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# Antidiarrheal Activity of Ethanol Extract of *Fragaria ananassa* and *Actinidia deliciosa* Fruit in Experimental Model

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## ABSTRACT

***Introduction*:** Diarrhea is the second leading causes of preventable mortality especially among infants and children of developing countries. The fruits of *Fragaria ananassa* (Rosaceae) and *Actinidia deliciosa* (Actinidiaceae) commonly known as garden strawberry and kiwi (Chinese gooseberry) respectively have been cultivated all over the world including Pakistan. They are widely consumed due to their pleasant taste and remarkable nutritious properties.

***Aim of Study*:** The aim was to investigate safety and efficacy as well as *in vivo* antidiarrheal effects of ethanol extract of *Fragaria ananassa* (EEFA) and *Actinidia deliciosa* (EEAD) in experimental models of diarrhea.

***Methodology*:** Animals were divided into five groups allocated as distilled water (control), loperamide (3 mg/kg) as reference standard, EEFA (800 mg/kg), EEAD (800 mg/kg), 1:1 combination of EEFA (400 mg/kg) and EEAD (400 mg/kg) as treated groups. *In vivo* antidiarrheal activity was determined by castor oil induced experimental model of diarrhea.

***Results*:** The results revealed that EEFA and EEAD and their combination possessed significant antidiarrheal activity in comparison to control in experimental model of mice.

***Conclusion*:** These results of the experiment postulating the antidiarrheal activity of *Fragaria ananassa* and *Actinidia deliciosa* by alleviating gastrointestinal motility and fluid accumulation thus supporting their safer use in the management of diarrhea.

**Keywords:** *Fragaria ananassa*, *Actinidia deliciosa*, antidiarrheal activity, castor oil, loperamide, charcoal.

## INTRODUCTION

Diarrhea is the condition of discharging frequent stools in liquid form which generally affects infants and children resulting in intensified motility as well as secretion of gastrointestinal tract where there is imbalance in electrolytes and intestinal fluid absorption of body get reduced. Diarrhea is classified into three categories with respect to duration of loose stools as acute (less than two weeks), persistent (two to four weeks) and chronic (more than one month) diarrhea [1]. According to WHO approximately 1.9 million children died every year globally from diarrhea

specifically in developing countries. Currently, various antidiarrheal drugs are available for the empirical management of diarrhea such as opioids, zinc supplements, third generation cephalosporin and oral rehydration therapy which are associated with a number of side effects as well as contraindications [2, 3]. Therefore, herbal medicines are gaining prompt attention of researchers towards their alternative use for the treatment of diarrhea especially by 85% population of developing countries [4].

*Fragaria ananassa* generally known as garden strawberry belonging to a low-growing perennial flowering plant of genus *Fragaria* of Rosaceae family. It is widely cultivated in various countries worldwide including Pakistan. Strawberries are mainly consumed in various dairy products and desserts due to its sweetened taste and juicy texture. They are rich in vitamin C and dietary minerals. However, various phytochemicals are also present in strawberries such as flavonoids, tannins, alkaloids and phenolic acids [5, 6]. Many researchers have identified the nutritious as well as medicinal properties of *F. ananassa* fruits and established their safe use against various maladies and pathologies such as tumor, cardiovascular disorders, hyperglycemia, obesity and numerous infections [7, 8]. Similarly, *Actinidia deliciosa* well-known as kiwi fruit or Chinese gooseberry belonging to the genus *Actinidia* of family Actinidiaceae. Kiwi fruits are native to Asia, russet-brown color oblong fruit heavily covered with stiff hairs. The fruit is mainly consumed in fresh form but are also used in the form of juices. Likewise, *A. deliciosa* are rich in vitamin C, minerals and a number of polyphenols such as flavonoids, alkaloids, tannins and carotenoids.

Globally, a number of herbal medicines claim to be effective for managing diarrhea including *Cissampelos pareira*, *Teucrium polium*, *Solanum hastifolium* and *amaranthus caudatus*, respectively [9-11]. This study is designed to investigate safety and efficacy as well as *in vivo* antidiarrheal effects of ethanol extract of *Fragaria ananassa* and *Actinidia deliciosa* in experimental models of diarrhea.

## MATERIALS AND METHODS

### Drug and Chemical

Loperamide (2 mg) under the brand name of Imodium and atropine were purchased from Patel Hospital. All other chemicals were used in this study were of analytical grade from Merck.

### Collection of Fruit Material

The fruits of *Fragaria ananassa* and *Actinidia deliciosa* were procured from local market. They were identified and authenticated by Dr. Mohtesheem ul Hassan, Pharmacognosy Department, Faculty of Pharmacy and Pharmaceutical Sciences under the voucher number FAF-06-19 for *Fragaria ananassa* and ADF-05-19 for *Actinidia deliciosa*, respectively.

### Preparation of Extract

About 500 g of both fruits of *Fragaria ananassa* and *Actinidia deliciosa* were purchased from local market and were soaked in 70% ethanol in two separate glass jars for 48 hours. with occasional shaking after every 2 hours and filtered. The filtrate obtained was subjected under reduced pressure of rotary evaporator at 60°C to get the yield of 18 and 21 grams of *Fragaria ananassa* and *Actinidia deliciosa*, respectively.

### Selection and Housing of Animals

Albino wistar mice (20-25 g) of either sex were taken from the animal house of Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi. All animals were subjected to acclimatization for 2 days before the start of experiment. They were kept in plastic cages under the ambient temperature of 30 ± 2°C provided with standard pelleted feed and water.

### Ethics Approval

The study was found in accordance with the international ethical guidelines and approved by Departmental Animal Ethics Committee, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan.

### Experimental Protocol

All animals were allocated in five groups with 5 animals in each group (n=5).

**Table 1. Castor oil induced diarrhea test.**

| GROUP | TREATMENT |
|---|---|
| Group I | Distilled water 10 ml/kg p.o |
| Group II | Loperamide 3 mg/kg body weight p.o [12] |
| Group III | Ethanol extract of *Fragaria ananassa* 800 mg/kg body weight p.o [13] |
| Group IV | Ethanol extract of *Actinidia deliciosa* 800 mg/kg body weight p.o [14] |
| Group V | EEFA plus EEAD 400 mg/kg each per body weight p.o [13, 15] |

**Table 2. Intestinal motility test.**

| GROUP | TREATMENT |
|---|---|
| Group I | Distilled water 10 ml/kg p.o |
| Group II | Atropine 5mg/kg i.p [16] |
| Group III | Ethanol extract of *Fragaria ananassa* 800 mg/kg body weight p.o [13] |
| Group IV | Ethanol extract of *Actinidia deliciosa* 800 mg/kg body weight p.o [14] |
| Group V | EEFA plus EEAD 400 mg/kg each per body weight p.o [13, 15] |

**EEFA:** Ethanol extract of *Fragaria ananassa*

**EEAD:** Ethanol extract of *Actinidia deliciosa*

**Castor Oil Induced Diarrhea**

Twenty-five mice were kept fasted for 18 hours before the commencement of the study though provided with free access to water (Table **1**). First group was labelled as control and given with distilled water. Second group was considered as reference standard and was on 3 mg/kg loperamide while rest of the three groups were taken as test groups and administered with 800 mg/kg of EEFA, 800 mg/kg of EEAD and 400 mg/kg of each EEFA and EEAD, respectively. One hour after the administration of respective treatments, 0.5 ml of castor oil was served to all mice orally and were kept in separate cages. The severity of diarrhea was estimated for 4 hours [17]. The total amount of diarrheal or wet feces of group I was considered as 100%. Percentage inhibition of diarrhea and defecation calculated by the following formula [18]:

$$\% \text{ Inhibition} = (\text{control} - \text{test})/\text{control} \times 100\%$$

**Gastrointestinal Motility Test**

All animals were kept fasted for 18 hours before the start of the study though provided with free access to water (Table **2**). First group was labelled as control and given with distilled water. Second group was considered as reference standard and was on 5 mg/kg i.p atropine while rest of the three groups were taken as test groups and administered with 800 mg/kg of EEFA, 800 mg/kg of EEAD and 400 mg/kg of each EEFA and EEAD, respectively. One hour after the administration of respective treatments, each mouse was provided with 0.5 ml of charcoal suspension orally freshly prepared in distilled water. After 30 minutes all mice were sacrificed and the small intestine was isolated from pylorus to caecum. The intestinal distance covered by the charcoal meal was determined from the pylorus towards the caecum. Percentage of intestinal distance moved by the charcoal meal and percent inhibition of intestinal transit was calculated by using following formula [19]:

$$\text{Percentage of intestinal fluid inhibition} = (T_0 - T_1/T_0) \times 100$$

Where,

$\mathbf{T_0}$ = complete length of intestine

$\mathbf{T_1}$ = length covered by charcoal in intestine

### Statistical Analysis

Values are presented as mean ± SEM (n=5). Data was analyzed statistically by using IBM SPSS (version 24) one-way ANOVA followed Post hoc Tukey's test for multiple comparison. P ≤ 0.05 and P ≤ 0.01 were considered to be significant and highly significant, respectively.

## RESULTS

### Effect of *Fragaria ananassa* and *Actinidia deliciosa* on Castor Oil Induced Diarrhea

The results illustrated the antidiarrheal effect of ethanol extract of the *Fragaria ananassa* and *Actinidia deliciosa* and their combination by using castor oil to induce diarrhea are shown in Table **3**. A moderate reduction was observed in number of diarrheal feces and defecation in all treatment groups in comparison with negative and positive control by Post hoc Tukey test. Ethanol extract of *Actinidia delicious* (EEAD) displayed highly significant (P ≤ 0.01) antidiarrheal effects in terms of defecation number among all groups with percentage inhibition of 56.25%. But in terms of cumulative decrease in amount of wet and solid feces, the combination of both *Fragaria ananassa* and *Actinidia deliciosa* are more significant in comparison to other treated groups as illustrated in Figure **1**.

**Table 3. Anti-diarrheal activity of ethanol extract of *Fragaria ananassa* (EEFA), ethanol extract of *Actinidia delicious* (EEAD), their combination (EEFA+EEAD) and Loperamide on castor oil induced diarrhea in mouse. Also percentage inhibition (PI) after 4 hours. Statistical significance at $^{*,!,‡,\#}$P ≤ 0.05 as significant, $^{**,!!,‡‡,\#\#}$P ≤ 0.01 as highly significant.**

| Group/Treatment | Mean Number of Feces in 4 hours | | Percentage Inhibition (%) | |
|---|---|---|---|---|
| | Wet/Loose Feces | Solid/Hard Feces | Diarrhea | Defecation |
| I/Distilled Water | 5.40 ± 0.51 | 3.20 ± 0.37 | ---- | ---- |
| II/Loperamide (3 mg/kg) | 1.40 ± 0.24$^{**}$ | 2.40 ± 0.24 | 74.07% | 25% |
| III/EEFA (800 mg/kg) | 6.40 ± 0.51$^{!!\,\#}$ | 3.60 ± 0.24 | -18.51% | -12.5% |
| IV/EEAD (800 mg/kg) | 3.60 ± 0.51$^{!‡‡}$ | 1.40 ± 0.24$^{**‡‡}$ | 33.33% | 56.25% |
| V/EEFA+EEAD (400 mg/kg + 400 mg/kg) | 3.40 ± 0.5$^{*!‡‡}$ | 1.80 ± 0.37$^{*‡}$ | 37.03% | 43.75% |

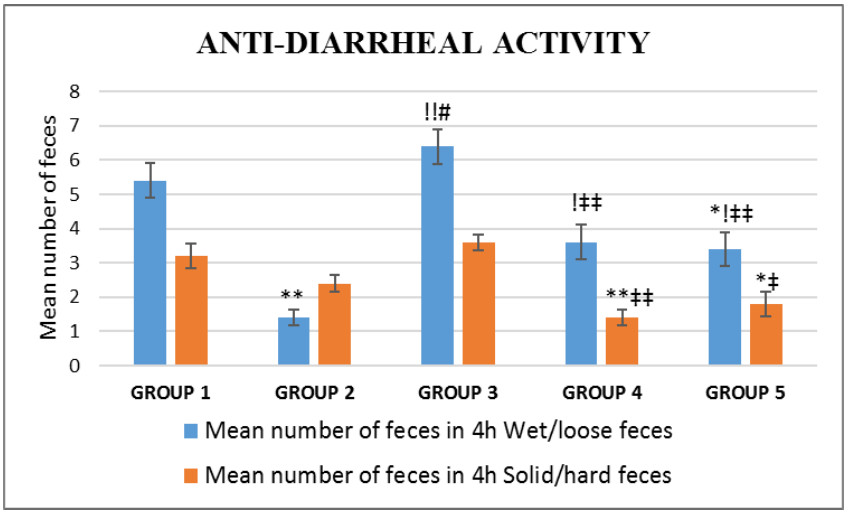


**Figure 1.** Antidiarrheal activity of EEFA and EEAD after 4 hours.

**Table 4. Percentage inhibition of motility of ethanol extract of *Fragaria ananassa* (EEFA), ethanol extract of *Actinidia delicious* (EEAD) and their combination (EEFA+EEAD).**

| Group/Treatment | Complete Length of Intestine (cm) | Length Covered by Charcoal (cm) | Percent Inhibition of Motility |
|---|---|---|---|
| I/Distilled Water | 101.91 ± 1.27 | 82.45 ± 0.55 | 19.09% |
| II/Atropine (5 mg/kg) | 104.38 ± 1.93 | 42.11 ± 2.37** | 59.65% |
| III/EEFA (800 mg/kg) | 103.41 ± 1.33 | 66.23 ± 3.40** | 35.95% |
| IV/EEAD (800 mg/kg) | 104.67 ± 2.77 | 38.91 ± 3.66** | 62.82% |
| V/EEFA+EEAD (400 mg/kg + 400 mg/kg) | 106.52 ± 3.28 | 44.08 ± 3.98** | 58.62% |

**Effect of *Fragaria ananassa* and *Actinidia deliciosa* on Gastrointestinal Motility Test**

Both ethanol extracts of *Fragaria ananassa* and *Actinidia deliciosa* showed highly significant ($P \leq 0.01$) reduction in the mean length covered by charcoal as compared to group I. However, the percentage of intestinal motility was found to be 35.95%, 62.82% and 58.62% as illustrated in Table **4** by EEFA, EEAD and their combination doses, respectively.

## DISCUSSION

In the current study, it is observed that both ethanolic extracts of *Actinidia deliciosa* and *Fragaria ananassa* have significant antidiarreal activity.

The extracts of *Actinidia deliciosa*, percentage inhibition of number of solid/hard feces were more improved among all groups even in comparison with reference standard (loperamide) in castor oil induce diarrheal test. Castor oil works by increasing the mucosal permeability of intestine that causes diarrhea [20]. The phytochemical analysis of both the fruits have shown significant amount of flavonoids and polyphenolic compounds presence, which have prominent antidiarrheal activity [20].

The *Actinidia deliciosa* has shown better antidiarrheal activity in comparison to *Fragaria ananassa*, might be because of presence of alkaloids and tannins in addition to flavonoids and polyphenolic compounds [20].

Charcoal was used to produce laxative effect *via* cholinergic receptors [21], this effect has been significantly opposed by EEAD and EEAD/EEFA combination. EEAD showed highly significant ($P \leq 0.01$) anticholinergic activity in gastrointestinal motility test in comparison to positive and negative control. Whereas, the EEAD/EEFA combination has displayed approximately identical peristalsis index to cholinergic antagonist (atropine). Similarly, minor anticholinergic activity was observed in EEFA only in comparison with negative control.

The qualitative phytochemical analysis of native kiwi fruit (*Actinidia deliciosa*) have revealed the presence of significant amount of polyphenolic compounds, tannins, alkaloids, flavonoids with trace amount of carbohydrates and protein in the ethanol extract of *Actinidia deliciosa*. Similarly, the qualitative phytochemical analysis of native strawberry (*Fragaria ananassa*) specie have shown significant amount of ellagic acid, tannins and flavonoids as well as polyphenolic compounds. In addition to these glycosides, carbohydrates, proteins, dietary fibers and few fatty acids were also detected.

The antimicrobial activity of the two was not studied to prove the role of fruits in infectious diarrhea. To ascertain the exact effective dose of the ethanol extracts of kiwi and strawberry and their mechanism of action as antidiarrheal agents require further studies.

## CONCLUSION

The results of above study have revealed that the ethanol extracts of both the fruits have nonspecific antidiarrheal activity. At given doses, both fruits have shown moderate reduction in diarrhea. Thus, it can be postulated that extracts have met few of the acceptance criterion and further study with slight

modification is required to find out most efficient dose dependent response.

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