



COMPARISON OF ANTI-DIARRHEAL ACTIVITY OF HYDROETHANOLIC EXTRACTS OF *CALOTROPIS PROCERA* AND *CALOTROPIS GIGANTEA* LEAVES

Murti Yogesh*, Singh Abhay Pratap and Pathak Devender

Rajiv Academy for Pharmacy, Mathura, P.O. Chattikara, Mathura-281001 India

*E-mail : ymurti@rediffmail.com

Received on: 15/04/12 Revised on: 18/05/12 Accepted on: 05/06/12

ABSTRACT

The 70% hydroethanolic extracts of *Calotropis procera* (CP) and *Calotropis gigantea* (CG) leaves have been evaluated and compared for anti-diarrheal activity using castor oil-induced diarrhea model in rats. Results showed statistically significant reduction in the frequency and severity of diarrhoea produced by castor oil in both CP and CG. The anti-diarrheal effect of CP was found superior to CG and inferior to loperamide. It is concluded that CP and CG possess marked anti-diarrheal activity supporting the folkloric claim that both plants are good remedies for acute nonspecific diarrhoea.

Keywords: Anti-diarrheal Activity, Leaves, Castor oil, *Calotropis procera* and *Calotropis gigantea*

INTRODUCTION

Diarrhoea is characterized by an increase in the frequency of bowel movements, wet stool and abdominal pains¹. It is commonly caused by gastrointestinal infections which results in the death of around 1.8 million people globally each year. Generally, the treatment of diarrhoea is non-specific, and is usually aimed at reducing the discomfort and inconvenience of frequent bowel movements². Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhoea but sometimes provoke side effects and increases resistance to the common antibiotics³⁻⁸. The need for newer, more effective, and most importantly, cheaper anti-diarrhoeal drugs has become a paramount issue to tackle this present situation⁹.

Calotropis procera and *Calotropis gigantea* belonging to family Asclepiadaceae are widely growing plants and have been reported to possess number of medicinal properties. The leaves of these plants have been used in traditional or folk medicine as antipyretic, antioxidant, analgesic, abortifacient, hepatoprotective, antimicrobial, antimalarial, anticancer, and antifertility agents¹⁰. Different parts of these plants are known for their anti-diarrheal activity, with different mode of actions^{11,12}. The present study is an effort to evaluate and compare the anti-diarrheal activity of hydroethanolic (70%) extracts of *Calotropis procera* (CP) and *Calotropis gigantea* (CG) leaves by castor oil-induced diarrhea model in rats. Phytochemical screening of plants extract has revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, sterols, terpenes, carbohydrates, lactones, proteins, amino acids, glycosides, and saponins etc.¹³.

MATERIALS AND METHODS

Experimental animals:

Animals were kept under standardized animal house conditions (temperature: 28-31°C, photoperiod: approximately 12 hrs. of natural light per day, relative humidity 50-55%) at the animal house of Rajiv Academy for Pharmacy, Mathura and provided with food and domestic tap water *ad libitum*. All animal experiments were conducted according to ethical guidelines of the committee of institute for Control and Supervision of Experiments on animals (Registration no. IAEC/RAP/2978/2010).

Plant Material:

Fresh *Calotropis procera* and *Calotropis gigantea* leaves were collected from the medicinal garden of Rajiv Academy

for Pharmacy, Mathura, U.P., India and compared with a voucher specimen maintained in the herbarium of NISCAIR, New Delhi. The leaves were cleaned by washing with running water and shade dried and then milled to coarse powder by mechanical grinder.

Preparation of extracts:

The leaf powders of both plants (2.0 kg each) were extracted by maceration for seven days with 70% ethanol (3 x 2.5 lit.) at room temperature and the combined hydroethanolic extracts were evaporated under reduced pressure separately to yield CP (60 g) and CG (67 g) as brownish green viscous residue.

Castor oil- induce Model¹⁴

Wistar albino rats (210-230 g) both males and females were used, after overnight food deprivation. For the experiment, the rats were housed in individual metabolic cages. The potential anti-diarrheic agents were administered orally in 125 mg/kg and 250 mg/kg doses in normal saline. Loperamide was taken as reference standard drug. Controls received the normal saline only. Each dose was given to 08 animals. One hour after dosage, 1 mL of castor oil was administered orally. Stools were collected on non-wetting paper sheets of uniform weight up to 8 hrs after administration of the castor oil. Every 15 min during the first 8 hrs, urine was drained off by gravity, and the net stool weight, termed as early diarrheal excretion, was recorded. The diarrhea-free period is defined as the time in minutes between castor oil administration and the occurrence of the first diarrheal output (latent period). Observation for defecation continued up to 8 hrs. on filter paper placed beneath the individual perforated rat cages. This paper was replaced every hour after noting its weight (M₁). Finally, the filter paper was exposed in the laboratory for drying and it was reweighed (M₂). The fecal water content was calculated as (M₂-M₁) g. The presence of characteristic diarrheal dropping with CP and CG was noted (Table: 1).

Statistical analysis

The experimental results are expressed as Mean \pm S.E.M., with eight animals in each group. Statistical significance test were performed by Student's t-test¹⁵. P values less than 0.001 and 0.01 were considered as significant.

RESULT AND DISCUSSION

The results of anti-diarrheal activity using castor oil-induced diarrhea model in rats showed that 70% hydroethanolic

extracts of *Calotropis procera* (CP) and *Calotropis gigantea* (CG) leaves dose dependently and markedly decreased the number of faecal boluses produced in the castor oil-induced diarrhoea test and also improved the severity of the diarrheal condition. The latent periods were also observed to increase with increase in drug dose. This is an interesting and therapeutically important finding which also provides scientific evidence in support of the claim that these plants are effective against acute nonspecific diarrheas. Further antidiarrhoeal potential of CP was superior to CG and inferior to the reference antidiarrhoeal drug, loperamide (evaluated in the castor oil-induced diarrhoea test).

Anti-diarrhoeal effect of CP and CG had a rapid onset and a fairly long duration of action (upto 8 hrs.). Further, the antidiarrhoeal action of CP and CG were dose dependent indicating phytoconstituents mediated genuine and specific action. CP and CG contains a variety of phytoconstituents of which flavonoids and alkaloids could be attributed to exhibit antidiarrhoeal potentials¹⁶. It is now known that prostaglandins are involved in castor oil-induced diarrhoea and prostaglandin synthesis inhibitors impair castor oil-induced diarrhoea¹⁷. CP and CG inhibit castor oil-induced diarrhoea in this study. Thus, it is possible that CP and CG produced its antidiarrhoeal activity by inhibiting gastrointestinal prostaglandin synthesis.

CONCLUSION

In conclusion, this study, scientifically demonstrated antidiarrhoeal activity of 70% hydroethanolic extracts of *Calotropis procera* and *Calotropis gigantea* leaves and justify the traditional use of these plants in the management of diarrhoea. No death and no signs of abnormal behavior were observed in the rats treated with CP and CG and could be useful for the development of new antidiarrhoeal drugs. However, further pharmacological and toxicological studies will be necessary.

REFERENCES

- Ezekwesili C., Obiora K. and Ugwu O., Evaluation of anti-diarrhoeal property of crude aqueous extract of *Occimum gratissimum* L. (Labiatae) in rats, *Biokemistri*. 2004, 16, 122-131.

- Brunton L.L., Agents for control of gastric acidity and treatment of peptic ulcer In: Goodman and Gilman's 'The pharmacological basis of therapeutics', McGraw-Hill, New York, 9th edn., 1996, 901-915.
- Maiti A., Dewanjee S. and Mandal S.C., *In-vivo* evaluation of antidiarrhoeal activity of the seed of *Swietenia macrophylla* King (Meliaceae), *Trop. J. Pharm. Res.* 2007, 6 (2), 711-716.
- Gupta, S., Antidiarrhoeal activity of diterpenes of *Andrographis paniculata* (Kalamegh) against *Escherichia coli* enterotoxin in *in-vivo* models, *Int. J. Crude Drug Res.* 1990, 28(4), 273-283.
- Patel J.D., Patel D.K., Shrivastava A. and Kumar V., Screening of plant extracts used in traditional antidiarrhoeal medicines against pathogenic *Escherichia coli*, *Scientific World*, 2008, 6(6), 63-67.
- Tripathi K.D., *Essential of medicinal pharmacology*, Jaypee brother's medical publishers (P), New Delhi, 5th edn. 2009, 610-623.
- Munson P.L., *Principles of pharmacology basic concepts & clinical applications*, ITP an international Thomson publishing company, 1996, 1083-1089.
- Soberon J.R., Sgariglia M.A., Sampietro D.A., Quiroga E.N. and Vattuone M.A., Antibacterial activities of plant extracts from northwestern Argentina, *J. Appl. Microbiol.* 2007, 102, 1450-1461.
- Alam M.A., Akter R., Subhan N., Rahman M.M., Majumder M.M., Nahar L. and Sarker S.D., Antidiarrheal property of the hydromethanolic extract of the flowering tops of *Anthocephalus cadamba*, *Braz. J. Pharmacog.* 2008, 18(2), 155-159.
- Ahmed K.K.M., Rana A.C. and Dixit V.K., *Calotropis* species (Asclepiadaceae)- A comprehensive Review, *Phcog. Mag.* 2005, 1(2), 48-52.
- Chitme H.R., Chandra M. and Kaushik S., Studies on anti-diarrhoeal activity of *Calotropis gigantea* R.Br. in experimental animals, *J. Pharm. Pharm. Sci.* 2004, 7(1), 70-75.
- Kumar S., Dewan S., Sangraula H. and Kumar V.L., Anti-diarrhoeal activity of the latex of *Calotropis procera*, *J. Ethnopharmacol.* 2001, 76, 115-118.
- Murti Y., Yogi B. and Pathak D., Pharmacognostical standardization of leaves of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae), *Int. J. Ayur. Res.* 2010, 1(1), 14-17.
- Vogel H.G., *Drug discovery and evaluation-Pharmacological Assays*, Springer, New York. 2nd edn., 2009, 875-876.
- Woodson R.F., *Statistical methods for the analysis of biomedical data*, Wiley series in probability and mathematical statistics, Wiley, Chichester, 1987, 315.
- Palombo E.A., Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function, *Phytother. Res.* 2006, 20, 717-724.
- Awouters F., Niemegeers C.J.E., Lenaerts F.M. and Janssen P.A.J., Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis, *J. Pharm. Pharmacol.* 1978, 30, 41-45.

Table: 1 Effect of CP and CG on castor oil-induced diarrhoea

Treatment	Dose (mg/kg)	Latent period (min)	Mean defecation	Mean number of wet faeces
Saline control	---	50.00±5.35	18.16±1.82	13.67±0.56
Loperamide	2.5	260.00±15.68*	6.34±0.31*	3.93±0.22*
CP	125	181.50±9.88*	12.57±0.43**	9.80±0.67**
	250	245.10±23.56**	8.83±0.90*	3.56±0.43**
CG	125	167.14±11.65*	15.34±0.89*	11.50±0.28*
	250	204±9.77*	10.45±0.33*	7.33±0.63**

All the values are given as Mean ± SEM; N=8; *P<0.01 & **P<0.001 as compared to control.

Source of support: Nil, Conflict of interest: None Declared