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Antidiarrheal and antidiabetic effect of ethanol extract of whole *Ageratum conyzoides* L. in albino rat model

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***Ageratum conyzoides* L. (Asteraceae) is a medicinal herb widely used for several diseases although data are unavailable for diarrhea and diabetes. This study investigated the efficacy of orally administrated ethanol extract of *A. conyzoides* L. on diarrheal and diabetic rat models. Castor oil induced diarrheal model and gastrointestinal motility test models (barium sulfate milk and charcoal suspension) of albino rats were undertaken to measure the antidiarrheal effect. Alloxan induced diabetic model was used for antidiabetic effect. Data were analyzed by one-way analysis of variance (ANOVA) using Tukey's post hoc test for multiple comparisons. The extract reduced the severity of castor oil induced diarrhea with significant ($p < 0.01$) inhibition of mean defecation numbers 65.89 ± 2.44, 52.1 ± 3.21 and 27.13 ± 4.67% with 2.0, 1.0 and 0.5 g/kg bw of extract, respectively. The number of stools at different hours in treated group was significantly ($p < 0.01$) decreased as compared to normal control and positive control group (Loperamide, 1.0 mg/kg). Distance of gastrointestinal motility was significantly ($p < 0.05$) reduced to 38.88 ± 1.06% from control distance 57.25 ± 1.08% in barium sulfate milk model and to 36.34 ± 0.41% from 57.99 ± 0.66% in charcoal suspension model by the highest dose 2.0 g/kg. In a diabetic model, the blood glucose level was decreased 17.6 ± 1.31%, by 2.0 g/kg, which was significant ($p < 0.05$) compared to that (52.00 ± 1.21%) of positive control (Glimepiride, 4.0 mg/kg). The results demonstrate the marked anti-diarrheal and moderate anti-diabetic role of whole *A. conyzoides* L. ethanol extract.**

Key words: Antidiarrheal, charcoal, antidiabetic, *Ageratum conyzoides*, barium sulfate.

INTRODUCTION

Diarrhea is one of the main causes of infant death especially in third world country (Zavala et al., 1998). Diarrhea affects the smooth life-style due to its huge discomfort, although it is not life threatening for adults (Saito et al., 2002). However, twenty percent of total children die from diarrhea before the age of five in developing countries. There are some synthetic drugs available for diarrheal treatment although most of them have side effects like uncomfortable bowl movement, uneasiness etc. A continuous search, therefore, for an alternative treatment is still urged (Nester et al., 1998).

Diabetes mellitus is a metabolic disorder causing hyperglycemia due to partial pancreatic dysfunction (World Health Organization (WHO), 1980). It happens by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the

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insulin produced. It results either from inadequate secretion of insulin, an inadequate response of target cells to insulin, or both of these factors (Li et al., 2004). Till now, the management of diabetes is a global problem because curative treatment is yet to be discovered. However, several synthetic drugs are used to control diabetes but the modern oral hypoglycemic agents create undesirable side effects. Therefore, alternative therapy is required; a need of the hour is to shift towards the different indigenous plant and herbal formulations (Satyanarayana et al., 2006).

Ageratum conyzoides L. (Asteraceae) is an annual herbaceous, branched, hairy and aromatic plant with a long history of traditional medicinal uses in several countries (Abbiw, 1990; Menut et al., 1993). It is a tropical plant commonly found in West Africa, Australia, some parts of Asia and South America. It is also currently found in several tropical and sub-tropical regions including Brazil (Cruz, 1985). The plant grows commonly in waste and ruined sites as weeds in many countries (Waterhouse, 1994; Kshasttriya et al., 1994).

A. conyzoides, except the hosting of begomovirus in Indonesia and Cameroon (Leke et al., 2012), has been widely used as herbal medication and folk remedies of different diseases. Various pharmacological and biological uses of this plant or plant-parts have been investigated by many researches. They have reported the tissue repair and collagen formation (Arulprakash et al., 2012), anticancer and antiradical scavenging (Adebayo et al., 2010), ovicidal and larvicidal (Wabo Poné et al., 2011; de Mendonça et al., 2005), antiprotozoan (Harel et al., 2011; Nour et al., 2010), antiaflatoxigenic and antioxidant (Patil et al., 2010), anticoccidial (Nweze and Obiwulu, 2009), haematopoietic (Ita et al., 2007), *in vitro* anti-helicobacter pylori (Ndip et al., 2007), antibacterial and wound healing (Chah et al., 2006), analgesic and anti-inflamamtory (Moura et al., 2005), antitumour (Rosangkima and Prashad, 2004), gastroprotective (Shirwaikar et al., 2003) and anti-rheumatism (Chopra et al., 2002) effects of *A. conyzoides*. In Trinidad and Tobago, this plant has been used in fertility problem of female and erectile dysfunction of male (Lans, 2007). The use of this plant in human immune deficiency virus/acquired immune deficiency syndrome (HIV/AIDS) disease is also documented (Igoli et al., 2005).

A. conyzoides has also been proved to be used in phytoremediation of arsenic contamination (Mahmud et al., 2008). Mangesh et al. (2009) conducted a research on antidiarrheal effect of the hydroalcoholic extract of *A. conyzoides* leaves. Hypoglycaemic and antihyperglycaemic activity of leaf aqueous extract of *A. conyzoides* has been reported previously (Rahmatullah et al., 2012; Nyunaï et al., 2010). However, antidiarrheal and anti-diabetic effects of whole *A. conyzoides* organic extract are yet to be studied. We investigated the antidiarrheal and antidiabetic effects of whole *A. conyzoides* ethanol extract in this research.

MATERIALS AND METHODS

Chemical and reagents

All chemicals and reagents used were of analytical grade. Absolute ethanol (99.5%), refined pure castor oil and alloxan monohydrate were purchased from Sigma-Aldrich, Munich, Germany (Cat No. A7413-10G). Barium sulfate and activated charcoal were purchased from Merck, India Limited. Alloxan monohydrate was prepared using 0.9% NaCl saline solution. Loperamide powder and glimepiride powder were kindly donated by GlaxoSmithKline, Chittagong, Bangladesh Ltd.

Collection of plant material

A. conyzoides whole plant was collected from the moist hillside of the University of Chittagong, Bangladesh, during the month of 15th March to 7th April, 2010. The plant was taxonomically identified by Dr. Shaikh Bokhtear Uddin (Taxonomist and Associate Professor, Department of Botany, University of Chittagong, Bangladesh) and identification was confirmed by Sarder Nasir Uddin (Taxonomist, Bangladesh National Herbarium, Ministry of Environment and Forest, Bangladesh). A voucher specimen of the plant has been preserved, with the accession number 36073.

Preparation of plant extract

Fresh *A. conyzoides* whole plants were washed, chopped into small pieces with chopper, air dried at room temperature (25 ± 1)°C and ground into powder (450 g) which was left to soak in 3 L absolute ethanol for 7 days at room temperature with occasional stirring. The ethanol extract was filtered through Cheese cloth and filter paper (Whatman No. 1) and concentrated through rotatory vacuum evaporator (RE200 Rotary evaporator, Bibby Sterling, UK) under reduced pressure below 50°C. The concentrated 25 g of blackish-green crude extract was collected in plastic petri dish and air dried to allow complete evaporation. The dried extract was preserved at 4°C until further use.

Animals and diet

Six-seven weeks old wistar albino male rats weighing 180 to 200 g were obtained from the animal house of BCSIR laboratories, Chittagong. The animals were acclimatized to room temperature (23 ± 0.5°C) with a relative humidity of 55 ± 5% in standard wire meshed plastic cages for 4 to 5 days prior to commencement of the experiment. During the entire period of study, animals were caged individually and supplied with a standard pellet diet and water *ad libitum*. All the animals were maintained and treated according to the guidelines stipulated by the Institutional Animal Ethics Committee (AEIIUC-PH-2011/06).

Antidiarrheal assay

Antidiarrheal assay was conducted by castor oil induced diarrheal model and gastrointestinal motility test model.

Castor oil-induced diarrhea

The method described by Shoba and Thomas (2001) was followed for this study. Twenty five wistar albino male rats were randomly divided into five equal group (n = 5) control group, positive control group and three individual treated groups. Control group received only distilled water, positive control group received loperamide 1 mg/kg as standard, and treated groups received *A. conyzoides* extract at the dose 2.0, 1.0 and 0.5 g/kg body weight, respectively. Rats were housed in separate cages having blotting paper placed below for collection of fecal matters. Diarrhea was induced by oral administration of castor oil (2 ml/rat). Extract and drugs were given orally 1 h before the administration of standard dose of 2 ml of castor oil. Diarrhea was defined as the presence of fluid material in the stool, which was stained by the absorbent paper placed beneath the cage. The number of diarrheal episodes in terms of both hard and soft pellet was counted at every hour over 5 h period for each rat. A numerical score based on stool consistency was assigned as follows: normal stool = 1, semisolid stool = 2 and watery stool = 3. Percent inhibition (PI) was calculated as follows:

$$PI = \frac{\text{Mean defecation (control group-treated group)}}{\text{Mean defecation of control group}} \times 100$$

Gastrointestinal motility test model

This model was involved in the following two chemical assays:

1. Barium sulfate milk assay: Gastrointestinal motility test with $BaSO_4$ milk was performed according to method described by Chatterjee (1993). Briefly, rats fasted for overnight were randomly divided into five groups (n = 5). Control group received only distilled water, positive control group received commercially available anti-diarrheal drug loperamide 1.0 mg/kg, and three individual treated groups received *A. conyzoides* extract 2.0, 1.0 and 0.5 g/kg bw, respectively. Thirty minutes later, 2 ml of 10% barium sulfate suspension was administered in all groups of rats. Rats were sacrificed after 30 min. The total length of small intestine and the distance travelled by barium sulfate milk was measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileo-cecal junction).
2. Charcoal suspension assay: Charcoal model was performed according to method described as $BaSO_4$ milk model. Briefly, overnight fasted rats were randomly divided into five groups (n = 5). Control group received only distilled water, positive control group received commercially available anti-diarrheal drug loperamide 1.0 mg/kg, and three individual treated groups received *A. conyzoides* extract 2.0, 1.0 and 0.5 g/kg bw, respectively. Thirty minutes later, 2ml of 10% charcoal was administered in all groups of rats. Rats were sacrificed after 30 min. The total length of small intestine and the distance travelled by charcoal was measured and expressed as a percentage of the total length of small intestine (from stomach to caecum).

Alloxan induced antidiabetic assay

Thirty rats were randomly divided into six experimental groups (marked as group I to VI), containing five rats in each group. Diabetes was induced in five groups (group II to VI) of rats by intraperitoneal injection of alloxan monohydrate (140 mg/kg bw). After 18 h of fasting, alloxan induced diabetic rats were treated orally as: group I (normal control) received only distilled water (1 ml); group II (diabetic control) alloxan induced diabetic rats received only distilled water (1 ml); group III (positive control) diabetic rats received reference antidiabetic drug glimepiride (4 mg/kg, purchased locally); group IV to VI (sample treated) diabetic rats treated with ethanol extract of *A. conyzoides* at the rate of 2.0, 1.0 and 0.5 g/kg bw.

Blood collection and glucose quantification

All the animals were anesthetized with diethyl ether (Sigma-Aldrich, India), and blood was collected from cardiac vessel using disposable syringe by heart puncture method (Hoff, 2000). The blood collected from cardiac vessel was kept undisturbed in room temperature for 20 min. Serum from blood after clotting separated out and collected in a clean tube by centrifugation at 1100 g for 15 min. The level of glucose in blood samples from each of the experimental and control rat was determined spectrophotometrically at 546 nm by using glucose kit (gluco-lequicolor, Sigma-Aldrich, Germany) essentially followed by glucose oxidase-peroxidase (GOD-POD) method (Trinder, 1969).

Statistical analysis

All the values in the tests were expressed as mean ± SD (standard deviation). Data were analyzed by statistical package for social science (SPSS) software (SPSS, Version 18.0, IBM Corporation, NY) using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. The values were considered significantly different at $p < 0.05$.

RESULTS

The ethanol extract showed dose-dependent inhibition of castor oil induced diarrhea in albino rats. This antidiarrheal effect in terms of defecation was significant ($p < 0.01$) at all the doses of extract in comparison to control group, however, the activity was less as compared to loperamide as shown in Figure 1. Highest inhibition of castor oil induced diarrheal severity was achieved 65.89 ± 2.44% at 2.0 g/kg of extract (Figure 2). The number of stools at 1st, 2nd, 3rd 4th, 5th and 6th h for ethanol extract treated group was significantly ($p < 0.01$) decreased as compared to control group (Figure 3). The ethanol extract showed dose-dependent inhibition of barium sulfate induced gastrointestinal motility in albino rat. This effect was significant even at lower dose of 0.5 g/kg over 30 min as compared to normal control, however, this activity was less as compared to loperamide as shown in Table 1.

The results of present study showed that the extract significantly (*$p < 0.05$) reduced the gastrointestinal transit of charcoal in animal model (Table 2). The motility (57.99 ± 0.66%) of control group has been reduced mostly by the highest dose 2.0 g/kg of extract. This effect was dose–dependent and comparable to positive control loperamide (1.0 mg/kg) over 30 min study. Table 3 shows the serum blood glucose level in normal control and all experimental groups. The alloxan-induced blood glucose level was significantly ($p < 0.05$) decreased at the doses

Table 1. Effect of *A. conyzoides* L. ethanol extract of on gastrointestinal motility with barium sulfate milk model on rats.

Treatment	Dose	Gastrointestinal motility		
		Length (stomach-caecum)	Length passed by $BaSO_4$	% motility mean±SD
Normal control (DW)	0.0 mg/kg	59.2 ± 3.11	103.6 ± 7.89	57.25 ± 1.08
Positive control (LP)	1.0 mg/kg	95.8 ± 5.89[a]	29.6 ± 2.88[a]	30.88 ± 1.04[a]
Treated ACEx	2.0 g/kg	95.6 ± 12.79[a]	37.0 ± 3.93[a]	38.88 ± 1.06[b]
	1.0 g/kg	103.4 ± 6.76[a]	43.2 ± 3.11[a]	41.77 ± 0.29[c]
	0.5 g/kg	100.0 ± 7.84[a]	51.8 ± 3.03[a]	51.90 ± 1.01[d]

DW: Distilled water; LP: Loperamide; ACEx: *Ageratum conyzoides* extract. All values are expressed as mean ± SD for 5 rats. Values with superscript [a-d]letters in the table for a given period of time are significantly different from each other (SPSS for windows, version 18.0, One-Way ANOVA followed by Tukey's *post hoc* test for multiple comparisons, $p < 0.01$).

Table 2. Effect of ethanol extract of *A. Conyzoides* L. on gastrointestinal motility with 10% charcoal suspension.

Group	Dose	Gastrointestinal motility		
		Length (stomach-caecum)	Length passed by charcoal	% motility Mean±SD
Control (DW)	0.0 mg/kg	95.2±5.16	55.2±3.34	57.99±0.66
Positive Control (LP)	1.0 mg/kg	96.8±8.52[a]	28.4±3.20[a]	29.29±0.37[a]
Treated ACEx	2.0 g/kg	92.6±13.50[b]	33.6±4.5[b]	36.34±0.41[b]
	1.0 g/kg	96.8±10.56[c]	39±4.63[c]	40.26±0.42[c]
	0.5 g/kg	95±14.15[d]	48.2±6.87[d]	50.78±0.53[d]

All values are expressed as mean ± SD for 5 rats. Values with superscript [a-d]letters in the table for a given period of time are significantly different from each other (SPSS for windows, version 18.0, One-Way ANOVA followed by Tukey's *post hoc* test for multiple comparisons, $p < 0.01$).

Table 3. Increase of glucose level in alloxan induced diabetic rats.

Group	Treatment	Dose	Blood glucose level (mg/dl)
G-I	Normal control (DW)	-	59.99±1.04
G-II	Diabetic control (ALXN+DW)	-	98.77±0.93[a]
G-III	Positive control (ALXN+GLMP)	10 mg/kg	47.45±1.21[b]
G-IV	Treated (ALXN+ACEx)	2.0 g/kg	81.38±1.31[c]
G-V		1.0 g/kg	94.39±0.71[d]
G-VI		0.5 g/kg	97.98±8.40[e]

ALXN: Alloxan; GLMP: Glimepiride; All values are expressed as mean ± SD for 5 rats. Values with superscript letters are different as follows: the values for superscript [a-d]different letters in the table for a given period of time are significantly different from each other, whereas, the value of superscript [e]letter is insignificant compared to the values of normal control and the superscript letters[a-d] (SPSS for windows, version 18.0, One-Way ANOVA followed by Tukey's *post hoc* test for multiple comparisons, $p < 0.05$).

of 2.0 and 1.0 g/kg of extract. However, these effects of extract were lower than glimepiride at a dose of 4 mg/kg (Figure 4).

DISCUSSION

In the present study, the ethanol extract of *A. conyzoides*

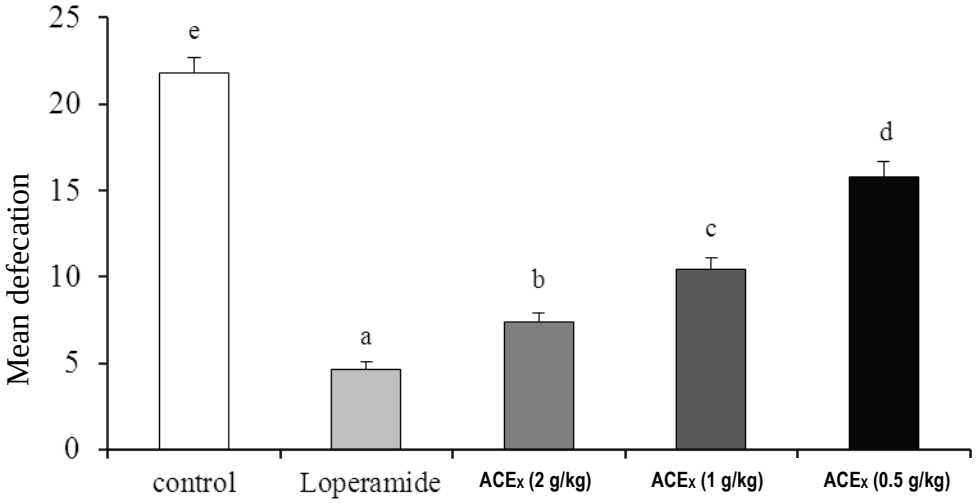

Figure 1. Effect of *A. conyzoides* ethanol extract and loperamide on castor oil induced diarrhea in terms of total no of feces in 5 h. Values are expressed as mean ± SD for 5 rats. [a-d]Different letters over the bars for a given period are significantly different from each other and from the value of control[e] (SPSS for windows, version 18.0, One-Way ANOVA followed by Tukey's post hoc test for multiple comparisons, **p < 0.01).

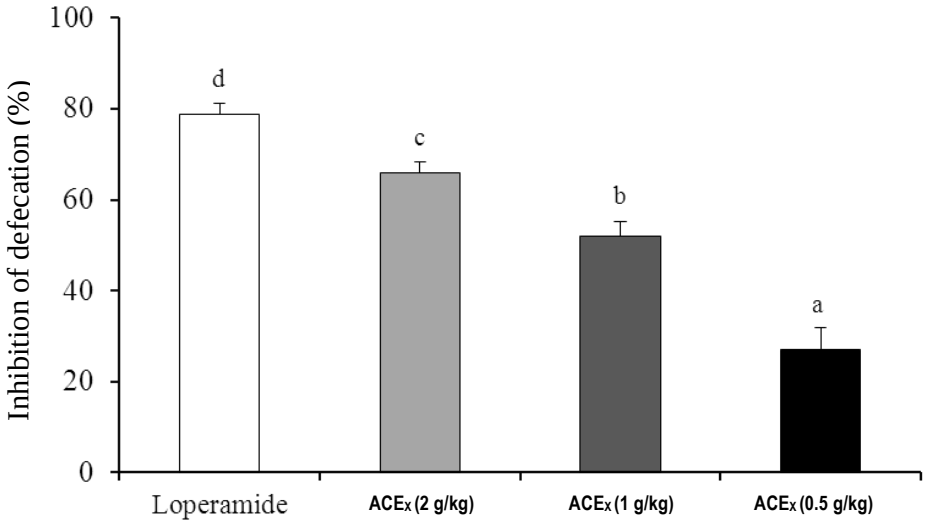

Figure 2. Inhibition (%) of defecation by *A. conyzoides* ethanol extract and loperamide on castor oil induced diarrhea. Values are expressed as mean ± SD for 5 rats. [a-d]Different letters over the bars for a given period are significantly different from each other (SPSS for windows, version 18.0, One-Way ANOVA followed by Tukey's *post hoc* test for multiple comparisons, **p < 0.01).

whole plant showed significant activity against castor oil, barium sulfate and charcoal induced changes of motility in gastrointestinal tract.

Castor oil induced diarrheal model is an autacoid-based normal to assess antidiarrheal agent. Castor oil causes diarrhea through its active metabolite ricinolic acid which prevents fluid and electrolyte absorption (Ammon et al., 1974; Brown and Taylor, 1996). Castor oil induced gastrointestinal hypermotility has been suggested to be indirectly mediated by the cholinergic system since it is inhibited by atropine, a known anticholinergic agent (Brown and Taylor, 1996). So the prevention of cholinergic transmission or its anticholinergic effect on gastric mucosa should be regarded as a probable

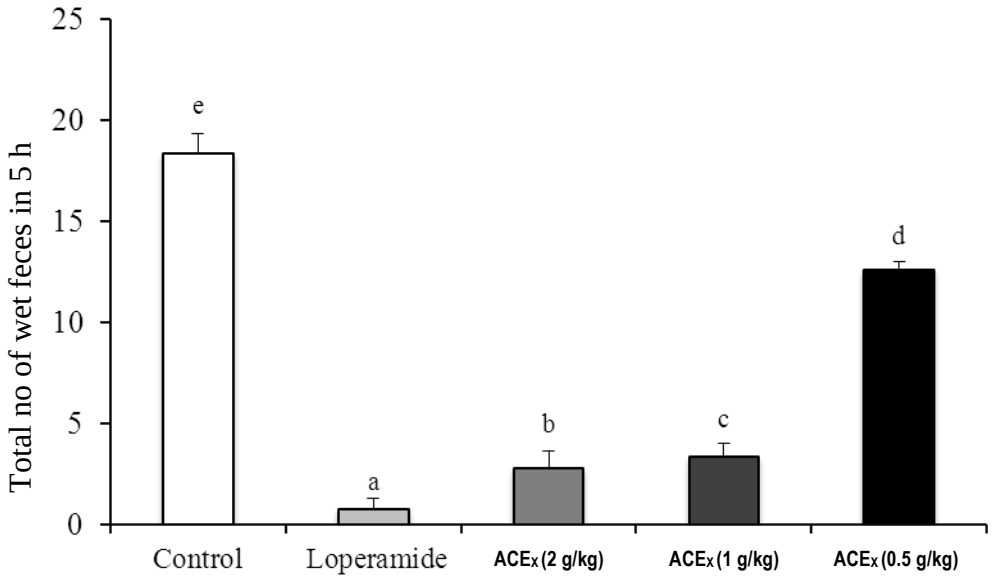

Figure 3. Effect of *A. conyzoides* ethanol extract and loperamide on castor oil induced diarrhea in terms of total no of wet feces in 5 h. Values are expressed as mean ± SD for 5 rats. [a-d]Different letters over the bars for a given period are significantly different from each other (SPSS for windows, version 18.0, One-Way ANOVA followed by Tukey's *post hoc* test for multiple comparisons, **p < 0.01).

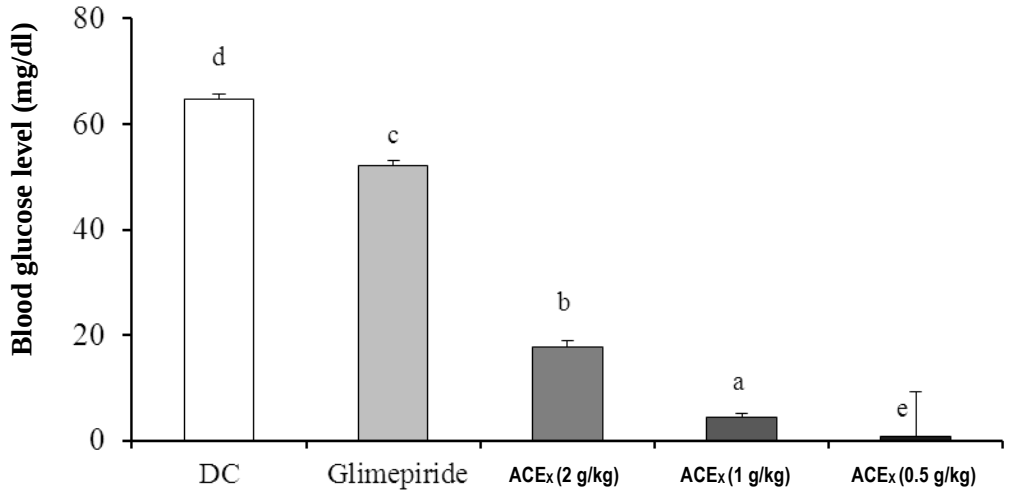

Figure 4. Effect of *A. conyzoides* ethanol extracts (2.0, 1.0, 0.5 g/kg,) of on blood glucose level of alloxan-induced diabetic rats. Values with superscript letters are different as follows: the values for superscript [a-d]different letters over the bars for a experimental period of time are significantly different from each other, whereas, the value of superscript[e] is insignificant compared to the values of diabetic control (DC) and the superscript letters[a-c] (SPSS for windows, version 18.0, One-Way ANOVA followed by Tukey's *post hoc* test for multiple comparisons, *p < 0.05).

mechanism of *A. conyzoides* extract (Mycek et al., 1997). Apart from this, the extract under investigation may contain certain components having affinity to μ (mu) receptor, which is an opioid receptor located on the GI mucosa and relieves diarrhea when activated by an agonist (Goodman and Gillman, 1996).

Barium sulphate increases the volume of the intestinal content by preventing the reabsorption of water. It also promotes the liberation of cholecystokinin from duodenal mucosa, which increases the secretion and motility of

small intestine and also prevents the reabsorption of NaCl and water. Barium sulphate induced diarrhea is presumed to be by osmotic properties and cholecystokinin production (Galvez et al., 1993). Further, the extract showed inhibition of peristalsis activity in normal as well as in charcoal suspension induced increase peristalsis as evident from distance traveled by charcoal suspension. This significant reduction in peristaltic activity is one of the important factors contributing to antidiarrheal activity of extract.

However, the results of this study showed that the extract reduced gastric contents and watery texture of diarrheal stools as well as gastrointestinal motility which is prevailed over other models of this study. Thus prevention of cholinergic transmission or its anticholinergic effect on gastric mucosa could be the leading mechanism for significant reduction (**$p < 0.01$) in frequency of diarrheal episodes. Thus further phytochemical studies are required to isolate antidiarrheal component(s) from the extract to establish its exact mode of antidiarrheal activity.

The results of this study reveal that the ethanol extract of *A. conyzoides* contains pharmacologically active substances with antidiarrheal properties. These properties may explain the rational for the effective use of the plant as an antidiarrheal agent in traditional medicine. Alloxan induced diabetes is believed to result from production of free radicals which damage the β cells of the pancreas (Asayama et al., 1984) and it is a useful experimental model to study the activity of hypoglycaemic agents (Szkudelski, 2001). Alloxan is a beta cytotoxin, induces "chemical diabetes" (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic cell. The action of this diabetogenic agent is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals that undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals cause rapid destruction of β cells and resulting in a diabetic state (Szkudelski, 2001).

The possible mechanism of action of extracts could be correlated with the reminiscent effect of the reference antidiabetic drug glimepiride that promote insulin secretion by closure of K + -ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion. Since alloxan is known to destroy pancreatic cells, the present findings appear to be in consonance with the earlier suggestion of Jackson and Bressler (1981) that sulphonylureas (for example, glimepiride) have extra-pancreatic anti-hyperglycemic mechanism of action secondary to their insulin secreting effect and the attendant glucose uptake into, and utilization by the tissues (Jackson and Bressler, 1981).

It has been suggested that supplementation of natural antioxidants may retard or halt oxidative damage which may lead to disease progression. Potential natural products having antioxidant effects have been proved to exert beneficial effects including diabetes mellitus (Maxwell, 1995). From phytochemical analysis, it was found that the major constituents of the *A. conyzoides* extract were mono- and sesquiterpenes, flavonoids, triterpenoids, sterols, alkaloids, coumarins, essential oils and tannins (Okunade, 2002). Over 150 plants extracts and some of their active principles including flavonoids are known to be used for the treatment of diabetes (Meiselman et al., 1976; Choi et al., 1991). Moreover, tannin-containing drug demonstrated antidiabetic activity (Klein et al., 2007). However, if the hypothesis of Marles and Farnsworth (1995) which indicates that plants which contain terpenoids and/or coumarins possess hypoglycemic activities in diabetic and normal mammals is worthwhile, it could be suggested that the hypoglycemic effect of *A. conyzoides* may be partly due to flavonoids, tannins, terpenoids and/or coumarins present in the plant (Adebayo et al., 2011; Wiedenfeld, 2011; Moreira et al., 2007; Abdelkader and Lockwood, 2011).

One or some of the other miscellaneous compounds of the plant may synergistically contribute to the hypoglycemic effect of the ethanol extract of *A. conyzoides*, a phenomenon that may validate the use of this plant in folk medicine against diabetes. More investigations are needed in order to clarify its mechanism of action.

Conclusion

Present study justifies the traditional use of the plant in diarrhea and diabetes indicating the anti-diarrheal and antidiabetic effect of whole *A. conyzoides* extract. Since the extract is reported to contain a range of compounds, it is difficult to ascribe these observed properties to any specific group of compounds. Hence, further studies are suggested to be undertaken to conduct a structure activity relationship to better understand the mechanism of such properties of *A. conyzoides*.

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