Evaluation of Antidiarrheal Activity of 95 % Ethanolic Extracts from the Leaves of *Andrographis paniculata Nees. (Kalamegha)*

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Abstract

Andrographic paniculata Nees.is commonly known as kalamegha or creat , is an annual herbaceous plant in the family Acanthaceae. It has been used as a traditional medicinal herb for centuries. The leaves of *Andrographic paniculata* Nees. (Sega gyi) were collected from Thonegwa Township, Yangon Division. In the present research, morphological study of vegetative and reproductive parts were observed so as to ascertain the correct identification. 95% ethanolic extract were prepared from the leaves and which were applied in vivo screening for antidiarrheal activity. The extract treated group has the modest reduction in intestinal transit and the percentage of *in vivo* antidiarrheal index is 42.2% in ethanolic extract treated group and 97.48% in dicotil treated group. In vivo, antidiarrheal induce of ethanoilc extract was nearly half of the dicotil groups. These extract produced a decreased in weight of the intestinal content. The secretion was more viscus. Thus the ethanolic extract of *Andrographis paniculata* was also found to possess antienteropooling activity.

Introduction

Andrographis paniculata Nees. belongs to the family Acanthaceae. Acanthaceae is a large homogenous family of about 250 genera and about 2500 species. About 1500 species are in the tropical and warm temperate regions (Hooker, 1885). Normally grown from seeds and is ubiquitous in its native areas. Because of its well known medicial properties, it is also cultivated quite easily because it can grow in all types of soil. Stem and branches acutely quadrangular, particularly in the upper region with four bulges arising the four corners jointed and nearly glabrous, branches cross armed, four sided, spreading or horizontal, cultivated as a rainy season crop (Kapoor, 2001). This plant is found throughout the plains of India and all parts have been extensively used in Unani and Ayurvedic medicine. It is also utilized The Government of Union of Myanmar is emphasized in the treatment of six major diseases. Diarrhea is included in these diseases. So, to fulfill the purposes, the present research has been carried out on the selected plants. *Andrographis paniculata* Nees. is used since immemorial time as Chinese and Ayurvedic medicine mainly for liver troubles, dysentery, women disorders, hypertension, diabetes, diarrhea etc. Deng, 1978 reported that *Andrographis paniculata* Ness. was used to treat 955 cases of diarrhea with overall effectiveness of 91.3 %.The present research aims to screen the valuable medicinal plant which have their respective curative power.

Materials and Methods

Collection and Preparation of *Andrographis paniculata* Nees.

The plant specimens of *Andrographis paniculata* Nees. studied in the present research were collected from Thone-gwa Township, Yangon Division. Leaves intended to use in isolation of compounds were harvested when the inflorescence axis starts to grow. The flowering and fruiting occurred throughout the year but were more abundant in November to February. The fresh specimens were used for morphological

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with the help of literature cited by Hooker (1879), Backer (1963), Cronquist (1981) and Hundley and Chit Ko Ko (1987).

Antidiarrheal Activity of 95% Ethanolic Extract

The antidiarrheal effect of 95% ethanolic extract of *Andrographis paniculata* Nees. leaves were studied against castor oil-induced diarrhea model in mice.

Preparation of 95% Ethanolic Extract

The leaves of *Andrographis paniculata* Nees. were collected from Thone- gwa area. The leaves were dried under shade. The dry leaves 500 g were coarsely powdered and subjected to successive solvent extraction with 95% ethanol 1000 ml for about one week and then filtered. Extraction and filtration process was carried out three times. The combined filtrate was concentrated under vacuum rotary evaporator to get 95% ethanolic extract.

Material Required

Total 66 albino mice of both sex, Intragastric dosing canala '18' guage, Castor oil, (Laxative agent), Different doses, of ethanolic extract of *Andrographis paniculata*, Loperamide (Dicotil), (standard refrence anti-diarrheal drugs), Distilled water, Charcoal powder, Dissecting box, Digital balance, Chloroform, Aluminium Mouse cages.

Animal Used in Experiment

Albino mice of either sex weigh (20 – 40 g) were obtained from the animal house at the Department of Medical Research (Lower Myanmar). The mice were given food and water adlibitum. All the animals were kept under laboratory conditions for an acclimatization period of 7 days before doing the experiments. All experiments were carried out in 11 groups of 6 mice each. Each mouse was placed separately in an aluminium mice cage.

Experimental Methods

Castor-oil incuded enteropooling

Castor-oil induced small intestinal transit

Castor-oil incuded enteropooling

Data analysis

The values were expressed as mean ± standard error. The statistical analysis of data was done by computer software of Exal using student" t " test method.

Results

Botanical Aspect

Morphological Characters of *Andrographis paniculata* Nees.

Annual dark green erect herb, 30.0 - 90.0 cm high; stem branched, quadrangular, narrowly winged in the upper part Leaves simple, opposite and decussate, petioles short, blades small, lanceolate to elliptic-lanceolate, tapering at both ends, margin entire, glabrous on both surfaces, dark green above, whitish green beneath, main nerves 4-6 pairs, taste bitter. Inflorescence terminal and axillary lax racemes, frequently combined into terminal panicles. Flowers bracteate, bracteolate, complete, bisexual, irregular, zygomorphic, pentamerous, hypogynous, small, white, pubescent; synsepalous, basally connate, subequal, lanceolate, sepaloid, green, pubescent outside, inferior; petals (5), synsepalous tubes short, slightly enlarged below the limbs; lobes longer than the tubes, bilabiate, cuneate, petaloid, white with purple spots, inferior; stamens 2, free, petalostemonous, exerted, filaments long, flat at the base, white with purple centre, hairy at the tip, anther dithecous, basifixed; carpels (2), syncarpous, bilocular, 1 - 2 ovules in each locule, axile placentation, style long, slender, stigma simple, deep purple, superior. Fruit erect, oblong capsules, longitudinally furrowed, pubescent when young. Seeds 4 - 12, oval, glabrous yellow.

Flowering and fruiting take throughout the year, more prominent from November to February.

Figure (1). Habit and the whole plant of *Andrographis paniculata*

Effect of Ethanolic Extract of *Andrographis paniculata* Nees. on Castor Oil induced Diarrhea

In the castor oil induced diarrhea experiment, the mice that received the distilled water showed typical diarrhea signs. The mean cumulated onset of diarrhoea was (18.33 ± 2.1) minutes. The mean cumulated frequency of diarrhoea of 4 hr was (14.67 ± 1.74). The cumulated frequency of defecation and onset of diarrhoea in *Andrographis paniculata* Nees. 1.5 g / kg treated on castor-oil induced diarrhoea in individual mice at various time interval is investigated. The mean onset of diarrhoea was (31.67 ± 3.07) minutes which was more longer than control. The mean cumulated frequency on diarrhoea at 4 hr. was (12.67 ± 1.73) which was not significant decreased in frequency of diarrhea showed that cumulated frequency of defecation and onset of diarrhoea in *Andrographis paniculata* Nees. 3 g/kg treated on castor-oil induced diarrhea in individual mice at various time interval. The mean onset of diarrhea was (57.5 ± 2.74) minutes which was more longer than control. The mean cumulated frequency on diarrhoea at 4 hr. was (12.83 ± 2.23).

That cumulated frequency of defecation and onset of diarrhea in *Andrographis paniculata* Nees. 6g/kg treated on castor-oil induced diarrhoea in individual mice at various time interval showed the mean onset of diarrhoea was (47.5 ± 1.08) minutes which was more longer than control. The mean cumulated frequency on diarrhea at 4 hr. was (7.83 ± 0.98) which was significant decreased in frequency of diarrhoea in treated with *Andrographis paniculata* Nees. 6 g/kg than control animal ($p < 0.005$). In the cumulated frequency of defecation and onset of diarrhoea in standard drug (dicotil 6 mg / kg) treated on costor-oil induced diarrhoea in individual mice at various time interval, the mean onset of diarrhoea was (73.33 ± 7.39) minutes. The mean diarrhoea at 4 hr. was (7.5 ± 0.29). The mean defecation of standard drugs dicotyl showed significant reduced ($p < 0.001$) when compared with that of control. The mean defecation effect of extract of *Andrographis paniculata* Nees. 6 g/kg body weight was nearly similar to that of the standard drug dicotil (6 mg / kg).

Table (1) showed the percent inhibition effect of the ethanolic extract of *Andrographis paniculata* Nees. on castor oil induced diarrhea in mice 30 minutes

after administration of castor oil, the diarrhoea was clinically appearent in all animal of control group, for the next 4 hour. The parcent inhibition of defecations of dicotil 6 mg/kg was (48.88%). A nearly similar percent inhibition of defecation over four hour was achieved with *Andrographis paniculat,* dose of 6 g/ kg orally.

Effect of Ethanolic Extract of *Andrographis paniculata* Nees. on Castor-Oil intestinal Transit.

The effect of ethanolic extract of *Andrographis paniculata* Nees. also showed down the propulsion of charcoal meal through the gastro intestinal track when compared with to the castor-oil treated mice. The mean percentage of intestinal length traveled by charcoal meal intestinal transit in ethanolic extract of *Andrographis paniculata* Nees. pre-treated (6 g / kg) and costor oil treated mice were (45.77 ± 5.56 %) and (77.91 ± 12.99 %) respectively**.** It shows significant reduced in % of intestinal transit of *Andrographis paniculata* Nees.6 g /kg than control (castor oil only treated mice). Dicotyl on its part produced a marked decreased in propulsive movement and the intestinal length traveled by charcoal meal was (68.07 ± 2.00%). The percentage inhibition of intestinal transit was also calculated. The mean inhibition of intestinal transit showed (77.91 ± 12.99) in control group, of (68.07 ± 2.00) in dicotil group and (45.77 ± 5.55) in *Andrographis* group. (Fig.3.13) show the percentage of intestinal transit of control group is 87.01 ± 5.7, the standard drug group is 87.01 ± 5.7, the standard drug dicotyl group is 31.93 ± 2.00 and ethanolic extract treated group is 54.23 ± 5.55.

The Effect of Ethanolic Extract of *Andrographis paniculata* Nees. on Castor Oil Induced Enterpooling

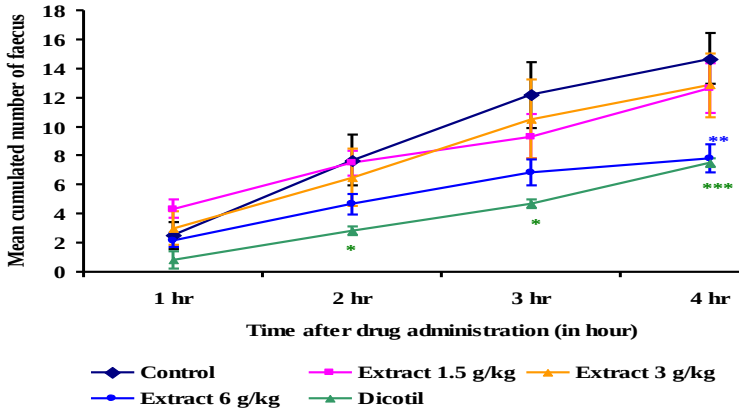
In the effect of distilled water on castor-oil induced enteropooling in dividual mice, the mean weight of intestinal content of control was 1.19 ± 0.26 g and the mean volume of intestinal content was 0.98 ± 0.07 ml. In the effect of standard drug dicotil (6mg/ kg) on castor-oil induced enteropooline in individual mice, the mean weight of intestinal content was (0.35 ± 0.05) g and the mean volume of intestinal content was (0.43 ± 0.56) ml. This result showed that there was significant reduced ($p < 0.01$) in the mean of result of intestinal content. There was significant reduced ($p < 0.0005$) in dicotil than the control, mean weight of intestinal content was (0.86 ± 0.11 g) and the mean volume of intestinal content was (0.75 ± 0.02 ml). **Table(2)** showed the antidiarrhoeal efficacies of *Andrographis pamiculata* Nees. 6 g/kg on *in vivo* mouse models laying defaecation time of onset the control group is (18.33 ± 2.11) min, the ethanolic extract treated group is (47.5 ± 10.87 min) and the dicotil group is (73.33 ± 7.39) min. In gut meal travel distance of control, ethanolic extract and standard drug are (43.92 ± 3.46 cm), (27.32 ± 2.80 cm) and (16.17 ± 1.08 cm). In vivo and diarrhoeal index of ethanoilc extract was 42.2 % and dicotil was 97.48%.

Table(1). Effect of *Andrographis paniculata* Nees. on castor oil Induced diarrhea in mice

Treatment	Mean defection in 4 hr	% Inhibition of defecation
Control (Castor oil only)	14.67±1.74	
Castor oil + ethanolic extract 1.5 g/kg	12.67±1.73	13.43
Castor oil + ethanolic extract 3 g/kg	12.83±2.23	12.54
Castor oil + ethanolic extract 6 g/kg	**7.83±0.98***	**46.63**
Castor oil + ethanolic dicotil	7.5±0.29**	48.88

***p<0. 005, **p<0.001**

Table (2). Anti-diarrheal efficacies of *Andrographis paniculata* Nees.6 g/kg on *in vivo* mouse models



Figure(2). Comparative effects of ethanolic extract of Andrographs paniculata Nees. 6 g/kg and standard drug dicotil on mice with castor oil induced diarrhoea at various time interval.

	Delaying defaecation time of onset in mice, in min	Gut meal travel distance in mice, in cm	Purging frequency in mice, in number of stool	In vivo antidiarrhoeal index, in percent
Control	**18.33±2.11**	**43.92±3.46**	**14.66±1.74**	**0**
Ethanolic extract (6 g/kg)	**47.5±10.87**	**27.32±2.80**	**7.83±0.98**	**42.2**
Dicotil (6mg/kg)	**73.33±7.39**	**16.17±1.08**	**7.5±0.29**	**97.48**

Each point represents as mean ±SEM from the experiments

Discussion and Conclusion

Collected plant samples were identifiedaccording to the standard method used in the Department of Botany, Yangon University, with the help of reference literature cited by Hooker (1885), Backer (1963) and Hundley (1987).

The plant *Andrographis paniculate* Nees. subject in this work is mainly found in tropical to subtropical forests especially in the damp soil and masshy places. In Myanmar mostly found in lower part of the country well grown in semi-shade, in waste places by roadside in the moist and dry lowlands, not common. It is annual

erect herb of extremely bitter taste in each and every parts of the plant body with quadrangular stems, often narrowly winged in the upper part (Cooke, 1958). The leaves are dark green, lanceolate, with a small winged petiole. (Textbook of Pharmacognosy, 1998).

Infloresences terminal and axillary, often laxly branched with racemiform branches, higher ones often forming a leafy terminal panicles (Backer, 1965). Flowers small, white arranged in terminal panicles, bracts small and lanceolate (Medicinal Plants of South East Asia). The plant also have the ornamental values.By observing the result shown in Table (1), 6 g/kg dose of *Andrographis paniculata* extract effected to the onset of diarrhoea. The doses of 1.5 g/kg and 3 g/kg of ethanolic extract of *Andrographis paniculata* did not effect to the severity and onset of diarrhoea. In the inhibition percentage of defecation, ethanolic extract 1.5 g/kg, 3 g/kg and 6 g/kg are 13.43%, 12.54% and 46.63% respectively and dicotyl 6 mg/kg dose is 48.88 %. Therefore, the percentage of defecation of ethanolic extract is nearly similar to standard drug dicotyl.

According to the literature reported by Horton *et al*., 1968 and Greenbergena *et al.*, 1978 that the autocoids and prostaglanding involved in castor oil have been implicated in the causation of diarrhea is man. The use of castor oil induced model in my research work is logical.

In the study of Castor oil induced intestinal transit, the effect of ethanolic extract of *Andrographis paniculata* Nees. also showed down the propulsion of charcoal meal through the gastro intestinal track when compared with to the castor-oil treated mice. It show significant reduced in percentage of intestinal transit of *Andrographis paniculata* Nees. 6 g/kg dose than control (only castor oil treated mice).

The extract significantly reduced the castor oil induced intestinal transit. This can be due to the fact that the extract increased the reabsorption of water by decreasing intestinal motility as observed in the decrease of intestinal transit by charcoal meal. (Mukherjee *et al.*, 1998)

The weight and volume of intestinal content induced by castor oil were studied by enteropooling method. The results showed that there was significantly reduced weight of wets feaces content of *Andrographis paniculata* Nees. than control ($P<0.05$). There was also significant reduced in mean volume of intestinal content of *Andrographis paniculata* than control ($P < 0.05$). The ethanolic extract of *Andrographis paniculata* Nees. produced a decreased in weight of the intestinal content and volume of intestinal content. The secretion were more viscous. Thus the ethanolic extract of *Andrographis paniculata* Nees. was also found to possess on antienteropooling activity.

The extract treated group has the modest reduction in intestinal transit and the percentage of *in vivo* antidiarrhoeal index is 42.2% in ethanolic extract treated group and 97.48% in dicotil treated group. *In vivo*, antidiarrhoeal induce of ethanoilc extract was near half of the dicotil groups due to the extract was cruded and may be contain little amount of active compound. Castor oil is a suitable model of diarrhea in mice, since it allows the observation of measurable changes in the number of stools, enteropooling and intestinal transit.

According to the above result, the extract resulted in a marked reduction in the number of diarrhea stools and the reduction in the weight and volume of the intestinal contents, as well as a modest reduction in intestinal transit. This signifies the usefulness of this model. These properties confirm the use of *Andrographis paniculata* Nees. as an anti-diarrheal drug as proposed by traditional healers and it is consumed very commonly by the local people in Thone-gwa

Township as remedy for fever common colds and diarrhea and the antidiarrhoeal effect of the extract of *Andrographis paniculata* leaves the period of our study.

References

Ashin Nagathein, 1979. **Pon-Pya-Say-Abidon**, Vol.1, Mingalar Press, Yangon.

Brandis, D., 1907. Indian Trees. Archihord Constable and Co., Ltd. London.

Chopra, R.N., 1956. Glossary of Indian Medicinal Plants, Drug Reseach Laboratory, New Delhi.

Cronquist, A. , 1981. Am integrated system of classification of flowering plants, Columbus University Press, New York. P-815

Dassanayake, M.D, 1985. Flora of Ceylon, Vol,I, American publishing Co. pvt. ltd. New Delhi.

Esau, K., 1953. Plant Anatomy. John Wiley and Sons, Inc. NewYork London.

Flora of Hong Kong, 2009. Hong Kong Herbanium Agriculture, Fisheries and Conservation Department Vol. IV

Harbone, J.B., 1984. Phytochemical Methods. A Guide to Modern Technigues of lant Analysis. (2nd Ed), New York.

Hooker, V.H. 1978. Flowering Plants of the World. Oxford University Press, London.

Kress, W.John, Robert A. Defilipps, Ellen Farr and Daw Yin Yin Kyi, 2003.

A Checklist of the Trees, Shrubs, Herbs and Climbers of Myanmar. National Museum of Natural History, Washington DC.

Metcalfe, C.R., and L. Chalk, 1971, Anatomy of the Dicotyledons. The Clarendon Press, Ely House, Volume I, London.

Pandey, S.N and A. Chadha, 1998. Plant Anatomy and Embryology. Vikas Publishing House Pvt. Ltd., New Delhi.

Quality Standards of India Medicinal Plants, Vol-1, Indian Council of Medical Research, New Delhi, 2003. P-102-108

Quality Control Methods for Medicinal Plant Materials WHO, Geneva, 1998.

Trease & Evans WC. Pharmacognosy. Balliere Tindall Press London: 1983.