INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Panchgavya Granules: A Novel Nutraceutical Approach to Immune Enhancement

Jahnavi Jadav, Dr. Navin Sheth, Dr. Ravi Manek*, Dr. Chetan Borkhataria

B. K. Mody Government Pharmacy College, Rajkot, Gujarat, India.

ARTICLE INFO

Published: 09 Sept 2025
Keywords:
Panchgavya granules, Immunomodulatory activity, Phagocytic index, Neutropenia, Chemotherapy, Toxicity evaluation, Nutraceutical approach.
DOI:
10.5281/zenodo.17088028

ABSTRACT

Panchgavya, a traditional Ayurvedic formulation comprising five cow-derived elements—milk, curd, ghee, urine, and dung—has long been valued for its immunomodulatory and therapeutic properties. This study aimed to formulate Panchgavya granules that include traditional Panchgavya Ghrita to evaluate their safety and immunomodulatory potential by various models in Albino Wistar rats. Granules were prepared using a two-phase process, ensuring uniform composition and palatability with improved shelf life. Acute oral toxicity was assessed as per OECD guideline 423, revealing no mortality or significant toxicity at doses up to 2000 mg/kg. The evaluation of immunomodulatory activities was conducted through the macrophage phagocytic index, triple antigen-induced paw oedema, hemagglutination titer, and cyclophosphamide-induced neutropenia models. Panchgavya granules demonstrated a significant dose-dependent increase in phagocytic index and hemagglutination titre, suggesting enhanced macrophage activity and humoral immune response. Furthermore, the granules attenuated paw oedema and improved leukocyte counts after cyclophosphamide administration, indicating their efficacy in modulating both cell-mediated and humoral immunity. The formulation also displayed favourable physicochemical properties, including good flowability and suspension stability. These findings suggest that Panchgavya granules are a safe and effective immunomodulatory nutraceutical formulation. Their synergistic action, driven by cow-derived bioactive constituents, presents a promising approach for immune enhancement, particularly in conditions requiring immunological support such as infections, autoimmune disorders, and cancer chemotherapy.

***Corresponding Author:** Dr. Ravi Manek
Address: *B. K. Mody Government Pharmacy College, Rajkot, Gujarat, India.*
Email ✉: ravimanek16@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

INTRODUCTION

The immune system plays a critical role in protecting the body from infections, diseases, and external pathogens. A well-functioning immune system is essential for overall health, and various natural formulations have been explored to enhance immune response. Ayurveda, the traditional Indian system of medicine, emphasises the use of natural compounds to support immunity. Among these, Panchgavya-based formulations have gained attention due to their potent immunomodulatory properties[1].

Panchgavya, derived from the Sanskrit words "Pancha" (five) and "Gavya" (cow), is a combination of five cow-derived products: Godugdha (cow milk), Gomaya (cow dung), Go-Ghrita (cow ghee), Takra (cow curd), and Go-mutra (cow urine)[2]. These components possess various therapeutic properties, including antimicrobial, antioxidant, detoxifying, and immunity-boosting effects[3, 4]. Classical Ayurvedic texts such as Charaka Samhita and Sushruta Samhita document their medicinal significance, particularly in treating chronic diseases and enhancing overall well-being[5, 6].

Panchgavya granules offer a novel and convenient approach to delivering these benefits[7]. Compared to traditional formulations such as liquids or powders, granules provide enhanced stability, improved palatability, and ease of administration. By incorporating herbal plants known for their immunomodulatory effects, such as Ashwagandha, Giloy, and Tulsi, Panchgavya granules create a synergistic effect that further enhances immune response. This combination strengthens the body's defence mechanisms while minimising potential side effects.

The advantages of Panchgavya granules over other formulations lie in their bioavailability, prolonged shelf life, and patient compliance. Granules ensure controlled dosage, reducing the chances of overconsumption or degradation, which is common in liquid formulations. Moreover, the incorporation of herbal extracts enhances the therapeutic efficacy, making them a promising alternative in disease management, particularly in conditions where immune enhancement is crucial, such as cancer, autoimmune disorders, and viral infections[8, 9].

Developing Panchgavya granules as a supportive therapy aligns with the growing demand for natural, holistic treatment approaches. Their immunomodulatory properties, combined with herbal medicine, offer a safe and effective strategy to boost immunity and manage diseases[10, 11].

2. MATERIALS AND METHODOLOGY

The formulation of Panchgavya granules involves a precise combination of natural ingredients to ensure optimal efficacy and stability[12]. The primary constituents include milk powder (400 g), which serves as a nutrient-rich base, and Panchgavya ghee (150 g), derived from traditional Ayurvedic practices, is utilised for its therapeutic attributes, including its role as a bioenhancer. Cocoa powder (50 g) is incorporated primarily to improve taste and mask the strong flavour of ghee. To achieve the desired sweetness and palatability, sugar (Sharkara) (400 g) is added, contributing to the granules' texture and taste.

The preparation of Panchgavya granules begins with the formulation of Panchgavya Ghee, which serves as a crucial component due to its Ayurvedic significance and therapeutic potential. The ghee is meticulously prepared following traditional methods to ensure its purity and efficacy. Once the Panchgavya Ghee is ready, it is incorporated into the granule formulation along with other ingredients in precise proportions.

The process begins with the preparation of cow dung extract, where fresh cow dung is mixed with an appropriate quantity of water to form a uniform paste. This paste is then filtered using a muslin cloth to remove coarse impurities, ensuring a refined extract. Once the extract is obtained, all five essential ingredients—cow ghee, cow milk, cow curd, cow urine, and cow dung paste—are collected for further processing.

Melted cow ghee serves as the base, into which hot cow milk, cow curd, cow urine, and cow dung paste are sequentially added. The mixture is heated until the froth disappears, signaling the separation phase, where a distinct two-finger roll-like structure is observed. The absence of a cracking sound when exposed to fire confirms the completion of the process. Finally, the mixture is filtered to remove impurities, yielding purified Panchgavya Ghrita, ready for use in granule preparation mentioned in Figure 1.

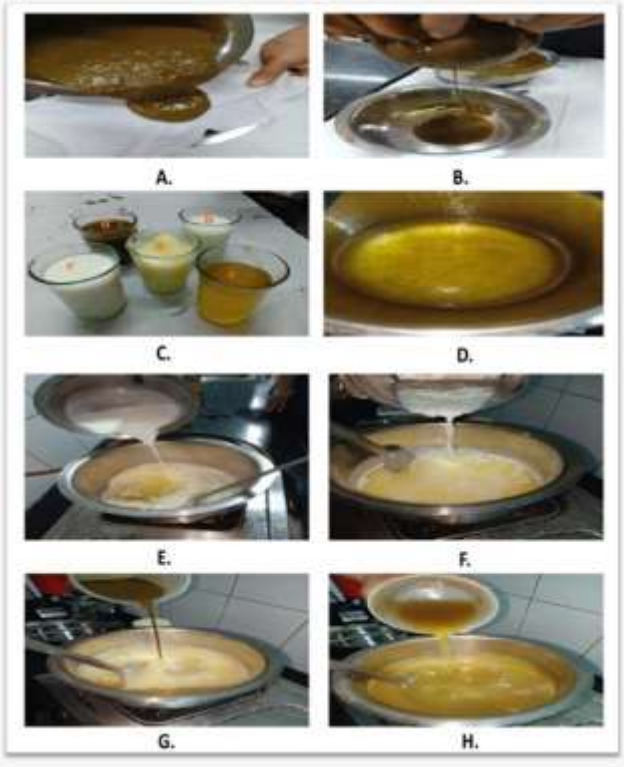


Figure: 1. Preparation of Panchgavya Ghee
A – Mixture of Gomaya and water, **B –** Filtration of Gomaya ras, **C** - a. Go Ghrita, b. Go Dugdha, c. Go Dahi, d. Gomaya rasa, e. Gomutra, **D -** Go Ghrita was melted, **E -** Hot Go Dugdha was added in Ghrita, **F -** Go Dahi was added, **G** - Gomaya rasa was added, **H** - Gomutra was added

2.1 Preparation of Panchgavya Granules

The preparation of Panchgavya granules involves two sequential phases to ensure uniform composition and stability. In Phase 1, the specified amounts of milk powder and cocoa powder are incorporated into Panchgavya Ghee and thoroughly mixed using a mortar to achieve a homogeneous blend. This step enhances the uniform dispersion of ingredients.

In Phase 2, a sugar syrup is prepared separately by dissolving the required quantity of sugar in water and heating it to the desired consistency. Once both phases are completed, they are combined to form a moist mass (slug), which is then passed through a 10-number sieve to obtain granules of uniform size. The granules are then subjected to

drying in a hot air oven, ensuring proper moisture reduction and improved shelf stability. The final dried Panchgavya granules are then ready for further use or analysis.

Figure: 2. Preparation of Panchgavya Granules

2.2 Acute Oral Toxicity Study:

An acute oral toxicity study of Panchgavya granules was conducted in female Albino Wistar rats following OECD guideline 423 over 14 days[13, 14]. Fifteen rats were randomly divided into five groups (n=3): one control and four treatment groups (A–D). The control group received only the vehicle, while Groups D, C, B, and A were administered 5, 50, 300, and 2000 mg/kg of Panchgavya granules, respectively.

Animals were observed daily for clinical signs, body weight, food consumption and water consumption. Animals were observed daily with a focus on mortality, clinical signs of toxicity, changes in general behaviour, skin, eyes, fur, mucous membranes, unusual respiration patterns, and somatomotor activity. Attention was directed to monitoring sleep, diarrhoea, tremors, convulsions, lethargy, and coma. After 14 days, all animals were fasted overnight and anaesthetised, and then blood samples were collected via retro-orbital plexus and used for haematology and clinical biochemistry analysis. The hematologic parameters recorded were white blood cell (WBC), neutrophil (NE), lymphocyte (LY), monocyte (MO), eosinophil (EO), basophil (BA), red blood cell (RBC), haemoglobin (HGB), haematocrit (HCT), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red blood cell distribution width (RDW). Clinical biochemistry values were alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (T-Bil), direct bilirubin (D-Bil), total protein (TP), globulin (Glo), albumin (Alb), blood urea nitrogen (BUN), creatinine (Cre), triglyceride (TG), and total cholesterol (TC)[15]. After taking the blood sample, the organs such as the heart, liver, lungs, spleen, kidneys, and reproductive organs were weighed,

and the relative weight of each organ was calculated.

No mortality or significant toxic effects were observed, indicating the formulation's safety. The LD_{50} of Panchgavya granules was estimated to be >2000 mg/kg, suggesting it is non-toxic according to GHS classification[16].

2.3 To perform immunomodulatory activity, animals were used:

Albino Wistar rats of both sexes were utilised in this study and maintained under standardised conditions. The experimental protocol was approved by the Institutional Animal Ethics Committee and conducted according to CPCSEA guidelines. The animals were housed at an ambient temperature of 25 ± 1°C, with a relative humidity of 45–65%, and maintained on a 12-hour light/dark cycle. Body weights were recorded before and after the experiment[17].

2.3.1 Macrophage Phagocytic Index in Rats:

In this model, 30 rates were used, divided into 5 groups (n=6 per group). The drug was given over seven days, as described in Table 1.

Table 1- Macrophage phagocytic index study design

Sr no	Group (n=6)	Treatment
1.	Normal	Drug vehicle P.O.
2.	Standard control	levamisole (2.5 mg/kg P.O.)
3.	Low Dose	250 mg/kg of Panchgavya granules formulation for 7 days
4.	Medium Dose	350 mg/kg of Panchgavya granules formulation for 7 days
5.	High Dose	500 mg/kg of Panchgavya granules formulation for 7 days

On day 8, the animals were injected intravenously with ink suspension (1 mL/250 g body weight) via the tail vein. Blood samples were collected from the retro-orbital plexus at 0- and 15-minute post-injection. Blood (50µL) was mixed with 4 mL of 0.1% sodium carbonate, and the solution was subsequently quantified for absorbance at 650nm using the spectrophotometer. The phagocytic index (K) was determined using the specified equation[18].

K= logOD1 – logOD2/15

where OD1 & OD2 are the optical densities at 0 and 15 min, respectively

2.3.2 Triple Antigen-Induced Immunological Paw Oedema:

Five groups of rats (n = 6 per group) were assigned for the study. A triple antigen suspension was made with alum precipitate in the following proportion: the triple antigen (1 mL), normal saline (4 mL), and potash alum (1 mL). The 10% sodium carbonate solution was used to adjust the pH to a value of 5.6–6.8. The test drugs were given on the sensitisation day and continued for 5 days. On day 5, one hour after receiving the experimental and standard treatments, rats were injected with 0.1 mL of the triple antigen-alum suspension beneath the plantar aponeurosis of the left hind paw. To monitor the changes in paw volume, an electronic plethysmograph was utilised at the intervals of 0, 24 and 48 hours after injection. The specified formula was used to estimate inhibitory activity[19].

% Inhibition = Vc - Vt / Vc * 100

where Vc is paw size at 24 hr and Vt is paw size at 48 hr

Table 2 – Triple antigen-induced immunological paw oedema study design

Days	Group (n=6)	Treatment

Day-1	**I-IV**	Sensitization of each animal with the suspension of triple antigen with alum precipitates subcutaneously in the nape of the neck in a dose of 1 ml/200g body weight.
Day 1-5	Drug administration	
	Group I (Control)	Drug Vehicle P.O.
	Group-II (Std. grp)	Levamisole (2.5 mg/kg) P.O.
	Group-III	250 mg/kg of granules formulation
	Group-IV	350 mg/kg of granules formulation
	Group-V	500 mg/kg of granules formulation
Day-6	**Group-I-V**	Challenge with 0.1ml of triple antigen suspension in the left hind paw

2.3.3 Haemagglutination Reaction:

2.3.3.1 Preparation of SRBC

Sheep blood was collected from the local slaughterhouse in a bottle containing Elsevier's solution. This is an isotonic balanced salt solution for red blood cells, and it is often employed as an anticoagulant or blood preservative for blood containing red blood cells during collection during preservation and transportation. The solution comprised 2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride.

Blood was centrifuged at 3000 RPM for 10 minutes using a cooling centrifuge. The serum was separated, and sheep red blood cells (SRBCs) were collected, washed thoroughly with sterile normal saline, and stored at refrigerated conditions until use. SRBCs from the same animal were used for both sensitisation and antibody titre determination.

Table 3- Haemagglutination reaction study design

Days	Group (n=6)	Treatment
Day 1-10	**Group-II**	Levamisole (2.5 mg/kg) P.O.
	Group-III	250 mg/kg of granules formulation P.O.
	Group-IV	350 mg/kg of granules formulation P.O.
	Group-V	500 mg/kg of granules formulation P.O.
Day-3	**Group-I-V**	Antigenically challenged by subcutaneous injection of 30% v/v SRBC (1 ml /200g)
Day-11	**Group-I-V**	Collection of blood via ocular puncture

2.3.3.2 Haemagglutination antibody titre:

Blood collection through retro-orbital puncture was conducted under light anaesthesia on day 11. The collected blood was then used to measure hemagglutination antibody (HA) titre. The microtiter plate was washed using distilled water and air-dried[20]. Distilled normal saline was added in 0.1 mL, followed by two-fold dilutions of the serum in sterile saline. Each dilution was set in 0.1 mL in 96-well microtiter plates, while 0.1 mL of 30 % v/v SRBC suspension was added. They were then left to incubate at room temperature for 2 hours and visually examined for agglutination against a white background. The HA titre was determined as the lowest serum dilution showing hemagglutination[21].

2.3.4 Cyclophosphamide-Induced Neutropenia:

The rats were administered the vehicle or test drug for 10 days. On the 10th day, a neutropenic dose of cyclophosphamide (100 mg/kg, i.p.) was given, recognised as day 0. All groups had blood samples taken through retro-orbital puncture and placed in tubes containing EDTA[22, 23]. Total leukocyte count (%) was assessed before and on day 3 post-cyclophosphamide injection. The results from the treatment groups were compared with those of the untreated control group[24].

Table 4- Cyclophosphamide-induced neutropenia study design

Days	Drug Administration	
Day 1-10	Group-II	Levamisole (2.5 mg/kg) P.O.
	Group-III	250 mg/kg of granules formulation P.O.
	Group-IV	350 mg/kg of granules formulation P.O.
	Group-V	50 mg/kg of granules formulation P.O.
Day-10	All group	Cyclophosphamide (100mg/kg IP)
Day-7th & 13th	Complete blood count (CBC)	

3. RESULT AND DISCUSSION:

3.1 Physico-chemical properties of Panchgavya granules:

The physicochemical properties of Panchgavya granules were analysed to assess their quality, stability, and efficacy. Various parameters were evaluated using standard methodologies, ensuring compliance with pharmaceutical and Ayurvedic formulation guidelines.

Table 5 - Organoleptic and Physicochemical Properties of Panchgavya Granules

Colour	Light brown
Odour	Predominant - milk
	very trace - cocoa and cow urine
Touch	Rough
Taste	Sweet, Milky
Bulk density	0.53 gm/ml
Tap density	0.5681 gm/ml
Suspension stability	Prepare (12% suspension w/v; 12gms in 100ml) at room temperature **Result:** No separation of layers with naked eyes observed in either medium in lukewarm condition (50-60 °C) for 30mins.
Flow property (Angle of repose)	**33.69° (Good flow) (funnel method/poured angle)** The slight hindrance in flow property of Panchgavya granules is unlikely due to sugar, cocoa, or milk powder, as the negligible loss on drying (0.04) indicates minimal hygroscopic influence.

3.2 Acute oral toxicity study

An acute oral toxicity study was performed as per OECD guidelines, and observations were recorded over a period of 14 days. No statistically significant changes were observed during the study. The results are shown in the table and figure below.

3.2.1 Evaluation Parameter:

Table 6 – Observational Parameter of acute oral toxicity study

Observation parameter	Normal														Treatment (Panchgavya Granules Formulation)												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Skin and fur	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lethargy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	-	✓	-	-	-	✓	-	-	-	-
Sedation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clonic convulsion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tonic extension	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Pilo erection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Muscle spasm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spasticity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

3.2.2 Effect of Panchgavya granules on body weight:

Table 7- Effect of Acute oral toxicity study of Panchgavya granules on body weight of rats:

Group (n=6)	Body weight in grams (MEAN ± SEM)		
	0 day	**7th day**	**14th day**
Normal	230 ± 3.65	243.0 ± 4.47	242.66 ± 2.10
Treatment (2 gm/kg)	235.0 ± 2.23	255.33 ± 7.03	260.66 ± 13.58

All values are represented as MEAN ± SEM, n=6. There was no statistically significant difference seen in body weight as compared to the normal group.

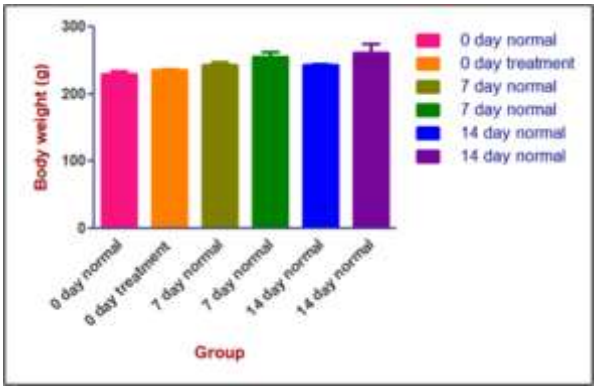


Figure: 3 Effect of Acute oral toxicity study of Panchgavya granules on body weight of rats

3.2.3 Effect of Panchgavya granules on food & water consumption of rats:

Table 8: Effect of Acute oral toxicity study of Panchgavya granules on food and water consumption of rats:

Groups (n=6)	Water consumption (ml/day/group)
Normal	101.0 ± 2.23
Treatment	108.45 ± 4.07
Groups (n=6)	**Food consumption (gm/day/group)**
Normal	32.04 ± 0.81
Treatment	34.02 ± 1.23

All values are expressed as Mean ± SEM. There was no statistically significant difference seen in food & water consumption as compared to the normal group.

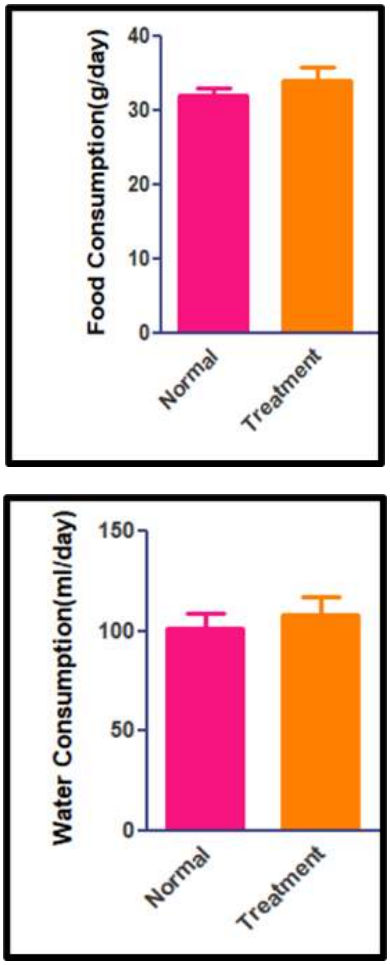


Figure: 4: Effect of Acute oral toxicity study of Panchgavya granules on food and water consumption of rats

3.2.4 Effect of Panchgavya granules on Haematological parameter of rats:

Table 9: Effect of Acute Oral Toxicity Study of Panchgavya Granules Formulation on Haematological Parameters of Rats

Parameter	Normal	Treatment
Haemoglobin (g/dL)	14.48 ± 0.29	14.47 ± 0.20
RBC (10^6/mm^3)	7.19 ± 0.31	8.22 ± 0.45
WBC (10^6/mm^3)	9328.33 ± 1073.64	9173.66 ± 786.14
Platelets (10^3/mm^3)	547333.3 ± 9035.73	558178.5 ± 22887.89
HCT (%)	40.95 ± 1.28	42.09 ± 0.34
MCV (Fl)	137.43 ± 79.91	136.56 ± 80.35
MCH (pg)	20.26 ± 0.60	19.16 ± 0.65
MCHC (g/dL)	35.45 ± 0.46	35.25 ± 0.35
RDW-CV (%)	14.15 ± 0.03	14.25 ± 0.04
Neutrophils (%)	21.66 ± 3.73	19.22 ± 5.78
Lymphocytes (%)	71.5 ± 3.96	71.56 ± 3.47
Eosinophils (%)	1.16 ± 0.16	1.16 ± 0.16
Monocytes (%)	5.16 ± 0.40	3.23 ± 0.38

*** All values are expressed as Mean ± SEM (n=6)**

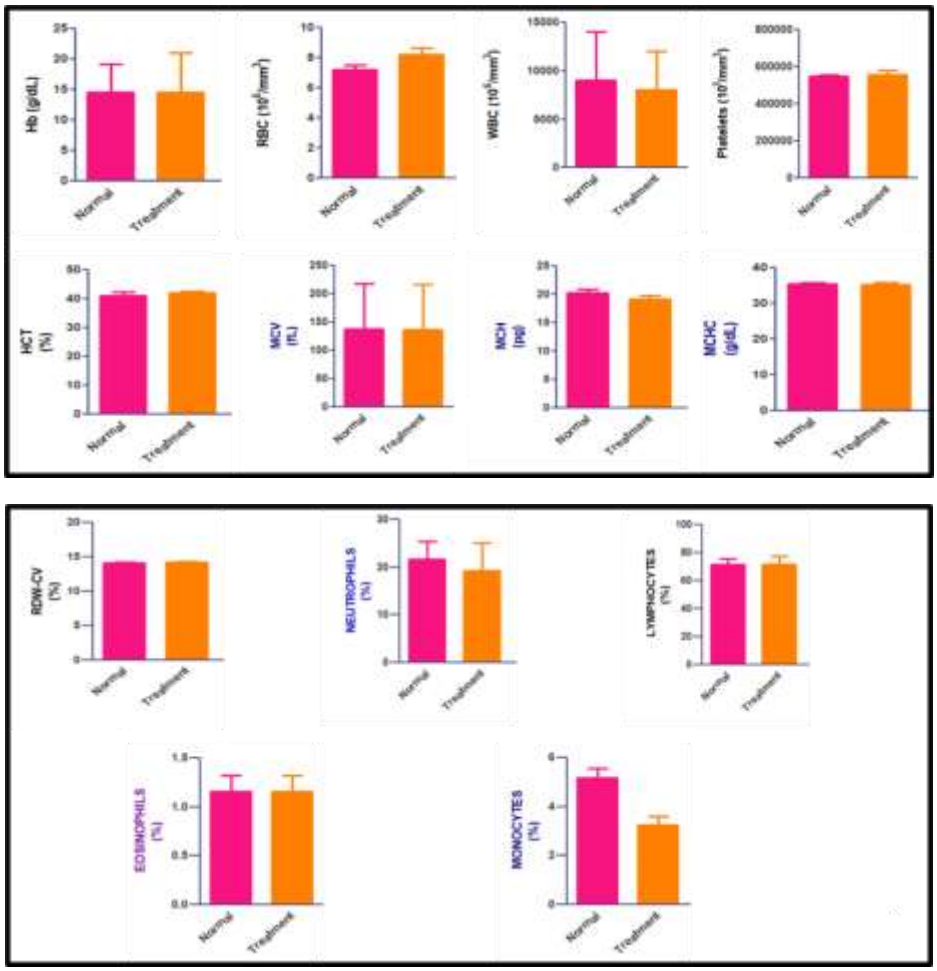


Figure: 5: Effect of Acute oral toxicity study of Panchgavya granules on Haematological Parameters of Rats

3.2.4 Effect of Panchgavya granules on Biochemical parameter of rats:

Table -10: Effect of Acute oral toxicity study of Panchgavya granules formulation on biochemical parameters of rats

Parameter	Normal	Treatment
Glucose (mg/dL)	134.78 ± 12.91	130.68 ± 10.99
Cholesterol (mg/dL)	131.57 ± 12.47	147.86 ± 15.62
TG (mg/dL)	91.93 ± 15.94	90.99 ± 15.68
Bilirubin (mg/dL)	1.04 ± 0.06	0.98 ± 0.09
SGOT (U/L)	224.14 ± 18.35	223.50 ± 25.33
SGPT (U/L)	60.7 ± 10.68	62.56 ± 7.89
Total protein (g/dL)	7.88 ± 0.14	7.81 ± 0.22
Creatinine (mg/dL)	0.99 ± 0.05	0.98 ± 0.08
BUN (mg/dL)	152.66 ± 8.43	150.89 ± 12.78
Uric acid (mg/dL)	2.73 ± 0.23	2.70 ± 0.22

***All values are expressed as Mean ± SEM (n=6)**

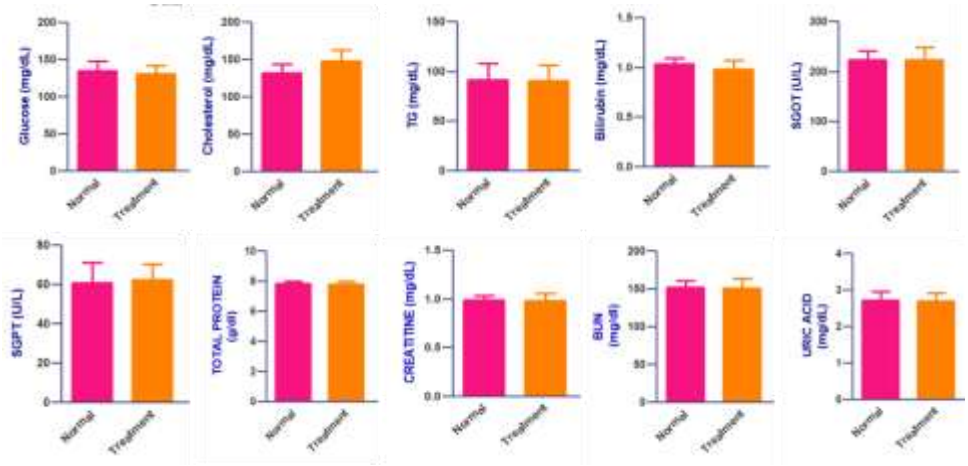
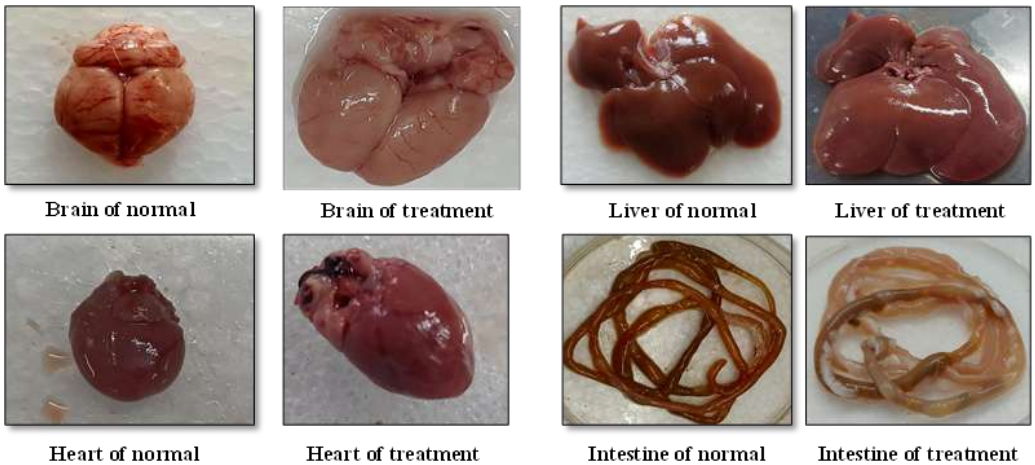


Figure: 6: Effect of Acute oral toxicity study of Panchgavya granules on formulation on biochemical parameters of rats.

3.2.3 Effect of Panchgavya granules on Gross necropsy of rats:



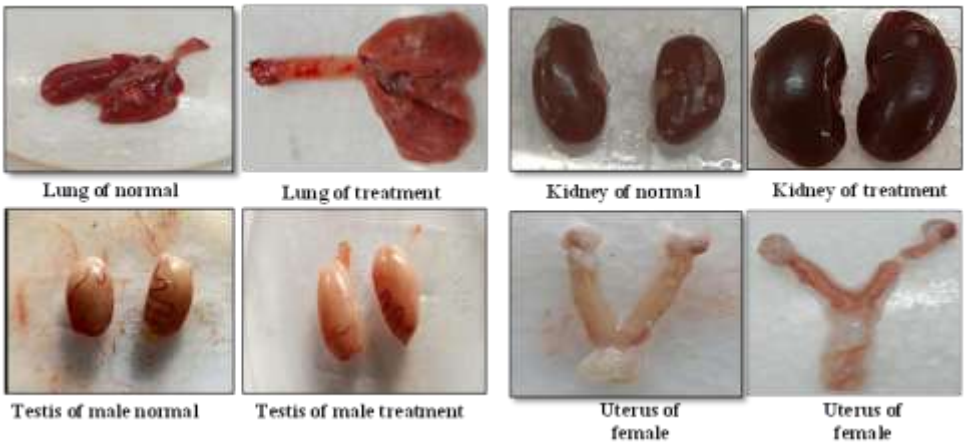


Figure: 7: Effect of Acute oral toxicity study of Panchgavya granules formulation on Gross Necropsy

There were no gross pathological abnormalities found in both the normal control group and the treatment control groups.

3.3 Macrophage Phagocytic Index in Rats

Administration of Panchgavya granules low dose (250 mg/kg, po), medium dose (350mg/kg, po) and High dose (500 mg/kg) produced increase in clearance of carbon particles from blood as indicated by a significant increase in phagocytic index (P<0.05) as compared to normal group.

Table 11- Effect of Panchgavya granules on macrophage phagocytic index

Treatments (n=6)	Phagocytic index	
	MEAN	SEM
Normal	0.001733	0.000517
Standard- Levamisole (2.5 mg/kg)	0.00735	0.002334*
Low Dose (250 mg/kg)	0.0042	0.000759*
Medium Dose (350 mg/kg)	0.006867	0.00259*
High Dose (500 mg/kg)	0.007967	0.001564*

All values are expressed as Mean ± SEM; n = 6 for each group

***Indicates significant difference from Normal group at (p<0.05)**

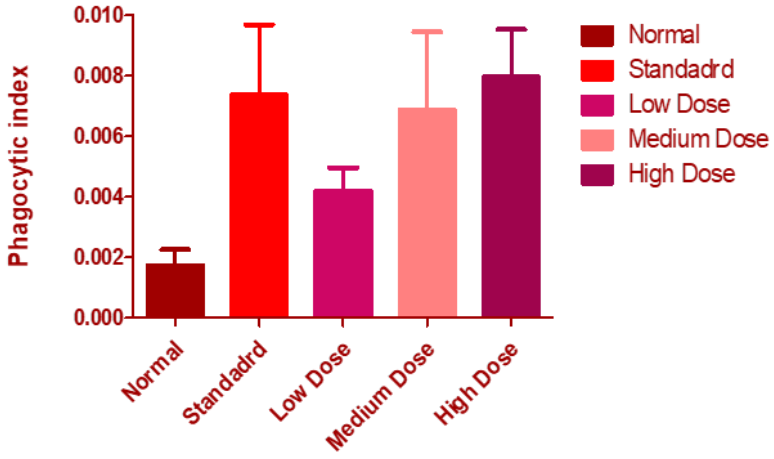


Figure: 6: Effect of Panchgavya granules on macrophage phagocytic index

*Indicates significant difference from Normal group at (p<0.05) (M/C remaining)

The clearance of particulate matter from circulation is primarily mediated by the reticuloendothelial system (RES), with macrophages playing a pivotal role. Colloidal carbon particles, such as those in ink, follow an exponential clearance model regulated by macrophage-driven phagocytosis. This essential

process, executed by macrophages, neutrophils, and monocytes, involves the internalisation of particulates into phagosomes, which subsequently fuse with lysosomes to form phagolysosomes, facilitating enzymatic degradation and oxidative breakdown. In this study, the administration of Panchgavya granules demonstrated a dose-dependent enhancement in phagocytic activity, evidenced by increased uptake and accelerated clearance of colloidal carbon particles, indicating improved particulate elimination efficiency.

The immunomodulatory potential of Panchgavya granules is attributed to their rich phytochemical profile, including alkaloids, flavonoids, carbohydrates, and glycosides, which stimulate the RES. As a potent immunomodulator, Panchgavya granules regulate the activation of B and T lymphocytes, macrophages, natural killer (NK) cells, neutrophils, and dendritic cells, reinforcing its role in immune modulation.

3.4 Triple antigen-induced immunological paw oedema:

Paw oedema increased in all groups at 24 hours. By 48 hours, a reduction in oedema was observed in the standard control, low-dose (250 mg/kg, p.o.), medium-dose (350 mg/kg, p.o.), and high-dose (500 mg/kg, p.o.) groups. But there is less inhibition of paw oedema at 48 hr in Panchgavya-treated groups and the standard group. While all doses of Panchgavya granules reduced paw volume at 48 hours, the changes were not statistically significant compared to the normal group. Notably, the high dose (500 mg/kg) and standard drug levamisole significantly reduced paw volume inhibition ($P < 0.05$).

Table 12 Effect of Panchgavya granules in triple antigen-induced immunological paw oedema

Treatments	Volume of oedema at different hours (ml)			% Inhibition of paw oedema at 48 hr.
	0 hour	24 hours	48 hours	
Normal	2.29 ± 0.05	4.27 ± 0.02	2.99 ± 0.04	29.97%
Standard	2.32 ± 0.07	5.12 ± 0.03	4.25± 0.02	16.99%*
Dose-1	3.60 ± 0.07	5.25 ± 0.06	3.72 ± 0.05	29.14%
Dose-2	3.75 ± 0.06	5.49 ± 0.08	3.99 ± 0.07	27.32%
Dose-3	3.62 ± 0.05	4.98 ± 0.05	4.01 ± 0.08	19.47%*

All values are expressed as Mean ± SEM; n = 6 for each group

***Indicates significant difference from Normal group at (p<0.05)**

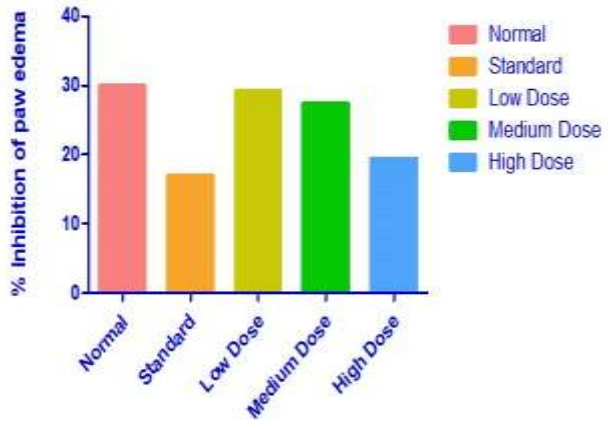


Figure: 7: Effect of Panchgavya granules % inhibition of paw volume at 48 hr

*Indicates significant difference from Normal group at ($p<0.05$) (M/C remaining)

Cell-mediated immunity is crucial in graft rejection, tumour defence, and protection against intracellular pathogens and chronic infections. Delayed-type hypersensitivity (DTH) responses involve the activation of sensitised T lymphocytes, which differentiate into lymphoblasts and secrete lymphokines to recruit immune cells to the antigenic site, sustaining the inflammatory response. In the challenge phase, animals received an intraplantar injection of the sensitising allergen, directing antigen-specific T cells to the site, where they released cytokines to mediate inflammation. Activated T lymphocytes triggered lymphoblast transformation and lymphokine secretion, leading to localised swelling and inflammation within 24 hours, marked by increased vascular permeability, vasodilation, and macrophage activation.

Panchgavya granule treatment resulted in a dose-dependent reduction in paw volume inhibition at 48 hours, indicating enhanced vascular permeability, vasodilation, and macrophage activation, along with increased class II MHC molecule expression. These findings suggest that Panchgavya granules enhance cell-mediated immunity, likely due to their immunomodulatory properties. The presence of flavonoids in cow dung may contribute to this effect by modulating β and T cell activation.

3.5 Haemagglutination antibody titre:

All doses of treatment, including low (250 mg/kg, p.o.), medium (350 mg/kg, p.o.), and high (500 mg/kg, p.o.), significantly reduced the hemagglutination antibody titre compared to the control group.

Table 13- Effect of Panchgavya granules on HA titre

Treatments	Haemagglutination
Normal	0.018 ± 0.02
Standard Levamisole (2.5 mg/kg)	0.05 ± 0.009*
Dose-1	0.098 ± 0.01*
Dose-2	0.092 ± 0.01*
Dose-3	0.088 ± 0.01*

All values are expressed as Mean ± SEM; n = 6 for each group

*Indicates significant difference from the Normal group at $p<0.05$

The indirect haemagglutination test was carried out to determine the effect of Panchgavya granules on the humoral immunity system. Administration of the standard drug (levamisole) showed a significant increase in HA titer (antibody titer) compared to the normal control group. HA titer in low dose, medium dose and high dose group at a dose of 250 mg/kg [0.018 ± 0.02, 350 mg/kg [0.092 ± 0.01], 500 mg/kg [0.088 ± 0.01] was significantly ($p< 0.05$) increased when compared with control group [0.18 ± 0.02] respectively. The increase in HA titer correlated with the activation of the humoral immune response.

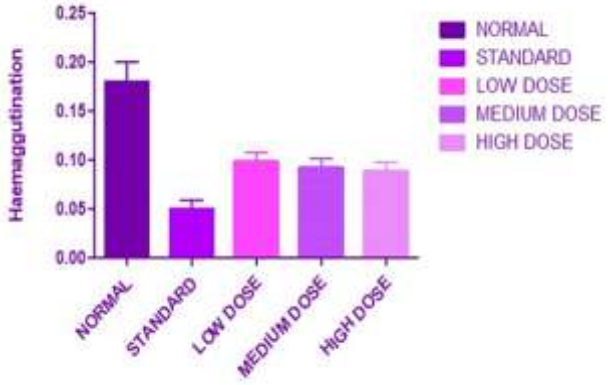


Figure: 8: Effect of Panchgavya granules on HA titre

***Indicates significant difference from Normal group at ($p<0.05$)**

Hemagglutination is a technique for detecting foreign particles based on the ability of certain enveloped viruses to adsorb onto red blood cells

(RBCs). Hemagglutinin, a viral envelope glycoprotein, induces RBC agglutination, forming a lattice structure instead of a distinct red dot. In the presence of antibodies, antigen-neutralisation leads to cross-linking, enhancing clearance via phagocytosis.

Test animals treated with conventional drugs and granules exhibited increased circulating antibody titers. The assay involved serial serum dilutions with standardised sheep RBCs (SRBCs), where agglutination indicated antibody binding. Data from Table 7 and Figure 3 demonstrate that pretreatment with both standard and granule elevated circulating antibody levels, confirming humoral immune activation. The rise in hemagglutination titer suggests enhanced secondary antibody production, indicating β-lymphocyte differentiation into memory cells against the SRBC antigen.

3.6 Cyclophosphamide-induced neutropenia:

Administration of Panchgavya granules and standard per oral had a significant reduction in neutropenia condition as demonstrated by a significant increase ($p<0.05$) in total leukocyte count level when compared to the normal group.

Panchgavya granules given before cyclophosphamide administration provided significant protection against cyclophosphamide-induced neutropenia compared to the control group.

Table 14- Effect of Panchgavya granules on cyclophosphamide-induced neutropenia

Treatment	Total leucocyte count (10^3 cells/mm^3)		% Reduction of Total leucocyte count
	Before cyclophosphamide	After cyclophosphamide	
Normal	12.60± 0.73	4.89 ± 0.71	61.19
Standard	11.01 ± 1.00	08.87 ± 1.08	19.43*
Dose-1	08.60 ± 1.06	06.39 ± 0.50	25.69*
Dose-2	11.15 ± 1.28	08.50 ± 1.09	23.76*
Dose-3	13.18 ± 1.78	10.53 ± 1.78	20.10*

All values are expressed as Mean ± SEM; n = 6 for each group

*Indicates significant difference from the Normal group at $p<0.05$

The percentage reduction in total leukocyte count was lower in the standard group and all Panchgavya granule-treated groups. This indicates a greater difference in leukocyte counts before and after cyclophosphamide (100 mg/kg, i.p.) administration compared to the standard and Panchgavya-treated groups.

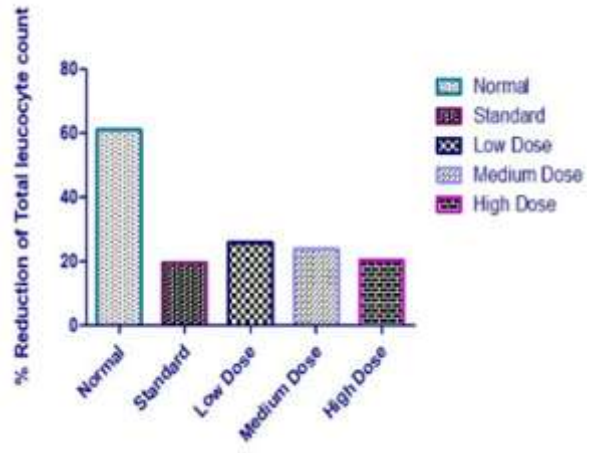


Figure: 9: % reduction of total leucocyte counts

*Indicates significant difference from Normal group at ($p<0.05$)

Cyclophosphamide, a potent anticancer agent, alkylates DNA but induces neutropenia due to bone marrow suppression. Its metabolism by microsomal enzymes generates reactive metabolites that produce free radicals, causing cellular damage. Immunostimulants counteract these effects by enhancing bone marrow function.

In this study, Panchgavya granules significantly reduced cyclophosphamide-induced neutropenia, indicating their potential role in hematopoietic support. Their protective effect against radiation-induced hematopoietic suppression is likely attributed to their rich antioxidant content, which helps mitigate oxidative stress and sustain haematopoiesis.

CONCLUSION:

The present study highlights the promising immunomodulatory potential and safety profile of Panchgavya granules formulated with cow-derived bioactive and traditional Ayurvedic herbs. Acute toxicity evaluations revealed no harmful effects at doses up to 2000 mg/kg, confirming their safety for oral administration. Across multiple experimental models—including macrophage phagocytic index, triple antigen-induced paw oedema, hemagglutination titre, and cyclophosphamide-induced neutropenia—the granules consistently exhibited dose-dependent immune-enhancing effects. Notably, there was a significant increase in macrophage activity, enhancement of humoral response, and partial reversal of neutropenia, suggesting both innate and adaptive immune stimulation. The granule dosage form further improves palatability, stability, and patient compliance, making it a suitable candidate for long-term use as a supportive therapy.

REFERENCES

1. Chauhan R. Cowpathy in cancer management. Recent Advances in “Immunobiotechnology: IBT Patwadangar (UK); 2010. p. 75-85.
2. Mahajan SP, Chavan SA, Shinde SA, Narkhede MB. Miraculous benefits of cow urine: a review. J Drug Deliv Ther. 2020;10:275-81.
3. Ghosh T, Biswas M. Evaluation of antibacterial and antifungal activity of cow urine against some seed borne microflora. International Journal of Current Microbiology and Applied Sciences. 2018;7(5):1714-27.
4. Krishnaveni S, Mamatha M. Effect of cow urine treatment on plant growth and anti-microbial activity on Gossypium hirsutum L. Plant Archives (09725210). 2021;21(2).
5. Ganesh P. All About Panchagavya for Human usage–A Review.
6. Joshi DR, Adhikari N. Benefit of cow urine, milk, ghee, curd and dung versus cow meat. Acta Scientific Pharmaceutical Sciences. 2019;3(8):169-75.
7. Bajaj KK, Chavhan V, Raut NA, Gurav S. Panchgavya: A precious gift to humankind. Journal of Ayurveda and integrative medicine. 2022;13(2):100525.
8. Bhojraj N, Sawarkar G. The effect of Panchagavya formulations in the case of CA Rectum. International Journal of Ayurvedic Medicine. 2020;11(3):572-4.
9. Nithya V, Thavasuraj S. Effect of Indigenous cow ark with Plectranthus amboinicus for anticancer activity–In vitro. Gedrag & Organisatie Review. 2020;33(3):705-11.
10. Gottimukkala KSV, Mishra B, Joshi S, Reddy MK. Cow urine: Plant growth enhancer and antimicrobial agent. Journal of Horticulture and Plant Research Vol. 2019;8:31.
11. Bhandari M, Ravipati AS, Reddy N, Koyyalamudi SR. Traditional ayurvedic

medicines: pathway to develop anti-cancer drugs. J Mol Pharm Org Process Res. 2015;3(3):1000130.
12. Arumugam DG, Sivaji S, Dhandapani KV, Nookala S, Ranganathan B. Panchagavya mediated copper nanoparticles synthesis, characterization and evaluating cytotoxicity in brine shrimp. Biocatalysis and agricultural biotechnology. 2019;19:101132.
13. Bedi O, Krishan P. Investigations on acute oral toxicity studies of purpurin by application of OECD guideline 423 in rodents. Naunyn-Schmiedeberg's archives of pharmacology. 2020;393(4):565-71.
14. Umeshgiri SGB, Nagraj A, Meghraj RH, Srinivasamurthy A. Evaluation of Acute Oral Toxicity of a Polyherbal Ayurvedic Formulation in Wistar Rats as per OECD 423. International Journal of Ayurveda and Pharma Research. 2025:16-21.
15. Pal R, Gulati K, Banerjee B, Ray A. Pharmacological and biochemical studies on the role of free radicals during stress-induced immunomodulation in rats. International immunopharmacology. 2011;11(11):1680-4.
16. Onisei T, Tihăuan B-M, Dolete G, Axinie M, Răscol M, Isvoranu G. In vivo acute toxicity and immunomodulation assessment of a novel nutraceutical in mice. Pharmaceutics. 2023;15(4):1292.
17. Afolayan FI, Erinwusi B, Oyeyemi OT. Immunomodulatory activity of curcumin-entrapped poly d, l-lactic-co-glycolic acid nanoparticles in mice. Integrative medicine research. 2018;7(2):168-75.
18. Ganeshpurkar A, Saluja AK. Protective effect of catechin on humoral and cell mediated immunity in rat model. International Immunopharmacology. 2018;54:261-6.
19. Khedekar S, Priya A. Immunomodulatory activity of Swarna Prashana in Charle's Foster albino rats. Journal of Ayurveda Medical Sciences J. 2016;1(2).
20. Mathivanan R, Kalaiarasi K. Panchagavya and Andrographis paniculata as alternatives to antibiotic growth promoters on haematological, serum biochemical parameters and immune status of broilers. The journal of poultry science. 2007;44(2):198-204.
21. Yadav SS, Prajapati P, Ashok B, Ravishankar B. Evaluation of immunomodulatory activity of "Shirishavaleha"–An Ayurvedic compound formulation in albino rats. Journal of Ayurveda and integrative medicine. 2011;2(4):192.
22. Kolathingal-Thodika N, Usha P, Sujarani S, Suresh NN, Priya P, Naseef PP, et al. A cyclophosphamide-induced immunosuppression Swiss Albino mouse model unveils a potential role for cow urine distillate as a feed additive. Journal of Ayurveda and Integrative Medicine. 2023;14(5):100784.
23. Morley A, Stohlman Jr F. Cyclophosphamide-induced cyclical neutropenia: an animal model of a human periodic disease. New England Journal of Medicine. 1970;282(12):643-6.
24. Ismail S, Asad M. Immunomodulatory activity of Acacia catechu. Indian J Physiol Pharmacol. 2009;53(1):25-33.

HOW TO CITE: Jahnavi Jadav, Dr. Navin Sheth, Dr. Ravi Manek, Dr. Chetan Borkhataria, Panchgavya Granules: A Novel Nutraceutical Approach to Immune Enhancement, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 9, 1119-1134. https://doi.org/10.5281/zenodo.17088028