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Safety and antidiarrheal activity of *Priva adhaerens* aqueous leaf extract in a murine model

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Abstract

Ethnopharmacological relevance—*Priva adhaerens* (Forssk.) Chiov., a wildy growing plant, is reported in central Uganda to be an effective traditional remedy for diarrhea. The objective of this study was to provide a scientific basis for the ethnopharmacological utility of this plant whose aqueous leaf and shoot extract was evaluated for acute toxicity and antidiarrheal activity using a murine model.

Materials and methods—Acute toxicity of the aqueous leaf and shoot extract was assessed after determining the major phytochemicals present in the extract. The aqueous leaf and shoot extract was assayed against castor oil-induced diarrhea, transit time, and enteropooling, in comparison to loperamide, a standard drug.

Results—The oral LD₅₀ value obtained for *Priva adhaerens* aqueous extract was greater than 5000 mg/kg in rats; the aqueous leaf and shoot extract possessed several important phytochemicals. Furthermore, the aqueous extract significantly, and dose-dependently, reduced frequency of stooling in castor oil-induced diarrhea, intestinal motility, and castor oil-induced enteropooling in rats.

Conclusion—This murine model shows that it is relatively safe to orally use the aqueous leaf and shoot extract of *Priva adhaerens*. The aqueous extract contains phytochemicals that are active for the treatment of diarrhea in a rat model.

Keywords

Acute toxicity; antidiarrheal; *Priva adhaerens*; murine model

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1.0 INTRODUCTION

Diarrhea, the passing of increased amounts (more than 300 g in 24 hours) of loose feces, and often caused by viruses or bacteria, can be acute or chronic. Etiological factors for diarrhea include the consumption of drinking water contaminated with bacteria, undercooked meat and eggs, or inadequate kitchen hygiene (Tumwine et al., 2002). Diarrhea can also be caused by food intolerance, food poisoning or as a side effect of certain medications. The World Health Organization (WHO) estimates that food-borne and water-borne diarrheal diseases taken together kill about 2.2 million people annually, 1.9 million of whom are children (United Nations Children's Fund/WHO, 2009; Black et al., 2010).

Diarrhea is the most common gastrointestinal symptom in Human Immunodeficiency Virus (HIV) infection, affecting 90% of patients, and becomes more severe as the immune system deteriorates (Katabira, 1999; Musiime et al., 2009). Diarrhea is also one of the main causes of high mortality in developing countries where over five million children die annually from severe diarrheal diseases (United Nations Children's Fund/WHO, 2009; Black et al., 2010), being the most common cause of morbidity and mortality among infants and children worldwide. In developing countries, diarrheal diseases account for an estimated 17.5-21% of all deaths in children under the age of 5 years, equivalent to 1.5 million deaths per year. Of all child deaths from diarrhea, 78% occur in the African and South-East Asian regions, which also are disproportionately burdened with infant and childhood HIV infections (United Nations Children's Fund/WHO, 2009).

In Uganda, about two decades ago, each child on average got six episodes of diarrhea yearly (Konde, et al., 1992), with one of those episodes being severe enough to result in death. In the last decade, diarrheal disease as a cause of mortality has decreased from approximately 29.5% to 22% in children below 5 years. Similarly, deaths per 1000 live births have declined from approximately 37.2% to 11.8% in children below 5 years (W.H.O., Global Health Observatory Data Repository). Despite great advances in the management of diarrheal diseases, persistent diarrhea remains a major problem in developing countries like Uganda due to its syndromic nature (Bitarakwate et al., 2003). Treatment of diarrhea includes the administration of appropriate antibiotics where indicated, and Oral Rehydration Therapy (ORT) has been very useful in the treatment (Casburn-Jones and Farting, 2004; Forsberg et al., 2007). The World Health Organization (WHO) initiated a diarrhea control program to study traditional medicine practices and other related aspects, together with the evaluation of health education and prevention approaches to combat the problems of diarrhea (Snyder & Merson, 1982; Anokbonggo et al., 1990; Eisterberg et al., 1999; George et al., 2012).

Herbal treatment for diarrhea is a widespread practice that needs further evaluation to ascertain the usefulness of specific herbal preparations used in different communities. In Uganda, 58% of mothers are estimated to use herbal extracts to treat diarrhea (Konde et al., 1992), and many traditional healers in different parts of Uganda also use herbal extracts in the treatment of diarrheal diseases (Anokbonggo et al., 1990). One of the listed herbs used in the remedy of diarrhea is *Priva cordifolia* (Sekagya et al., 2006); *Priva adhaerens* (Forssk.) Chiov. is used as an antidiarrheal agent in many parts of central Uganda (Buganda). *Priva*

adhaerens belongs to the family Verbenaceae, and is an erect annual herb, up to 1m tall, branched, with conspicuously elongated fruiting branches. The stem is quadrangular and pubescent with hooked hairs. There are no published scientific data on the antidiarrheal activity of *Priva adhaerens*; however, as mentioned, it is listed among plants with medicinal value for the remedy of diarrhea in Uganda, and the aim of the present study, therefore, was to establish the safety and effectiveness of *Priva adhaerens* in the treatment of diarrhea in a rat model of induced diarrhea.

2.0 MATERIALS AND METHODS

2.1 Collection and authentication

Shoots and leaves of the wildy growing plant *Priva adhaerens* were collected from abandoned farmland in Mityana District, in central Uganda. A sample of the plant material was taken to the Herbarium of the Botany Department, Makerere University, Kampala, for identification. A voucher specimen has been preserved in our laboratory for further reference (voucher number 41912). The leaves obtained were shade-dried for two weeks and then ground to fine powder after which extraction was done using water.

2.1.1 Extraction of plant materials—Fine powder (100 g) of air-dried leaves of *Priva adhaerens* was subjected to the Soxhlet extractor for continuous hot extraction with distilled water. The extract was filtered and the filtrate freeze-dried. As quality control, the aqueous extract was also obtained by maceration through soaking the powder overnight in distilled water, filtering in the morning, then freeze-drying the extract; the results of both methods of extraction revealed the same results for phytochemical analysis. The dried extract was then used to determine acute toxicity and antidiarrheal activity in rats. The stock dose concentration was 200 mg/ml, calculated by dissolving 2 grams of the extract in 10 ml of distilled water for the dose-response studies carried out.

2.2 Experimental design and animals

This study was a between-groups experimental one, designed to show the difference between group means indicating the size of the effect of the treatments administered. Albino Wistar rats of either sex weighing between 150-200 g were used for experiments. The animals were maintained at the animal house of the Department of Pharmacology, Faculty of Veterinary Medicine, Makerere University, and were group-housed. The rats were fed with standard animal pellets and had access to clean water *ad libitum*. The animals were maintained under standard conditions of humidity, temperature and 12 h light: 12 h darkness cycle. Experimental treatments and animal sample sizes involved in acute toxicity testing, castor oil-induced diarrhea, gastrointestinal motility test, and castor oil-induced enteropooling are detailed in sections 2.4 to 2.7 below. The research was reviewed and approved by the Institutional Review Committee, Mbarara University (approval number, 06/09-12); and Uganda National Council for Science and Technology (approval number, HS1299), prior to its conduct. All procedures were in accordance with the approved Animal Welfare Protocols and NIH guidelines for the humane care and use of animals.

2.3 Preliminary phytochemical screening

Preliminary phytochemical screening for detection of various constituents was carried out using standard procedures (Harborne, 1973; Odebiyi and Sofowora, 1991; Evans, 2009). Briefly, alkaloid detection was carried out by extracting 1 g powdered sample with 5 ml methanol and 5 ml of 2N HCL, then treating the filtrate with Mayer's and Wagner's reagents. The samples were scored positive based on turbidity or precipitation. Testing was done for flavonoids by heating 1 g powdered sample with 10 ml ethyl acetate over a steam bath (40-50°C) for 5 min; filtrate was treated with 1ml dilute ammonia. A yellow coloration demonstrated a positive test for flavonoids. The presence of tannins was confirmed by boiling 0.5 g powdered sample in 20 ml distilled water, followed by addition of 3 drops of 5% FeCl₃ to the filtrate. Development of brownish-green or blue-black coloration was taken as positive for the presence of tannins. Saponin content was determined by boiling 1 g powdered sample in 10 ml distilled water for 15 min and after cooling, the extract was shaken vigorously to record froth formation. Cardiac glycosides were identified by extracting 2 g sample in 10 ml methanol; 5 ml of this methanolic extract was treated with 2 ml glacial acetic acid containing 1 drop of 5% FeCl₃ solution. This solution was carefully transferred to the surface of 1 ml conc. H₂SO₄. The formation of a reddish-brown ring at the junction of the two liquids was indicative of cardenolides/ cardiac glycosides (Odebiyi and Sofowora, 1991; Evans, 2009). Baljet test was done to determine the presence of cardiosselective glycosides (digitoxose); sodium picrate was added to a small amount of the extract and a positive test shown as a yellow to orange color. In Borntrager's test, the powdered extract was macerated with ether, and after filtration, aqueous ammonia was added. A pink, red or violet color in the aqueous layer after shaking indicates the presence of free anthraquinone derivatives; if the tested extract contained either very stable anthraquinone glycosides or reduced derivatives of the anthranol type, Borntrager's test would be negative (Evans, 2009). Fehling's test was done by adding Fehling's solution drop by drop to a heated solution of the aqueous extract; the presence of a brick-red precipitate indicated the presence of a reducing sugar.

2.4 Acute toxicity test

Lethal effective dose on 50% of the experimental animals (LD₅₀) following oral administration of the aqueous leaf and shoot extract of *Priva adhaerens* was estimated in Wistar albino rats (150-200 g) following Lorke's method (Lorke, 1983). Recognizing that WHO guidelines (WHO, 1998) do not require pre-clinical toxicity testing for herbal products that have been used by communities without demonstrated harm, we nonetheless wanted to ascertain the safety of the aqueous extract of *Priva adhaerens* as used in traditional medicine. Because of the ethnomedical use of this plant (diarrheal treatment), it is likely that even a mild or moderate acute toxic effect could exacerbate diarrhea and potentially produce a fatal outcome, especially in young children. We chose Lorke's method which gives a more robust estimation of the median lethal dose (LD₅₀) than the fixed-dose procedure in the OECD guideline for acute oral toxicity. Therefore, we wanted to establish a more robust value for LD₅₀; at the same time however, we wanted to demonstrate that indeed, doses that were far less than the toxic doses studied, had antidiarrheal activity, as

shown in this rat model, especially because there was no prior toxicology study on *P. adhaerens*. Undeniably, we used more animals than the OECD 420 guideline.

Dose levels used ranged from 5000 -13000 mg/kg of the aqueous extract. The 13000 mg/kg dose was made by dissolving the dry extract of *Priva adhaerens* in a ratio of 1.3 g: 100 ml of distilled water, and then administering this in a volume not exceeding 2 ml/kg body weight of the rat. The other doses were made in a similar manner. This volume of administration followed guidelines for enteral administration, especially of aqueous solutions (Brown et al., 2000; Turner et al., 2011). The acute toxicity LD₅₀ was then calculated as the geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all. Toxicity signs such as death, changes in physical appearance, and behavioral changes were observed for 72 h after administration of aqueous leaf and shoot extract of *Priva adhaerens*.

2.5 Castor oil-induced diarrhea

The method described by Awouters et al. (Awouters et al., 1978) and Beck et al. (Beck et al, 1977) was followed; healthy albino Wistar rats, both male and female, weighing 150-200 g were randomly selected and divided into five groups of six animals each. The rats were fasted for 18 hr prior to the test, with free access to water. Rats in group 1 received 20 ml/kg of normal saline (negative control), while rats in group 2 received loperamide (5 mg/kg) as positive control treatment. Rats in group 3 received 100 mg/kg, rats in group 4 received 200 mg/kg and rats in group 5 received 400 mg/kg *Priva adhaerens* extract. The animals were housed singly in cages lined with transparent paper. One hour later after pre-treatment with the extract, the animals were then administered 1 ml of castor oil orally. Thereafter, they were observed for 4 hrs for the presence of diarrhea. Diarrhea for the purpose of this study was taken to mean watery (wet), unformed stool.

2.6 Gastrointestinal motility test (Charcoal meal)

Wistar albino rats, both male and female, weighing between 150-200 g were randomly divided into 5 groups of 6 rats each, (Rajput et al., 2011). They were fasted for 18 hrs prior to test, but were allowed water *ad libitum*. Rats in group 1 were treated with 20 ml/kg normal saline and served as control, while rats in groups 2, 3, and 4 received different doses of the extract (100, 200, and 400 mg/kg). Rats in group 5 received atropine sulphate (5 mg/kg.). Thirty minutes after drug administration, 1 ml of charcoal meal (5% deactivated charcoal in 10% aqueous tragacanth) was administered orally to all animals in the study then after a further 30 minutes, all the rats were sacrificed and the abdominal cavity opened. The small intestines were dissected out from the pylorus to the cecum and the total distance travelled by the charcoal plug along the small intestine was estimated for both the control and the treated groups. The percentage distance travelled by the charcoal meal from the pylorus to the cecum was noted.

2.7 Castor oil-induced enteropooling

In this method (Rajput et al., 2011), rats were fasted for 18 hr prior to the experiment. The rats were divided into five groups of 6 rats per group. Normal saline (20 ml/kg orally) was given to the first group. The second group received 5 mg/kg of loperamide, while the last

three groups received graded doses of *Priva adhaerens* extract at 100, 200 and 400 mg/kg orally, respectively. Thirty minutes later, all the rats were treated with 1 ml of castor oil. After a further 30 minutes, each rat was sacrificed, the abdominal cavity opened, and the whole length of the intestine from the pylorus to the cecum, was dissected and the contents measured. Percentage reduction of the intestinal secretion (volume) was calculated.

2.8 Data analysis

Results were expressed as mean $\pm$ SEM (standard error of the mean). Data were analyzed using one-way analysis of variance (ANOVA). In these tests, homogeneity of variances was tested using Bartlett's test, and differences in the means tested using ANOVA followed by Bonferroni multiple comparison test as the post hoc test. Values of $p < 0.05$ were considered statistically significant for the treatment differences. Statistical analysis was done using GraphPad Prism[®] version 6.0 (GraphPad[®] Software, Inc, La Jolla, CA, USA).

3.0 RESULTS

3.1 Phytochemical screening

Phytochemical screening, by identifying the presence of chemicals in the extract, provides a reference for benchmarking in subsequent studies on the same plant. This procedural screen allows quality control and standardization in the event that a phytomedicine is developed. Screening results showed the presence of most of the phytochemicals in the aqueous leaf and shoot extracts; screening was positive for saponins, glucides and reducing compounds, catechol tannins, alkaloids, and cardenolides or steroidal glycosides. Anthraquinones (anthracenosides and anthrocyanosides), and flavonoids were absent in these aqueous extracts.

3.2 Acute toxicity

Following phytochemical screening, we conducted an acute toxicity study to estimate the median lethal dose (LD₅₀), and to estimate the doses that we would use to carry out the antidiarrheal testing. Signs of toxicity that were observed included excessive urination due to relaxation of bladder sphincter muscles with constriction of detrusor muscles, convulsions, defecation, GIT muscle twitches, and pupillary dilation; death was the end-point of acute toxicity. No animal died after administration of the oral dose of 5000 mg/kg body weight of *Priva adhaerens*; conversely, all animals given the oral dose of 13000 mg/kg body weight, died (Table 1). These two doses allowed the oral lethal dose on 50% of the rats to be calculated (Figure 1) as 8320 mg/kg body weight.

3.3 Antidiarrheal activity

After establishing the LD₅₀, and the treatment doses to use, the next step was to do the dose-dependent assay of antidiarrheal activity in the rats using the aqueous extract of *Priva adhaerens*. We used the crude aqueous extract and not the “pure” main chemical compound(s) isolated from the plant extract because our aim was to simulate the manner in which the plant is used traditionally in ethnomedicine to treat diarrhea. In addition, herbal extracts (or phytomedicines) contain several compounds that may be pro-drugs, may have additive or synergistic effects with each other, or even have different pharmacokinetic

profiles. Our goal in this study therefore, was to evaluate the “whole” first, and later, in other studies perhaps, test the main compound(s) isolated, alone or in combination.

Treatment of the rats produced a significant difference in the effect of treatment on castor oil-induced diarrhea [$F_{(4, 29)}=8.786$, $p<0.0001$]. The aqueous extracts of *Priva adhaerens* at 200 mg/kg and 400 mg/kg body weight significantly reduced the number of wet feces in five hours, compared to negative control animals (Table 2). There was no statistical difference between the loperamide (positive control) group and the extract-treated groups, implying that the extracts produced similar effect as the loperamide positive control (Figure 2 and Table 2).

Treatment of the rats did not produce any significant difference in the effect of treatment on castor oil-induced diarrhea measured by wet fecal weight (Figure 3 and Table 2).

On gastrointestinal transit time in the rats, measured by the distance traveled by charcoal, treatment of the rats produced a significant difference in the effect of treatment on distance traveled by charcoal [$F_{(4, 29)} = 55.23$, $p<0.0001$] (Table 2). The aqueous extracts of *Priva adhaerens* significantly reduced the distance traveled by charcoal in a dose-dependent manner, with the 200 mg/kg and the 400 mg/kg doses studied showing the greatest reduction in gastrointestinal motility compared to the negative control group.

Lastly, treatment of the rats produced a significant difference in the effect of treatment on castor oil-induced enteropooling [$F_{(4, 29)} = 50.15$, $p<0.0001$]. *Priva adhaerens* aqueous extracts significantly reduced the volume of intestinal contents (enteropooling) in a dose-dependent manner compared to negative control (Table 2).

4.0 DISCUSSION

The present study sought to assess the safety and antidiarrheal activity of the aqueous extract of *Priva adhaerens* leaves and shoots. Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by an excess loss of fluid in feces. Field surveys done about 25 years ago showed that more than half of traditional healers in five districts representing different regions of Uganda used herbal medicines with water as the main vehicle (Anokbonggo et al., 1990). *Priva adhaerens* is one of the herbs still used traditionally in Uganda in managing diarrhea, especially in central Uganda (Buganda region). In some types of diarrhea, the secretory component predominates, while other types of diarrhea are characterized by hypermotility. The aqueous leaf extract of *Priva adhaerens* did not show any toxic effects at the dose of 5000 mg/kg; this dose did not alter the behavior of normal animals, nor did it cause any deaths in this group of animals. Generally, substances that are not toxic at 5000 mg/kg, are considered relatively safe (Lorke, 1983). *Priva adhaerens* aqueous extract was considered, therefore, to be safe at doses 5000 mg/kg. Our findings also showed that the extract inhibited castor oil-induced diarrhea in rats significantly, comparing the inhibition favorably with that of the standard antidiarrheal drug loperamide.

It has been observed that the most active component of castor oil, ricinoleic acid produces an irritating action on the intestinal mucosa, that stimulates peristaltic activity of the small

intestines (Rouf et al., 2003; Rouf et al., 2007). The irritation causes changes in the permeability of the intestinal mucosa to electrolytes. Castor oil is also believed to cause release of prostaglandins, which stimulate motility and secretion, thereby decreasing the absorption of sodium and potassium ions (Zavala et al., 1998; Rouf et al., 2003). The aqueous extract of *Priva adhaerens* significantly reduced the intestinal transit, as was observed by a decrease in the intestinal motility of the charcoal meal. This also compared well with the antimuscarinic drug atropine, and different doses decreased the propulsive movements in the charcoal meal.

The aqueous extract of *Priva adhaerens* also significantly reduced intestinal fluid accumulation (enteropooling) as was observed by the reduced volume of intestinal contents, which compared favorably with loperamide. This effect suggests the usefulness of this extract in the management of diarrhea. The inhibition of castor oil-induced enteropooling, may be due to the ability of the extract to increase reabsorption of the electrolytes and water, also observed with the standard drug loperamide. It can also be assumed that the antidiarrheal action of the extract was mediated by an antisecretory mechanism.

Secretory diarrhea is associated with an activation of chloride (Cl^-) channels causing Cl^- ion efflux from the cell; the efflux of Cl^- results in massive secretion of water into the intestinal lumen and profuse watery diarrhea (Ammon and Soergel, 1985). The extract might inhibit the secretion of water into the lumen by reversing this mechanism. Loperamide regulates the gastrointestinal tract by inhibiting the propulsive activities, predominantly in the jejunum. Other effects on intestinal motility may be mediated through inhibition of prostaglandin stimulation of gut motility and/or through calcium antagonist actions (W.H.O., 1990). Apart from regulating the gastrointestinal tract, loperamide is also reported to reduce colonic flow, and consequently increase colonic water absorption, but does not have any effect on colonic motility. Because of the similarity in the effects of loperamide and the dose-dependent decrease in wet fecal matter, as well as the dose-dependent reduction in castor oil-induced enteropooling, it is possible that the aqueous leaf extract of *Priva adhaerens* could have the same mechanism of action as loperamide.

Phytochemical analysis of the extract of *Priva adhaerens* showed the presence of saponins, mucilages, reducing compounds, glucides, tannins, alkaloids, and steroidal glycosides, which have been reported to be present in other plants with antidiarrheal properties (William et al., 2009). It is possible that the bioactive compounds in the leaf extract of *Priva adhaerens* are responsible for the antidiarrheal effects recorded for the extract. Earlier studies have shown that anti-diarrheal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids and reducing sugars (Longanga et al., 2000). Tannins, saponins, and alkaloids in the extract, may be responsible for the antidiarrheal activity of the *Priva adhaerens* extract. One of the limitations of our study was that we did not perform histology of the organs after termination of the study. However, we predicted that since diarrhea is usually treated acutely, and since the doses we used in our study were several times lower than the LD_{50} , histological examination would be more relevant for a chronic or long-term study of *Priva adhaerens*.

In conclusion, *Priva adhaerens* is generally safe for acute use at moderate doses, and exhibits antidiarrheal activity. The dose-related antidiarrheal activity shown by the aqueous extract of *Priva adhaerens* leaves suggests the presence of some active phytochemicals, which act through one or more antidiarrheal mechanisms. The possible clinical significance of these findings therefore, is that this murine model has established the acute safety of *Priva adhaerens*, at least in rats, and has established that the aqueous leaf extract possesses antidiarrheal activity. Controlled clinical trials of *Priva adhaerens* to establish its safety and efficacy are warranted to develop recommendations further justifying its use in traditional medicine, but long-term pre-clinical toxicity should be investigated, as should its mechanism of antidiarrheal activity.

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Figure 1. Graph of log dose vs. probits to determine the LD_{50} of the aqueous extract

LD_{50} calculation was done using the formula $Y = 7.851X - 25.77$.

Whereas $y = 5$, $5 = 7.851X - 25.77$

$X = (5 + 25.77) / 7.852$, $X = 3.92$

$LD_{50} = \text{Antilog of } 3.92$

$LD_{50} = 8317.64 \text{ mg/kg body weight}$. Therefore, the LD_{50} of *Priva adhaerens* aqueous leaf extract was estimated to be 8320 mg/kg of body weight.

Figure 2. Antidiarrheal activity of the aqueous extract of *Priva adhaerens* in castor oil-induced diarrhea

Priva adhaerens aqueous extract significantly reduced the number of wet feces in 5 hours compared to the negative control group. Values shown for number of wet feces are mean $\pm$ SEM; * $p < 0.05$, comparing negative control group with loperamide (positive control) group, with group 2 extract, and with group 3 extract; $n = 6$.

Figure 3. Weight of wet fecal matter (gm.)

Values shown for weight of fecal matter are means $\pm$ SEM; there was no statistically significant difference between the treatment groups for weight of wet fecal matter after induction of diarrhea by castor oil.

Table 1

Results of the lethal doses of aqueous plant extract for the determination of LD50 after oral administration (n=6)

Group	Dose (mg/kg)	Log dose	Number dead	% dead	Probits
1	5,000	3.69	0/6	0	3.45
2	7,000	3.85	1/6	16.67	4.19
3	9,000	3.95	3/6	50	5.15
4	11,000	4.04	4/6	66.67	5.56
5	13,000	4.11	6/6	100	6.96

No animal died after administration of the oral dose of 5000 mg/kg body weight of *Priva adhaerens* ; conversely, all animals given the oral dose of 13000 mg/kg body weight, died; the intermediate dose of 7000 mg/kg caused the death of 1 of 6 animals, the 9000 mg/kg dose caused the death of 3 of 6 animals, and the 11000 mg/kg dose caused the death of 4 of 6 animals; n = 6.

Table 2

Effect of aqueous extract of *Priva adhaerens* on castor oil-induced diarrhea assayed as the number of wet feces in 5 hr., wet fecal weight in Wistar albino rats, gastrointestinal (GIT) transit time (distance traveled by charcoal), and volume of intestinal contents (enteropooling)

Treatment groups	Dose	Number of wet feces in 5 hr.	95 % CI of difference	Weight of wet fecal matter (g)	95 % CI of difference	Distance traveled by charcoal (mm)	95 % CI of difference	Volume of intestinal contents (ml)	95 % CI of difference
Negative control	20 ml/kg	3.32 ± 0.30	2.485 to 4.155	4.07 ± 1.27	0.02 to 8.12	68.20 ± 2.59	60.99 to 75.41	2.38 ± 0.21	1.71 to 3.04
Positive control	Diverse (see text)	1.20 ± 0.29*	0.376 to 2.024	1.94 ± 0.53	0.24 to 3.63	24.20 ± 2.52**	17.21 to 31.19	0.45 ± 0.04*	0.34 to 0.57
Group 1 extract	100 mg/kg	2.16 ± 0.37	1.130 to 3.190	3.45 ± 1.20	-0.36 to 7.26	60.60 ± 1.778 ^a	55.66 to 65.54	1.08 ± 0.11***	0.78 to 1.38
Group 2 extract	200 mg/kg	1.36 ± 0.37*	0.330 to 2.390	2.55 ± 0.80	0.001 to 5.10	54.40 ± 1.50*** ^a	50.23 to 58.57	0.78 ± 0.05*	0.64 to 0.93
Group 3 extract	400 mg/kg	0.96 ± 0.28*	0.187 to 1.733	1.94 ± 0.62	-0.04 to 3.90	37.60 ± 3.23*** ^{a b c}	28.62 to 46.58	0.52 ± 0.04***	0.40 to 0.64

Priva adhaerens aqueous extract significantly reduced the number of wet feces in 5 hours compared to the negative control group. Values shown for number of wet feces in 5 hr. are mean ± SEM

* p<0.05, comparing negative control group with loperamide (positive control) group, with group 2 extract, and with group 3 extract; n=6. After induction of diarrhea by castor oil, there was no statistically significant difference between the treatment groups for weight of wet fecal matter (columns 5 and 6). Values shown for weight of fecal matter are means ± SEM. *Priva adhaerens* aqueous extracts also significantly reduced the distance traveled by charcoal in a dose-dependent manner, with the 200 mg/kg and the 400 mg/kg doses showing increasing efficacy (columns 7 and 8). As expected, atropine sulphate (the positive control) showed the best efficacy in reducing the distance traveled by charcoal, compared to all the groups. Values shown for distance traveled by charcoal are mean ± SEM; **p<0.05, comparing negative control with atropine sulphate (positive control) group; ***p<0.05, comparing negative control with group 2 and with group 3 extracts; ap<0.05, comparing positive control with extracts from groups 1, 2, and 3; bp<0.05, comparing group 1 extract with group 3 extract; cp<0.05, comparing group 2 extract with group 3 extract; n=6. Lastly, *Priva adhaerens* aqueous extracts significantly reduced the volume of intestinal contents (enteropooling) in a dose-dependent manner compared to negative control (columns 9 and 10). Values shown for volume of intestinal contents are mean ± SEM; *p<0.05, comparing negative control with loperamide (positive control) group, with group 1, with group 2, and with group 3 extracts; **p<0.05, comparing positive control with group 1 extract; ***P<0.05, comparing group 1 extract with group 3 extract; n=6.