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Scientific Evaluation of Safety and Efficacy of Panchagavya Formulation: A Scoping Review

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Abstract

Within the realm of Ayurveda, the ancient Indian medicinal system, the Panchagavya formulation has been utilized to address various human ailments. The Panchagavya blend, derived from five key bovine products (cow milk, curd, ghee, urine, and dung), has been proposed as an alternative, preventive, and healing method for both poultry and human health. Immunostimulant, antioxidant, hepatoprotective, anti-stress, anti-epileptic activities, nootropic effects, anxiety, and mental retardation are the well-investigated areas of interest. According to Ayurveda practitioners, the Panchagavya formulation has therapeutic potential in a wide range of health conditions including leukoderma, alopecia, colds, rheumatoid arthritis, allergies, cough, leucorrhoea, flu, dietary and gastrointestinal disorders, aging-related issues, renal disorders, wound healing, heart disease, skin infections, tuberculosis, chickenpox, worm infestations, hepatitis, leprosy, and various bacterial and viral infections. Enhanced body immunity is attributed to the presence of minerals, hormones, and acids such as citric, carbolic, and succinic, as well as salts like manganese, sulfur, phosphate, chloride, and sodium, along with vitamins A, B, C, D, and E. Individual components like cow's milk, curd, ghee, dung, and urine possess notable therapeutic properties within the Indian traditional medicine system; their combination likely yields synergistic pharmacological effects, as reported in recent Indian literature. Cow ghee possesses anti-cancer, anti-inflammatory, and wound healing effects. Cow milk exhibits anticancer and antihypertensive properties, while cow dung demonstrates antimicrobial and antioxidant capabilities. Curd and yogurt exhibit antihypertensive and immunomodulatory characteristics. The notable features of cow urine include its antioxidant, antibacterial, anticancer, anti-hypertensive, and wound healing effects. Nevertheless, there aren't sufficient scientific studies to support Ayurveda's medicinal claims for the Panchagavya formulation. This review delves into the available literature elucidating the diverse treatment benefits of the Panchagavya formulation in addressing various human disorders and diseases claimed by Ayurveda. The review also discusses the potential, drawbacks, current level of social acceptance, and uncritical evaluation of the Panchagavya formulation.

Keywords: Antiepileptic, Antioxidant, Anti-stress, Anxiety and mental retardation, Hepatoprotective, Immunostimulant, Nootropic, Panchagavya formulation, Traditional Indian Pharmacology.

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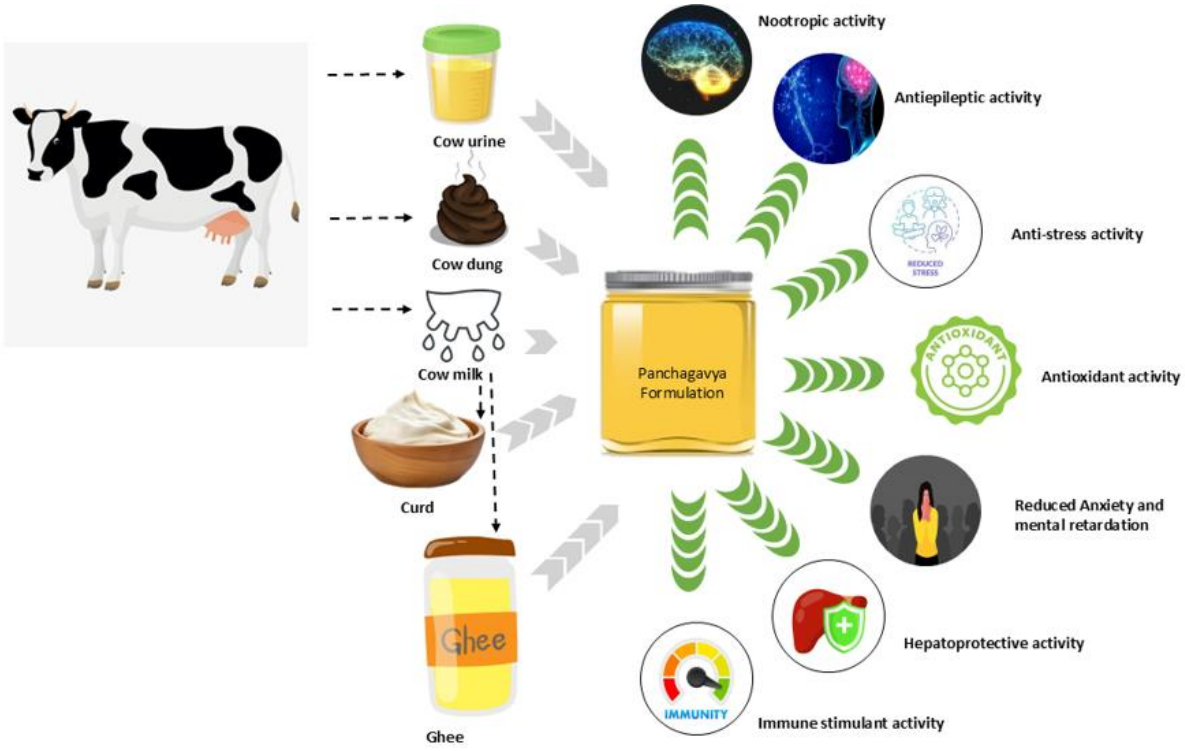

Figure 1.
Schematic representation of the therapeutic uses of Panchagavya formulation as per Indian Ayurveda.

1. Introduction

Panchagavya formulation (PGF), a blend created by combining five primary elements derived from the cow, exhibits notable therapeutic properties as per the Indian Ayurvedic system of medicine. These elements include curd, milk, ghee, urine (Gomutra), and cow dung, which have been historically recognized as integral components of PGF. References to PGF can be found in various ancient texts related to the traditional Indian Ayurvedic system of medicine [1]. Within Ayurveda, PGF has been proposed as an alternative treatment for both therapeutic and preventive measures in safeguarding human health [2-4]. PGF has been observed to effectively treat a wide range of human illnesses while also enhancing the body's resistance and immunity against numerous infections [5]. PGF, a specific formulation containing hormones, amino acids, vitamins, proteins, minerals, and other nutrients, is detailed in traditional Ayurvedic texts. These texts prescribe specific methods for preparing PGF, including the purification of cow urine and dung before blending the five ingredients in specified proportions and cooking them over low heat until the preparation aligns with traditional Ayurvedic recommendations [6, 7].

The therapeutic applications of PGF encompass a diverse array of conditions, including leukoderma, alopecia, colds, rheumatoid arthritis, allergies, cough, leucorrhea, flu, dietary and gastrointestinal disorders, aging-related issues, renal disorders, wound healing, heart disease, skin infections, tuberculosis, chickenpox, worm infestations, hepatitis, leprosy, and various bacterial and viral infections. Each component of PGF possesses unique qualities that have been utilized not only in healthcare but also in agriculture, livestock, and poultry [2].

The constituents of PGF are rich in minerals, hormones, and acids such as citric, carbolic, and succinic, as well as vitamins A, B, C, D, and E, and minerals like manganese, sulfur, nitrogen, chloride, phosphate, and sodium. These essential components alleviate a multitude of human medical ailments and enhance immunity by modulating the immune system, stimulating lymphocyte proliferation, improving cellular and humoral immune responses, and acting as anti-aging agents by preventing free radical generation and repairing damaged DNA [3, 5].[8]

While traditional Indian literature has long extolled the medicinal virtues of PGF, scientific evidence supporting these claims is relatively limited. Scientific investigations can explore the therapeutic potential of PGF, as it has been utilized as a medicine in the traditional Indian Ayurvedic system for a long time [8]. The reluctance to accept PGF among medical professionals and society stems from the inclusion of two components: cow urine and cow manure. Several other individuals

have rejected it because of the repulsive odor and flavor of the PGF formulation. Therefore, PGF has experienced a decrease in societal acceptance in contemporary times. Nevertheless, Ayurveda practitioners continue to prescribe PGF as a remedy for mental and neurological diseases, citing its inclusion in the old Indian Ayurvedic textbook, Charaka Samhita [9]. To comprehend the effectiveness and present state of scientific research, it is necessary to conduct a thorough analysis of literature reports on the advantages of PGF for the identification of ambiguous situations.

We have compiled study reports from scientific and non-scientific sources found in various accessible databases spanning from 1987 to 2025. This review aims to shed light on the health benefits and medicinal properties of PGF based on available scientific data. It also discusses significant scientific studies regarding the medicinal properties of PGF components. The therapeutic efficacy of PGF in addressing various human ailments may be attributed to the synergistic effects of its constituent elements. Overall, this literature review highlights the scientifically elucidated diverse therapeutic effects of PGF in accessible scholarly works.

1.1. Immuno Stimulant Activity

The immunostimulant activity of PGF has been examined by Gajbhiye et al. by feeding mice orally. The oral uptake of PGF (500 mg/kg) enhanced neutrophil adhesion to nylon fibers, highlighting its immunostimulatory activity. HA titer assays observed the enrichment of antibodies using sheep RBCs as the immunogen Gajbhiye, et al. [10]. Patel, et al. [11] reported the prophylactic ability of PGF against bacterial infection, employing Caenorhabditis elegans. Worms fed with PGF beforehand competed with pathogenic bacteria and had a reliable survival rate against four bacterial pathogens compared to the control group. PGF was observed to be most effective against Staphylococcus aureus, resulting in 27% ($p=0.0001$) worm survival. The protective effect of PGF against microbial infection may partly stem from its immunomodulatory potential [11].

1.2. Antioxidant Activity

PGF contains both non-polar and polar antioxidants. Athavale, et al. conducted an investigation into the antioxidant potential of PGF using HPTLC-DPPH bioautography. The radical scavenging activity was assessed by determining IC50 (Half-maximal Inhibitory Concentration) values. However, the PGF sample did not exhibit a definitive IC50 value in the DPPH assay. This discrepancy may be due to the presence of residual chemical compounds that compete with DPPH reduction or engage in preferential internal scavenging reactions, thereby hindering electron transfer to DPPH. The data from the antioxidant study revealed unconventional activity patterns, suggesting the possibility of heightened activity at lower concentrations, which warrants further detailed investigation. Athavale, et al. [12]. Chinniah et al. [13] have analyzed the antioxidant activity of PGF utilizing the DPPH assay and compared it with the activity of ascorbic acid. The antioxidant activity exhibited by PGF was determined to be inferior to that of ascorbic acid. Additionally, they performed a FRAP assay to evaluate the Fe^{3+} lowering capacity of PGF. Like the DPPH antioxidant experiment, the FRAP assay confirms that PGF has the capacity to reduce Fe^{3+} to Fe^{2+} ions, strictly dependent on concentration [13].

1.3. Hepatoprotective Activity

The hepatoprotective efficacy of PGF has been studied in rodents with hepatotoxicity caused by carbon tetrachloride (CCl_4). Oral ingestion of PGF resulted in a significant reduction in CCl_4 induced hepatotoxicity in rats. Sprague Dawley strain male albino rats were selected for the experiment. The PGF dosage reported in Ayurvedic texts and by expert Ayurvedic physicians has been used for the study. The liver was swiftly excised and serially sectioned from the immolation and subjected to histological examination. The serum marker enzymes viz – SGPT (Serum Glutamic Pyruvic Transaminase), ALP (Alkaline Phosphatase), ACP (Acid Phosphatase), and SGOT (Serum Glutamic-Oxaloacetic Transaminase) levels were elevated in the CCl_4 treated group with respect to the control group. The PGF treated group showed a considerable drop in serum marker enzyme levels compared with the CCl_4-treated group. Silymarin and PGF restored the liver to normalcy. Carbon tetrachloride has been biologically transformed via the cytochrome P-450 system to generate trichloromethyl free radicals, resulting in lipoperoxidation. Treatment with PGF drastically curtailed the rise in serum marker enzymes viz. ALP, ACP, GOT, and GPT. The results have been compared with silymarin. Enzymes ALP, ACP, GOT, and GPT indicate the induction of hepatotoxicity. An increase in the thymidylate synthetase and thymidine kinase levels in the liver implies regeneration influenced by coupled phospholipids [14].

1.4. Anti-Stress Activity

Kumar et al. [15] investigated the anti-stress effects of Panchagavya, combined with an alcoholic extract of Aloe barbadensis Mill (Xanthorrhoeaceae), using the Tail-immersion model. Sixty mice were randomly divided into 10 groups, each receiving different combinations of PGF and Aloe barbendansis Mill extract to assess their synergistic antinociceptive effects in Swiss albino rats. The combination of PGF with Aloe barbadensis Mill ethanolic extract demonstrated enhanced and unified antinociceptive activity, potentially attributed to the presence of steroids, sesquiterpenes, terpenes, flavonoids, and vitamins. Future studies are expected to reveal additional pharmacological activities such as anthelmintic and anti-stress properties [15].

1.5. Antiepileptic Effect

Epilepsy is a fundamental disorder of the brain characterized by excessive and abnormal neural activity disrupting cerebral grey matter, leading to seizures. Amit et al. clinically investigated the effectiveness of PGF in paediatric epilepsy convulsions. An open-label randomized interventional drug efficacy study was conducted. Sixty untreated patients diagnosed

with idiopathic epilepsy were enrolled in the study. These patients were randomly divided into two groups, each consisting of 30 individuals. Group I received PGF, while Group II received Tegretol. Both groups were monitored for one year to assess the efficacy of the drugs. Group I exhibited significant improvements in seizure frequency, extent of amnesia, and convulsions. Conversely, Group II showed notable reductions in seizure frequency and duration; however, minimal improvements were observed in amnesia. PGF demonstrated promising effects in managing paediatric epilepsy without complications or side effects [16].

PGF has subdued jaundice, mania, fever, and epilepsy according to the Ayurvedic Formulary of India (AFI). The aftermath of oxidative stress, cognitive impairment, and convulsions in Pentylenetetrazole (PTZ) in rats has been examined by Joshi et al. PGF with different dosages has been administered orally for seven days to male Wistar rats. Sodium valproate has been established as the positive control group. Latency to generalized tonic-clonic seizures (GTCS), clonus, and myoclonic jerks have been observed for seizure severity. Cognitive impairment was assessed via increased passive avoidance and plus maze tests. Glutathione and malondialdehyde levels have been measured in rat brains [17].

Joshi, et al. [17] investigated the pharmacokinetic and pharmacodynamic interactions of PGF with carbamazepine (CBZ) and phenytoin (PHT) in rats. PGF was administered along with a sub-therapeutic dose of CBZ and PHT, and behavioral parameters were evaluated. Serum levels and oxidative stress markers of CBZ and PHT were measured. PGF reversed cognitive impairment, oxidative stress, and tonic hind limb extension induced by maximal electroshock seizures (MES). Co-administration of PGF with CBZ and PHT enhanced cognitive function, reduced oxidative stress, and improved antiepileptic effects. Although a slight increase in serum levels of CBZ and PHT was observed after co-administration with PGF, it was statistically insignificant. Notably, co-administration of PGF with minimal doses of CBZ and PHT provided extensive protection against seizures [18].

The antiepileptic efficacy of PGF was assessed in three different doses using the Increasing Current Electroshock Seizure (ICES) animal model. Mice were treated with the drugs for seven days and then subjected to the ICES test. Administration of PGF for seven days at a dose of 5 g/kg demonstrated antiepileptic activity in the ICES animal model without any reported adverse effects on animal memory compared to the standard Phenytoin [19].

Pre-treatment with PGF has significantly ameliorated oxidative stress and cognitive functions triggered by seizures, demonstrating its protective response against PTZ-induced seizures. Additionally, PGF is widely recognized as an anticonvulsant in traditional Indian Ayurveda. Among the numerous formulations mentioned in Ayurveda for addressing psychiatric disorders, PGF stands out as one of the most commonly used. Ghee-based formulations are prevalent remedies across cultures, with several formulations employed in clinical settings yielding positive outcomes. PGF has been recommended for managing epileptic syndromes and has shown clinical efficacy in conditions such as schizophrenia, attention deficit hyperactivity disorder, post-traumatic dementia, various types of epilepsy, and depression. Moreover, Pawar et al. observed memory impairment induced by antiepileptic drugs in animal studies [20].

The organoleptic, physicochemical, phytochemical, and pharmacological study of PGF utilized for Ayurvedic treatment of epilepsy has been conducted by Snehal, et al. [21]. The color, odor, taste, texture, and appearance of PGF are green, ghee-like, bitter, unctuous, and viscous semisolid, respectively. The pH of the PGF formulation was measured at 6.52 ± 0.01, with a viscosity of $250{,}175 \pm 16.3$ cp and a specific gravity of 0.7223 ± 0.01. The acid value, saponification value, peroxide value, iodine value, and refractive index were determined to be 1.651 ± 0.01, 36.160 ± 0.01, 61.3623 ± 0.01, 21.5227 ± 0.01, and 1.6393 ± 0.01, respectively. The presence of phenolics, flavonoids, steroids, proteins, and amino acids has been detected in PGF. Thus, the formulation has a potential anticonvulsant effect observed in PTZ (Pentylenetetrazole)-induced convulsions [22].

1.6. Anxiety and Mental Retardation

Gururaja MP [23] conducted a study to validate traditional claims regarding ancient knowledge alongside recent preclinical pharmacological aspects of PGF. Swiss albino mice were divided into four groups, each comprising six mice. The anti-anxiety activity was assessed using the Open Field Test, Light Dark Transition Model, and Elevated Plus Maze Model. Mice treated with PGF demonstrated consistent results, including increased entries and duration of stay in the elevated plus maze model, as well as increased entry numbers and duration of stay in the lighted chamber during the Light & Dark Transition experiment. The open field test revealed enhanced time spent in the central square, increased number of rearing, and increased number of squares crossed, thus supporting the anti-anxiety potential of PGF [23].

FibiMol PP [24] clinically investigated the efficacy of PGF on cognitive functions in Down's syndrome. Mental retardation is a prominent feature of Down's syndrome. PGF was administered and assessed using the Vineland's Social Maturity Scale and the Seguin Goggard Form Board test for Intelligence Quotient. A significant improvement of 51.9% was observed after the first month, followed by a further improvement of 55.9% after the second month [24].

Shihabudheen EM [25] clinically examined the effects of PGF on obsessive-compulsive disorder (OCD). Twenty patients were orally administered 25 ml of PGF daily for 30 days. Assessment was conducted using the Yale-Brown Obsessive-Compulsive Scale (YBOC). The overall improvement in compulsions was 14%, and in symptoms, it was 18.5%. The drug was found to be effective in addressing the obsessive and compulsive aspects of OCD clinically [25].

Arathi, et al. [26] did a study to assess the efficacy of oral administration of Brahmidrakshadi decoction and nasal administration of PGF in improving patients with depression. Upon completion of the 30-day treatment, a clinical and statistical evaluation was conducted. The statistical study revealed a high incidence rate of depression among women, particularly within the age group of 30 to 60 years. A notable disparity in the clinically recognized symptoms of depression was evident during the follow-up period [26].

1.7. Anticancer Activity

The anticancer efficacy of PGF was assessed on the breast cancer cell line MDA-MB-231 by Chinniah, et al. [13]. The anticancer activity exhibited a positive correlation with increasing concentrations of PGF. The IC50 value for PGF was determined to be 7.68 µg/ml. Fluorescence imaging revealed the cytotoxic effects of PGF. AO/EB (Ethidium Bromide/Acridine Orange) staining demonstrated a significant number of dead cells, whereas the control exhibited no dead cells. The IC50 value for the non-cancerous Vero cell line was 987.56 µg. In comparison, the inhibitory concentration for Vero cells was substantially higher than that for breast cancer cells when evaluating the effects of PGF [13].

1.8. Antibacterial Activity

The antibacterial efficacy of PGF against six microbial pathogens, specifically the gram-positive bacteria E. faecalis, L. monocytogenes, B. subtilis, and the gram-negative bacteria P. aeruginosa, S. sonnei, and P. vulgaris, was determined using the agar diffusion method by Chinniah, et al. [13]. The antibacterial efficacy of PGF against E. faecalis was quantified at 10 ± 0.8 mm, against L. monocytogenes at 8 ± 1.2 mm, and against B. subtilis at 12 ± 1.4 mm, in response to 200 micrograms/mL of PGF. The inhibitory zone assessed for gram-negative bacteria was 11 ± 1.5 mm for P. aeruginosa, 8 ± 1.2 mm for S. sonnei, and 10 ± 0.9 mm for P. vulgaris. In comparing the antibacterial efficacy of PGF against gram-positive and gram-negative bacteria, the gram-negative bacteria exhibit slightly greater susceptibility to PGF. In addition, they assessed the antibiofilm activity of PGF utilizing the 24-well plate method. The confocal laser scanning microscopy demonstrated that PGF possesses the capability to prevent the proliferation of microbial suspensions. The maximum biofilm formation was recorded at 200 µg/ml [13].

1.9. Nootropic Effect

The cognitive enhancement potential of PGF was explored using diazepam-induced amnesia in mice and Morris Water Maze (MWM) analysis in rats. PGF effectively mitigated diazepam-induced amnesia, as demonstrated in the Elevated Plus Maze (EPM) test, where PGF at a dose of 5 g/kg showed significant efficacy. Additionally, the combination of piracetam and PGF at a dose of 3.5 g/kg exhibited notable memory enhancement in the MWM test [27].

Furthermore, the combination of Bacopa monnieri (BM) and Convolvulus pluricaulis (CP) with PGF surpassed the cognitive enhancement potential of BM and CP alone. The investigation focused on assessing the learning activity and memory of water and methanolic extracts of BM and CP leaves. Nootropic potential was evaluated using the Actophotometer, radial arm maze, phenobarbital sodium-induced sleeping time, and Elevated Plus Maze. The aqueous and methanolic extracts of BM and CP, when combined with an appropriate amount of PGF, demonstrated a significant improvement in memory and learning capacity. It was confirmed that these herbs exhibit excellent memory and learning abilities when used in conjunction with PGF [28].

As explained above, the bio-product PGF has been used from time immemorial for spiritual and treatment purposes. PGF was influential in disparate maladies affecting the brain's functioning, on the grounds that it can surpass the lipid blood-brain barrier. PGF purges channels inside the body and revitalizes mind functions. The drug was perceived to be persuasive in improving children's cognition and autism with irreversible damage to cognitive tasks. PGF was deemed to be effective in the positive and negative symptoms of schizophrenia. PGF has a potent revitalizing effect in OCD (obsessive-compulsive disorder), a debilitating multidimensional disorder. PGF is a satisfying option as far as epilepsy is concerned, as well as organic brain disorders such as post-traumatic amnesia. Henceforth, PGF admirably regulates copious psychiatric conditions like Down syndrome, cognitive function, schizophrenia, OCD, and biological brain dysfunction following a stroke. The sequential combinations of PGF have yet to be completed by further research. However, some recently published literature reports cited the pharmacological activities of the 5 ingredients of PGF.

1.10. Cow Urine (Gomutra)

Antioxidant and antimicrobial characteristics of cow urine distillate (CUD) and cow urine (CU) have been studied by Edwin et al. Freshly obtained samples of cow urine showed more satisfactory results than distilled samples. Antioxidant models such as DPPH radical scavenging and superoxide scavenging have been selected for the study. The reference standard selected was ascorbic acid. The IC50 values of fresh cow urine were recorded from the DPPH and NBT methods. However, the cow urine distillate displayed IC50 values of 5.1 µg/mL and 5.0 µg/mL from DPPH and NBT, respectively. The antimicrobial activity has been examined using Escherichia coli by the agar well method, Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae, and Staphylococcus epidermidis. The inhibition zone developed by cow urine distillate appeared to be 18-21 mm. Nevertheless, fresh cow urine exhibited an inhibition zone of 22-25 mm. The antimicrobial activity exhibited by fresh cow urine was observed to be more admirable than that of the distilled one. The reports revealed the antimicrobial and antioxidant potential of cow urine, supporting the presumption of traditional Ayurvedic practitioners [29].

Sathasivam, et al. [30] assessed the antifungal and antibacterial properties of distilled cow urine against pathogenic microorganisms. The efficacy of cow urine distillate was examined against Salmonella typhi, Klebsiella pneumoniae, Bacillus subtilis, and Pseudomonas aeruginosa using the disc diffusion method. Discs soaked in 5, 10, and 15 µL of cow urine distillate were prepared separately for the investigation. Significant antibacterial activity was observed against Pseudomonas aeruginosa and Salmonella typhi. Distilled cow urine also exhibited antifungal potential against Aspergillus flavus and Aspergillus niger. Aspergillus niger demonstrated a higher growth inhibition compared to Aspergillus flavus. Notably, 5% cow urine distillate was found to be effective against Aspergillus niger (5.2%). The investigative study

concluded that distilled cow urine possesses considerable antifungal and antibacterial properties that could effectively combat various bacteria and fungi [30].

Jain, et al. [31] conducted a questionnaire survey to assess the efficacy of cow urine in healing cancer-afflicted patients across various regions of India. Sixty-eight individuals with cancer were enrolled in the study from April 8-15, 2007. The survey, based on a developed questionnaire, revealed that 30.87% had throat cancer, 14.70% had breast cancer, followed by lung cancer, lymphoma, and bone cancer (2.94%), cervix and uterine cancer (5.88%), both throat and buccal cancer (5.88%), buccal cavity cancer and sinus (4.41%), and other types of cancer (8.82%). Symptoms such as burning sensation, inflammation, irritation, difficulty swallowing, and pain were categorized as mild, moderate, or severe. The data showed that on the initial day, the proportion of severe, moderate, and mild symptoms was 1.58%, 15.8%, and 82.16%, respectively, which changed to 36.34%, 55.3%, and 7.9% by the eighth day. Patients who underwent cow urine therapy in the preceding 2-3 months showed the most significant improvement [31].

The wound healing potential of cow urine, particularly regarding wound contraction, was investigated using an excision wound model in Wistar albino rats. The excision wound healing process demonstrated reduced wound size across all groups daily. On the 4th day of external application, a significant decrease in wound size was observed in rats. The study found that the external application of cow urine expedited the wound healing process [32].

Sachdev, et al. [33] examined the impact of Cow urine distillate (Gomuthra arc) on alloxan-induced diabetes in Wistar albino rats. In Ayurveda, distilled cow urine is known as Gomuthra arc (GoA). They monitored various parameters, including vitamin C levels, malondialdehyde release, and blood sugar. The results showed that GoA significantly lowered blood glucose levels in diabetic rats, although the effect was less pronounced compared to glibenclamide. Additionally, it substantially reduced vitamin C and malondialdehyde levels in diabetic rats. Acute toxicity analysis involving repeated administration of a fixed dose of cow urine over thirty-two times showed no observable toxicity. The study suggests that GoA has a considerable therapeutic potential in managing diabetes and is safe for chronic consumption. However, further investigations are necessary to elucidate the mechanistic action of Cow urine distillate [33]. Research conducted by the Cow Science Research Center (Go-Vigyan Anusandhan Kendra, Nagpur) revealed the therapeutic potential of cow urine in combating cancer. Extensive research on urine therapy involving cow urine for cancer reversal was conducted and confirmed. The study emphasized the role of cow urine in limiting cancer growth and enhancing the efficacy of anticancer drugs. Their findings were granted a U.S. Patent (No. 6896907) for the treatment of cancers (Amar Ujala, July 19, 2005) [34].

Haemorrhoids, a prevalent anorectal condition, are characterized by distal transposition and symptomatic swelling of the anal mucosa. Vascular hyperplasia and inflammation may manifest in Haemorrhoids. Talokar, et al. [35] conducted a clinical assessment of haemorrhoid complications by administering cow urine extract orally. Improvement in symptoms and signs of the condition served as the primary criteria for assessing patient progress. Cow urine acts on the large intestine, facilitating smoother stool excretion and providing relief to haemorrhoid patients. Oral administration of cow urine has mitigated the time-consuming, painful, and costly complications of Haemorrhoids [35].

Raad, et al. [36] investigated the antibacterial activity and protein content of cow urine against a selection of non-pathogenic and pathogenic bacteria. The disc diffusion assay was conducted using fourteen bacterial cultures, encompassing both non-pathogenic and pathogenic strains, with ten randomly chosen cow urine samples. Total protein content was determined using the Folin-Lowry method, and proline identification in urine samples was carried out using paper chromatography. Among the ten urine samples tested, the highest activity was observed against Gram-negative bacteria compared to Gram-positive bacteria. All tested cow urine samples exhibited evidence of bioactive compounds and antibacterial activity [36].

Hoh and Dhanashree [37] explored the potential antifungal properties of cow urine against clinically isolated Candida species. Thirty-seven Candida species were subjected to disk diffusion tests to evaluate their susceptibility to fluconazole, voriconazole, and amphotericin B. It was found that 18.9% of the isolated Candida species were resistant to voriconazole, while 35.1% and 24.3% showed growth inhibition against fluconazole and amphotericin B, respectively. The inhibitory effect of distilled cow urine on Candida species exhibited concentration-dependent behavior, with both resistant and sensitive effects observed against the isolated species in comparison to routine antifungal agents [37].

Dave, et al. [38] conducted a study to assess the Minimum Inhibitory Concentration (MIC) and antibacterial activity of cow urine and goat urine against major dental microorganisms. Distilled samples of cow and goat urine were tested against Lactobacillus acidophilus (L. acidophilus) and Streptococcus mutans (S. mutans). Statistical analysis was performed using ANOVA (One Way Analysis of Variance) with SPSS version 19 to acquire the statistical data. MIC values for dental caries bacteria ranged from 5 to 10 g/ml in both urine samples, indicating potent antimicrobial action. Goat urine exhibited the lowest MIC values (5 g/ml) against S. mutans and L. acidophilus. At the highest concentration (50 g/ml), a statistically significant inhibition zone ($p < 0.05$) was observed for S. mutans. Goat urine demonstrated more effective bacterial repellence compared to cow urine. Both cow and goat urine samples showed therapeutic potential against microorganisms contributing to tooth decay [38].

Rachana and Sreepada [39] assessed the anti-inflammatory and antioxidant properties of raw and distilled cow urine. They evaluated the effectiveness of various radical scavenging models, including a method involving the induction of edema in a rat's paw using carrageenan to assess anti-inflammatory activity. A saline solution of carrageenan (1% w/v) was injected into the sub-plantar area of the rats' left paw to induce paw edema. Paw volume was monitored hourly before and after the carrageenan injection using a plethysmometer. The study concluded that distilled cow urine lacks the antioxidant and anti-inflammatory properties observed in raw cow urine [39].

Adhikari, et al. [40] evaluated the synergistic antibacterial and antioxidant properties of Tulsi and neem leaf extract. Antioxidant as well as antibacterial activity has been performed using agar disc diffusion and DPPH radical scavenging

method, respectively, against Staphylococcus aureus (gram-positive), besides gram-negative (Proteus Vulgaris, Klebsiella, and Escherichia coli) bacteria. The Antibacterial and Antioxidant potential depends upon concentration. Extract of Neem and Tulsi in cow urine exhibits a voluminous inhibition zone against P. vulgaris (10.14%) and E. coli (12.75%), whilst synergistic antibacterial investigation. Ultimately, cow urine, neem, and tulsi exhibit potent antibacterial and antioxidant properties both synergistically and individually, with the effectiveness varying based on concentration. Tulsi methanol extract particularly demonstrates significant inhibition of free radicals. Consequently, further investigation into combined formulations is deemed necessary [40].

Vipin et al. conducted an investigation to identify the bioactive compounds responsible for the antimicrobial activity of cow urine using FTIR and GC-MS analysis. They separated various bioactive compounds in cow urine through thin-layer chromatography (TLC). The highest inhibitory activity was observed against Staphylococcus aureus (15 mm ZOI), with satisfactory inhibition against Klebsiella pneumoniae. The urine fractions demonstrated maximum inhibitory potential against Gram-positive bacteria. FTIR analysis was employed for functional group identification, revealing the presence of amide, alkene bending, intramolecular bonded alcohol, polysulfide stretch, alkyl halide, and phosphate ion. GC–MS analysis identified significant chemical entities such as fatty alcohols, including 1-Heneicosanol, n-Heptadecanol-1, n-Nonadecanol-1, and 1-Hexadecanol, as well as other compounds like octamethyl-3–5 bis (trimethyl siyloxy) pentadecanal, 1,1,1,3,5,7,7,7 Hexadecamethyl, Cyclooctasiloxane, Tetra Siloxane, 6H-Pyrazolotetrazine, 2(1H)-pyrimidinone, hexahydro-2,3-dimethyl-, 1-triethylsilyloxyheptadecane, 5-chloro-4,6-diphenyl, 1,4-dioxane-2,6-dione, and 2-pentanone 4-hydroxy-4-methyl-. ICP-MS analysis was utilized to quantify essential inorganic elements such as Na, Ca, Cr, Fe, Mg, Al, K, Zn, and Au. Long-chain fatty alcohols were found in higher concentrations compared to other chemical compounds, potentially contributing to the antibacterial activity [41]. Both non-volatile and volatile compounds may contribute to the inhibitory action, as indicated by proton-transfer-reaction mass spectrometry (PTR-MS), which detected methanol, acetone + propanal, dimethylsulfide, 4-methyl-phenol (m/z 109), trimethylamine (m/z 60), and ethanol qualitatively [42]. The bacterial inhibitory effect of long-chain fatty alcohols against Staphylococcus aureus was attributed to the leakage of K+ ions from the cell membrane [43]. The inhibition of bacterial action against Salmonella gallinarum may be linked to the presence of hexadecanoic acid or palmitic acid [44]. Additionally, a fatty aldehyde (Pentadecanal) identified in the urine fraction was observed to be effective against Staphylococcus epidermidis [45]. NMR-based metabolomics was employed to detect urinary metabolites related to nitrogen uptake efficiency, with compounds such as p-cresol sulfate, phenylacetylglutamine, urea, and hippurate indicating nitrogen consumption efficiency [46].

1.11. Cow Dung

In Waziri and Suleiman [47] investigated the antimicrobial properties of extracts derived from dried cow dung. They prepared and tested the antimicrobial activity of cow dung ash extract against Escherichia coli (E. coli), Bacillus subtilis (B. subtilis), Cyanobacteria (C. bacteria), and Staphylococcus aureus (S. aureus). The pH of the extract was 11.7, and the elements were present in the following increasing order of concentration: Zn < Al < Fe < Ca < Mg < Na < K. The most sensitive microorganism was S. aureus with a minimum inhibitory concentration (MIC) of 0.082 mg/mL, while the least sensitive was B. subtilis with an MIC of 4.3 mg/mL. The findings support the potential use of the extract in reducing infections and meeting dietary Na and K requirements [47].

Jirankalgikar, et al. [48] utilized in vitro antioxidant activity testing to characterize cow dung through HPTLC (High-Performance Thin Layer Chromatography). Additionally, they conducted measurements of ferric reducing antioxidant power (FRAP), DPPH (2,2-Diphenyl-1-picrylhydrazyl) bioautography, in vitro antioxidant activity by HPTLC, and free radical scavenging action using DPPH. Significant variances were observed in the HPTLC profiles. HPTLC-DPPH bioautography revealed the presence of numerous antioxidant molecules, which were further confirmed through quantitative assessment of antioxidant potential. The findings suggest that cow dung contains both phenolic and non-phenolic bioactive compounds. The obtained chromatograph provides a convenient means for assessing the characteristic profiling of cow feces [48].

1.12. Cow Milk

Kadooka, et al. [49] presented findings from a study investigating the effects of probiotic Lactobacillus gasseri (LG2055) on body weight, abdominal adiposity, and other body parameters in adults with a tendency towards obesity. This multicenter, placebo-controlled intervention trial randomly assigned participants with elevated body mass index (BMI) and abdominal visceral fat area to either receive fermented milk (FM) containing LG2055 or FM without LG2055. Participants were instructed to consume 200 g of FM daily for 84 days. Computed tomography was used to assess abdominal fat area. The group receiving FM with LG2055 (Active FM group) experienced significant reductions in both abdominal visceral and subcutaneous fat areas, decreasing by approximately 4.6% and 3.3%, respectively, compared to baseline. Serum adiponectin levels also increased significantly ($P<0.01$) in both the active and control groups, with a rise of 12.7% and 13.6%, respectively. The probiotic LG2055 demonstrated beneficial effects on body weight, abdominal obesity, and other measured parameters, indicating its potential for managing metabolic disorders [49].

According to Praveesh, et al. [50] cow milk has anti-hypertensive and anti-cancer responses. Lactobacillus plantarum and Lactobacillus casei were used for the cow milk fermentation. Fermented milk with the combination of Lactococcus casei and Lactobacillus plantarum had a high ACE-inhibitory activity of 79%. The discoveries disclose that the hydrolysate of cow's milk has antioxidant potential with an IC50 value of 231 g/ml. Milk hydrolysate demonstrated notable antioxidant potential in the total reducing assay method. Additionally, the growth of Bacillus subtilis, Staphylococcus aureus, and Escherichia coli was inhibited by the fermented milk hydrolysate [50].

1.13. Cow Ghee

Charde, et al. [51] developed Madhu ghrita by combining equal parts of cow ghee and honey. Incision and excision wound models were employed to evaluate the wound healing properties of Madhu ghrita. Male Wistar rats were used to assess parameters including keratinization, epithelialization, fibrosis, neovascularization, and collagenation. The formulation was also tested for its ability to reduce inflammation using carrageenan-induced paw edema in male Wistar rats. Madhu ghrita significantly improved the tensile strength and accelerated the healing of untreated wounds. It reduced the time taken for wound closure, thereby promoting wound healing and enhancing tensile strength. Histopathological examination revealed enhanced proliferation of epithelial tissue, promoting angiogenesis, and strengthening fibrous connective tissue. Madhu ghrita demonstrated considerable anti-inflammatory activity compared to ibuprofen gel, which served as the reference standard [51].

The study reports on the wound-healing properties of a gel containing aloe vera and cow ghee in rats, which are interesting. They assessed histopathological changes in the healed tissues, incision wound tensile strength, and excision wound contractions to evaluate wound healing. Wound contraction (in excision wounds) and tensile strength (in incision wounds) demonstrated recovery comparable to that seen with framycetin sulfate. Histological analysis revealed favorable characteristics such as fibrosis, cohesion, keratinization, and epithelization, indicating the wound healing potential of the formulation. The study provided valuable insights into the purported wound-healing capabilities of the tested formulation [52].

Kansal, et al. [53] aimed to elucidate the biochemical mechanisms underlying the therapeutic efficacy of cow ghee and soybean oil against carcinogen-induced mammalian cancer in rats. Cow ghee, in comparison to soybean oil, reduced the enzymatic activity of cytochrome P450s (CYPs), which are responsible for activating liver carcinogens. Rats consuming cow ghee exhibited superior detoxifying activity of QR (quinone reductase) and UDPGT (uridine diphosphate-glucuronosyl transferase) in the liver, as well as QR and GGTP (γ-glutamyl transpeptidase) in mammary tissue, compared to those fed soybean oil. While hepatic GGTP activity decreased in rats on a soybean oil diet, it remained unchanged in the cow ghee-fed group. Dietary cow ghee downregulated the metabolism of carcinogen activation enzymes and enhanced detoxification processes in both the liver and mammalian tissues [53].

Yr, et al. [54] conducted an analysis of the physicochemical characteristics of cow ghee before and after hydrogenation, aiming to compare quality, shelf-life, palatability, and acceptability. The Fourier-transform infrared (FTIR) spectra revealed a peak at 966 cm^{-1}, indicating the presence of trans compounds in both hydrogenated cow ghee (HCG) and cow ghee (CG). The FTIR data confirmed the presence of trans fatty acids in both HCG and CG. The increased solidification temperature, specific gravity, and melting range of HCG indicated a higher content of solidified fat compared to CG. Moreover, HCG exhibited lower saponification value and ester value, along with a higher acid value, suggesting a higher content of free fatty acids. The low iodine value of HCG indicated a lower degree of unsaturation. The presence of an OH-group in both CG and HCG was confirmed by the Hydroxyl value. Importantly, there was no significant increase in nickel and heavy metal content due to adherence to good laboratory practices. To enhance quality properties and adhere to traditional ghee processing parameters, good manufacturing practices are essential, including appropriate sealing in packages infused with inert nitrogen and incorporating antioxidants for optimal distribution [54].

1.14. Curd/Yogurt

Patil, et al. [55] investigated the role of yogurt and curd in preventing and influencing diabetes through inflammation-induced protein glycation. Methylglyoxal and glyoxal present in curd were identified as agents responsible for protein glycation. Rats were administered 1.5 g of curd and yogurt daily for a period of 45 days. Various tissues were examined for specific and general Advanced Glycation End Products (AGEs). Liver tissue exhibited a significant increase in general fluorescence, with curd-fed rats showing a 6.5-fold increase at 12 hours, yogurt-fed rats showing a 10.5-fold increase, and curd-fed rats at 24 hours showing an 11.5-fold increase. Specific fluorescence levels also increased in the kidney and liver. Additionally, intense formation of AGEs was observed in the lungs, pancreas, lens, soleus muscle, intestines, and heart. Rats demonstrated reduced Superoxide Dismutase (SOD) values, elevated Thiobarbituric Acid Reactive Substances (TBARS), and increased catalase levels, indicating oxidative stress, with the highest stress observed in rats administered curd for 24 hours, followed by yogurt-fed rats and rats administered curd for 12 hours. Levels of IL-10, TNF-α, and IL-6 were found to increase, while IFN-γ and IL-4 levels decreased. The Oral Glucose Tolerance Test (OGTT) confirmed impaired glucose tolerance in rats administered curd for 24 hours and in rats consuming yogurt. The consumption of curd for 12 hours in diabetic animals resulted in elevated AGE levels. Thus, regular consumption of curd leads to oxidative stress, inflammation, and protein glycation, contributing to the development of diabetes [55].

Irvine, et al. [56] investigated the long-term impact of yogurt supplemented with Lactobacillus rhamnosus Fiti on the immune function of HIV/AIDS patients. This observational retrospective study spanned three years and involved assessing the CD4 count of 68 participants during and before the intake of probiotic yogurt. These participants were compared to a control group consisting of 82 individuals who did not consume yogurt. The average CD4 count among participants was found to be 0.13 cells/μL/day (95% CI: 0.07-0.20, P=<0.001). Individuals who consumed yogurt experienced an increase in CD4 count, with a rate of 0.28 cells/μL/day. Even when concurrently receiving antiretroviral medication, the rise in CD4 count for yogurt consumers remained at 0.17 cells/μL/day. Comparatively, the administration of antiretroviral medication alone resulted in an increase of CD4 count at a rate of 0.27 cells/μL/day. The consumption of probiotic yogurt, locally prepared by women in Tanzania, significantly boosted CD4 count in HIV patients [56].

1.15. Related Investigations Regarding PGF

Nariya, et al. [57] analyzed the physical and chemical characteristics along with HPTLC of PGF. Generating reports and profiles of PGF was beneficial for controlling batch-to-batch variations and quality control. Secondary metabolites such as phenolic compounds, steroid/triterpenes, and flavonoids were observed in the sample [57].

The antagonistic potential of lactobacilli isolated from dairy products (local) was examined by Karami et al. [57] in three common strains of Bacillus subtilis, Pseudomonas aeruginosa, and Staphylococcus aureus. Twenty dairy product samples were collected locally. The antimicrobial disc diffusion method was adopted for Mueller-Hinton agar medium-plated B. subtilis (ATCC-12711), P. aeruginosa (ATCC-27853), and S. aureus (ATCC-6538). Only three isolated lactobacilli strains from local dairy samples, including Lactobacillus sake, Lactobacillus alimentarius, and Lactobacillus colloides, have been demonstrated to have inhibitory effects on the understudied pathogens, according to the data. Except for L. alimentarius and L. colloides, which revealed comparatively moderate activity (15 mm ZOI) against B. subtilis and P. aeruginosa respectively, all three isolates displayed significant activity (15 mm ZOI) [58].

Esophageal cancer is a male-dominant, aggressive tumor with a five-year survival rate of 15-20%, esophageal squamous cell carcinoma (ESCC) being one of the predominant subtypes. Sawarkar, et al. [59] described a case study involving a 70-year-old man with esophageal squamous cell carcinoma (ESCC), who experienced dysphagia, an inability to burp, persistent nausea, and intermittent difficulty swallowing water for two months. The administration of PGF, in conjunction with other Ayurvedic medicinal therapies, demonstrated notable symptom alleviation, suppressed cancer proliferation, and revitalized tissues. The PGF demonstrated favorable outcomes without negative effects, underscoring its effectiveness in enhancing the quality of life for this senior patient with ESCC [59].

Sawarkar, et al. [60] conducted a case study on an 83-year-old woman with acute burn wounds on her right leg and abdomen, characterized by pain, discharge, foul odor, edema, and skin discoloration. The treatment commenced with PGF in conjunction with other Ayurvedic drugs, resulting in notable outcomes, including complete wound healing and remission of accompanying symptoms. The data indicate that PGF manages burn wounds successfully and safely, even in elderly individuals [60].

Sawarkar, et al. [61] conducted a case study examining the impact of PGF on the adverse effects induced by chemoradiation therapy in cancer patients. A 31-year-old female patient diagnosed with right infiltrative ductal carcinoma of the breast was recommended to receive chemoradiation therapy following surgical intervention. She experienced palpitations, anorexia, nausea, emesis, intense restlessness, and diaphoresis. She received PGF in conjunction with other Ayurvedic medicinal therapies for a duration of six months. Post-treatment, the patient had considerable alleviation of all symptoms caused by chemotherapy and radiotherapy. The case study demonstrates that PGF is beneficial in mitigating the effects caused by chemotherapy and radiotherapy [61].

The scientific research provides evidence for the medicinal properties of the Ayurvedic medicated ghee formulation known as PGF. Various disorders can be treated by a range of administration procedures. However, this medicated ghee is highly nutritious because it contains proteins, vitamins, amino acids, and minerals. The components utilized in the creation of PGF also possess individual therapeutic properties for the treatment of diverse human illnesses. The bio-drug formulation yields tremendous therapeutic effects when administered under medical supervision. Unsubstantiated and unsupported criticism of PGF without any credible evidence continues to be prevalent and is being published in non-prestigious journals, which is negatively affecting the reputation of Ayurvedic PGF therapies. The Ayurveda and scientific community must thoroughly explore mystical conceptions using scientific means and produce valid scientific findings, supported by corroborating data, which should be published in reputable journals. Only the validation of scientific evidence through peer review can provide insight into the misconceptions and drawbacks of PGF. While all synthetic medications in the world have adverse effects, no known side effects have been identified for PGF. However, further in-depth research is necessary to fully understand this matter. This review aims to enhance the scientific comprehension of PGF and explore strategies for achieving a more favorable social acceptance status in the future.

2. Conclusion

The traditional Ayurvedic therapy using PGF has shown remarkable therapeutic efficacy in treating various human ailments. However, due to its composition, including cow dung and cow urine, it has been less commonly prescribed and socially accepted. To establish the promising healing potential of PGF scientifically and with concrete evidence, comprehensive investigations are necessary. These investigations should focus on validating the formulation, examining the toxicity profile and safety of active chemical drug molecules, assessing its pharmacological potential, and elucidating the mechanism of therapeutic activity of the drug molecules.

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