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Research Article

Phytochemical Screening And Pharmacological Evaluation of Anti Diarrheal Efeect of Aswagandha and Plambago Zeylanica Plant Extract for Preventive and Curative Activity

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Abstract

Aswagandha (solanaceae) and plambago zeylanica (plambaginaceae) were used for anti-diarrheal activity. The present study was to evaluate the anti-diarrheal activity by curative and preventive method of aswagandha and plambago zeylanica by using castor oil induced diarrhea model. Animals were divided into 4 groups as control, standard, and two test groups. The crude extract of aswagandha was found to be more significant ($p < 0.05$) between the groups in curative method whereas the crude extract of plambago zeylanica in preventive method was found to be more significant ($p < 0.001$) between the groups.

Keywords: Antidiarrheal Activity; Ashwagandha; Plumbago Zeylanica

Introduction

Diarrhea is defined as an alteration in normal bowel movement, which leads to increase in daily stools, which contains 60-95% of water [1].

There are nearly 1.7 billion cases of diarrhea disease year globally. Diarrheal disease is the second leading cause of death under 5 years old.[2] Diarrhea appeared by several mechanism such as increasing the gut motility, along with increased secretion of ions and a decrease in the absorption of fluid, and thus a loss of electrolytes, particularly Na+ and water [2].

According to World Health Organization (WHO) estimation for the year 1998, there were about 7.1 million deaths due to diarrhea [3].

Plumbago zylanica and Ashwagandha are highly studied plants for various pharmacological activities in the present paper the antidiarrheal activity is studied here [12,13].

Classification

Diarrhea generally is divided into two types

- Acute diarrhea- lasts from a few days up to a week.
- Chronic diarrhea- lasts more than three weeks [4].

Based on pathophysiology, it is divided into [41].

- Osmotic diarrhea
- Secretory diarrhea
- Motility-related diarrhea
- Inflammatory diarrhea

Materials

Drug and chemicals

Ethanol was bought from SD fine chem lab while loperamide as standard manufactured by cipla laboratory was brought from local pharmacy. Castor oil was purchased from s-d fine chemical laboratory. All the chemicals and reagents were analytical grade [5].

Plant Materials

Aswaganda and Plambago zeylanica was collected and dried for one week and powdered by using pulveriser [6].

Animals

Albino male sprague dwaley rats weighing between 120-150 gms were purchased from S.N enterprises Uppal after the AIEC clearance, which used as experimental model for investigation of the anti-diarrheal activity. All the animals housed under standard laboratory condition at 25 ± 2 C and 12hrs dark: light cycle, acclimatized for 10 days before experiment standard diet and water were provided constantly [6].

Methodology

Preparation of extract

The plant was dried for one week and grounded into fine powder by pulverization.

The powdered plant was uniformly moistured with 500 ml ethanol and the mixture was subjected to Soxhlet apparatus.

The process was carried until whole solvent becomes colorless and semisolid extract was preserved in air tight container [6].

Phytochemical screening

Phytochemical screening of ethanolic extract was carried out to detect the presence of carbohydrates, alkanoids Flaronoids, steroids, Glycosides, phenols, gums, protiens, amino acids, fats [7,8].

Acute toxicity

Safety studies was carried out by using fixed dose study under OECD guidelines (420) [9].

Animals are tested with sighting dose (2000 mg) and are observed for 14 days. Observation should include changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and CNS.

TLC

TLC of alcoholic extract was carried out on silica gel 'G' plates using methanol, H_2O, acetic acid (30:60:5) as the mobile phase. After solvent travels the plate, they are dried and are placed under UV light (254mm and 365mm) shows fluorescent spots (dark blue zone and blue to green) [9].

Castor oil induced diarrhea in rats

The castor oil induced diarrhea model was carried out by curative and preventive method. Male albino rats (120-150 gms) were fasted overnight [10].

Diarrhea induced by castor oil by curative method

Four groups each containing 6 animals are administered with 0.5 ml of castor oil, after 15 mins of cathartic agents, groups are treated with vehicle (saline), loperamide 5 mg/kg (standard drug), aswagandha, plambago zeylanica. Immediately after ingesting different treatments, animals are placed individually in box containing elevated cages, which are lined with blotting paper and are observed for 4 hrs. The weight of the blotting paper is noted before and after the experiment. The wet and dried fecal matter is noted and compared between the groups [10,11].

Diarrhea induced by castor oil by preventive method

Four groups each containing 6 animals each were orally treated with vehicle (saline), loperamide 5mg/kg (standard drug), aswagandha, plambago zeylanica. After 30 mins 0.5 ml of castor oil is given. Immediately after ingesting castor oil, animals are individually placed in elevated cages, which are lined with blotting paper and are observed for 4 hrs. The weight of the blotting paper is noted before and after the experiment. The wet and dried fecal matter is noted and compared between the groups [10,11].

Statistical analysis

The results are represented as mean ± standard error of mean (SEM). The one-way ANOVA test with Turkey Kramer multiple comparison test using graphed prism software. P-value is noted.

Results and Conclusion

Phytochemical evaluation

- **Aswagandha:** The phytochemical evaluation of ethanolic extract of aswagandha showed presence of all chemical constituents like carbohydrates, proteins, amino acids, glycosides, steroids, tannins, oils, flavonoids as mentioned in table 1.
- **Plambago zeylanica:** The phytochemical evaluation of ethanolic extract of aswagandha showed presence of all chemical constituents like carbohydrates, proteins, amino acids, glycosides, steroids, tannins, oils, flavonoids as shown in table 2.
- **Acute toxicity studies:** The plants under the study were subjected to safety study by fixed dose method and scored by blind screening method were found to safe which was evaluated by the tests for somatic and autonomic activities. Awareness, mood, motor activity, CNS excitation, posture, motor incoordination, muscle tone, reflexes, MISC, dead are noted during the study of aswagandha and plambago zeylanica.

S. NO	Tests	Ethanol	1-Butanol	N- Heptane	Acetone
1.	Carbohydrates:				
	I. Benedict's Test	+++	+	-	-
	Ii. Fehling's Test	+++	+	-	-
	Iii. Molisch Test	+++	-	+	-
2.	Test For Gums	+	+	+	+
3.	Test For Proteins:	+			
	I. Biuret Test	+	-		-
	Ii. Millon's Test	+	+		-
4.	Test For Amino Acids: Ninhydrin Test	+	-	-	-
5.	Test For Alkaloids:	+			
	I. Dragondroff's Test	+	+	+	+
	Ii. Mayer's Test	+	-	-	-
6.	Test For Phenols: Acetic Acid Test	+	-	-	-
7.	Test For Oils: Solubility For Benzene	+	+	+	+
8.	Test For Saponin Glycosides: Foam Test:	+	-	-	+
9.	Test For Steroids: Salkowski Test:	+++	+	+	+
10.	Test For Flavonoids:	+++	-	-	-

Table 1: Result of the phytochemical evaluation of Ashwagandha.

S.NO	Tests	Ethanol	1-Butanol	N- Heptane	Acetone
1.	Carbohydrates:				
	I. Benedict's Test	+++	+	-	-
	Ii. Fehling's Test	+++	+	-	-
	Iii. Molisch Test	+++	-	+	-
2.	Test For Gums	+	+	+	+
3.	Test For Proteins:	+			
	I. Biuret Test	+	-		-
	Ii. Millon's Test	+	+		-
4.	Test For Amino Acids:Ninhydrin Test	+	-	-	-
5.	Test For Alkaloids:	+			
	I. Dragondroff's Test	+	+	+	+
	Ii. Mayer's Test	+	-	-	-
6.	Test For Phenols: Acetic Acid Test	+	-	-	-
7.	Test For Oils: Solubility For Benzene	+	+	+	+
8.	Test For Saponin Glycosides: Foam Test:	+	-	-	+
9.	Test For Steroids: Salkowski Test:	+++	+	+	+
10.	Test For Flavonoids:	+++	-	-	-

Table 2: Result of the phytochemical evaluation of Plumbago zylanica.

- **TLC Results:** Ethanolic extract of aswagandha, plumbago zeylanica were subjected to TLC studies to identify the presence of flavonoids, which are the most important constituents for the establishment of pharmacological activity in the present trial. The solvent system separated the individual flavonoids with the Rf value for aswagandha 2.87 cm and for plambago zeylanica 10cm.

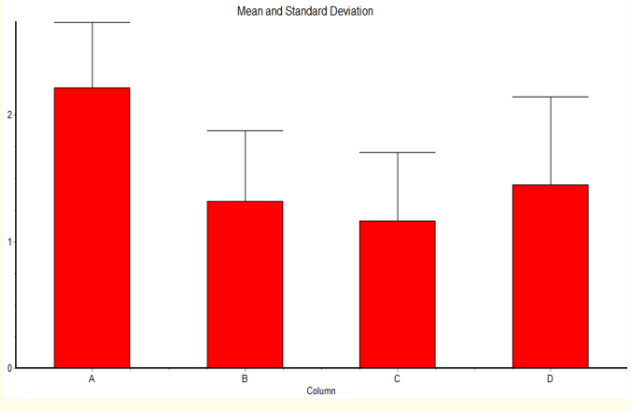
Castor oil induced diarrhea model

Curative method

Curative method between groups, under study aswagandha was found more significant as antidiarrheal were more potential with curative property when compared to other groups. P-value was found to be P-value 0.0023 while ** P-value is less than 0.05 between groups. Results are shown in table 3 and Graph 1.

S.no	Group	Mean ± SEM
1	Untreated	2.215 ± 0.213
2	Standard	1.32 ± 0.226
3	Aswgandha	1.66 ± 0.22
4	Plambago zeylenica	1.45 ± 0.28

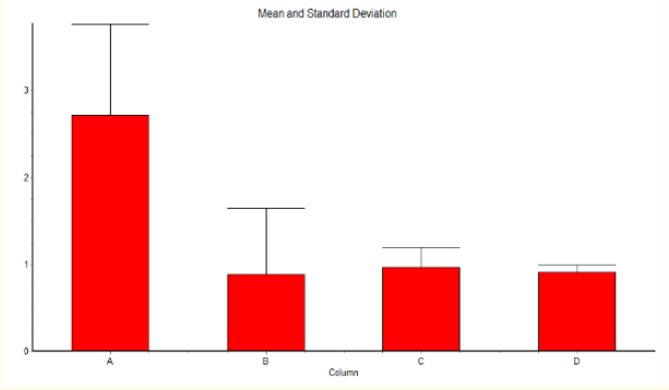
Table 3: Showing comparison of the curative method of castoroil induced diarrhea model results between various groups of the study.



Graph 1: Showing comparison of the curative method of castoroil induced diarrhea model results between various groups of the study.

Preventive method

In preventive method between groups under study plambago zeylindica was found to be significant in showing preventive activity in comparison to other groups. P-value was found to be P- value is 0.0002 while *** P- value < 0.001 between the groups.



Graph 2: Showing comparison of the preventive method of castoroil induced diarrhea model results between various groups of the study.

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