



SYNERGISTIC ANTHELMINTIC ACTIVITY OF DIFFERENT COMPOSITIONS OF PANCHAGAVYA AND *BAUHINIA VARIEGATA* LINN

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ABSTRACT

Panchagavya, which is an important component of many rituals and Indian traditional systems, is an incredible source for many medicinal substances whose synergistic action has been reported but their scientific data are not available. The main objective is to investigate synergistic anthelmintic activity of panchagavya with ethanolic extract of *Bauhinia variegata* Linn (EEBV). Earthworms were divided into 11 groups & in each group, six earthworms were taken and they were treated with PG1, PG1+10% EEBV, PG1+50% EEBV & PG1+75% EEBV, PG2, PG2+10% EEBV, PG2+50% EEBV & PG2+75% EEBV, Control group with Cow Urine and Standard group with Piperazine Citrate (50 mg/ml and 100 mg/ml concentration) & investigated the role of different composition of Panchagavya and its ethanolic extract of *Bauhinia variegata* Linn (EEBV) for synergistic anthelmintic activity. After drug administration, effect of PG 1 , PG 2 , PG 1 + EEBV (all composition) and PG 2 + EEBV (all composition) were found to be significant at the level $p < 0.01$ as compared to Standard and Control group. The synergistic activity of PG with EEBV might be due to tannin which interferes with energy generation in helminth worm by inhibiting oxidative phosphorylation. Effect of tannin might be potentiated in presence of PG. Other possible mechanism might be that presence of PG could potentiate binding of free protein in GIT of host animal and causes death. This work will open new avenue for the study of various preparations used in worship because this study has showed the synergistic anthelmintic activity. Further studies may reveal some more pharmacological activities like antinociceptive, anti-stress etc. This will give impetus for the study of various materials used in worship of God which will reveal the logic of materials used in worship.

Key words: Ethanolic extract of *Bauhinia variegata* Linn (EEBV), Anthelmintic activity, Panchagavya.

INTRODUCTION

Infection by *helminths* (worms) may be limited solely to the intestinal lumen or may involve a complex process with migration of the adult or immature worm through the body before localization in a particular tissue. Complicating our understanding of the host– parasite relationship and the role of chemotherapy in helminth-induced infections is the complex life cycle of many of these organisms. Whereas some helminths have a simple

cycle of egg deposition and development of the egg to produce a mature worm, others must progress through one or more hosts and one or more morphological stages, each metabolically distinct from the other, before emerging as an adult.

Cow is described as “Kamdhenu” (one which fulfills all the wishes) since Vedic times in Indian civilization. According to Ayurveda various cow products like urine, dung, milk, ghee and curd are used to treat various disease conditions in human beings. These five products of cow are called as Panchagavya. Panchagavya is an important component of many rituals and pooja. Many useful elements have been found in Panchagavya like Urea, Uric acid & Minerals and bioactive substances

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and hormones like Urokinase, Epithelium growth factor, Colony stimulating factor, Growth hormone, Erythropoetin, Gonadotropins, Kallikrin, Trypsin inhibitor, Allantoin, Anti-cancer substance, Nitrogen, Sulphur, Ammonia, Copper, Iron, Phosphate, Sodium, Potassium, Manganese, Carbolic Acid, Calcium, Salts, Vitamin A, B, C, D, E, Lactose Sugar, Enzymes, Water, Hippuric Acid, Creatinine etc. Moreover, the root, bark and leaves of *Bauhinia variegata* Linn (EEBV) are depurative, anthelmintic, anti-ulcer, anti-tumours, analgesics, anti-inflammatory, hepatoprotective, immunomodulatory, wound healing & used for skin disease & leprosy etc. Tremendous interest is generated in the therapeutic value of cow product due to the patent awarded by USFDA. This was awarded for the synergetic activity of cow urine distillate with some antibiotic and anticancer agents. But no patent awarded to other constituents of Panchagavya but there is a synergistic action of Panchagavya components either alone or combination with drug of herbal, animal or mineral origin (Gosavi and Jhon, 2012; Sathasivam *et al.*, 2010).

MATERIALS AND METHODS

Collection of plant

Leaves of *Bauhinia variegata* Linn (EEBV) were collected from Narayan Baag, Jhansi (UP), India and got identified by National Vrakshayurved Research Institute (NVRI), Gwalior road, Jhansi Accession No. 21966 by Dr. Neelima Sharma (Research Officer Incharge) in May 2013. Fresh plant parts were used for macroscopical examination whereas sample which was air dried and powdered was used for microscopical studies. An exhaustive pharmacognostic study was carried out using standard methodology (WHO, 1998; OECD 2010).

Collection of Panchagavya

Various cow products like urine, dung, milk are collected from DRMS colony, Jhansi and curds & Ghee were prepared.

Collection of *Pheretima posthuma*

Earthworms are collected from the water lodge area of Jhansi and identified in Zoology department of Bipin Bihari degree college Jhansi.

Preparation of extract

The plant leaves were dried in shade and powdered with the help of an electric grinder. Complete dried leaves powder (75 g) was packed in a soxhlet apparatus and subjected to hot continuous percolation with 40-60°C temperature for 12 hrs using 250 ml of ethanol (95% v/v) as solvent.

Drug

Piperazine citrate was purchased from GSK Ltd,

Indore, India

Chemicals

All Chemicals and reagents of analytical grade were purchased from Qualigen Fine Chemicals like Chloroform, Sodium hydroxide pellets, Glacial acetic acid, Ethanol, Methanol, n-Hexane, Formic acid, Silica gel G etc

Objective of Research

The main objective of research is to reveal the logic of using Panchagavya in rituals. Further, to study the synergistic anthelmintic activity of Panchagavya with ethanolic extract of *Bauhinia variegata* Linn since no scientific data are available.

Experimental procedure

Earthworms were divided into 11 group. In each group six earthworms were taken and they are treated with PG1, PG1+10% EEBV, PG1+50% EEBV & PG1+75% EEBV, PG2, PG2+10% EEBV, PG2+50% EEBV & PG2+75% EEBV, Control group with Cow Urine and Standard group with Piperazine Citrate (50 mg/ml and 100 mg/ml concentration) and time period of paralysis and death of worms were recorded with the help of the stop watch.

Anthelmintic Activity

The anthelmintic assay was carried as per the method of Ajaiyeoba *et al.*, with necessary modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (Raj Kapoor B *et al.*, 2006; Pandey S and Agarawal RC, 2009; Pandey S and Agarawal RC, 2010; Ambiga S *et al.*, 2007). Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro. Each 10 ml formulation containing different composition of Panchagavya and ethanolic extract of *Bauhinia variegata* Linn (EEBV) (10%, 50%, 75% in Panchagavya) were prepared and six worms (same type) were placed in it. Time for paralysis was noted when no movement could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Piperazine citrate (50mg/ml, 100mg/ml) was used as standard while cow urine as control.

RESULTS

On the basis of observation table 11 it was found that effect of PG 1 & PG 2 as well as PG 1 +EEBV and PG 2 +EEBV (all compositions) were found to be

significant at the level $p < 0.01$ as compared to Standard (50mg/ml & 100mg/ml) and Control group. The composition of PG1 and PG 1 +EEBV as well as PG2 and

PG 2 +EEBV exhibited anthelmintic activity in synergetic manner thereby reducing the time of paralysis (P) and death.

Table 1. Anthelmintic activity using *in-vitro* Anthelmintic assay

	PG 1	PG 1 + EEBV			PG 2	PG 2 + EEBV			Control	Standard	
		10%	50%	75%		10%	50%	75%		50mg/ml	100mg/ml
Paralysis (Time in min)	6.47±1.11 ** bb	4.47±0.85 ** bb	3.7±0.231 ** bb	3.17±1.66 ** bb	5.20±0.381 ** bb	3.33±0.22 ** bb	2.53±0.016 ** bb	2.125±0.683 ** bb	5.2±0.712	16.50±0.281	10.21±0.21
Death (Time in min)	9.50±0.21 2 ** bb	5.20±1.21 ** bb	4.3±0.81 ** bb	2.10±1.22 ** bb	7.10±1.612 ** bb	4.20±2.11 ** bb	4.00±0.321 ** bb	2.29±0.112 ** bb	4.11±0.516	20.41±0.612	14.18±0.417

P values are mean±SEM of 6 animals. Statistical significance test for comparison was done by ANOVA, followed by Dunnett's test. Comparison made between standard Vs PG 1, PG 1+EEBV 10%, PG 1+EEBV 50%, PG 1+EEBV 75%, PG 2, PG 2 +EEBV10%, PG 2 +EEBV50%, PG 2 +EEBV 75%.

**P value <0.001, * p value <0.05

Comparisons with control ^{bb} $p < 0.01$, ^b $p < 0.5$

DISCUSSION

The anthelmintic activity in the present study emanated that PG1; PG1+EEBV (at 10%, 50% & 75%); PG2 as well as PG2+EEBV found to be highly effective than standard piperazine citrate. The synergistic activity of PG with EEBV might be due to the presence of cystine protease which attack on the structural protein & digest the nematodes which might be due to availability of twice the number of molecules of different constituent of PG 2

for drug-receptor interaction than PG1.

CONCLUSION

The present study of synergistic action of different composition of constituents of Panchagavya (PG) as well the ethanolic extract of *Bauhinia variegata* Linn (EEBV) has opened new area in the field of anthelmintic.

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