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EVALUATION OF ANTIDIARRHEAL ACTIVITY OF ETHANOLIC ROOT EXTRACT OF *GLYCYRRHIZA GLABRA* LINN. IN RATS

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ABSTRACT
Diarrheal diseases are a major public health problem in developing countries. The present study was undertaken to evaluate the effects of ethanolic extract of roots of *Glycyrrhiza glabra* on castor oil-induced diarrhea and magnesium sulphate-induced diarrhea method were examined. Each model consisted four groups, control, standard (loperamide), *Glycyrrhiza glabra* root extract 150 mg/kg and 300 mg/kg were used. It was noticed that the fecal weight was reduced in the doses 150 and 300 mg/kg significantly in comparison to control. The extract dose (300 mg/kg) was significantly choose for reducing the diarrheal condition in experimental rats. The extract was found to inhibit peristaltic movements of secretions in castor oil induced and magnesium sulphate induced model confirming its antidiarrheal activity, which might be due to its high flavonoid content. The results provide evidence that the ethanolic extract of *Glycyrrhiza glabra* root possesses antidiarrheal activity and could be accounted for pharmacological effects and Herbal medicine.

KEYWORDS: *Glycyrrhiza glabra*, Diarrhea, castor oil, Antidiarrheal.

1. INTRODUCTION
Due to unhygienic livelihood condition, peoples of the third world counties are very prone to several common diseases including diarrhea. According to the World Health Organization (WHO), diarrhea is the second leading reason of death of children less than five years of age.[1] Diarrheal diseases are a major public health problem in developing countries.[2,3] Diarrhea is intestinal disorders characterized by the passage of three or more loose and/or liquid stools daily and is one of the leading causes of mortality and morbidity in developing countries.[4,5] The pathophysiology of diarrhea is due to increased secretion and decreased fluid absorption leading to increased fluidity, stool volume and frequency, excessive loss of body water and electrolytes.[6] The present antidiarrheal drugs are known for their adverse effects like abuse potential of codeine, loparamide induced common side effects like abdominal cramps and rashes. In young children serious complications like paralytic ileus and toxic megacolon was seen due to loparamide, probable cause being absorption of toxins.[7]

Therefore the search for safe and more effective agents from plant origin has continued to be an important area of active research. However, plants have long been a very important source of new drugs. Many plant species have been screened for substances with therapeutic activity. For the treatment of diarrhea, medicinal plants are a potential source of antidiarrheal drugs.[8] Moreover, many international organizations including WHO have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices.[9–11] At present, around 25% of drugs are isolated from plants and there are numerous evidences available about the use of medicinal plants including their pharmacological and biochemical properties.[12]

Glycyrrhiza glabra Linn, is a herb belonging to the family Leguminoceae. *Glycyrrhiza glabra*, also known as Liquorice and sweetwood, is native to the Mediterranean and certain areas of Asia. Historically, the dried rhizome and root of this plant were employed medicinally by the Egyptian, Chinese, Greek, Indian, and Roman civilizations as an expectorant and carminative. *Glycyrrhiza glabra* Linn is a hardy perennial shrub, attaining a height up to 2.5m.[13] It is widely used in ayurvedic formulations. The phytochemical screening of the *Glycyrrhiza glabra* root, leaves and other part revealed the presence of alkaloids, glycosides,

carbohydrates, starches, phenolic compounds, flavonoids, proteins, pectin, mucilage, saponins, lipids, tannins, sterols and steroids.[14,15] It showed memory enhancement, antidepressant, antimicrobial, anticancer, antioxidant, protective, anti-inflammatory, antiulcer, anti-diabetic, hypolipidemic and many other pharmacological effects.[16-19]

In the view of above literature study, we have attempted to investigate the traditional polyherbal formulation for their antidiarrheal activity. In the traditional system of medicine, the roots and rhizomes of G. glabra have been employed clinically for centuries for their antiinflammatory, antiulcer, expectorant, antimicrobial and anxiolytic activities.[20-21] No scientific work has so far evaluated the antidiarrheal activity of this plant. The main objective of this work is to evaluate the activities of Glycyrrhiza glabra root extract on castor oil-induced and Magnesium sulphate induced diarrhea in rats.

2. MATERIAL AND METHODS

2.1 Collection and Authentication of plant

Glycyrrhiza glabra roots were collected from the state of Madhya Pradesh District Bhopal during the month of May. The plant has been identified and authenticated by Dr Saba Naaz, Head of the Department Botany at the Safia college of science, Bhopal (M.P.). The plant part specimen was submitted as herbarium with Voucher specimen no 120/Bot./SaifSc./Bpl/2019.

2.2 Drying, size reduction and storage of plant material

The plants parts were dried under shade. It was pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve No.20 to maintain uniformity and packed into airtight container and stored in cool and dry place. This material was used for the further study.

2.3 Preparation of *Glycyrrhiza glabra* leaves extract

Extraction of *Glycyrrhiza glabra* was done by Soxhlet extraction method.

Soxhlet Extraction: Soxhlet apparatus was used for the extraction and ethanol solvent was selected as a solvent for extraction and calculated percentage yield of the extract.

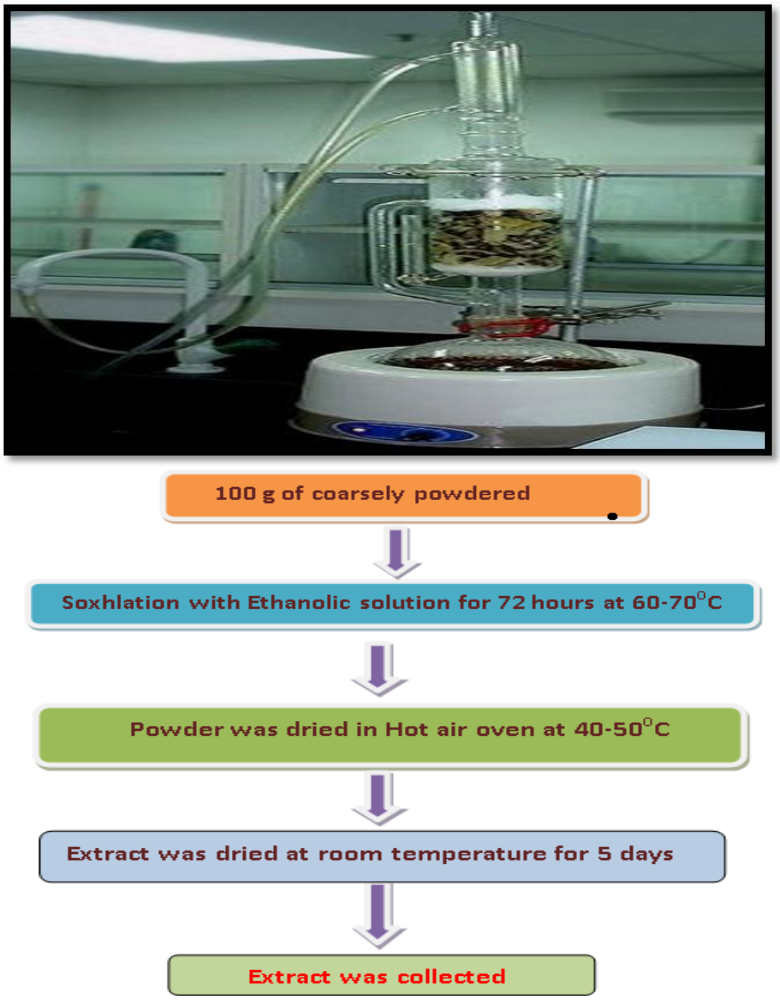

Figure: Process of Soxhlet Extraction of Plants Parts.

2.4 Phytochemical Analysis of Crude Extracts

The crude extracts of plants obtained by solvent extraction were subjected to various qualitative tests to detect the presence of common chemical constituents as: alkaloid, glycoside, carbohydrate, phytosterols, saponins, tannin, flavonoids and protein etc.

2.5 Experimental Work

Animals: Adult Wistar rats of 150-200 g were used for the study. The rats were obtained from the Ravishankar college of pharmacy Bhopal, (M.P.) for experimental purpose. The animals were maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ±5%) and 12 h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile husk as bedding. They had free assessed to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Ravishankar college of pharmacy Bhopal, (M.P.) **(Proposal no:)** according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animal, Govt. of India. **(Approval no. 1733/PO/Ere/ S/13/CPCSEA).**

Preparation of dose: The ethanolic extract of *Glycerrhiza glabra* root part was dissolved in suspending agent (1% CMC) before orally administered to the Rats. Standard drug was dissolved in suspending agent (1% CMC) before orally administered to the Rats.

In vivo Antidiarrheal activity

Castor oil induced diarrhea: Rats of either sex were fasted for 18 h and divided into four groups.

Group	Treatment
1. Normal control	Received 10% Tween 80 (5ml/kg) by gavage
2. Disease control	Received castor oil (1 ml)
3. Standard control	Received loparamide (3mg/kg) + castor oil (1 ml)
4. Test control (A)	Received extract (150mg/kg) + castor oil (1 ml)
Test control (B)	Received extract (300mg/kg) + castor oil (1 ml)

After 30 min, diarrhea was induced by oral administration of castor oil (1 ml) to each rat. Rats were observed for a period of 4h during which the total number of fecal outputs and the number of diarrheic feces excreted were recorded.

Magnesium sulfate induced diarrhea: Rats of either sex were fasted for 18h and divided into four groups.

Group	Treatment
1. Normal control	Received 10% Tween 80 (5ml/kg) by gavage
2. Disease control	Received magnesium sulfate (2 mg/kg)
3.Standard control	Received loparamide (3mg/kg) + magnesium sulfate (2 mg/kg)
4. Test control (A)	Received extract (150mg/kg) + magnesium sulfate (2mg/kg)
Test control (B)	Received extract (300mg/kg) + magnesium sulfate (2mg/kg)

After 30 min, diarrhea was induced by oral administration of magnesium sulfate (2 mg/kg) to each rat. Rats were observed for a period of 4 h during which the total number of fecal outputs and the number of diarrheic feces excreted were recorded.

Evaluation: Every hour, total weight of faecal output, total weight of wet faeces, total number of faecal output, and number of wet faeces were recorded. A numerical score based on stool consistency was assigned as follows:

Normal stool= 1, semi-solid stool= 2 and watery stool= 3. And % inhibition of diarrhea was calculated as follows:

$$\% \text{ inhibition of diarrhea} = \frac{\text{Mean Number of wet defecation (control – test)}}{\text{Mean wet defecation of control}} \times 100$$

Statistical analysis: The data were expressed as mean ± SEM. Results were analyzed statistically by One-way ANOVA (analysis of variance) followed by Dunnett's t-test using standard statistical software. All the groups were compared with the control group in each model. The difference was considered significant if $p<0.05$.

3. RESULTS

Morphology and Phytochemical investigation

Morphological characteristics of *Glycyrrhiza glabra* root shown in Table 1 and 2. The percentage yields of the ethanolic extracts were found to be 13.25% w/w. The ethanolic extract showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins and saponins in *Glycyrrhiza glabra* root part. Phytochemical

screening of ethanolic extract of *Glycyrrhiza glabra* shown in Table 3.

Table 1: Morphological characteristics of *Glycyrrhiza glabra*.

S. No.	Character	Observation
1	Color	Brownish black
2	Odor	None
3	Taste	Characteristic
4	Size	10-20 cm. length

Table 2: Consistency and color of ethanolic extract of *Glycyrrhiza glabra.*

Extract	Color	Consistency
Ethanolic	Blackish	Semi solid

Table 3: Phytochemical screening of ethanolic extract of *Glycyrrhiza glabra.*

S. No.	Identification Test	Test name	Results
1	Alkaloids	Mayer's test	-
		Dragendroff's test	+
		Wagner's test	+
2	Glycosides	Killer-killani test	-
3	Carbohydrates	Molisch's test	+
		Fehling test	+
4	Tannins & Phenols	Gelatin test	+
		Ferric chloride test	+
5	Flavonoids	Shinoda test	+
		Alkaline reagent test	+
6	Steroids	Libermamm-Burchard test	+
		Salkowski test	+
7	Saponins	Foam test	+
8	Protein	Xanthoprotic	-
9	Gums & Mucilage	With 95% alcohol	-

(+) = Present, (–) = Absent

Acute Toxicity Studies (LD_{50})

In both phase I and Phase II procedures, none of the animal mortal or any signs of behavioral changes or show any toxicity upon the single administration of PFEE (2000 mg/kg p.o.). Hence,the dose of 150mg/kg and 300 mg/kg were chosen.

Table 4: Results of Acute oral toxicity study of EEGG.

Group name	Anima l mark	Dose mg/kg	Body weight (gm)			Observation	Mortality (If any)
			1 day	7 days	14 days		
Control	H	Normal saline (0.91%)	153	148	146	No sign of toxicity & all animals Survived.	No mortality occurs.
	B		155	150	152		
	T		138	135	132		
Test	HT	2000 mg/kg of ethanolic extract GG (Once dosing at start of acute oral toxicity study)	205	208	202		
	BT		190	185	180		
	NM		175	165	168		

Effects of the ethanolic extract of *Glycyrrhiza glabra* on castor oil-induced diarrhea

Diarrhea induced by castor oil and magnesium sulphate. Castor oil is a very effective laxative hydrolyzed into ricinoleic acid, and stimulated fluid secretion, inhibited water and electrolyte absorption, reduced active Na+ and K+ absorption, and decreased Na+, K+-ATPase in the small intestine and colon. Castor oil also increased peristaltic activity and produced permeability changes in the intestinal mucosal membrane to electrolytes and water. Furthermore, ricinoleic acid can also lead to the release of endogenous prostaglandins, which play an important role in the modulation of GIT, stimulate motility and secretion, and cause diarrhea. In our study, the results showed that HATP could, in a dose-dependent manner, reduce castor oil-induced diarrhea as well as the number of diarrheal faeces and total weight of faeces, which could be taken as antidiarrheal activities.

Loperamide is used as standard drug for diarrheal treatment. The therapeutic effect of Loperamide is due to its anti-motility and anti- secretary activity.

Table 09: Results of Castor oil induced diarrhea

Group	Dose (mg/kg)	Time of onset of diarrhea (min)	Total no. of faeces in 4hrs	Weight of stool (gm)	% Inhibition of defecation
Normal control	10% Tween 80 (5 ml/kg)	60.0±0.6	1.8±0.2***	0.05±0.02***	-
Disease control	Castor oil (1 ml)	143.8±2.0	6.9±1.0	0.72±0.01	-
Standard control	Loparamide (3 mg/kg) + castor oil (1 ml)	198.2±0.2***	1.6±0.2***	0.01±0.02***	76.81 %
Test Control	Ethanolic extract (150 mg/kg) + castor oil (1 ml)	150.0±0.1***	2.8±0.4**	0.32±0.02***	26.92 %
	(300 mg/kg) + castor oil (1ml)	175.0±0.1***	3.8±0.4**	0.20±0.02***	44.92 %

Results are Mean ± SEM and significantly different when compared with that of the control at * P<0.05, **P<0.01, ***P<0.001.

Effects of the ethanolic extract of *Glycyrrhiza glabra* on Magnesium sulfate -induced diarrhea

Magnesium sulfate has been reported to induce diarrhea by increasing the volume of intestinal content through prevention of reabsorption of water. It has been demonstrated that it promotes the release of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium, chloride and water.

Table 10: Results of Magnesium sulfate induced diarrhea.

Group	Dose (mg/kg)	Time of onset of diarrhea (min)	Total no. of faeces in 4hrs	Weight of stool (gm)	% Inhibition of defecation
Normal control	10% Tween 80 (5ml/kg) by gavage	60.0±0.6	1.8±0.2***	0.12±0.14***	-
Disease control	castor oil (1 ml)	136.8±2.0	6.80 ± 0.34	0.68±0.02	-
Standard control	loparamide (3mg/kg) + castor oil (1ml)	202±0.1***	1.43 ± 0.77	0.10±0.98	78.97 %
Test control	Ethanolic extract (150mg/kg) + castor oil (1ml)	160.0±0.3***	1.32 ± 0.98	0.50±0.01*	30.88 %
	(300mg/kg) + castor oil (1 ml)	186.0±0.3***	3.00 ± 0.98	0.39±0.01*	55.88 %

Results are Mean ± SEM and significantly different when compared with that of the control at *P<0.05, **P<0.01, ***P<0.001

4. DISCUSSION

Diarrhea is a very common ailment and national problem in many tropical countries and the cause of 4-5 million deaths throughout the world annually. Apart from modern medical therapy, the use of herbal drugs in the treatment of diarrheal diseases is a common practice in many countries of Asia including India and Bangladesh. A number of medicinal plants have been reported to be effective against diarrhea and dysentery, as they are used in traditional herbal practice. Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diarrhea and dysentery. The present study was undertaken to substantiate out the scientific rationale behind the local use of *Glycyrrhiza glabra* root in diarrhea.

The antidiarrheal activity of the ethanolic extract of the root of *Glycyrrhiza glabra* was evaluated by employing castor oil-induced diarrhea and magnesium sulphate-induced methods. The results of the present study showed that the ethanolic extract of *Glycyrrhiza glabra* in castor oil-induced diarrhea at 150 and 300mg/kg body weight doses significantly lowered several typical parameters of diarrhea, producing a statistically significant reduction in the severity and frequency of diarrhea produced by castor oil. Furthermore, our preliminary investigations have revealed that the extract was safe up to 5 g/kg dose level in acute oral toxicity studies.

In the preliminary phytochemical screening, the extract

was positive for flavonoids, saponins, tannins, phenols, terpenoids, and alkaloids. These secondary metabolites are effective as antioxidant, antineoplastic, anti-ulcer, anti-inflammatory, and immune stimulating agents. Flavonoids are thought to increase mucosal prostaglandin content, decrease histamine secretion from mast cells by inhibition of histidine decarboxylase, inhibit Helicobacter pylori growth, act as free radical scavengers, and inhibit H+/K+- ATPase. Saponins may activate mucous membrane protective factors, and tannins render the outermost layer of the mucosa less permeable, for instance, to chemical irritation. In addition, terpenoids and alkaloid compounds are also reported to have potent activity against gastric ulcers.

As per literature review in acute oral toxicity study there were no behavioral changes seen up to 4hrs and no mortality was observed up to the end of 24hrs even at the maximum tested dose level of 2000 mg/kg per oral. The extract dose (300 mg/kg) was significantly choose for reducing the diarrheal condition in experimental rats. For the treatment of rats standard drug for diarrhea loparamide was used. However, it is well documented that castor oil produces diarrhea due to its most active component ricinoleic acid through a hypersecretory response. Therefore it can be assumed that the antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretions which are altered in this intestinal condition. Antidiarrheal action of the extract was mediated by an antisecretory mechanism. The present study sought to assess the antidiarrheal activity of root extracts of *Glycyrrhiza glabra.*

In addition, loperamide inhibits the release of acetylcholine and prostaglandins since these two substances are known secretagogues (substances that, by stimulating cAMP, cause the secretion of ions) We can say that our extracts would act in the same mechanism to reduce or treat diarrhea by reducing the volume of the secretion of the intestinal continents and electrolytes.

5. CONCLUSION

In conclusion we can say that extract dose (300 mg/kg) of *Glycyrrhiza glabra root* show significantly reducing the condition of diarrhea with the help of flavonoids, saponin, alkaloid, tannins, phenols and some other phyto-constituents. The plant extract was also found to have optimal safety margin based on the limit test at 2000 mg/kg dose level acute toxicity test. Therefore, the plant is potentially useful to develop plant based products after further studies to identify the active principle and the mechanism of action.

These results would justify the use of this plant in traditional medicine for the treatment of diarrhea.

Conflict of Interests

The authors declare that there is no conflict of interests.

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