



Research Article

PHYTOCHEMICAL STUDY, ACUTE DRUG TOXICITY AND ANTI-DIARRHOEAL ACTIVITY OF ETHANOL EXTRACT OF WHOLE PLANT OF *AINSLIAEA APTERA*

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ABSTRACT

The present study deals with the investigation of the Phytochemical screening and Acute drug toxicity and Anti Diarrhoeal activity of Ethanolic extract of whole plant of *Ainsliaea aptera* (EEAA). The genus *Ainsliaea* is a medicinal herb that belongs to the family of Asteraceae (Compositae). This Genus has been used as a folk medicine for the treatment of rheumatism, traumatic injuries, edema, and stomachache so far, several sesquiterpenes, triterpenes and flavonoids, have been isolated from this genus. The genus *Ainsliaea* Dc. is distributed in South East Asia ranging from Afghanistan to Japan. The present study deals with the investigation of the Phytochemical screening, Acute drug toxicity and Anti Diarrhoeal activity of Ethanolic Extract of Whole Plant of *Ainsliaea aptera* (EEAA). Preliminary Phytochemical screening done for the presence of carbohydrate, protein, alkaloids, flavonoids, tannins, glycosides, phytosterol, and saponins were carried out using standard test procedures. The procedure was followed as per OECD423 guidelines for acute drug toxicity. The methods used for evaluating the Anti-Diarrhoeal activity were castor-oil induced diarrhoea model in rats and charcoal meal test/intestinal motility test in mice at dose 800 mg/kg, p.o. in rats and the corresponding doses in mice. The parameters observed were the onset of defecation, cumulative faecal weight in the castor oil induced diarrhoea model and the distance travelled by charcoal in the intestinal motility test. Preliminary Phytochemical results showed the presence of carbohydrates, proteins, Triterpenoid, Flavonoids, Phytosterols, Tannins and Saponins and absences of Alkaloids and Glycoside in the drug. Test substance related mortality was not observed at 2000 mg/kg which revealed the nontoxic nature of EEAA. This finding probably suggests that the ethanol extract is relatively safe or non-toxic in rat/mice at the doses used for this study. Study showed non-significant effects on both, first defecation time and cumulative fecal weight at the dose of 800 mg/kg in the castor oil induced Diarrhoea model. Similarly, a non-significant reduction in the distance travelled by charcoal was found at the dose of 800 mg/kg in the charcoal meal test. There was no significant effect of EEAA at dose 800mg/kg in castor oil induced diarrhoea and the plant show no significant effect on intestine motility in intestinal motility test. The Ethanolic Extract of Whole Plant of *Ainsliaea Aptaera* (EEAA). The Present study showed non-significant Anti-Diarrhoeal, non-significant Anti-Motility Activities at dose 800 mg/kg. The present study showed the presence of carbohydrates, proteins, Triterpenoid, Flavonoids, Phytosterols, Tannins and Saponins and absences of Alkaloids and Glycoside in the drug. Test substance related mortality was not observed at 2000 mg/kg which revealed the nontoxic nature of EEAA.

Keywords: *Ainsliaea Aptaera*, Diarrhoea, Motility, Castor-Oil Induced Diarrhoea, Charcoal Meal Test, EEAA.

INTRODUCTION

Plants are important sources of therapeutic drugs and play a significant role in the survival of the tribal and ethnic communities. According to the world health organization (WHO), about three-quarters of the world population rely upon traditional remedies (mainly herbs) for their health care¹. In fact, plants are the oldest friends of mankind. They not only provided food and shelter but they also served the humanity to cure different ailments. Medicinal plants have provided the modern medicine with numerous plant derived therapeutic agents. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. These drugs are invariably single plant extracts or fractions thereof or mixtures of fractions/extracts from different plants, which have been carefully standardized for their safety and efficacy². *Ainsliaea aptera* (Asteraceae) is native of Southeast Asia. The genus *Ainsliaea* consists of 72 species most of which are indigenous and well distributed in Southeast Asia. The roots of *Ainsliaea aptera* is widely used in traditional medicine as Stomach-ache³. However, the plant has not been scientifically evaluated for any pharmacological activity. So, in present study we explore the whole plant for Phytochemical Screening, Acute Drug Toxicity and Anti-diarrhoeal Activity.

MATERIAL AND METHOD

Plant collection and Identification

The plant material was collected from the Bagi, a village of Tehsil Chirgaon, Dist. Shimla (H.P) in the month of September-October and was authenticated by Dr. H. B. Singh, Chief Scientist and Head Raw Materials Herbarium & Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR) New Delhi

Preparation of Extract

The collected plant material of *Ainsliaea aptera* was shade dried at room temperature, and then pulverized in mixer grinder to coarsely powdered drug and passed through mesh size 40 sieves. Powder drug was extracted with 75% of ethanol in a Soxhlet apparatus. The extract was concentrated in a rotator flash evaporator. The residue was dried in desiccators over calcium carbonate slit. This was stored in the refrigerator and was used throughout the experiment.

Drugs and Chemicals

Loperamide (Torrent, Ahmedabad, India), Castor oil (S.D. Fine Chem. Ltd.), Activated charcoal, Atropine sulphate and chemicals used for Phytochemical screening. All chemicals used are analytical grade. Male and female mice (20-35g) and rats (150-200) were used in this study. The animals were housed in the animal house facility of the Department of Pharmacology, Rayat Institute of Pharmacy. The animals were housed in groups of 6 in polypropylene cages with soft husk as bedding, fed with normal commercial pellet diet (Ashirwad Industries, Ropar, India) given water *ad libitum* and maintained under laboratory conditions (temperature 24 - 28°C, relative humidity 60 - 70%, and 12 h light-dark cycle). The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and was carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India

Phytochemical Screening

Preliminary phytochemical screening for the presence of carbohydrate, protein, alkaloids, flavonoids, tannins, glycosides, phytosterol, and saponins were carried out using standard test procedures⁴.

Acute drug toxicity

The procedure was followed as per OECD423 guidelines. The extract was administered orally at a dose 2000 mg/kg body weight to different groups of rats. After the administration of extract, food was withheld for further 3-4 h but not water. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks.

Assessment of Anti-Diarrhoeal Activity Castor oil Induced Diarrhoea in Rats

The method of Awouters et al. (1975)⁵ was adopted. Rats were divided into three groups of 6 animals each. Group I served as control group that received 0.5% CMC (5ml/kg). Group II served as standard group and received standard drug loperamide (3mg/kg, p.o). Group III served as drug treated group and received EEAA (800mg/kg, p.o). Rats were fasted overnight. Food was withdrawn during the experimental period, however, water was provided *ad libitum*. After the pre-treatment period of 1 h, castor oil (15 ml/kg) was administered orally to all the rats. The rats were then housed individually in cages on a filter paper and observed for a period of 4 h. The filter paper was changed after every 1 hr. The first defecation time was noted for each rat. After every 1 h the faecal matter was collected, weighed and consistency of faecal matter was recorded.

Charcoal Meal test or Small intestine transit model in mice

The method of Yegnanarayan and Shrotri (1982)⁶ was used. Mice were divided into three groups of 6 animals each. Group I

served as control group that received 0.5% CMC (5ml/kg). Group II served as standard group and received standard drug Atropine sulphate (5mg/kg, i.p). Group III served as drug treated group and received EEAA (800mg/kg, p.o). Mice were fasted overnight. After a pre-treatment period of 45 min, activated charcoal (10%) suspended in gum acacia was administered orally to all the mice at the dose of 25 ml/kg. The animals were sacrificed 15 min after charcoal administration, the intestine was removed from pyloric sphincter to caecum and the total length was measured (in cm). The distance travelled by charcoal was measured (in cm) and expressed as the percent of the total length of the intestine.

STATISTICAL ANALYSIS

All the results are expressed as mean $\pm$ standard mean error (SEM) followed by one way ANOVA along with tukey's multiple comparison tests by using graph pad prism version-5.0 software. The $p < 0.05$ was considered to be statically significant.

RESULT

Phytochemical Screening

Whole plant of *Ainsliaea aptera* was collected and analysed for different Phytoconstituent by use of standard procedure. Preliminary Phytochemical results showed the presence or absence of certain Phytochemicals in the drug. The results are given in Table 1.

Acute Toxicity Studies

Acute drug toxicity studied was conducted as per OECD guidelines 423. Test substance related mortality was not observed at 2000 mg/kg which revealed the nontoxic nature of EEAA. This finding probably suggests that the ethanol extract is relatively safe or non-toxic in rat/mice at the doses used for this study.

Effect of Ethanolic Extract of Whole Plant of *Ainsliaea Aptera* (EEAA) on Castor oil induced Diarrhoea

In the control group, castor oil administration produced watery diarrhoea with the first defecation time noted at 10.67 ± 0.89 min. and total cumulative fecal weight of 4.29 ± 0.43 g whereas in treatment of 800 mg/kg of EEAA showed non-significant effects on both, first defecation time and cumulative fecal weight. Loperamide (3 mg/kg, p.o) treatment showed highly significant effects on both, the first defecation time and cumulative fecal weight ($p < 0.001$). The consistency of faeces in these animals was solid in nature and all parameters were shown in Table 2.

Effect of Ethanolic Extract of whole plant of *Ainsliaea Aptera* (EEAA) on intestinal motility in the charcoal meal test

The distance travelled by charcoal in terms of percent of the total length of intestine was found to be 45.65 % in the vehicle-treated control animals. The EEAA (800 mg/kg) produced non-significant effects on intestinal motility as compared to the control group. The animals which received the standard drug, atropine sulphate showed a significant reduction in the distance travelled by charcoal in intestine 11.67 % ($p < 0.001$). Table 3. depicts non-significant anti motility activity of EEAA.

Table 1: Qualitative Chemical Evaluation

Phytoconstituent	Result
Carbohydrate	Present
Protein	Present
Alkaloid	Absent
Glycoside	Absent
Triterpenoid	Present
Flavonoids	Present
Phytosterols	Present
Tannin	Present
Saponins	Present

Table 2: Effect of Ethanolic Extract of *Ainsliaea aptera* (EEAA) on Castor oil induced Diarrhoea

Groups	Dose	First Defecation Time (In Min)	Cumulative Weight (In gm)
Control (CMC 0.05%)	5 ml	10.67±0.89	4.29 ± 0.43
Loperamide	3 mg/kg	165.00 ± 37.53***	0.19 ± 0.09***
EEAA	800 mg/kg	40.00 ± 1.15 ^{ns}	3.88 ± 0.05 ^{ns}

Values are expressed as Mean ± SEM; ***p<0.001, ns: non-significant Compared with control.

Table 3: Effect of Ethanolic Extract of *Ainsliaea aptera* (EEAA) on intestinal motility in the charcoal meal test

S. No.	Treatment and dose	Distance travelled by charcoal (IN %)
Control CMC (0.05%)	5ml (p.o)	45.65
Standard (Atropine sulphate)	5mg/kg (i.p)	11.67***
EEAA	800mg/kg (p.o)	31 ^{ns}

Values are expressed as Mean ± SEM; ***p<0.001, ns: non-significant Compared with control

DISCUSSION

In this present study, Preliminary Phytochemical results showed the presence of carbohydrates, proteins, Triterpenoid, Flavonoids, Phytosterols, Tannins and Saponins and absences of Alkaloids and Glycoside in the drug. Test substance related mortality was not observed at 2000 mg/kg which revealed the nontoxic nature of EEAA.

Diarrhoea is a national problem, especially among children and causes too much morbidity and mortality. During the past decade, oral rehydration therapy has reduced mortality from acute diarrhoeal disease, whereas chronic diarrhoea still remains a life-threatening problem in those regions in which malnutrition is a common co-existing and complicating factor. Many plants available in India have been found to be effective against diarrhoea and dysentery and are used by local people and in traditional folklore medicine⁷. Researches have shown that prior treatment with plant extracts had a protective effect on the intestinal tract⁸.

In the present study, the EEAA was evaluated for its anti-diarrhoeal potential against castor oil induced diarrhoea model in rats however the EEAA does not show any significant anti diarrheal effect on castor oil induce diarrhoea and anti-motility effects charcoal meal test in mice. Loperamide, the standard drug, generally produces rapid and sustained inhibition of peristaltic reflex through depression of longitudinal and circular muscle activity. It is known to reduce the daily fecal volume and decreases intestinal fluid and electrolyte loss. The pharmacological effect of loperamide is due to its antimotility and antisecretory properties⁹. In the present study too, loperamide proved the claims by causing a decrease in the cumulative fecal weight, delay in the onset of defecation, a change in consistency of stools from watery to solid and decrease in weight and volume of intestinal content when compared to the vehicle treated control animals. The main chemical constituents present in plants are tannins and tannic acid, flavonoids and triterpenoids. Gastric secretion mainly

inhibits by Tannins and Tannic acid by denature the proteins. Denaturation of proteins is done by forming Protein tannate, a complex which coats the intestinal mucosa¹⁰. Tannins are reported to possess various physiological effects like anti-irritant, anti-secretory, anti-phlogistic, antimicrobial, and anti-parasitic¹¹. whereas the plant product. However, the plant fail to show Antidiarrhoeal activity this may be due to presence of saponin and triterpenoids content in the extract which counter balance the antidiarrhoeal effect which are produced by the flavonoids and the tannin also present in the plant. The parasympathetic and sympathetic fibers of the autonomic nervous system stimulate activity of the gastrointestinal tract. The peristaltic movement of gastrointestinal is sparked by the local reflexes, which is myogenic in character and having no neural connections to the brain and spinal cord. The peristalsis activity of the organ is also triggered by the extrinsic nerves to the intestine which has minor role in nature¹². Cholinergic stimulation often causes diarrhoea by increasing gastrointestinal motility. Atropine sulphate, the standard drug used in the experiment, is a parasympatholytic drug, which acts by blocking the actions of acetylcholine at muscarinic receptors. The present study demonstrated a significant anti-motility effect of atropine at the dose of 5 mg/kg (i.p.). The extract at the dose of 800 mg/kg did not show significant anti-motility effect. This finding reveals the absence or loss of anti-motility effect and anti diarrhoeal activity at this dose (800 mg/kg).

CONCLUSION

The present study thus proves that the ethanol extract of whole plant of *Ainsliaea aptera* (EEAA) possesses non-significant anti-diarrhoeal activity. Anti diarrhoeal activity generally due to the inhibitory effect on gastrointestinal propulsion and anti-secretory effect due to the inhibition on fluid secretion. But in this study EEAA does not show any inhibitory effect on gastrointestinal propulsion and on fluid secretion. However, further studies are necessary to find out the dose more than dose used (800 mg/kg), in this study which are responsible for Anti diarrhoeal activity because traditionally it claims for used in

Stomach ache. However, the plant fail to show Anti diarrhoeal activity this may be due to presence of saponin and triterpenoids content in the extract which counter balance the anti-diarrheal effect which are produced by the flavonoids and the tannin also present in the plant.

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