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SHORT COMMUNICATION

ANTI-DIARRHEAL ACTIVITY OF METHANOLIC EXTRACT OF *TEPHROSIA PURPUREA*

KHALID HUSSAIN JANBAZ[1], M. IMRAN QADIR[2]*, ASMA JAN[1] and ANWARUL HASSAN GILANI[3]

[1]Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan
[2]College of Pharmacy, GC University, Faisalabad, Pakistan
[3]Department of Biological and Biomedical Sciences, The Aga Khan University, Stadium Road, Karachi, Pakistan

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Tephrosia purpurea, (Syn: *Galega purpurea*, Linn.; Family: Fabaceae) is locally known as Bansa (Punjabi), Sarphunka (Hindi) and Jangli kulthi (Sindhi). It grows wild throughout Indo-Pak-Bangla Desh subcontinent on hard and stony grounds (1-3). It is branched and sub-erect herbaceous perennial plant. The plants are propagated through seeds (4, 5). Phytochemical investigations revealed the presence of β-sitosterol, quercetin, lupeol, rutin, delphinidine chloride, cyanidine chloride, isolonchocarpin, lanceolatins A and B, pongamol, karangin, kangone, 5,7-dimethoxy-8-flavanone, 2-methoxy-3,9-dihydroxycoumestone, flevichapparins B and C, methylkaranjic acid and purpurin among the plant constituents (6, 7). Resistance to the present compounds against management of different diseases has lead to search for the new candidates (8, 9). *Tephrosia purpurea* is reputed to possess diuretic, antipyretic, anti-inflammatory, anti-ulcer, anti-asthmatic, anti-leprosy and anthelmintics properties (10, 11). The aim of this study was to evaluate anti-diarrheal activity of methanolic extract of *Tephrosia purpurea*.

MATERIALS AND METHODS

Plant material

Whole plant of *Tephrosia purpurea* (Tp) was collected from the local herbal market of Multan, Pakistan. These plant material was identified/ authenticated by the kind cooperation of an expert taxonomist (Prof. Dr. Altaf Ahmad Dasti) at the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan and voucher specimen was deposited in the herbarium.

Preparation of crude extract

The herbal material was shade dried and rendered free of any dust particles or adulterated materials by manual picking. It was subsequently grinded to coarse powder by an electrically driven grinding machine. About 500 g of the coarsely grinded powder material was soaked in 80% aqueous methanol for eight days with occasional shaking (11). The material was passed through double layered muslin cloth to get rid of organic debris and the fluid portion was filtered through Whatman grade 1 filter paper. The collected filtrate was subsequently concentrated to thick semi solid mass at 37°C on a rotary evaporator (R210, BUCHI, Switzerland) under reduced pressure and was dried further through freeze drying and transferred to final containers to kept in refrigerator (-4°C). The approximate yield was 4.0%. Different dilutions of the crude extract (Cr) were made fresh on the day of experiment.

Animals and housing conditions

Animals (♂/♀) used in this study were rabbits (1.0-1.8 kg), guinea-pigs (500-600 g), Bulb-c mice (20-30 g) and Sprague-Dawley rats (200-300 g). These were housed under controlled environmental condition (23-25°C) at the animal house of The Aga Khan University, Karachi. The animals were given

* Corresponding author: e-mail: mrimranqadir@hotmail.com

standard diet and tap water *ad libitum*. The animals were deprived of food 24 h prior to the experiments but were given free access to water. Rabbits were sacrificed following a blow on back of head, while rats and guinea-pigs were killed by cervical dislocation to be used for *in vitro* studies, whereas mice were used for the *in vivo* studies. All the experiments performed complied with the rulings of Institute of Laboratory Animal Resources, Commission on Life Sciences (NRC, 1996), approved by the Ethical Committee of The Aga Khan University, Karachi.

***In vivo* experiments**

Anti-diarrhoeal effect in mice

Mice of either sex (16-36 g) being maintained at 25°C were fasted for 24 h before the experiment. Animals were housed in individual cages and were divided into five groups each containing five animals. Group 1st animals served as negative control

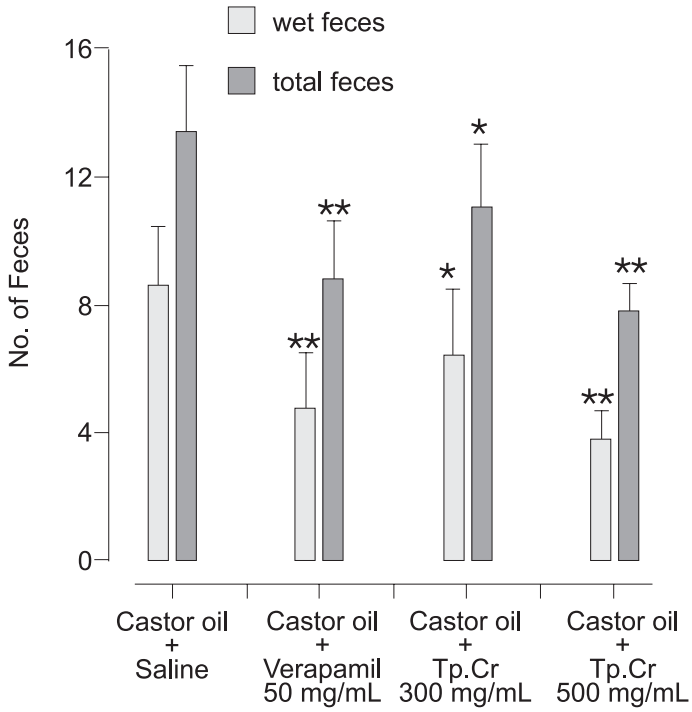

Figure 1. Anti-diarrheal activity of *Tephrosia pupurea* crude extract (Tp.Cr) and verapamil on castor oil-induced diarrhea in mice; *p < 0.05, **p < 0.005

Table 1. Anti-diarrheal activity of and Tp.Cr and verapamil on castor oil-induced diarrhea in mice.

Treatment	Total count	Total wet count	Diarrhea	Effect (%)
Saline + C. oil 10 mL/kg + 10 mL/kg	8.60 ± 0.81	13.20 ± 0.93	5	0
Verapamil + C. oil 50 mg/kg + 10 mL/kg	4.80 ± 0.73	8.80 ± 0.80	1	80
Tp.Cr + C. oil 300 mg/kg + 10 mL/kg	6.40 ± 0.92	11.00 ± 0.89	3	40
Tp.Cr + C. oil 500 mg/kg + 10 mL/kg	3.80 ± 0.37	7.80 ± 0.37	1	80

Tp Cr = *Tephrosia pupurea* crude extract, C. oil = castor oil

and were treated with an oral dose of normal saline (10 mL/kg) and followed after 1 h by an oral dose of castor oil (10 mL/kg). Group 2nd animals were designated as positive control and were treated similarly to group 1st except normal saline was replaced by an oral dose of verapamil (50 mg/kg). Group 3rd, 4th and 5th animals were designated as test groups and were given oral doses of plant extract at the dose of 100, 300 and 500 mg/kg, respectively.

The animal cages were inspected after 5 h for the presence of diarrhoeal spots, the lesser numbers of diarrhoeal spots in group 3rd, 4th and 5th animals was an indication for possible anti-diarrhoeal effect.

Statistical analysis

The data were expressed as the mean ± standard error of mean (SEM, n = number of animals) and the median inhibitory concentrations (IC_{50}) with 95% confidence intervals (CI). The statistics applied was Student's *t*-test except in case of castor oil induced diarrhea where χ^2-test was used and $p < 0.05$ was taken as significant difference. Concentration-response curves (CRCs) were analyzed by non-linear regression using Graph Pad program (Graph PAD, San Diego, CA, USA).

RESULTS

Effect of Tp.Cr on castor oil-induced diarrhea in mice

In order to assess the anti-diarrhoeal potential of Tp.Cr, *in vivo* studies were conducted on mice. Castor oil was administered orally to mice to induce diarrhoea and subsequently, different doses of Tp.Cr were administered orally to see the possible anti-diarrhoeal activity. In the control group of animals the frequency of diarrhoea induction was high and almost all of the treated animals were found to develop diarrhoea. The mice treated with verapamil were found to be highly protected (80%) from diarrhoea and only one mouse was found to develop diarrhoea. The group of mice to whom 300 mg/kg Tp.Cr was administered partial protection (40%) from diarrhoea was observed, whereas group of mice treated with 500 mg/kg of Tp.Cr exhibited 80% protection from diarrhoea, which is comparable to the protection provided to the verapamil treated group (Table 1, Figure 1).

CONCLUSIONS

The oral administration of the methanolic extract of the whole plant of *Tephrosia pupurea,* Linn. provided protection against castor oil-induced diarrhea in mice.

REFERENCES

1. Khare C.P.: Indian Medicinal Plants. p. 650, Springer-Verlag, Berlin, Heidelberg 2007.
2. Balandrin M.F., Klocke J.A. Wurtele E.S., Bollinger W.H.: Science 228, :1154 (1985).
3. Bhandari, M. M.: Flora of the Indian desert. pp. 118-123, Scientific Publishers, Jodhpur 1990.
4. Bishop M.: Hawaiian Ethnobotany Online Database (2007).
5. Arnold M.D., Harry L.: Poisonous Plants of Hawaii. Charles E. Tuttle Co., Tokyo 1968.
6. Chang L.C., Gerhäuser C., Song L., Farnsworth N.R., Pezzuto J.M., et al.: J. Nat. Prod. 60, 869 (1997).
7. Duke J.A.: Handbook of phytochemical constituents of GRASH herbs and other economic plants, CRC Press, Tokyo 1992.
8. Qadir M.I., Malik S.A.: Rev. Med. Virol. 20, 23 (2010).
9. Qadir M.I., Malik S.A.: AIDS Res. Hum. Retroviruses 27, 57 (2011).
10. Parjapati N.D., Purohit S.S., Sharma A.K., Kumar T.: A Handbook of Medicinal Plants: A Complete Source Book, p. 506, Agrobios (India), Jodhpur 2003.
11. Williamson E.M., Okpako D.T., Evans F.J.: Selection, Preparation and Pharmacological Evaluation of Plant Material, John Wiley & Sons, Chichester 1998.

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