*Indian Journal of Animal Sciences* **78** (12): 1325–1328, December 2008

# Indigenous practices for the treatment of diarrhoea in goats and their scientific validation

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Received: 23 October 2007; Accepted: 16 June 2008

ABSTRACT

Three indigenous technical practices on treatment of diarrhoea, viz. juice of *urhul* (*Hibiscus rosa sinensis*) flower, paste of bark of *pojo* (*Litsaea anthapoly*) plants and *takala* (*Cassia tora*) flower juice, used by rural farmers of country were validated as antidiarrhoeal in goats. The plant materials were collected and methanolic extract were prepared and evaluated in clinical cases of diarrhoea in goats. The antibacterial property of the extracts was tested against isolated ETEC strains of *Escherichia. coli* (O26, O32 and O2). The qualitative chemical analysis of each plant extract revealed the presence of flavonoids, glycosides, tannins, carbohydrates, alkaloids in *urhul* flowers; glycosides, tannins, alkaloids, carbohydrates and saponins in *pojo* bark extract and flavinoids, glycosides, tannins, saponins, carbohydrates in *takala* leaves extracts. Antibiogram study of extracts in different concentration (1, 2, 4, 8, 16 mg/disc) of *pojo* bark extract, showed 8–14 mm zone of inhibition in 4, 8, 16 mg concentration; *takala* leaves showed 8–9 mm zone of inhibition at 16 mg per disc concentration only. *Urhul* flower did not show anti-*E. coli* activity. Diarrhoic goats were given the methanolic extract @ 50, 30 and 20 mg/kg body weight of *urhul* flowers, *takala* leaves and *pojo* bark after preparing suspension in gum acacia (5%). Faecal, dehydration and recovery scores improved in all extracts but was the best in *pojo* bark followed by *urhul* flowers and *takala* leaves. All the extracts were considered safe, as there were no untoward reactions noticed during treatment in goats.

**Key words**: Diarrhoea, Goat, I T K

The indigenous technical knowledge (ITK) and practices revolve around traditional values of the resources include housing, medicine, many other practices, including medicinal plants, ethno-medicine, ethno-veterinary etc. (Samal *et al.* 2004). In an estimate, there are 1748 and 2500 species of medicinal plants reported in Indian Himalayan region (IHR) and India respectively (Samant *et al.* 1998). The knowledge and practice of utilization and conservation of medicinal plants, ethno-medicinal plants and ethno-veterinary treatment by local farmers/communities are amazingly vast (Samal *et al.* 2001). Therefore, there is need to identify potential ITKs and their scientific validation in order for its better utilization in terms of their better therapeutic values. In this paper, three ITKs on treatment of diarrhoea in goats have been selected for scientific validation.

## MATERIALS AND METHODS

Three indigenous technical knowledge, which were well adopted and practiced by the local farmers of Jharkhand and Maharashtra states were selected for scientific cross-validation under National Agriculture Technology Project (NATP-ITK). These technologies were- Treatment of diarrhoea by juices of *urhul* (*Hibiscus rosa sinensis*) flower in goats, where disclosure claimed that diarrhoea in goats is controlled by *urhal* flower (2–3 flowers) juice administration twice a day for 3 days and used by maximum farmers of Simdega district of Jharkhand State; second ITK was on control of diarrhoea in goats and sheep with paste of *pojo* (*Litsaea anthapoly*) plants disclosed from Ranchi (Jharkhand) claiming that diarrhoea was treated by paste of *pojo* tree bark given @ ½ cup daily for 3 days. Third ITK was on curing of diarrhoea in goats by using *takala* (*Cassia tora*) flower juice. Farmers of Sholapur district in Maharashtra used this practice, where *takala* flower (*Cassia tora*) juice was given @ ½ cup to the goat for 2–3 days. For scientific validation, the concerned plant materials were collected, dried and their methanolic extract were prepared separately. These extracts were evaluated in clinical cases of diarrhoea in goats predominantly caused by *Escherichia. coli*.

*Urhul* (also known as *gurhal*) is an evergreen woody glabrous 5–8 feet high showy herb. It is native of China, grown as an ornamental plant throughout India. *Urhul* flowers (*gurhal*) were collected from local area of Mathura district

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of Uttar Pradesh and taxonomically identified as *Hibiscus rosa sinensis*. *Pojo* (*Litsaea anthpoly*) is a tree found in Ranchi (Jharkhand). Bark of *pojo* tree was collected from Ranchi, from where this indigenous technology is well practiced. Both flower and leaves are used against eczema, ringworm and cough traditionally (ICAR document 2004). In experiment, *takala* leaves were used in place of flower, because flowers are seasonal in nature and appeared for a small period of time. During rest of the period, leaves were used for treatment in native tract.

The plant material was shade dried for 2 weeks at 30–40°C and crushed to powder in electric grinder. About 500 g of the plant material was uniformly packed into a thimble of a soxhlet extractor. It was exhaustively extracted with methanol for 48 h or 22 cycles or till the solvent in the siphon tube of an extractor becomes colorless. The completion of the extraction was confirmed by taking the solvent from the thimble and evaporated to check the absence of residue. The methanolic extracts thus obtained were evaporated to dryness by using rotary evaporator under reduced pressure, to get syrupy mass. The residue was dried over anhydrous sodium sulphate to remove traces of alcohol. The physical characters of the extract were noted and the percentage yield was calculated on such basis. The extracts were preserved in an airtight container to avoid loss of volatile principles for further studies.

In extract, test for steroids and triterpenoids (Liberamann – Buchard test), carbohydrate (Fehling test), alkaloids (Mayer's test), glycosides (Lugol's test), protein (Biuret test), tannins and phenols (ferric chloride test), fixed oil and fat (spot test), flavanoids (Shinoda test), gums and mucilage (potasium. permanganate test) and saponin (froth test) were conducted, as per standard protocol.

*E. coli* was taken as a representative test organism to assay the antibacterial activity of these plants. The organism was isolated from clinical cases of diarrhoea in goats and was biochemically characterized (Cowans and Steel 1977). Further, the strains were serologically characterized as ETEC O26, O32 and O2 from the Central Research Institute, Kasauli (HP).

The extracts were spot checked for antibacterial activity by using disc diffusion method. The extracts were reconstituted in alcohol in a stock solution concentration of 800 mg/ml and different concentration (1, 2, 4, 8, 16 mg) extract was poured on sterilized disc. These impregnated discs were dried, with control disc of alcohol. Standardized inoculum ($1\times10^6$cfu) of *E. coli* culture was spread on sterile Muller Hinton agar plates and impregnated discs were placed, incubated at 37°C for 15 h. Results were expressed in zone of inhibition (ZOI) in mm. Antibiotic resistance of isolated strains was also tested by using standard antibiotic disc of gentamycin, kanamycin, amikacin, tobramycin, cephadroxil, cefuroxime, norfloxacin, enrofloxacin, ampicillin+ cloxacillin, amoxacillin, cephatrioxone and lincomycin.

Clinical trial was conducted in 15 goats suffering with non-endoparasitic, bacterial diarrhoea using each extract. Clinical cases of diarrhoea caused by *E.coli* were selected for the study. Diarrhoic goats were given the methanolic extract of *urhul* flowers @ 50mg/kg body weights, *pojo* bark @ 20 mg/kg body weight and methanolic extract of *takala* @ 30 mg/kg body weight each after preparing their suspension in gum acacia (5%). This preparation was administrated once daily for 3 consecutive days. The clinical efficacy assessment was based on revival of appetite, faecal consistency, dehydration/rehydration status, which was categorized on scoring basis. Appetite score categorized as good 1, low 2, no appetite 3; faecal consistency as watery 1, semi solid 2, loose ball 3, normal 4; dehydration score +1,+2,+3,+4 on the basis degree of dehydration; and recovery score as poor 1, partial 2, moderate 3, complete 4.

Changes in serum biochemicals were also estimated to observe safety/toxicity of tested extracts. The serum biochemicals such as glucose, total protein, albumin, globulin, AST, and creatinine, Na, P and chloride were estimated by UV spectrophotometer by using diagnostic kits and as per standard technique recommended. Beside, any toxic or untoward reactions were noted in experimental diarrhoeic goats.

## RESULTS AND DISCUSSION

Antibiogram study of extract in different concentrations (1, 2, 4, 8, 16 mg/disc) was conducted against isolated *E. coli* from clinical cases of diarrhoea in goats. It revealed that extract of *urhul* flower in these concentrations did not exhibit antibacterial property against *E. coli*. Using same concentrations (1, 2, 4, 8, 16 mg/disc) of *pojo* bark extract, 8 – 14 mm zone of inhibition in 4, 8, 16 mg/disc concentration was observed. *Takala* leaves extract showed 8 – 9 mm zone of inhibition at 16 mg/disc concentration only. It indicated that *pojo* bark extract and *takala* leaves possess antibacterial property against *E. coli* (Table 1).

Methanolic extract of *urhul* flowers @ 50 mg/kg body weight improved the faecal score from 1.8±0.141 to 3.0±0.27 and overall recovery score were reached to 3.0±0.25. Faecal and recovery score were significantly improved and suggested that plant extract is moderately effective (75%) at this dose rate. Serum glucose, sodium and potassium levels were slightly increased post therapy. Apparently, drug did not show any untoward effect, as also supported by non-appreciable changes in biochemicals, viz. total protein, albumin, globulin, AST, and creatinine levels. Antidiarrhoeal property of *Hibiscus rosa sinensis* plant was reported (Gilani *et al.* 2005) along with its other medicinal uses like anti-inflammatory, antipyretic activity and contraceptive (Kabir *et al* 1984, Debasis *et al* 1998). *Pojo* bark extract efficacy indicated that faecal consistency score was significantly improved up to 3.34±0.04. Recovery score was 3.38±0.5 on day 3 post administration drug in given concentration, which

Table 1. Antibiogram profile of different plant extract against *E.coli* isolates

| Parameters Concentration of extract (per disc) | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | *Urhul (Hibiscus rosa sinensis)* flower | | *Pojo (Litsaea anthapoly)* bark | | *Takala (Cassia tora)* leaves | |
| | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| Control 0 mg | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 mg | 0 | 0 | 12 | 14 | 9 | 8 |
| 8 mg | 0 | 0 | 9 | 13 | 0 | 0 |
| 4 mg | 0 | 0 | 8 | 10 | 0 | 0 |
| 2 mg | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 mg | 0 | 0 | 0 | 0 | 0 | 0 |

Table 2. Therapeutic efficacy of different extract in clinical cases of diarrhoea in goats

| Parameters | Pretreatment | Post treatment | | | F-values |
|---|---|---|---|---|---|
| | | Day I | Day II | Day III | |
| *Urhul (Hibiscus rosa sinensis)* flower | | | | | |
| Appetite score[A] | 1.15±0.15 | 1.1±0.105 | 102.06±0.2 | 1.0±0.0 | 0.500 |
| Fecal score[B] | 1.8±0.141 | 1.8±0.141 | 2.3±0.49 | 3.0±.27 | 8.11** |
| Temperature ($0^F$) | 102.2±0.22 | 102.2±0.2 | 102.5±0.23 | 102.17±0.2 | 0.151 |
| Dehydration score[C] | 1.13±0.15 | 1.13±0.23 | 1.13±0.20 | 0.0±0.0 | 3.26* |
| Recovery score[D] | - | 1.5±0.324 | 1.1±0.105 | 3.0±0.27 | 7.88** |
| *Pojo (Litsaea anthapoly)* bark | | | | | |
| Appetite score[A] | 1.66±0.19 | 1.56±0.18 | 1.44±0.17 | 1.20±0.0 | 4.47** |
| Fecal score[B] | 1.25±0.15 | 1.22±0.15 | 2.62±0.17 | 3.34±0.34 | 23.32** |
| Temperature ($0^F$) | 101.8±0.13 | 101.3±0.17 | 102.2±0.17 | 101.44±0.20 | 1.67 |
| Dehydration score[C] | 1.23±0.25 | 1.13±0.23 | 1.13±0.23 | 0.0±0.0 | 3.69* |
| Recovery score[D] | - | 1.11±0.17 | 1.22±.05 | 3.38±0.15 | 87.52** |
| *Takala (Cassia tora)* leaves | | | | | |
| Appetite score[A] | 1.76±0.29 | 1.66±0.18 | 1.44±0.17 | 1.20±0.2 | 4.35* |
| Fecal score[B] | 1.25±0.15 | 1.22±0.15 | 2.62±0.17 | 3.24±0.34 | 30.26** |
| Temperature (°F) | 101.8±0.17 | 101.3±0.17 | 2.00±0.17 | 101.44±0.20 | 1.23 |
| Dehydration score[C] | 1.24±0.35 | 1.13±0.13 | 1.13±0.32 | 0.0±0.0 | 3.06* |
| Recovery score[D] | - | 1.11±0.17 | 1.32±.05 | 2.88±0.16 | 9.68** |

[A]Good 1, Low 2, No appetite 3; [B] Watery 1, semi solid 2, loose ball 3, normal 4; [C] +1,+2,+3,+4; [D]poor 1, partial 2, moderate 3, complete 4. *P>0.05, **P>0.01

was categorized as moderately (80%) (Table 2). Marginal increment was observed in serum glucose, sodium and potassium levels in post treatment, which were lowered in diarrhoeic cases. Extracts were considered to be safe, as there was no untoward reaction noticed during treatment and neither any appreciable change in biochemicals, viz. total protein, albumin, globulin, AST, and creatinine levels. Therapeutic testing of *takala* leaves extract @ 30 mg/kg body weight, possessed moderate (75%) efficacy in bacterial diarrhoea in goats as recovery score was 2.88±0.16 on day 3, which were lesser in comparison to previous 2 extracts, nevertheless, it showed antidiarrhoeal property. No untoward reaction was noticed during treatment in goats and neither any significant change was noticed in serum biochemicals in this group also.

The results of in *vitro* and clinical trials suggested that *urhul* flower extract showed antidiarrhoeal property *in vivo* however, did not exhibited antibacterial property *in vitro*. It appeared that antidiarrhoeal property might be associated with astringent activity of flowers. *Pojo* bark extract resulted in good clinical efficacy in goats and also exhibited antibacterial activity against *E. coli* isolates. Similarly, *takala* leaves extract showed moderate efficacy in clinical trials but lesser antibacterial activity. Other worker have also reported the antibacterial property of *Cassia tora* plant (Patel and Patel 1957). Trials on antidiarrhoeal effect of other medicinal plants have been largely restricted in laboratory animals models such as *Strychnos potatorum* seed extract (Biswas *et al.* 2002), *Punica granatum* seed extract (Das *et al* 1999), *Holarrhena antidysenterica* bark (Kavitha *et al.* 2004),

*Dalbergia lanceolaria* bark (Majmudar 2005), *Jatropha curcus* root (Majmudar 2000), *Benin hispida* (thumb) Cogn., fruit extract (Swamy *et al.* 2005) and *Rhus javanica* ripen fruit extract (Tangpu and Yadav 2004).

It is inferred that 3 indigenous technical practices on treatment of diarrhoea, viz. juices of *urhul* (*Hibiscus rosa sinensis*) flower, paste of bark of *pojo* (*Litsaea anthapoly*) plants and *takala* (*Cassia tora*) flower juice, used by rural farmers of country were validated as antidiarrhoeal in goats. Methanolic extracts of bark of *pojo* (*Litsaea anthapoly*) tree and *takala* (*Cassia tora*) leaves exhibited antibacterial activity against *E. coli* isolates. The antidiarrhoeal property of these plant extracts may be utilized in drug development for diarrhoea in animals. It is also to be noted that *pojo* bark and *takala* leaves showed antibacterial activity against a highly resistant strains of *E.coli*. The results reflected that plant extracts are potential alternative measures to combat resistant bacteria, but need full exploitation of medicinal plants for development of herbal antibacterial drug.

## ACKNOWLEDGEMENTS

Author is thankful to Indian Council of Agricultural Research (ICAR) for financial assistance to conduct this study under National Agriculture Technology Project (NATP), and to the Director Central Institute for Research on Goats, Makhdoom, for providing other assistance. Authors also wish to thank Director, Central Research Institute, Kasauli (H.P.), for characterization of *E coli* strains.

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