Assessment of Anthelmintic Activity of Cassia fistula L.

¹M. Irshad, ²Man Singh, ¹Moshahid A. Rizvi

¹Genome Biology Lab, Department of Biosciences, Jamia Millia Islamia (Central University), New Delhi-110025, India ²Chemistry Research Lab, Deshbandhu College, University of Delhi, New Delhi, 110019, India

Abstract: There is an increasing demand of herbal medicine worldwide, a great majority of medicinal plants were assessing for their efficacy. World Health Organization estimated that two billion of people harbour parasitic worm infection. In view of this, attempts have been made to study anthelmintic activity of *Cassia fistula* L fruit pulp and seeds. Both extracts were found not only paralysed, also killed the *Pheretima posthuma*. The concentrations of 100 mg/ml of extracts caused more significant paralysis as well as death of worms as compared to reference drug Piperazine citrate at dose of 10 mg/ml. The correlation coefficient between paralysis and death time of *Pheretima posthuma* by seeds and pulp were 0.9986 and 0.9976 respectively. It was concluded that *Cassia fistula* can be used as anthelmintic.

Key words: Anthelmintic • Cassia fistula L. • Pheretima posthuma.

INTRODUCTION

Plant products have long been used for healthcare. The interest in medicinal plants reflects the recognition and the validity of many traditional claims regarding the value of natural products in healthcare [1, 2]. Cassia fistula L (Leguminosae), a semi-wild Indian Laburnum, is distributed in various countries including Asia, South Africa, Mexico, China, West Indies, East Africa and Brazil [3]. This plant is widely used by tribal people to treat various ailments including ringworm and other fungal skin infections [4]. Indian people using the fruit as anti-inflammatory, antipyretic, antimicrobial, abortifacient, demulcent, purgative, refrigerant and good for chest complaints, eye ailments, heart and liver ailments and rheumatism [5-9]. The pulp of the ripe fruits has a mild, pleasant purgative action and also used as an anti-fungal drug [10]. Seeds are used to treat skin diseases, flowers and fruits are used to treat skin diseases, fever, abdominal pain, leprosy by traditional people [11]. Besides its pharmacological uses, its extract is also recommended for pest and disease control [12-14]. Cassia fistula plant parts are known to be an important source of secondary metabolites. The chemical constituent of Cassia fistula seeds were identified oxyanthraquinones, chrysophenol and chrysophanein [15,16], fistulic acid,

3-formyl-1-hydroxy-8-methoxy anthraquinone, 3B hydroxy-17-norpimar-8(9)-en-15-one, rhein and sennidine like compound present in pod [17-21]. The available informations on this plant have a vast virgin area of research to find out the real natural treasure. The present experiment was performed on adult Indian earthworm (*Pheretima posthuma*) due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [22-24]. It is also easy availability and has been widely used for preliminary investigation of anthelmintic compounds *in vitro* [25-27]. The study has an important implication on designing a potential herbal drug for various ailments.

MATERIALS AND METHODS

Collection and Preparation of Extract: The fresh ripe fruits of Cassia fistula L were collected from the campus of Delhi Institute of Pharmaceutical Science and Research (DIPSAR), New Delhi, India. A voucher specimen was stored in laboratory for future reference. The fruit pulp and seeds were separated and ground to coarse powder. The powder of pulp and seeds were first defatted with hexane and then extracted with methanol in Soxhlet apparatus for period of 72h. Further it was filtered and dried by rota vapour.

Corresponding Author: Moshahid A. Rizvi, Genome Biology Lab, Department of Biosciences,

Jamia Millia Islamia (Central University), New Delhi-110025, India

Phytochemical Studies: The extracts were subjected to various phytochemical tests to determine the active constituents present in the methanolic extracts. The tests were performed for alkaloids, flavanoids, glycosides, polyphenols, saponin and tannins by standard methods [28, 29].

Anthelmintic Activity: Methanolic extracts of Cassia fistula L fruit pulp and seeds were tested for their anthelmintic activity against Pheretima posthuma (earthworm). Various concentrations (20-100mg/ml) of each extracts were used to evaluate the time of paralysis and time of death of earthworms. Piperazine citrate (Glaxo Smithkline), 10mg/ml was taken as standard reference and distilled water as control. The anthelmintic assay was carried out by standard method [30]. Indian adult earthworms collected from moist soil and washed with normal saline to remove all faecal matter, were used for the anthelmintic study. The earthworms of 4-6 cm in length were used for all the experimental protocol. Test samples of the extract was prepared at the concentrations 20-100 mg/ml in distilled water and six earthworms approximately equal size were released in 25 ml test solutions. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colours. All experiments were carried out in accordance with the guideline of the Institutional Biosafety and Ethical Committee.

Statistical Analysis: All graph drawing and statistical calculations were performed using Microsoft excel. The experiments were repeated several times to confirm the reproducibility of the results. All values are expressed as mean±SEM. T-test was performed to determine significance and less than 0.05 (P<0.05) was regarded as statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of methanolic extract pulp and seeds revealed the presence of phenols, flavanoid tannins, alkaloid and glycosides in both. The results were supported by literature [31-33]. The anthelmintic activities of tested drugs were shown in Table 1. The tested drug not only demonstrated paralysis, but also caused death of worms. The methanolic extract of seeds and pulp of C. fistula showed anthelmintic activity in dose-dependent manner and shortest time of paralysis and death were found at dose of 100 mg/ml. As compare to seed, pulp exhibited more potent activity at all concentration (Fig. 1). The standard drug Piperazine citrate showed predominant effect at the dose of 10mg/ml on the earthworm and cause flaccid paralysis. However, at the dose of 100 mg/ml of seed and pulp extracts showed paralysis as well as death of worms more efficient as compared to reference drug Piperazine citrate at dose of 10 mg/ml. The correlation coefficient between paralysis and death time of Pheretima posthuma by seeds and pulp were 0.9986 and 0.9976, respectively. The anthelmintic activity of C. fistula may be due to phenolic compound, tannins that can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause

Table 1: Anhelmintic actity of Cassia fistula.

Methanolic Extract	Dose (mg/ml)	Peritema posthuma	
		Paralysed time (min) Mean±SEM	Death time (min) Mean±SEM
Seeds	20	82±0.232	118±0.115
	40	70±0.116	98±0.200
	60	52±0.118	77±0.115
	80	36±0.200	52±0.230
	100	20±0.118	27±0.118
Pulp	20	70±0.200	102±0.200
	40	64±0.230	89±0.200
	60	43±0.116	64±0.118
	80	28±0.118	43±0.118
	100	17±0.115	24±0.200
Piperazine citrate	10	21±0.200	28±0.115
Control	0	NIL	NIL

All values represent Mean ±SEM; n= 6 in each group.

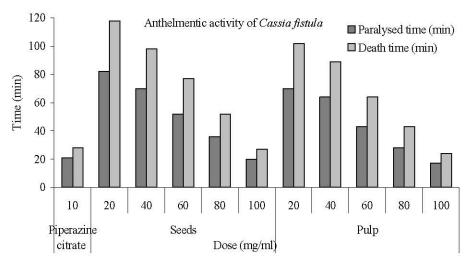


Fig. 1: Comparative studies of anthelmintic activity of Seeds and Pulp

death [34,35]. Hence, Piperazine citrate increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis [36]. The experimental results concluded that *C. fistula* showed significant (p<0.05) anthelmintic activity. Experimental support obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic and treat intestinal worm infections. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity.

In conclusion, the traditional use of fruit of *Cassia fistula* as an anthelmintic has been confirmed in the fruit pulp and seeds. As compare to Piperazine citrate, both, fruit pulp and seeds displayed cidal activities against the helminth used in this study. Further studies are to recognize active constituents and the mechanisms of action.

ACKNOWLEDGEMENT

The authors are acknowledged the LSRB, DRDO for financial support.

REFERENCES

 Awasthi, S., M. Irshad, M.K. Das, S.S. Ganti and M.A. Rizvi, 2009. Anti-Inflammatory Activity of Calotropis gigantea and Tridax procumbens on Carrageenin-Induced Paw Edema in Rats. Ethnobot. Leaflets, 13: 568-577.

- Nair, R., T. Kalariya and C. Sumitra, 2005. Antibacterial activity of some selected Indian Medicinal flora. Turkey J. Biol., 29: 41-47.
- Moshahid, A.R., M. Irshad, E.H. Gamal and B.Y. Salaem, 2009. Bioefficacies of *Cassia fistula:* An Indian labrum. Afr. J. Pharm. and Pharmacol., 3(6): 287-292.
- Rajan, S., D.S. Baburaj, M. Sethuraman and S. Parimala, 2001. Stem and stem bark used medicinally by the Tribals Irulas and Paniyas of Nilgiri District, Tamilnadu. Ethnobot., 6:19-24.
- Patel, D., D. Karbhari, D. Gulati and D. Gokhale, 1965. Antipyretic and analgesic activities of Aconatum spicatum and Cassia fistula. Pharmaceutcal. Biol., 157: 22-27.
- Biswas, K. and A.B. Ghosh, 1973. In Bharatia Banawasadhi, Calcutta University. Advancement of learning, Calcutta, India, pp. 2-336.
- 7. Kirtikar, K.R. and B.D. Basu, 1975. Indian Medicinal Plants, Jayyed Press, New Delhi, 2nd Ed, vol. 4.
- Satyavati, G.V. and M. Sharma, 1989. Medicinal Plant in India. ICMR, New Delhi.
- Elujoba, A.A., A.T. Abere and S.A. Adelusi, 1999. Laxative Activities of *Cassia* pods sourced fro Nigeria. Nig. J. Nat. Prod and Med., 3: 51-53.
- Kasuko, I. and O. Nagayo, 1951. Effects of vegetable drugs on pathogenic fungi I. Effect of anthraquinone-glycoside containing crude drugs upon the growth of pathogenic fungi. Bull Pharma. Res. Inst., 2: 23-29.
- 11. Perry, L.M., 1980. Medicinal plants of East and South East Asia. MIT Press, Cambridge.

- Jaipal, S., Z. Sing and R. Chauhan, 1983. Juvenile hormone like activity in extracts of some common Indian plants. Indian J. Agr. Sci., 53: 730-733.
- Sharma, B.K. and A.K. Basandrai, 1999. Efficacy of some plant extracts for the management of Karnal bunt *Neovossia (Tilletia) indica* of wheat *Triticum* aestivum. Indian J. Agr. Sci., 69: 837-839.
- 14. Raja, N., S. Albert and S. Ignacimuthu, 2000. Effect of solvent residues of *Vitex negundo* Linn. and *Cassia fistula* Linn. on pulse beetle, *Callosobruchus maculates* Fab. and its larval parasitoid, *Dinarmus vagabundus* (Timberlake). Indian J. Exp. Biol., 38: 290-292.
- Kuo, Y.H., P.H. Lee and Y.S. Wein, 2002.
 Four new compounds from the seeds of Cassia fistula. J. Nat. Prod., 65: 1165-1167.
- Khanna, R.K. and S. Chandra, 1996. Forest/Domestic waste as a source of natural dyes. J. Econ. Bot., 20: 497-500.
- 17. Lal, J. and P.C. Gupta, 1972. Galactomannon from the seeds of *Cassia fistula*. Planta. Med., 22: 70-77.
- 18. Modi, M.R.K. and M.L. Khorana, 1952. A study of *Cassia fistula* pulp. Indian J. Pharm., pp. 61-63.
- Misra, T.N., R.S. Singh, H.S. Pandev and R.P. Pandev, 1996. Chemical constituents of hexane fraction of Cassia fistula pods. Fitoterapia LXVII, 57: 173-174.
- Misra, T.R., R.S. Singh, H.S. Pandey and B.K. Singh, 1997. A new diterpene from *Cassia fistula* pods. Fitoterapia LXVIII, pp: 58-375.
- Kapadia, G.J. and M.L. Khorana, 1966. Studies of active constituents of *Cassia fistula* pulp. I. Colorimetric estimation of free rhein and combined sennidin-like compounds. Lloydia., 25: 55-58.
- Thorn, G.W., R.D. Adams, E. Braunwald, K.J. Isselbacher and R.G. Petersdorf, 1977. Harrison's Principles of Internal Medicine, McGraw Hill Co., New York.
- Vigar, Z., 1984. Atlas of Medical Parasitology.
 P.G. Publishing House, 2^d Ed, Singapore.
- Chatterjee, K.D., 1967. Parasitology, Protozoology and Helminthology. In Guha Ray Sree Saraswaty Press Ltd. Calcutta, 6th Ed.

- Dash, G.K., P. Suresh, D.M. Kar, S. Ganpaty and S.B. Panda, 2002. Evaluation of Evolvulus Isinoids Linn. For anthelmintic and antimicrobial activities. J. Nat. Rem., 2:182-185.
- Szewezuk, V.D., E.R. Mongelli and A.B. Pomilio, 2003. Antiparasitic activity of Melia azadirach growing in Argentina. Mol. Med. Chem., pp. 54-57.
- Shivkar, Y.M. and V.L. Kumar, 2003. Anthelmintic activity of latex of *Calotropis procera*. Pharma Biol., 41: 263-265.
- 28. Harborne, J.B., 1973. Phytochemical methods, Champan and Hall, London, pp. 113.
- Obadoni, B.O. and P.O. Ochuko, 2001. Phytochemical studies and comperative efficacy of the crude extracts of some homeostatic plants in Edo and Delta state of Nigeria. Global. J. Appl. Sci., 8: 203-208.
- Ajaiyeoba, E.O., P.A. Onocha and O.T. Olarenwaju, 2001. In vitro anthelmintic properties of Buchholzia coriaceae and Gynandropsis gynandra extract. Pharm Biol., 39: 217-220.
- 31. Rastogi, R.P. and B.N. Mehrotra, 1991. Compendium of Indian Medicinal Plants, CSIR, New Delhi, pp: 2-147.
- 32. Vasi, I.G. and V.P. Kalintha, 1980. Chemical examination of the fruit pulp of *Cassia fistula* Linn. J. Inst. Chemist., 22: 85-86.
- 33. Kashiwada, Y., H. Lizuka, K. Yoshioka, R.F. Chen, G. Nonaka and I. Nishioka, Tannin and related compounds. XCIII. Occurrence of enantiomeric proanthocyanidins in the leguminosea Plant, Cassia fistula L; Cassia javanica L. Chem. Pharm. Bull., 38(4): 888-893.
- Athnasiadou, S., I. Kyriazakis, F. Jackson and R.L. Coop, 2001. Direct anthelmintic effect of condensed tannins towards different gastrointestinal namatodes of sheep: in vitro and in vivo studies. Vet Parasitol., 99: 205-219.
- 35. Bate-Smith, E.C., 1962. The phenolic constituent of plants and their taxonomic significance, dicotyledons. J. Linn. Soc. Bot., 58: 95-103.
- 36. Martin, R.J., 1985. γ-Aminobutyric acid and Piperazine activated single channel current from Ascaris sum body muscles. Br. J. Pharmacol., 84: 445-461.