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# Evaluation of anti-diarrheal and anti-diabetic activities of the stem, barks and leaves of the plant *Vernonia cinerea* (Family: Asteraceae)

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**ABSTRACT**

The aim of this study was to evaluate the anti-diarrheal, anti-diabetic activity of barks and leaves of the plant *V. cinerea*. The acute toxicity test was also carried out on the stems, barks and the leaves of the plant *V.Cineria* was sun dried and extracted using methanol. Later the crude methanolic extract was fractionated into three different fractions using carbon-tetrachloride, di-chloromethane and water. The anti-diarrheal activity was tested using the non polar carbon-tetra-chloride fraction in a dose dependent manner and it was found that the fraction does not possess any anti-diarrheal activity. The anti-diabetic study was done in rats using the alloxan-induced diabetes method. The carbon tetrachloride fraction of methanolic stem-bark and leaves extract of *V. cinerea* in all the doses used caused a time dependent and significant ($p < 0.05$) reduction of the blood glucose levels of the alloxan-induced diabetic rats when compared to the negative control group. The highest activity of *V. cinerea* extract in this experiment was observed at the dose of 500 mg/kg. Acute toxicity was done by following the method of Lorke. The acute toxicity in rats produced no death or signs of toxicity even at the highest dose of the extract (3500 mg/kg).

## INTRODUCTION

Diarrheal diseases are one of the leading causes of morbidity and mortality in developing countries and are responsible for the death of millions of people each year (Carlos and Saniel, 1990). Similarly diabetes mellitus is the most common endocrine disease causing millions of death world-wide. Despite immense technological advancement in modern medicine, many people in the developing countries still rely on the healing practices and medicinal plants for their daily health care needs (Ojewole, 2004). Therefore, the World Health Organization encouraged studies for the treatment and prevention of diarrheal diseases and diabetes treatment depending on traditional medical practices (Atta and Mouneir, 2004). *Vernonia cinerea* (Family: Asteraceae) is a terrestrial annual erect herb. It grows up to 80 cm high. It can be found in roadside, open waste places, dry grassy sites and in perennial crops during plantation. It is located especially in different Asian countries such as India, Bangladesh and Nepal. *V. cinerea* is an important medicinal plant having application in abortion, cancer and various gastrointestinal disorders (Yusuf *et al.,* 1994).

The crude methanol extract of *V. cinerea* is found to have antidiarrhoeal activity (Ganesh *et al.,* 2011) but there is no study on non-polar fraction. Chloroform extract of stem-bark and leaves of *Vernonia cinerea* showed diuresis property but methanolic extract exhibited antidiuresis (Adeboye *et al.,* 1997). Toxicity study of the plant on mice was carried out plant but the result was inadequate for definite conclusion (Latha *et al.,* 2007).

Therefore, the present study was designed to investigate the antidiarrhoeal property and antidiabetic activity of non-polar carbon tetrachloride fraction of methanolic. The acute toxicity test was also performed.

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## MATERIAL AND METHODS

### Collection and Identification of plant materials

Fresh plant of *Vernonia cinerea* was collected from Gabtoli, Dhaka, Bangladesh in October, 2007. This plant was identified by Bangladesh National Herbarium. The reference sample for the plant was DACB Accession Number 32126.

### Preparation of plant extract

The stem-bark and leaves were sun dried for 5 days. The plant materials were then oven dried for 24 hours at low temperature. 960 gm of powdered material (Stem-bark and leaves) was macerated with 7.5 L of methanol in two 4 L round bottom flask. The containers were sealed with cotton plug and aluminum foil at room temperature for 15 days with occasional shaking. The mixture was filtered through cotton and then evaporated to dryness ($45^0$C) under reduced pressure by rotary evaporator. The obtained crude extract was 49.54 grams.

15 gm of methanolic extract was triturated with 270 ml of methanol containing 30 ml distilled water. The crude extract was dissolved completely. It was mother solution. This solution was partitioned successfully by three solvents of different polarity. The mother solution was taken in a separating funnel. 100 ml of Carbon-tetrachloride was added here and the funnel was shaken and kept undisturbed. Then the organic portion was collected and repeated thrice. Dichloromethane ($CH_2Cl_2$) extract was collected with the help of aqueous mother fraction adding 50 ml of water keeping the other procedure unchanged. Finally $CCl_4$, $CH_2Cl_2$ and aqueous extract were obtained.

### Anti-diarrheal Activity Screening:

Antidiarrheal test of the nonpolar carbon tetrachloride fraction of methnolic extract was conducted by using castor oil induced diarrhea in swiss albino mice by the method followed by Nwodo and Alumanah (Nwodo *et al.*, 1991). Of the experimental groups, Group-I or the Control received only distilled water containing distilled water. Group-II or the positive control received standard anti-motility drug, loperamide at a dose of 3mg/kg-body weight as oral suspension. The test groups (Group-III and Group- IV) were treated with suspension of leaves extract of *V. cinerea* at the oral dose of 250 mg/kg-body weight and 500 mg/kg-body weight. The mice were fed with the samples 1 hour prior to the oral administration of castor oil at a dose of 1ml per mouse respectively for individual animal of each group placed in separate cages having adsorbent paper beneath. Then the animals were examined for the presence of diarrhea every hour for 6 hours study after the castor oil administration.

### Anti-diabetic study

Alloxan monohydrate solution of 10 mg/ml was prepared in ice-cold citrate buffer (0.1M); pH of the ice was kept at 4.5 and was administered to the rats within 5 mins at a dose of 50 mg/kg bodyweight intraperitonially. The fasting blood sugar levels of each of the rats were checked every day with an autoanalyzer (Glucometer, Bioland G-423 S) glucose kit. After 8 days, animals with fasting blood sugar levels of 250 mg/dl and above were considered to be diabetic and were used for the study and assigned into five groups of five rats each. Group I served as the negative control and received tween 80 solution (solvent used to dissolve the extract) (10 ml/kg), group II–IV received the carbon tetrachloride fraction of methanolic *V. cinerea* extract at the dose of 250, 500 and 1000 mg/kg respectively while group V served as the positive control and received the standard reference drug glibenclamide (2 mg/kg) all by gastric lavage. The blood glucose levels of the rats were measured at 0, 1, 2 and 3 h after administration of drug and extracts. Blood samples were collected by tail snip and the blood glucose measured with an autoanalyzer (Glucometer, Bioland G-423 S) glucose kit. At the end of the experiment percentage reduction of the glucose levels of the rats at the 3rd hour was calculated using the formula below:

% reduction in glucose level= $[(V_0 - V_t)/ V_0 ]\times 100\%$

Where $V_{0=}$ value at zero hour and $V_t$ = value at subsequent hours.

### Acute toxicity test

Acute toxicity was done by following the method of Lorke (Lorke, 1983). Twenty rats of both sexes were randomly grouped into four groups (A-D) of five rats per group and were dosed with 100, 500, 1000 and 3500 mg/Kg of the extract orally by gastric gavage. They were observed over a period of 48 h for signs of toxicity and mortality.

### Statistical analysis

Results were presented as mean ±Standard Error of Mean (SEM) and the statistical analysis was done by using one way analysis of variance (ANOVA) followed by Tukey Post-hoc test. A p-value of $p < 0.05$ was considered to be statistically significant.

## RESULTS

### Anti diarrhoeal activities

The carbon tetrachloride fraction of methanolic extract of *V. cinerea* did not show antidiahoeal properties. It affected neither latent period nor number of stools. The result of antidiarrhoeal activities is shown in Table 1.

### Anti-diabetic study

The result of the effect of *V. cinerea* on the fasting blood glucose levels of alloxan-induced diabetic rats is presented in Table 2. The result showed that there was no significant change in the blood glucose levels of rats in group I that received tween 80 solutions (negative control). The methanolic stem-bark and leaves extract of *V. cinerea* in all the doses used including the reference drug caused a time dependent and significant ($p < 0.05$) reduction of the blood glucose levels of the alloxan-induced diabetic rats when compared to the negative control group with the extracts at the doses of 250, 500 and 1000 mg/kg. This decreased the blood glucose levels by 28.6%, 35.2% and 26.7% respectively at the 3rd hour while the reference drug (glibenclamide, 5 mg/kg) decreased

**Table. 1:** Experimental profile to observe the effect plant of extract of *V. cinerea* on castor oil induced diarrhoea in mice.

| Animal group/Treatment | Dose (/kg, per oral) | Latent period (h) | Period of study (h) | No. of stools (Mean± SE) | Total no. of stools Mean± SE |
|---|---|---|---|---|---|
| I - Control | 0 | 1.20 ± 0.1 | 1 | 2.1 ± 0.4 | 14± 0.1 |
| | | | 2 | 2.6 ± 0.5 | |
| | | | 3 | 3.3 ± 0.3 | |
| | | | 4 | 3.8 ± 0.3 | |
| | | | 5 | 2.9 ± 0.1 | |
| | | | 6 | 2.0 ± 0.2 | |
| II - Loperamide** | 3 | 3.81 ± 0.2 | 1 | 0.3 ± 0.1 | 4.0± 0.2 |
| | | | 2 | 0.4 ± 0.1 | |
| | | | 3 | 0.6 ± 0.2 | |
| | | | 4 | 1.0 ± 0.1 | |
| | | | 5 | 0.9 ± 0.1 | |
| | | | 6 | 0.8 ± 0.1 | |
| III - 250 mg/kg extract of *V. cinerea* ) | 250 | 1.31 ± 0.2 | 1 | 2.1 ± 0.2 | 13.75± 0.2 |
| | | | 2 | 2.5 ± 0.3 | |
| | | | 3 | 2.8 ± 0.2 | |
| | | | 4 | 3.0 ± 0.1 | |
| | | | 5 | 2.6 ± 0.2 | |
| | | | 6 | 2.5 ± 0.1 | |
| IV - 500 mg/kg extract of *V. cinerea* ) | 500 | 1.21 ± 0.2 | 1 | 2.4 ± 0.1 | 14 ± 0.3 |
| | | | 2 | 2.8 ± 0.1 | |
| | | | 3 | 3.0 ± 0.2 | |
| | | | 4 | 3.8 ± 0.3 | |
| | | | 5 | 2.5 ± 0.1 | |
| | | | 6 | 2.5 ± 0.3 | |

Statistically significant (**P < 0.01)

**Table. 2:** Effect of *V. cinerea* on the fasting blood glucose level of alloxan-induced diabetic rats.

| Group | Treatment | Fasting blood glucose level (mg/ml) | | | | % reduction at at the 3h |
|---|---|---|---|---|---|---|
| | | 0 h | 1 h | 2 h | 3 h | |
| I | Alloxan+ Tween80 (1%) | 380.0±5.7* | 385±6.2* | 382.2±5.5* | 383.7±5.5* | - |
| II | Alloxan+ MEVC (250mg/kg) | 325.2±5.7* | 207.3±4.1* | 255.2±4.6* | 216.2±3.2* | 28.6 |
| III | Alloxan+ MEVC (500mg/kg) | 395.2±5.5* | 370.3±3.8* | 350.2±4.0* | 245.4±6.2* | 35.2 |
| IV | Alloxan+ MEVC (1000mg/kg) | 334.2±4.3* | 289.6±4.1* | 261±4.8* | 234.5±4.1* | 26.7 |
| V | Alloxan+glibenclamide(5mg/kg) | 310.2±1.2* | 164.4±1.2* | 115.0±0.6* | 98.7±1.2* | 64.71 |

MEVC = Methanolic extract of stem-bark and leaves of *V. cinerea*; *$p$<0.05 when compared with the negative group.

The blood glucose levels by 64.71% at the 3rd hour. Also the extract at the different test doses caused various degrees of reduction (28.6% - 35.2%) of the blood glucose levels of the test rats at 1st, 2nd and 3rd hours when compared to the negative control rats. The highest activity of *V. cinerea* extract in this experiment was observed at the dose of 500 mg/kg while the reference drug glibenclamide (5 mg/kg) had a superior activity when compared with *V. cinerea* extract.

**Acute toxicity test**

The acute toxicity in rats produced no death or signs of toxicity even at the highest dose of the extract (3500 mg/kg).

## DISCUSSION

In this study non-polar carbon tetrachloride fraction of methanolic extract did not show any antidiarrheal activity which opposes the previous findings in case of methanolic extract of *V. cinerea* (Ganesh *et al.*, 2011). This could possibly due to the fact that the compounds responsible for antidiarrhoeal activity was not extracted in carbon tetrachloride fraction. Since castor oil induces diarrhoea by preventing fluid and electrolyte absorption according to Goodman and Gillman (1996) the compound in *V. cinerea* responsible for antidiarrhoeal activity is polar in nature and might enhance fluid and electrolyte absorption through the gastrointestinal tract according to Goodman and Gillman 11th edition, 2006. Castor oil, used to induce diarrhea in this study, is hydrolyzed in the upper small intestine to ricinoleic acid (Altman, 2007). Ricinoleic acid is believed to act by irritating the gastrointestinal tract mucosa and reducing sodium ion and chloride ion permeability, resulting in increased intestinal motility followed by diarrhea (Zavala *et al.*, 1998). The result of the anti-diabetic study showed that there was no significant change in the blood glucose levels of rats in group I that received tween 80 solutions (negative control). The carbon tetrachloride fraction of methanolic stem-bark and leaves extract of *V. cinerea* in all the doses used including the reference drug caused a time dependent and significant ($p < 0.05$) reduction of the blood glucose levels of the alloxan-induced diabetic rats when compared to the negative control group. The highest activity of *V. cinerea* extract in this experiment was observed at the dose of 500 mg/kg while the reference drug glibenclamide (5 mg/kg) had a superior activity when compared with *V. cinerea* extract. The exact mechanism by which the plant extract lowered the blood glucose level is not yet

clear but Malviya (Malviya *et al.*, 2010) attributed the anti-hyperglycemic effects of medicinal plants to be due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or a decrease in the intestinal absorption of glucose. *V. cinerea* might have worked through this mechanism or by stimulation of surviving beta cells to release more insulin just like glibenclamide. The acute toxicity test of *V. cinerea* in rats produced no death or signs of toxicity even at the dose of 3500 mg/kg which shows that the extract was well tolerated and the test doses safe in the animals.

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