
Therapeutic Efficacy of medicinal Plants against Experimentally Induced Shigellosis in Guinea Pigs

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The therapeutic efficacy of higher plants viz. *Camellia sinensis* L. (Theaceae) and *Chamaesyce hirta* Millsp. (Euphorbiaceae) was studied against experimentally-induced shigellosis in guinea pigs. The aqueous extract of black tea, polyphenon 60 (mixture of catechins), quercetrin (flavonoid glycoside) and quercetin (aglycone of quercetrin), the two latter compounds being obtained from *Chamaesyce hirta* showed promising *in vitro* antibacterial activity against *Shigella flexneri* 2452 and when tested in the *in vivo* model of experimental shigellosis, all of them completely cured the guinea pigs within three days. In the untreated group, all the guinea pigs died within 24 h.

SHIGELLOSIS is an acute bacterial enteritis of humans and non-human primates with two intestinal manifestations, diarrhoea and dysentery. It remains a lethal disease among infants in developing countries and multi-drug resistant strains are fast emerging. The developing countries have to face the cost of newer antibiotics and their side effects rate not to be overlooked especially in children. To overcome these limitations, it is imperative to improve research on new antibacterial agents for which higher plants present a promising future, since they are cost effective and may have less side effects.

Camellia sinensis L. (Theaceae) and *Chamaesyce hirta* Millsp (Euphorbiaceae) are two plants among many that have been reported to be useful against diarrhoea and dysentery. Their *in vivo* antibacterial activity against enteropathogens have been studied (data not presented), which prompted the authors to study their efficacy under *in vivo* conditions using an appropriate model.

Animal models using rabbits and guinea pigs have been developed successfully^{1,2} to study the infection caused by *Shigella* spp. The present study aims at proving the efficacy of *Camellia sinensis* L. and *Chamaesyce hirta* Millsp against experimental shigellosis in guinea pigs.

MATERIALS AND METHODS

Preparation of bacterial inoculum:-

Shigella flexneri 2452 (clinical sample) was inoculated onto that Trypticase Soy Broth (TSB) and incubated overnight. The culture obtained was diluted with TSB to give a 10⁹ cfu/ml inoculum.

Plant extracts used for the study are as follows-

- (A) Aqueous extract of black tea, 25 mg/ml
- (B) Polyphenon 60 (Mitsui Norin Co., Japan), 15 mg/ml
- (C) Quercetrin (Flavonoid glycoside of *Chamaesyce hirta*), 15 mg/ml
- (D) Quercetin (aglycone of quercetrin), 10 mg/ml.

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Table 1: Effect of plant constituents in experimental shigellosis by *Shigella flexneri* 2452

Group	Treatment agent and Schedule	No. of animals survive
A ₁ (2)	5 ml of aqueous ext. of black tea (25 mg/ml) at 6 hour intervals for 2 days.	All survived
A ₂ (2)	5 ml of polyphenon 60 (15 mg/ml) following similar schedule As A ₁	All survived
A ₃ (2)	5 ml of Quercetrin (15 mg/ml) following similar schedule	All survived
A ₄ (2)	5 ml of Quercetin (10 mg/ml) following similar schedule	All survived
Control (3)	No treatment given	died within 24 hours

*** Figures in parenthesis indicate number of guinea pigs used for study.

Experimental Shigella infection in guinea pigs

Three in bred guinea pigs (350 g) were allowed to fast for 24-36 h during which period they were provided with water containing tetracycline (0.5 mg/ml). On an average, the animals consumed 100 ml of water over the period of starvation. At time zero, cimetidine (50 mg/kg) was administered intravenously. At 15 and 30 min, 10 ml solution of 5% sodium bicarbonate in water was administered orally by a gastric tube. Immediately after the second sodium bicarbonate dose, the bacteria mixed with the broth (10 ml) were given by gastric tube followed by intraperitoneal administration of 2 ml tincture of opium. The animals were observed every half an hour after inoculation of the bacteria. The animals were returned to normal diet and observed for a week for the development of the disease condition.

Efficacy of the plant extracts against experimental shigellosis in guinea pigs

Eight in bred guinea pigs (350 mg) were taken for this study. They were distributed in four groups. Individual animals received 5 ml of each of the extracts at a particular concentration by oral route using a gastric tube, after observing the first signs for the initiation of the disease (Table 1).

RESULTS

Guinea pigs (3) which were orally fed with *Shigella flexneri* 2452, showed signs of indisposition about 2 h after the feeding. There was no diarrhoea but the animals became less active and total lack of movement in the hind limbs was observed. The animals became totally immobile after 4-5 h and they refused to eat. The animals died within 24 h. The dead animals were immediately cut open and the small intestine was observed for fluid accumulation. The small intestine showed the presence of greenish yellow viscous fluid with traces of blood.

The animals that were fed with the plant extracts at 6 h intervals started showing improvement of their symptoms after 5-6 h. Within 24 h, the animals showed normal movements. There was no diarrhoea. Feeding of plant extracts was stopped on the third day and the animals were kept under observation for a week. They remained normal throughout. (Table 1)

DISCUSSION

The above study reveals the efficacy of plant constituents of two Indian medicinal plants, *Camellia sinensis* and *Chamaesyce hirta* against *Shigella* infection induced in guinea pigs. Since guinea pigs

are easier to handle and are cost effective, they can be used as an effective animal model for shigellosis. The similarities in the pathogenesis of shigellosis in guinea pigs and the natural hosts have already been worked out.² Even though diarrhoea was not observed in the animals, the presence of the greenish yellow viscous fluid in the small intestine indicate the effective multiplication of the pathogens in the region as reported.²

All the preparations of the plant components showed good efficacy in the recovery of the animals infected with *Shigella flexneri*. Interestingly, quercetrin, the flavonol glycoside from *Chamaesyce hirta* did not show any antibacterial activity *in vitro* but exhibited therapeutic effects against *in vivo* infection. A plausible explanation for this could be that although quercetrin is an inactive component, during its absorption through the intestine, the bacterial enzymes might have hydrolysed it to its aglycone, quercetin which appears to be the active component possessing antibacterial property.^{3,4}

The guinea pig model appears to be a better system for studying the therapeutic efficacy of medicinal plants. There was total recovery of the animals after three days of treatment. Since the black tea also has nutritional value (minerals, vitamins etc.), it can be very well used against dysentery. *Shigella flexneri* forms the major pathogen apart from *Shi-*

gella dysenteriae, causing dysentery in the developing and the underdeveloped countries. It has been suggested that plant extracts having antibacterial activity when mixed with the ORS during rehydration therapy might provide better therapeutic efficiency for a quick recovery from diarrhoea and dysentery.⁵ A clinical trial using these extracts, however, is of utmost importance to establish such a hypothesis.

ACKNOWLEDGEMENTS

The financial assistance of the University Grants Commission, Government of India is very well appreciated.

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