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Research Article

Novel and Synergistic Antinociceptive Activity of Different Compositions of Panchagavya and *Aloe barbedansis* Mill using Tail Immersion Model

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Abstract

The main objective of research was to reveal the logic of using Panchagavya in rituals. Further, to study the synergistic anti-stress activity of Panchagavya with ethanolic extract of *Aloe barbedansis* Mill (Xanthorrhoeceae) by using Tail Immersion Model since no scientific data was available. Sixty mice were randomly divided into ten groups. The first, second, third, fourth , fifth , sixth, seventh, eighth, ninth and tenth groups received PG 1, PG1+ EEAB 10%, PG1+ EEAB 50%, PG1+ EEAB 75%, PG2, PG2+ EEAB 10%, PG2+ EEAB 50%, PG2+ EEAB 75%, Standard Alprazolam, Control Urine every day administered at the dose 4ml/kg body weight regularly at 9:00 am for 21 days and investigated the role of different composition of Panchagavya Aloe barbedansis Mill (Xanthorrhoeceae) for synergistic antinociceptive activity by using Tail Immersion Model in Swiss albino mice. Statistical analysis was carried out by using ANOVA, followed by Dunnet's test. Panchagavya with ethanolic extract of *Aloe barbedansis* Mill (Xanthorrhoeceae) were found to be exhibit enhanced and synergistic antinociceptive activity than standard and control which might be due to the steroids, terpenes, sesquiterpens, flavonoides, vitamins present in them. These investigations confirm that different composition of Panchagavya and Aloe barbedansis Mill (Xanthorrhoeceae) exhibits synergistic antinociceptive activity by using Tail Immersion Model. This work will open new avenue for the study of various preparations used in worship because this study has showed the synergistic antinociceptive activity.

1. INTRODUCTION

Ayurveda is a centuries old traditional medicinal system in India. 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[1-15] .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 and Minerals and bioactive substances 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 [16]. Moreover, the root, bark and leaves of *Aloe barbedansis* Mill (Xanthorrhoeceae) are depurative, anthelminthic, anti-ulcer, anti-tumours[17], analgesics, anti-inflammatory[18,19], hepatoprotective, immunomodulatory, wound healing[20,21] and used for skin disease and 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. No patent has been 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[22, 23].

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2. MATERIAL AND METHOD

2.1 Plant material

Leaves of *Aloe barbedansis* Mill (Xanthorrhoeceae) were collected in May 2013 from Narayan Baag, Jhansi (UP), India and was identified by National Vrakshayurved Research Institute (NVRI), Gwalior road, Jhansi Accession No. 21966.

The plant leaves were washed with distilled water, chopped into small pieces, air-dried and grinded into powder. The powder was extracted with 95% ethanol at 60 °C for 12 h and evaporated into concentrated extract. This concentrated leaf extract was used for further experiments.

2.2 Collection of Panchagavya

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

2.3 Animals

Healthy albino mice of either sex weighing between 20-30 g were taken for the study. All the animals were procured from Defence Research and Development Establishment (DRDE), Gwalior, India. Animals were acclimatized by keeping them in animal house. They were housed individually in polypropylene (32x24x16 cm) cages containing husk as bedding material and maintained under controlled conditions of temperature (23±20C), humidity (55±5%) and 12h light and 12h dark cycle. The animals were feed with standard pellet diet and water ad libitum. The Committee for the Purpose of Control and Supervision of Experimental Animal (CPCSEA) of Smt. Vidyawati College of Pharmacy, Jhansi (CPCSEA Reg.No. 966/PO/a/2006/CPCSEA) had approved Anti-stress activity of different composition of Panchagavya and ethanolic extract of Aloe barbedansis Mill (Xanthorrhoeceae) by using Tail Immersion Model.

2.4 Drugs and Treatment
Alprazolam purchased from Windlas Biotech Ltd., Dehradun was used as Standard and all chemicals and reagents used were of analytical grade

2.5 Acute Toxicity Studies
Healthy albino mice of either sex were orally fed with increasing doses (1, 3, 5, 7, 9) of ethanolic extract mixed with Panchagavya for 20 days. The dose upto 4g/kg (P.O.) body weight did not produce any sign of toxicity and mortality.

2.6 Experimental Procedure
The animals were divided into 10 groups of 6 animals each.
Group I: PG1; Group II: PG1+ EEAB 10%; Group III: PG1+ EEAB 50%; Group IV: PG1+ EEAB 75%; Group V: PG2; Group VI: PG2+ EEAB 10%; Group VII: PG2+ EEAB 50%; Group VIII: PG2+ EEAB 75%; Group IX: Standard Alprazolam; Group X: Control Urine.

2.7 Antinociceptive Activity
All the selected animals were divided in to 10 group. In each group 6 mice were taken and they are treated with PG1, PG1with plant extract (PG1+10% EEAB, PG1+50% EEAB and PG1+75% EEAB), PG2, PG2 with plant extract (PG2+10% EEAB, PG2+50% EEAB and PG2+75% EEAB), Control group with Cow Urine and Standard group with Diclofenac sodium. All treated animal are administered at the dose level of 4mg/Kg body weight P.O.standard group are treated with Diclofenac sodium at dose level 9mg/kg body weight and animal were treated per day in the morning at 9 AM. Treated animal observed at the day of 1st, 6th, 11th, 16th and 21st day for regular dosing.

2.8 Statistical Analysis
Results were expressed as mean ± SEM. The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Dunnet's test and two way analysis. P<0.01 was considered statistically significant.

Table 1: Antinociceptive Activity using Tail Immersion Model (Time in Sec)

Day	PG 1	PG 1 + EEAB			PG 2	PG 2 + EEAB			Control	Standard
		10%	50%	75%		10%	50%	75%		
1	3.50±0.16••	4.01±0.16••	4.50±0.33••	4.62±0.32**	4.17±0.39••	4.2±0.41••	4.96±0.39**	4.96±0.24**	3.15±0.321	4.50±0.39
6	4.20±0.14••	4.20±0.31••	4.88±0.26••	5.32±0.41**	4.56±0.34••	4.62±0.32••	5.1±0.45**	5.72±0.31**	4.15±0.521	5.1±0.214
11	5.40±0.18••	5.25±0.11••	6.31±0.39••	7.1±0.46**	5.6±0.32••	5.71±0.35••	6.87±0.37**	7.68±.39**	6.01±0.241	6.09±0.116
16	6.350.12••	7±3.21••	7.2±0.43••	7.58±0.38**	7.5±0.36**	7.96±0.52**	8.12±0.45**	8.4±0.54**	7.03±0.321	7.16±0.455
21	7.5±0.11••	7.8±0.21•	8.49±0.32**	9.01±0.42*8	8.46±0.42**	9.15±0.42**	10.2±0.24**	11.0±0.45**	8.19±0.281	8.15±0.392

P value is mean±SEM of 6 animals. Statistical significance test for comparision was done by ANOVA, followed by Dunnet's test. Comparison made between standard vs PG 1, PG 1+ EEAB 10%, PG 1+ EEAB 50%, PG 1+ EEAB 75%, PG 2, PG 2 + EEAB 10%, PG 2 + EEAB 50%, PG 2 + EEAB 75%.
***P value <0.001, * p value<0.05*

3. RESULTS
It was observed from table 1 that on the 1st day after drug administration, effect of PG 1 and PG2 as well as PG 1 + EEAB and PG 2 + EEAB (all compositions) were found to be significant when compared to Standard group and control group; on the 6th day, effect of PG 1 + EEAB (75%) and PG 2+ EEAB (50% and 75%) were found to be significant at the level compared to Standard group; on 11th day, effect of PG1 + EEAB (50% and 75%) and PG2 + EEAB (50%,75%) were found to be significance as compared to Standard group; on 16th day, effect of PG1 + EEAB (50% and 75%), PG 2 and PG 2+ EEAB 75% were found to be significant when compared to Standard group and on 21th day, effect of PG1+ EEAB (75%) and PG2 as well as PG 2+ EEAB (all compositions) were found to be significant at the level compared to standard group.

4. DISCUSSION
Pain sensation is said to be of the reason why people seek medical attention and therefore antinociceptives are commonly prescribed to relieve pain. the finding of present study by eddy hot plate model that antinociceptive activity increases with time as well as present composition of different composition of Panchagavya with EEAB (ethanolic extract of *Aloe barbedansis* Mill) of PG1, PG1+ EEAB, PG2+ EEAB, control as well as standard. However, it is also found that PG2 exhibited more potent antinociceptive activity than PG which appear to be due to twice the number of molecule of constituents of PG2 available for drug receptor interaction as compared to PG1. Further PG 1+EEAB (50% on 16th ,21th day ,75 % on 21th day) and PG 2 +EEAB (10% on 21th day ,50% 16th and 21th days , 75 % on 6th ,11th ,16th and 21 th days) using Tail Immersion Model found to be more potent than standard i.e Diclofenac sodium. The synergistic activity of PG + EEAB might be due to the steroids, terpenes, sesquiterpens, flavonoides, vitamins as present in the PG as well as EEAB which appear to classify work by the abolition of the rate limiting step by the enzyme phospholipase A (PLA_2) to liberate arachidonic acid from cell membrane which is then involved in the upregulation of cyclo-oxygenase and lipooxygenase enzyme production[18, 24, 25].

5. CONCLUSION
The present study of synergistic action of different composition of constituents of Panchagavya(PG) as well the ethanolic extract *of Aloe barbedansis* Mill (EEAB) has opened new area in the field of antinociceptives. Further studies may reveal some more pharmacological activities like anthelmintic, 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.

REFERENCES

1. Panicker A., Trivedi M., Shark R.K., Kumar N., Patel A. Effect of Cow urine ark and *Aloe vera* extract on sero biochemical parameter in vitro leghorn chicken. An International Journal of Pharmaceutical Science 2012; 3163-3170.
2. Achiliya G.S., Kotagale N.R., Wododkar S.G., Dorle A.K. Hepatoprotective activity of Panchagvya ghrita agains carbon tetrachloride induces hepatoxicity in rats. Indian Journal of Pharmacology 2003; 01(03): 308-311.
3. Joseph B., Sonkar ganesh P. Antifungal efficacy of Panchgavya. International Journal of Pharmatech Research 2011; 0 3(01):585-588.
4. Koneru A.,Satyanarayan S.,Mukkani K.,Abedula K., K. Pravin Kumar. Anticonvulsant activity of Panchagavya ghrutam: A polyherbal Ayurvedic formulation. Journal of Pharmacy Research 2009; 02(0 5): 795-797.
5. Lalitha G., Shivraj R., Nirmal A. Antioxidant and antibacterial activity of Panchagavya. Journal of Herbal Science and Technology 2010; 06:04.
6. Kekudo T.R., NishantB.C. Cow urine a potent agent with antimicrobial and antihelmentic activity. Journal of Pharmacy Research 2010; 03(05): 1025-1027.
7. Girish S. A., Sudhir G Wadekar, K. Dorke Avinash. Neuropharmacological action of Panchagavya formulation containing Emblica officinalis gaerth and Glycerrhiza glabra Linn in mice. Indian Journal Express Biol 2004; 42(05):499-503.

8. Wate S.P. Antioxidant potential of Cow urine and its fraction. International Journal of Univerasal Pharmacy and Life Science 2011; 01(01):95-96.
9. Vots S., Miglanic S. Synergestic antimicrobial effect of Cow urine and Azadirachta indica on infectious microbes. International Journal of Pharmaceutical Scince and Research 2011; 02(7):1781-1785.
10. Rani R., Kansal V.K. Effect of cow ghee and Soyabean oil on Carcinogenic-metabolizing enzyme in Rats. Indian J. Med Res 2012; 136:460-465.
11. Gosavi D.D., John P.S. Effect of Panchagavya ghritam on some Neurological parameter in Albino rats. Asian Journal of Pharmaceutical and Clinical Research 2012; 05(01): 154-156.
12. Jozwi K.A., Bognick E., Kolotoj A. Activity of selective glycosidase of whole milk of Cow related to feeding season. Animal Science Paper and Research 2004; 22(04):673-676
13. Giannino M.L., Marzotto M., Delloglio F., Feligini M. Study of Microbial divercity in raw milk. International Journal of Food Microbiology, 2009; 130: 188-195.
14. Pravesh B.V., Angoyar Kanni J., Paloniswomy M. Antihypertensive and anticancer effect of milk. International Journal of Pharmacy and Pharmaceutical Science, 2011; 03(0 5):452-456.
15. Agutera P., Briceno G., Mora M. Effect of liquid Cow manure. Research Journal of Nature 2010; 10(02):158-169.
16. Baby Joseph and Sankarganesh P. Antifungal Efficacy of Panchagavya. International Journal of Pharm Tech Research 2011; 3(1):585-588.
17. Naveena, Bharat B.K., Selvasubramanyam, Antitumor activity of Aloe vera against Ehrlich associated carcinoma in swice mice.Int.Journal of Pharma and Bioscience, 2011; 2(2):400-409.
18. Mwale M, masika PJ, Analgesic and Antiinflammatory activity of *Aloe vera*. African Journal of Pharmacy and Pharmacology 2010; 04(05):291-297.
19. Vazaques B, avila G, segura D, Escalente B, Anti-inflammatory activity of Aloe vera gel. Journ of Ethanopharmacology 55:69-75.
20. Maenthisang R, Chaiyakunapruk N, nirutrapan S, The efficacy of of *Aloe vera* used for burn wound healing. A systematic review,Burns 33:713-718.
21. Harith Yadav KC,J.Ravi,Basha SI,deshmukh GR,Gujja R, Wound healing activity of Aloe vera in rodent,Int Journ of Pharma and Bio Science 2012; 03(02):63.
22. Gosavi D. and Jhon S. Effect of Panchagavya Ghritra on some neurological parameters in albino rats. Asian journal of Pharmaceutical Science Clinical Research 2012; 5: 154-156.
23. Sathasivam A., Muthuselvam M., Rajendran R. Antimicrobial Activities of Cow Urine Distillate against Some Clinical Pathogens. Global Journal of Pharmacology 2010; 4: 41-44.
24. Mohammad Reza Shahraki, Hamide Mirshikari², Elham Shahraki³. The Anti-Nociceptive Effect of Aloe. Vera Aqueous Extract in Fructose-Fed Male Rats. Basic and Clinical Neuroscience 2010; 1(3):39-43.
25. Asim Kumar Ghosha, Manasi Banerjeea, Tapan Kumar Mandalb, Akhilesh Mishrab, Mrinal Kanti Bhowmik. A Study on Analgesic Efficacy and Adverse Effects of *Aloe Vera* in Wistar Rats. Pharmacologyonline 2011; 1: 1098-1108