



Immunostimulant activity of a medical preparation panchagavya

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ABSTRACT

Panchagavya finds mention in the Shushruta samhita for its wide variety of applications in the treatment of mania, epilepsy, apart from being suggested for treatment of hepatic diseases such as jaundice and hepatitis. It is a blend of five products derived from the cow namely cow dung, urine, milk and ghee (clarified butter). These are mixed in proper ratio and then allowed to ferment. In this study we prepared Panchagavya (PG) as per the guidelines suggested both by traditional texts and that used by other authors. Mice were fed with this preparation of PG and significant weight gain was observed compared to controls. Further the animals fed with PG displayed increments in both humoral immune response, as assessed by haemagglutination assay and cell mediated immunity as assessed by the neutrophil adhesion test. Our study therefore highlights the effect of PG on diet and in immune-stimulation, both aspects important for combating diseases during re-convalescence, thus validating the traditional use of PG in treatment of hepatic diseases.

Key words : Panchagavya, weight gain, humoral immunity, cellular immunity., apoptosis.

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INTRODUCTION

Panchagavya (PG) finds mention in many ancient texts in the scripts of Vedas and in texts related to the ancient Indian system of medicine Ayurveda. When literally translated from sanskrit, “Pancha” means Five and “Gavya” means substance or ingredient. Ayurveda has mentioned that the five individual constituents of PG possess medicinal properties and can be used singly or in combination for treatment of different human ailments. PG signifies the blend of five ingredients obtained from the cow namely dung, urine, milk, ghee (clarified butter) and curd. Jirankalgikar et al, have used different physicochemical, spectroscopic, chromatographic parameters, and antioxidant activity to establish the presence of anti-oxidants in PG¹. As a blend of all five ingredients, Sushrut samhita, an authentic Ayurvedic text mentions the use of PG in the treatment of mania, epilepsy, fever and hepatitis. Gosavi & Premendran, have reported that PG alleviates electroshock induced epilepsy in rats². Clinical trials in humans with Down syndrome, significant improvement was observed in mental retardation^{3,5}. Similarly, there was a significant blunting effect on schizophrenic properties such as hallucinations, delusions,

avolition and anhedonia^{4,5}. Modern biochemistry has identified that cow dung can be used as pH balancer and purifier. The biochemical estimation of cow urine has shown that it contains sodium, nitrogen, sulphur, Vitamin A, B, C, D, E, minerals, manganese, iron, silicon, chlorine, magnesium, citric, succinic, calcium salts, phosphate, lactose, carbolic acid, enzymes, creatinine and hormones^{6,7}. Fractions of cow urine obtained by solvent extraction possess antimicrobial activity due to presence of aforesaid components⁸. Milk fat has been reported to have anti-bacterial and anti-fungal. Immunoglobulins, lactoferin, lysozyme, lactoperoxidase and vitamin B12 binding protein present in cow's milk possess antimicrobial effects⁹. Cow urine also enhances the phagocytic activity of macrophages and thus is helpful against bacterial infections. It also facilitates the synthesis of interleukin-1 and interleukin-2^{10,11} augments B- and T-lymphocyte blastogenesis, and IgA, IgM and IgG antibody titers in response to infections^{6,12}. Recently, Council for Scientific and Industrial research (CSIR) India has obtained US patent (No. 689690 / and 6410059) for cow urine distillate claiming a novel pharmaceutical composition¹³ and is an effective anti-microbial agent¹³. In addition to these effects on hematopoietic components, Wate et al, also showed that cow urine has analgesic properties¹⁴. Thus all observations

reported thus far seem to point out that PG has analgesic and immunomodulatory properties, since cow urine is an integral part of PG formulation. Indeed PG works as a good immuno-adjuvant for Newcastle's disease in chickens. Chickens fed with PG, display delay in symptoms of clinical manifestation of the disease and improved response to a vaccine of Newcastle's disease¹⁵. Epilepsy afflicted subjects displayed an increase in appetite and good absorption after being orally fed PG and a decrement in epilepsy symptoms¹⁶. These studies point out that PG may also aid appetite. The present study was carried out to assess the effectiveness of PG as an appetite stimulatory agent and an immuno-stimulatory agent, both important parameters in convalescence.

MATERIALS AND METHODS

Ethical Considerations:

The use of animals in this study was approved by the Institutional Animal Ethics Committee of Haffkine Institute for Training, Research & Testing, Parel, Mumbai vide certificate number HITRT/IEC/07/2011 dated 24th Jan 2011 CPCSEA/166/99

Animals:

Male Albino Wistar rats procured from an approved breeder were used for the study. Animals were housed properly under standard conditions of temperature (23 ± 2°C), 12 h light/dark cycles and fed with standard pellet diet and water ad libitum. Animals were kept in isolation for 2 weeks prior to the start of the study for acclimatization and to ascertain that the animals were not afflicted with any disease to begin with.

Preparation of Panchagavya:

Collection of the five ingredients: Fresh cow urine, dung and milk were collected from local cow farm using sterile container and stored in refrigerator for further uses. Curd and ghee (clarified butter) were obtained from the local market. In brief, cow dung was added to an equal volume of water (1 kg of dung to 1L of water). This mixture was filtered through a fine muslin cloth. The filtrate is hereafter named as dung-water. Cow ghee (clarified butter) obtained commercially was heated until it stopped bubbling. The purity was confirmed when a cotton wick dipped in the clarified butter started burning without any crackling. To 250 ml of purified ghee, 500 ml of dung water, 250 ml of curd, 250 ml of milk, 250 ml of urine and 1L of sterile distilled water were added and the mixture was brought to a slow boil and allowed to cool gradually to room temperature. The mixture was then filtered through a fine muslin cloth. The procedure of heating to a boil, cooling and filtration was repeated once again and the mixture was ready to use^{17,18,19}.

Preparation of Herbal Extract from pomegranate

The pericarp of the pomegranate (*Punica granata*) was peeled and dried. The Methanolic extract was prepared using a standard Soxhlet extraction apparatus for 48 hrs. The extracts were filtered through Whatman filter paper

no. 1 and were dried in vacuum. The resulting extract (powder) was weighed and was suspended in sterile distilled water.

Panchagavya / Pomegranate extract administration

The animals were divided into three groups consisting of six animals each. A group of six rats were fed orally with a volume of Panchagavya (PG) which was 1/10th the weight of the animal (~0.5 g/Kg). Another group of 6 animals was taken as control group which were administered an equal volume of distilled water (DW). A third group of 6 animals were fed Panchgavya + Pomegranate extract (PG+P).

Weight measurement

Animals were carefully checked for any signs of morbidity or malaise daily and were weighed every three days after their acclimatization period of 2 weeks (which was considered to be Day =1). They were then on regular chow diet supplemented with Distilled water (squares); Panchagavya (circles); Panchagavya + Pomegranate extracts (triangles). Data represents average weight of 6 animals in one experiment and the experiment was duplicated.

Neutrophil adhesion test

Blood was collected by retro-orbital puncture on the 14th day into heparinized vials and analyzed for total leucocyte counts (TLC) and differential leucocyte counts (DLC). After initial counts, blood samples were incubated with 80 mg/ml of nylon fibres for 15 min at 37°C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and the percentage of neutrophils in the treated and untreated blood was determined and the difference was taken as index of neutrophil adhesion. Percent neutrophil adhesion was calculated as below.

$$\text{Neutrophil adhesion (\%)} = \left[\frac{(\text{Niu} - \text{Nit})}{\text{Niu}} \right] * 100$$

Where Niu = Neutrophil index of untreated blood sample.
Nit = Neutrophil index of treated blood sample²⁰

Haemagglutination assay

Fresh Sheep RBC (SRBC) were obtained from Bombay Veterinary College, Goregaon authentic source. On the 15th day, all mice (including controls) were inoculated with 0.5 x 10⁹ cells/ml SRBC in sterile PBS (0.25 ml/100 gm of mice weight) intra-peritoneally and blood was drawn after another 14 days i.e. on the 29th day for the haemagglutination assay. Serially diluted serum aliquots from the immunized animals were added to fresh SRBC in a 96 well plate and were scored for mat formation at the bottom of the tube. Hemagglutination measures the relative concentration of antibody in serum and is expressed as a titer^{20, 21}.

RESULTS AND DISCUSSION

PG has been recommended for the treatment of jaundice and diabetes in traditional medicine. This suggests that the intake of PG is for reconvalescence following a digestive

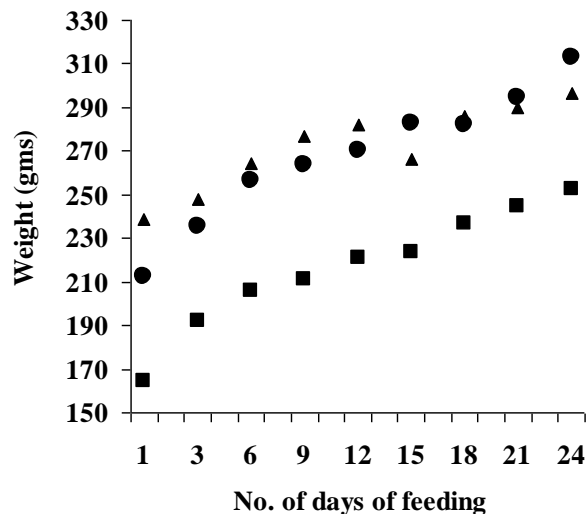


Figure 1: Weight gain in animals. Animals were weighed after their acclimatization period of 2 weeks (which was considered to be Day =1). They were then on regular chow diet supplemented with Distilled water (squares); Panchagavya (circles); Panchagavya + Pomegranate extracts (triangles). Data represents average weight of 6 animals in one experiment and the experiment was duplicated.

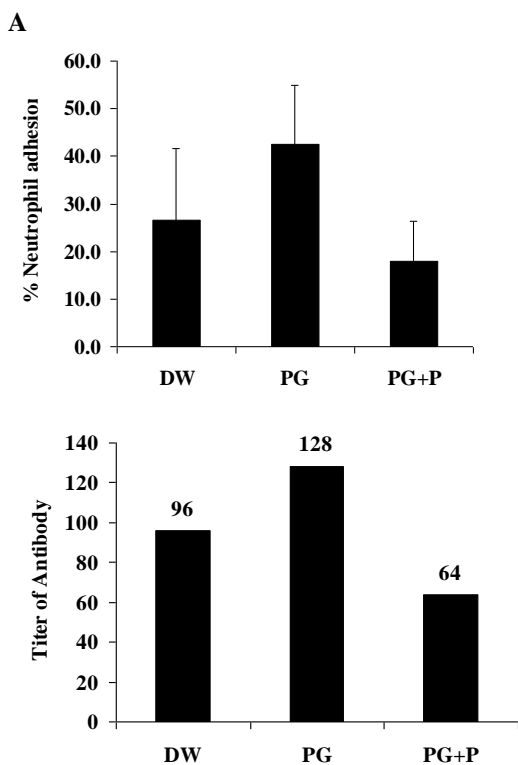


Figure 2: (A) Percentage of neutrophil adhesion. (B) Antibody titre from haemagglutination test. Data represents average from 6 animals fed with .DW: Distilled water; PG: Panchagavya; PG+P: Panchagavya + Pomegranate extracts. Each experiment was performed in duplicate.

tract disorder. Thus, in order to understand the effect of PG as a appetite stimulatory agent, animals were fed with one of the following combinations: Distilled water alone, PG alone or PG + Pomegranate extracts (PG+P). Animals fed with PG or PG+P had increased weight gain compared to controls (Figure 1). The gain in weight was noticeable as early as the third day after start of PG administration. Addition of pomegranate extract had no further effect on the weight gain. Neutrophils play an important role in host immune system. They are attracted by a number of stimuli, which generally signal tissue injury of unknown reason. We evaluated the immunomodulatory effects of PG by evaluating neutrophil adhesion to nylon fibres. Animals with PG alone in the diet had increased neutrophil adhesion, suggesting that PG possess immuno-stimulatory activities. However, when animals were given PG+P, the decrease in neutrophil adhesion was greater suggesting that pomegranate extracts had immuno-inhibitory effects which masked and reversed the immuno-stimulatory effect of PG (Figure 2A). In order to verify whether the immuno-stimulatory effects of PG were also reflected in antibody production, we assessed antibody production by HA titre assays using sheep RBCs as the immunogen. As demonstrated in Figure 2B, animals fed with PG had increased titres of antibody production.

DISCUSSION

Panchagavya was prepared by using all the above ingredients as per the procedures mentioned by Dr. K. Natarajan in Panchagavya- A Manual¹⁷ and by R Giridhar in How to Make Panchagavya, through a fermentation process of 19 (Nineteen) days¹⁸ and according to the protocols published in Bhaishadnya Ratnavali by Shastri¹⁹ and those recommended by the Go-Vigyan Anusandhan Kendra. This method of preparation of PG is standard and has been used by other authors^{22, 23}. The beneficial effects of PG have been investigated by authors at doses typically 300 mg/Kg of PG²². In our study we have used PG at a dose of 500 mg/Kg. Weight gain is usually associated with the unwanted complexities of the metabolic syndrome, obesity or type 2 diabetes. However, under certain physiological conditions such as during re-convalescence after jaundice or after protracted therapy regimens, weight gain without the aforesaid complexities is necessary for the improvement of the general health of the patient. The present study shows that PG alone or PG+P induces a good appetite leading to weight gain in animals without becoming visibly obese, suggesting that these agents may be used for controlled weight gain. The observed weight gain could be due to increase in appetite or due to improvement in digestion and adsorption of diet components. Achliya et al, have reported that PG acts as a hepato-protective agent and reverses CCl₄ induced hepatic damage, thus suggesting that the weight gain could be probable because of better digestion and absorption of nutrients from food²² without any significant toxicity and with some anti-diabetic activity²³. Most plant and animal origin ingredients show immunomodulatory effects by either modulating the cellular and humoral immune

system. However, their activity should be subjected to systematic studies to validate their clinical utility. Even foreign bodies, thermal or chemical burns, bacterial infection and other types of injuries can provoke an intense neutrophil response. Thus vast number of cells can be mobilized due to any set of chemotactic stimuli. Neutrophils are highly effective at killing certain bacteria and their ability to digest cellular debris and exogenous particulate matter provides an important step in the healing process. In the present investigation, when Panchagavya was administered orally, the adhesion of neutrophils to nylon fibers significantly increased and the HA titre was also significantly increased indicating that the Panchagavya potentiates humoral as well as cellular immunity. Ross et al, have reported that the aqueous suspension of the pericarp of the pomegranate enhanced inhibition of leucocyte migration and increased antibody titer to typhoid-H antigen²⁴. When pomegranate extracts were used in combination with PG, we observed a decrease in neutrophil adhesion in agreement with that observed by Ross et al., suggesting that the inhibitory effect of pomegranate was stronger than the stimulatory effect of Panchagavya alone; also perhaps suggesting that the pathways by which the two agents effused immunomodulation were synergistic. One major caveat of this paper is the quality of Panchagavya. While the method of preparation of Panchagavya through this method is standard, the quality of individual components can cause variability in the potency of panachagavya to elicit biological responses. This caveat has been highlighted by the study of Deshmukh et al, where the anti-fungal effect of cow urine from outdoor grazing cow is better than an indoor fed cow²⁵. Thus fresh ingredients from a healthy cow are of paramount importance. Nonetheless, our present study highlights the effect of PG on diet and in immune-stimulation, both aspects important for combating diseases and reconvalescence, thus validating the traditional use of PG for treatment of diseases such as jaundice.

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