal properties, although further research using various antidiarrheal models and solvents is warranted. Furthermore, the study assessed the acute toxicity of the plant extract and found it to be safe, with an LD_{50} exceeding 2000 mg/kg, ensuring the safe use of plant extracts in traditional medicine.

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