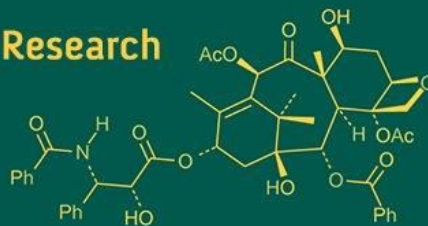
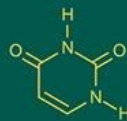
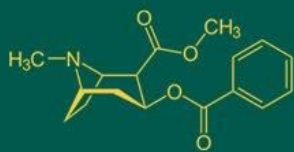


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28-Day repeated dose toxicity study of polyherbal gel formulation in Wistar rats

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Abstract

Background: The selected herbs for the polyherbal gel preparation include *Centella asiatica*, *Curcuma longa*, *Azadirachta indica*, *Ocimum tenuiflorum* and *Glycyrrhiza glabra* are traditionally used to treat wounds and skin conditions. This study aimed to evaluate the safety profile of the novel polyherbal gel formulation developed for treating oral ulcerative lesions in goats.

Methods: The polyherbal gel formulation was prepared with optimized concentrations of plant extracts, Carbopol 934, HPMC, propylene glycol-400, ethanol, methyl paraben, sodium benzoate, triethanolamine and water. In subacute toxicity study, the Polyherbal gel was administered orally at doses of 250, 500, and 1000 mg/kg daily for 28 days to Wistar rats. Behavioural changes, body weight and selected biochemical and hematological parameters were determined at the end of the experiment. Sections of livers and kidneys, lung, spleen, heart, brain, testes and ovary were removed for histopathological studies.

Result: The results of the study, It ensures that the safety evaluation in Wistar rats demonstrated no signs of toxicity at the highest dose, classifying the formulation as non-toxic with no adverse effects in a 28-day repeated dose oral toxicity study.

Keywords: Polyherbal gel, Oral ulcers, Toxicity study, NOAEL

Introduction

It is well known that a variety of dosage forms, primarily PHF (Polyherbal formation), are used in the preparation of Ayurvedic herbals. Polyherbalism offers certain advantages not found in a single herbal formulation because of its synergistic effects. A single multi-constituent formulation can yield better therapeutic results (Parasuraman *et al.*, 2014) [8]. Gels have been utilized in the current situation to deliver drugs to the body. For the treatment of oral ulcerative lesions, no therapy can be considered fully effective, despite the commercial availability of numerous formulations such as suspensions, ointments, and solutions. The use of polyherbal oral mucosal gel can increase the therapy's effectiveness. To prolong the duration of the medication's stay at the application site, the gel formulation's base must possess a satisfactory level of mucoadhesion. These medication delivery systems create close contact with the oral mucosal membrane, which speeds up the release of drug molecules at the site of absorption and improves the drug's bioavailability (Bhinge *et al.*, 2017) [3]. But, it's crucial to test novel formulations for a specified period to assess potential toxic effects due to phytochemical interactions. Therefore, a Preclinical toxicity study of new drugs through *in vivo* testing is essential to ensure safety before being tested in clinical trials on volunteers or patients. Hence, the present study explores the safety profile of the polyherbal gel developed with five herbs.

Materials and Methods**Extraction and Polyherbal Gel Formulation**

The plants were selected based on their wound healing property, tissue regeneration property, antimicrobial property, antioxidant property, anti-inflammatory property and analgesic property include *Centella asiatica*, *Curcuma longa*, *Azadirachta indica*, *Ocimum tenuiflorum* and *Glycyrrhiza glabra*. Polyherbal gel was developed using alcoholic extracts of each plants, Carbopol 934, HPMC, propylene glycol-400, ethanol (q.s), methyl paraben,

sodium benzoate, triethanolamine and water (q.s). The gel preparation followed the method devised by Mate *et al.* (2021) ^[5]. Excipients and preservatives were added following the inclusion levels mentioned by Sheshkey *et al.*. Calculated amount of soaked gelling polymers, plant extracts and excipients were mixed thoroughly in the mortar and pestle. On addition of triethanolamine, the required bubble-free, homogenous gel consistency was achieved.

Experimental Animals

The safety study was conducted in Laboratory Animal House, Veterinary College and Research Institute, Tirunelveli with the IAEC approval (Lr.No. 2016/IAEC/VCRI, TNI/2023, dated 26.07.2023). Wistar rats were purchased from the Laboratory Animal Medicine Unit, Directorate of Centre for Animal health studies, TANUVAS, Madhavaram Milk Colony, Chennai. Temperature and relative humidity were maintained between 22 and 26 °C and 30 and 70 per cent respectively. Light and dark cycles were controlled to give approximately a sequence of 12 hours of light and 12 hours of dark (Abebe, 2023) ^[1].

Animals were provided with *ad libitum* supply of feed and purified drinking water during the entire study period. Animals were acclimatized for seven days before the treatment and observed for clinical signs daily. On the day of initiation of the experimental trial, body weights were recorded and clinical examinations of animals were performed. Animals were identified by tail marking throughout the treatment period.

Experimental design

The repeated dose 28-day oral (gavage) toxicity study of polyherbal gel was conducted as per the OECD TG 407 (OECD 407, 2008) using 18 male and 18 female Wistar rats. The rats were randomly distributed to six different groups using the RAND function in Excel and it was ensured that the variation in body weight of the animals was not $\geq 20\%$ (Tiwari *et al.*, 2020) ^[12]. The treatment schedule for each group is detailed in the Table 1.

Table 1: Experimental Design of 28 Day Repeated Dose Oral Toxicity Study in Wistar Rats

S. No.	Groups	Treatments	Dose (mg/kg b.wt/day/p.o)	No. of animals	Recovery group
1	T ₁	Vehicle control	0	3M+3F	3M+3F
2	T ₂	Low dose	250	3M+3F	
3	T ₃	Mid dose	500	3M+3F	
4	T ₄	High dose	1000	3M+3F	3M+3F

General Observations

Clinical signs were observed on animals once daily. Animals were examined for changes in skin, fur, eyes, mucous membranes, presence of secretions and excretions. Changes in gait, posture and response to handling, presence of clonic or tonic movements and stereotypic or bizarre behavior were observed and recorded. The animals were observed twice daily for morbidity and mortality. (Kharchoufa *et al.*, 2020) ^[4].

Body Weight

Body weight (g) was recorded weekly during the treatment period. On the day 28 of treatment, the animals were kept on

water only fasting and on day 29 fasting weights were recorded before necropsy to calculate organ to fasting body weight ratio.

Feed Consumption

Feed consumption (g) was recorded for 24 h once daily for four weeks during the treatment period using an electronic weighing balance. Cage wise animal feed consumption (g) was recorded from the amount of feed offered and feed left over for 24 hours once every week. The feed consumption was normalized to 100g body weight of the animal.

Necropsy

Rats from the control and treatment groups were sacrificed at the end of the treatment period on day 29. The animals were kept for overnight fasting (12 - 14 h) and euthanized by intraperitoneal administration of xylazine and ketamine at the dose rate of 10mg/kg b.wt and 80 mg/kg b.wt, respectively (Struck *et al.*, 2011) ^[11]. Necropsy was performed on all animals and tissues were collected for histopathology in 10 percent neutral buffered formalin.

Blood Collection

Terminal blood collection was done by cardiac puncture. For the analysis of haematological parameters blood samples were collected in potassium EDTA-coated vacutainer and were analysed using autoanalyser (Exigo). For serum biochemistry analysis, blood was collected in plain vacutainer. It was allowed to clot for about 20 min and then serum was separated by centrifuging 2655 x g for 10 min and were analysed using automated biochemical analyser (Selectra PRO XS, Netherland).

Gross Pathology

Gross pathological observations were made on all animals both externally and internally. The external examination included the examination of external surfaces and orifices while the internal examination included the examination of abdominal, thoracic and cranial cavities, various organs and tissues after opening the body.

Organ Weight

The organs such as liver, kidneys, heart, brain, spleen, ovary and testes were trimmed off to remove any adherent tissue or fat and weighed wet as soon as possible to avoid drying. The organ weights were converted into relative organ weights (Organ weight to body weight ratio). After weighing, the organs were fixed in 10 percent neutral buffered formalin.

Histopathology

The tissue samples were trimmed into a thickness of about 3-4 mm. Tissues were processed, embedded in paraffin, sectioned at 3-5 mm thickness and stained with haematoxylin and eosin (H&E) for histopathological examination (Slaoui and Laurence, 2011). The prepared slides were examined under the microscope to record histopathological findings in different organs. The severity grade of findings was recorded as minimal, mild, moderate, marked and severe. The lesions, if any, were identified to understand the toxic changes in organs.

Results and Discussion

The animals were observed for behavioural and any outward manifestations of the given formulation. From the results, it is evident that there was no observable behavioural and skin morphological changes observed in rats.

Tables 2 and 3 present the body weight of male and female rats, respectively, expressed as mean±standard error. No significant ($p>0.05$) increase in body weight was observed in the high-dose group (1000 mg/kg b.wt). Similarly, no significant changes in body weight were noted in other groups.

Table 2: 28 day Repeated Dose Oral Toxicity Study of PHG - Body Weight of Male Wistar Rats

Group	0 Day	7 th day	14 th day	21 st day	28 th day	35 th day	42 nd day
T ₁	152±5.20	174.3±5.49	199±4.62	222.7±4.91	247±4.93	-	-
T ₂	147±5.29	18.3±5.81	192±4.62	216±3.79	242.7±3.93	-	-
T ₃	164.7±4.33	186.7±3.93	210±5.69	234.3±5.55	260.7±3.48	-	-
T ₄	156.3±8.19	178.7±8.09	205.7±7.97	230±6.25	255.3±7.31	-	-
T ₅	172±5.20	194.3±5.49	217.30±4.91	242.7±4.91	267±4.93	291±4.93	317±5.51
T ₆	176.3±8.19	198.7±8.09	225.7±7.97	250.3±6.49	275.3±7.31	296.7±7.36	322.3±7.69

Table 3: 28 Day Repeated Dose Oral Toxicity Study of PHG - Body Weight of Female Wistar Rats

Group	0 Day	7 th day	14 th day	21 st day	28 th day	35 th day	42 nd day
T ₁	144.3±5.21	161±4.73	179±4.62	195.7±5.21	215.7±5.04	-	-
T ₂	158±5.51	174±5.77	191.7±4.33	210.7±4.37	231.7±5.46	-	-
T ₃	156±5.51	171.7±3.76	190.3±5.36	209.3±5.84	230.7±4.81	-	-
T ₄	148.7±4.91	166.3±3.53	184.7±3.84	203.7±5.78	224±6.08	-	-
T ₅	164.3±5.21	181±4.73	199±4.62	216±5.20	233.7±4.98	250.7±5.36	267.3±5.46
T ₆	168.7±4.91	186.3±3.53	204.7±3.84	223.3±5.49	244.3±5.78	261.7±6.06	279.3±6.94

T₁ - Control; T₂ - Low dose, 250 mg/kg b.wt; T₃ - Medium dose, 500 mg/kg b.wt;

T₄ - High dose, 1000 mg/kg b.wt; T₅ - Recovery group, Control;

T₆ - Recovery group, High dose, 1000 mg/kg b.wt

Values are expressed as Mean±Standard Error of the Mean (SEM); (n=3)

P>0.05; No statistical significance was observed among all the parameters mentioned.

These findings contribute to the assessment of the repeated dose toxicity, and the observed increase in body weight in the high-dose group may warrant further investigation to understand its implications. Overall, the study provides valuable information on the potential effects of the polyherbal formulation at different dose levels.

Tables 4 display the feed intake (g/day/animal) of the male and female rat treatment groups, respectively. Notably, there were no significant differences in feed consumption among any of the treatment groups.

Table 4: Feed Intake of both Male and Female Wistar Rats

Repeated Dose 28 Day Oral Toxicity Study of PHF - Feed Intake in Male Wistar Rats (g/day/animal)							Repeated Dose 28 Day Oral Toxicity Study of PHF - Feed Intake in Female Wistar Rats (g/day/animal)						
Week	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	Week	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
1	18.1	17.4	19.1	18.8	20.6	19.6	1	13.4	15.8	14.3	16.2	18.1	15.8
2	18.6	18.3	17.4	19.2	19.7	18.7	2	15.8	16.2	15.6	16.8	17.6	14.6
3	17.8	16.9	19.7	17.6	18.9	21.4	3	14.2	14.7	15.3	15.7	15.4	17.3
4	17	17.7	18.6	18.3	18.4	20.6	4	16.1	16.8	14.9	15.3	17.7	15.9
5	-	-	-	-	19.9	19.5	5	-	-	-	-	16.9	18.5
6	-	-	-	-	18.9	18.7	6	-	-	-	-	18.4	17.8

No statistical analysis was done for feed intake

(T₁: Control; T₂: PHG 250mg/kg bw/day; T₃: PHG 500mg/kg bw/day; T₄: PHG 1000mg/kg bw/day; T₅: Control recovery group, T₆: PHG 1000mg/kg bw/day recovery group)

Hematology values viz. hemoglobin, PCV, RBC, WBC, platelet, MCHC, MCH, MCV, MPV, RDW, granulocyte, lymphocyte and monocyte obtained for male and female rats are detailed in tables 5 and 6 indicate no significant

differences among the treatment groups, suggesting that the polyherbal formulation had no notable impact on hematological parameters.

Table 5: 28 Day Repeated Dose Toxicity Study of PHG: Hematology of Male Wistar Rats

Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	F value
Hb (g/dl)	12.83±0.37	13.43±0.13	13.77±0.24	13.8±0.37	12.87±0.24	13.63±0.32	2.25
PCV (%)	38.93±1.36	41.73±0.55	41.9±0.55	42.23±2.27	38.87±1.41	42.03±2.27	1.21
RBC (10 ⁶ /μl)	6.75±0.20	7.44±0.11	7.44±0.48	7.497±0.09	6.72±0.29	7.28±0.08	1.72
WBC (10 ³ /μl)	3.96±0.42	3.33±0.33	4.4±0.76	4.77±0.39	3.80±0.40	4.80±0.35	1.47
Platelet	883.3±65.55	791.7±68.1	881.7±16.23	946.7±64.41	883.30±50.30	937±58.05	1.21
MCHC (g/dl)	31.83±0.44	31.77±0.31	32±0.1	32.47±0.73	31.73±0.52	32.63±0.32	0.47
MCH (pg)	19±0	18.33±0.33	18.37±0.31	18.5±0.05	18.77±0.15	18.57±0.09	1.73

MCV (fl)	57.63±0.49	56.4±0.36	56.57±0.21	58±0.58	57.47±0.48	57.63±0.37	3.29
MPV (fl)	4.56±0.18	4.5±0.15	4.6±0.05	4.9±0.05	4.47±0.19	4.80±0.06	1.94
RDW (%)	15.63±0.12	14.87±0.20	14.97±0.31	15.43±0.08	15.53±0.12	15.40±0.15	3.28
Granulocyte	1.167±0.27	1.1±0.25	1.233±0.39	1.4±0.25	1±0.26	1.37±0.23	0.18
Lymphocyte	4.7±0.65	4.2±1.002	4.5±0.28	4.9±0.49	4.60±0.61	5.03±0.27	0.20
Monocyte	0.2±0.05	0.17±0.03	0.23±0.03	0.27±0.09	0.27±0.09	0.33±0.09	0.55

Table 6: 28 Day Repeated Dose Toxicity Study of PHG: Hematology of Female Wistar Rats

Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	F value
Hb (g/dl)	12.87±0.43	12.9±0.68	13±0.50	13.43±0.40	12.87±0.29	13.40±0.42	0.25
PCV (%)	37.9±1.68	39.2±1.25	39.23±2.03	39.63±1.84	37.83±1.71	39.53±1.73	0.19
RBC (10 ⁶ /μl)	6.73±0.34	6.71±0.12	6.76±0.42	6.79±0.20	6.60±0.26	6.77±0.15	0.014
WBC (10 ³ /μl)	4.13±0.46	3.13±0.89	3.2±0.40	3.73±0.17	4.07±0.37	3.77±0.17	0.73
Platelet	810±27.07	724±100	798.7±1.08	871.7±16.18	803.30±18.28	855.30±13.62	0.64
MCHC (g/dl)	32.47±0.81	32.4±0.47	32.53±0.37	32.97±0.54	32.33±0.70	32.80±0.58	0.19
MCH (pg)	18.37±0.09	18.2±0.2	19.6±0.75	19.77±0.38	18.23±0.15	19.13±0.41	2.71
MCV (fl)	57.13±1.39	57.8±0.2	60.2±1.12	61.53±1.61	56.93±1.40	61.50±1.33	2.88
MPV (fl)	4.87±0.03	4.77±0.08	4.77±0.44	4.93±0.23	4.77±0.03	4.80±0.20	0.10
RDW (%)	14.8±0.1	14.5±0.65	14.73±0.03	15.17±0.42	14.73±0.12	14.93±0.47	0.49
Granulocyte	0.83±0.08	1.167±0.088	0.83±0.40	0.6±0.26	0.87±0.09	0.57±0.22	0.87
Lymphocyte	3.97±0.60	3.33±0.52	4.3±0.3	4.33±0.33	3.80±0.58	4.50±0.51	1.02
Monocyte	0.13±0.09	0.17±0.09	0.17±0.03	0.2±0.11	0.17±0.03	0.17±0.07	0.098

T₁ - Control; T₂ - Low dose, 250 mg/kg b.wt; T₃ - Medium dose, 500 mg/kg b.wt,

T₄ - High dose, 1000 mg/kg b.wt; T₅ - Recovery group, Control;

T₆ - Recovery group, High dose, 1000 mg/kg b.wt;

Values are expressed as Mean±Standard Error of the Mean (SEM); (n=3)

P>0.05; No statistical significance was observed among all the parameters mentioned.

Similarly, serum biochemistry parameters *viz.* creatinine, ALT, AST, albumin, globulin, total protein, cholesterol and glucose for males and females are outlined in Tables 7 and 8

indicate no significant differences were observed across the treatment groups.

Table 7: 28 Day Repeated Dose Toxicity Study of PHG: Serum Biochemistry of Male Wistar Rats

Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	F value
Creatinine (mg/dl)	0.94±0.09	0.95±0.08	0.95±0.1	0.99±0.05	0.87±0.12	0.93±0.03	0.08
ALT (U/L)	49.33±8.25	47.33±2.96	49.33±6.64	49.67±2.72	49.67±8.99	48.67±2.19	0.03
AST (U/L)	113.4±8.31	106.7±6.77	94.33±2.33	91.33±4.48	111.70±8.41	91.33±5.46	3.05
Albumin (g/dl)	2.54±0.29	2.75±0.3	3.02±0.06	3.07±0.08	2.53±0.23	2.97±0.09	1.29
Globulin (g/dl)	4.13±0.17	3.35±0.58	3.25±0.2	3.36±0.3	4.03±0.18	3.57±0.20	1.36
Total protein (g/dl)	6.67±0.18	6.1±0.29	6.27±0.18	6.43±0.3	6.57±0.09	6.53±0.24	0.99
Cholesterol (mg/dl)	62.67±4.98	61.67±6.89	62.33±5.84	71.33±1.45	63.67±4.98	72.33±1.45	0.77
Glucose (mg/dl)	76.33±3.48	71±2.30	72.67±5.61	78.67±3.67	77.33±3.48	79.33±1.76	0.77

Table 8: 28 Day Repeated Dose Toxicity Study of PHG: Serum Biochemistry of Female Wistar Rats

Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	F value
Creatinine (mg/dl)	1.03±0.04	0.77±0.2	0.98±0.12	0.99±0.06	0.94±0.04	0.87±0.07	0.88
ALT (U/L)	48.33±0.88	45.33±3.75	52±4.04	52.67±0.88	47.67±1.86	50±1.16	1.45
AST (U/L)	103.6±3.3	106.1±7.5	102±6.24	94.47±2.43	100.3±1.45	95.33±1.76	0.89
Albumin (g/dl)	2.49±0.3	2.24±0.11	2.72±0.3	2.78±0.32	2.47±0.37	2.73±0.37	0.80
Globulin (g/dl)	3.97±0.43	4.06±0.12	3.61±0.21	4.09±0.34	3.97±0.49	3.80±0.23	0.52
Total protein (g/dl)	6.47±0.21	6.3±0.05	6.33±0.12	6.87±0.09	6.43±0.30	6.90±0.06	3.7
Cholesterol (mg/dl)	61±1.52	65.67±2.96	67±1.52	67.33±0.88	61±1.53	66±2.08	2.40
Glucose (mg/dl)	77±2.64	70±5.13	72.67±9.02	74.33±2.6	76.67±1.76	75.67±2.33	0.28

T₁ - Control; T₂ - Low dose, 250 mg/kg b.wt; T₃ - Medium dose, 500 mg/kg b.wt;

T₄ - High dose, 1000 mg/kg b.wt; T₅ - Recovery group, Control;

T₆ - Recovery group, High dose, 1000 mg/kg b.wt

Values are expressed as Mean±Standard Error of the Mean (SEM); (n=3)

P>0.05; No statistical significance was observed among all the parameters mentioned.

The relative organ weight (liver, kidney, heart, lung, spleen, brain, ovary/testes) of the treatment groups and control groups were expressed as mean±SE of both male and

females in the tables 9 and 10, respectively. There was no significant changes noticed in the control and polyherbal treated groups.

Table 9: 28 Day Repeated Dose Toxicity Study with PHG: Relative Organ Weights in Male Wistar Rats

Organ	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	F value
Liver	5.47±0.12	5.15±0.13	5.22±0.1	5.34±0.21	5.78±0.10	5.79±0.04	0.95
Kidney	1.12±0.06	1.01±0.05	1.03±0.03	1.04±0.04	1.02±0.04	0.95±0.02	1.12
Heart	0.39±0.04	0.43±0.05	0.41±0.05	0.35±0.01	0.45±0.02	0.42±0.02	0.60
Lung	0.73±0.03	0.90±0.07	0.94±0.07	0.85±0.04	1.03±0.04	1.11±0.04	2.8
Spleen	0.38±0.06	0.34±0.01	0.41±0.05	0.42±0.03	0.46±0.02	0.49±0.02	0.86
Brain	0.72±0.03	0.68±0.04	0.67±0.01	0.66±0.01	0.78±0.05	0.85±0.04	1.12
Testes	1.24±0.03	1.24±0.02	1.11±0.03	1.19±0.20	1.16±0.04	1.15±0.05	0.34

Table 10: 28 Day Repeated Dose Toxicity Study with PHG: Relative Organ Weights in Female Wistar Rats

Organ	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	F value
Liver	4.29±0.24	3.91±0.21	3.83±0.32	3.89±0.22	5.36±0.07	5.46±0.23	0.69
Kidney	0.85±0.04	0.80±0.02	0.82±0.05	0.88±0.01	0.84±0.01	0.85±0.03	1.03
Heart	0.35±0.03	0.37±0.01	0.40±0.00	0.40±0.02	0.44±0.02	0.47±0.02	1.85
Lung	0.77±0.04	0.78±0.02	0.87±0.03	0.83±0.01	0.77±0.03	0.81±0.01	2.80
Spleen	0.36±0.02	0.34±0.02	0.34±0.01	0.38±0.05	0.51±0.04	0.52±0.02	0.27
Brain	0.67±0.01	0.71±0.00	0.74±0.04	0.74±0.01	0.79±0.01	0.76±0.01	2.57
Ovary	0.17±0.04	0.25±0.01	0.22±0.01	0.24±0.01	0.16±0.02	0.15±0.01	3.36

T₁ - Control; T₂ - Low dose, 250 mg/kg b.wt; T₃ - Medium dose, 500 mg/kg b.wt;

T₄ - High dose, 1000 mg/kg b.wt; T₅ - Recovery group, Control;

T₆ - Recovery group, High dose, 1000 mg/kg b.wt

Values are expressed as Mean±Standard Error of the Mean (SEM); (n=3)

P>0.05; No statistical significance was observed among all the parameters mentioned.

The recovery group revealed no significant differences were observed between the control recovery group and the high dose (1000mg/kg/day) recovery group in hematology, serum biochemistry, body weights and relative organ weights.

At the end of the study period, the animals were sacrificed and gross pathological examination exhibited no changes in all the organs. The histopathological evaluations were

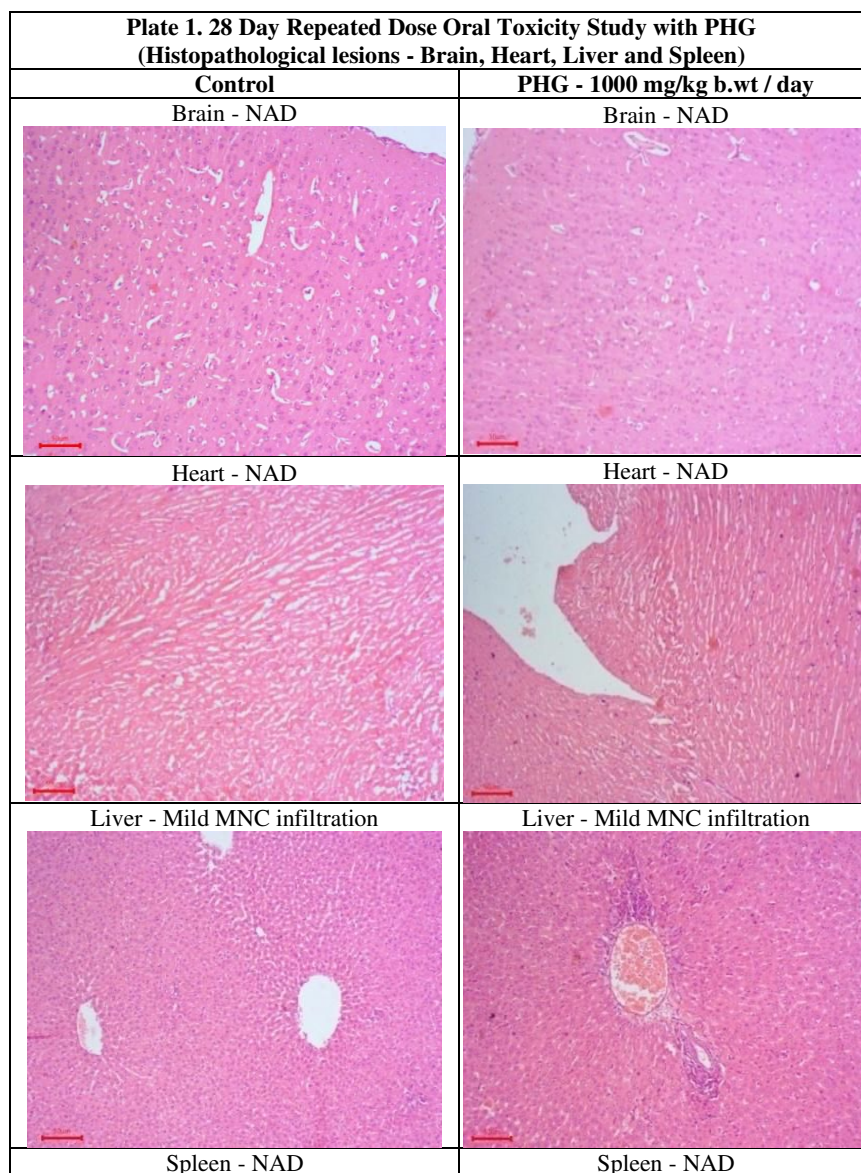
carried out from the control and high dose groups on both male and female rats. The lesions were showed in plate 1 and 2 and the identified lesions were systematically scored and presented in Tables 11 and 12. The histopathology revealed mild changes in the lung and liver, and these changes were unrelated to the administered polyherbal formulation indicating their incidental nature.

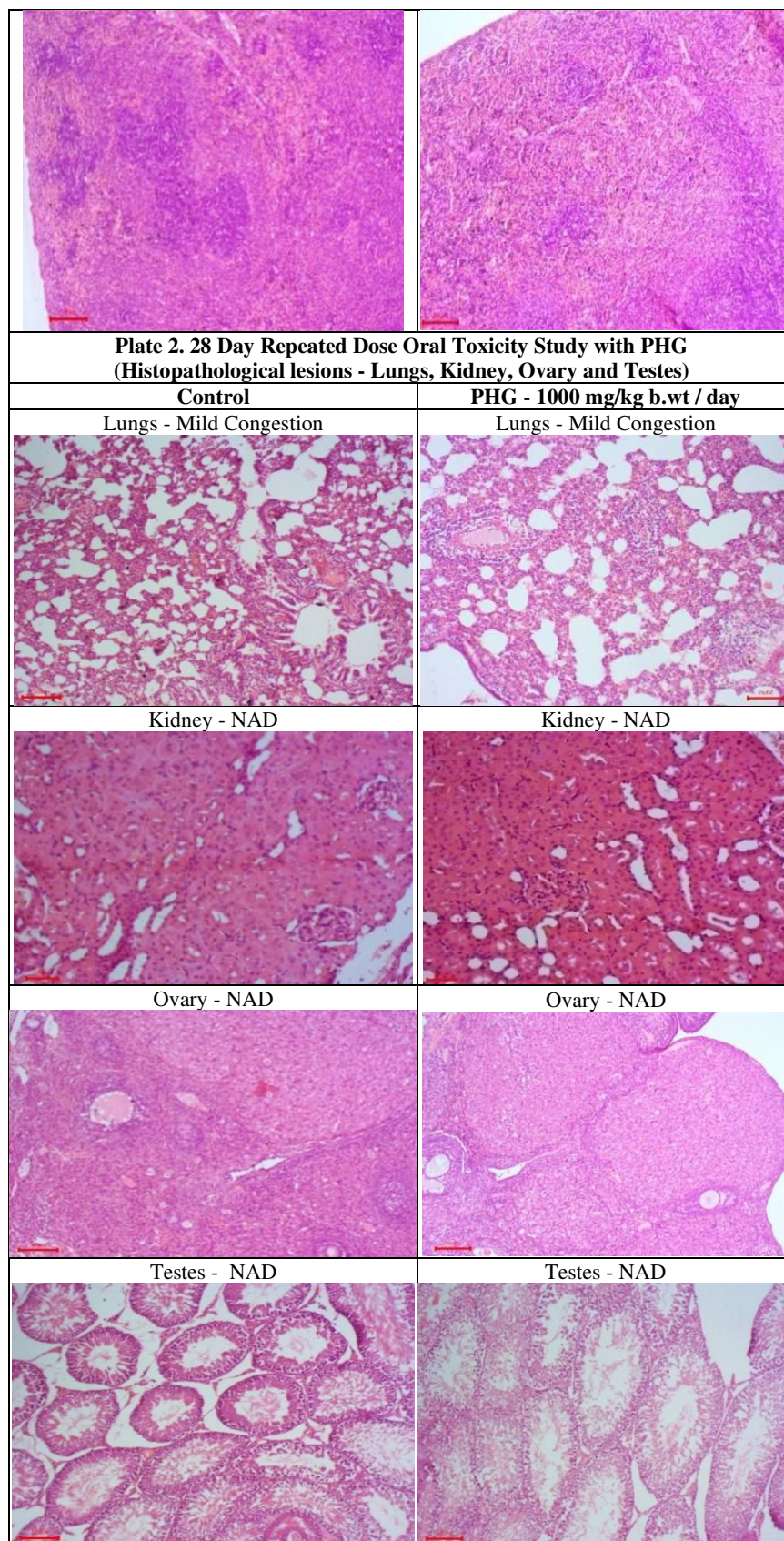
Table 11: 28 Day Repeated Dose Oral Toxicity Study with PHG: Summary of Histopathological Observations of Organs of Male Rats

Histological changes		Control	PHG: 1000 mg/kg/day
Liver			
a)	Hepatocellular vacuolar degeneration	1/3	0/3
b)	Mononuclear cell Infiltration	2/3	1/3
c)	Kupffer cell hyperplasia	2/3	0/3
Lungs			
a)	Alveolar congestion and edema	1/3	0/3
b)	Perivascular and peribronchiolar mononuclear cell infiltration	2/3	0/3
c)	Hyperplasia of Bronchus associated lymphoid tissue (BALT)	2/3	0/3
Kidneys			
a)	Tubular dilatation and vacuolar degeneration of tubules	2/3	1/3
b)	Interstitial mononuclear cell Infiltration	2/3	1/3
Spleen			
a)	Mild lymphoid depletion	0/3	0/3
b)	Sinusoidal dilatation and congestion	1/3	0/3
Brain			
a)	Neuronal degeneration and hypertrophy	1/3	0/3
b)	Gliosis and perivascular cuffing	2/3	1/3
Testes			
a)	Germ cell degeneration and depletion	1/3	1/3
b)	Sertoli cell cytoplasmic vacuolation	2/3	1/3

Table 12: 28 Day Repeated Dose Oral Toxicity Study with PHG: Summary of Histopathological Observations of Organs of Female Rats

Histological changes		Control	PHG: 1000 mg/kg/day
Liver			
a)	Hepatocellular vacuolar degeneration	1/3	1/3
b)	Hepatocellular hypertrophy	2/3	1/3
c)	Mononuclear cell Infiltration	1/3	1/3
d)	Kupffer cell hyperplasia	0/3	0/3
Lungs			
a)	Alveolar congestion and edema	2/3	1/3
b)	Perivascular and peribronchiolar mononuclear cell infiltration	1/3	2/3
Kidneys			
a)	Tubular dilatation and vacuolar degeneration of tubules	1/3	1/3
b)	Interstitial mononuclear cell Infiltration	2/3	1/3
Spleen			
a)	Mild lymphoid depletion	0/3	0/3
b)	Sinusoidal dilatation and congestion	1/3	1/3
Brain			
a)	Neuronal degeneration and hypertrophy	0/3	0/3
b)	Gliosis and perivascular cuffing	1/3	0/3
Ovary			
a)	Vacuolation of granulosa cells	2/3	1/3
b)	Decreased number or absence of oocytes, follicles and corpora lutea.	2/3	1/3





The results of the 28-day repeated dose oral toxicity study with PHG in Wistar rats once again confirmed the lack of untoward clinical signs and absence of mortality in all treated groups during the 28-day treatment period aligns with similar studies involving polyherbal formulations. There was no significant changes in body weight, feed consumption patterns in both control and treated groups of animals, hematology and clinical biochemistry Organ

weight and in histomorphology of organs collected suggest that the PHG did not induce significant systemic disturbances. This is consistent with previous findings by Ogbonnia *et al.* (2010) where polyherbal formulations exhibited no adverse effects on body weight or feed consumption.

Transitioning to the recovery period, it becomes apparent that the control recovery group and the 1000mg/kg/day

recovery group exhibited no significant differences across a range of key parameters. The determination of LD50 values greater than 2000 mg/kg in various studies underscores the non-toxic nature of polyherbal formulations. Moreover, the establishment of No Observed Adverse Effect Levels (NOAELs) higher than 1000 mg/kg body weight per day aligns with the results of the present study, which demonstrated a similar level of safety reports with the PHG (Tiwari *et al.* 2020) ^[12]. The findings from the study conducted revealed no significant treatment-related changes in gross and histopathological examinations (Bhide *et al.*, 2022).

Conclusion

The 28-day repeated dose oral toxicity study with PHG in Wistar rats reaffirmed its safety profile, with no adverse clinical signs or mortality. There were no significant changes in body weight, feed consumption, hematology, clinical biochemistry, organ weight, or histomorphology, aligning with previous polyherbal formulation studies. The recovery period further demonstrated no significant differences between control and recovery groups. No Observed Adverse Effect Level (NOAEL) above 1000 mg/kg body weight was ensured. This means that at or below this dosage, there were no observable adverse effects, indicating a favorable safety profile.

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Conflict of Interest

No conflict of interest

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