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Design, Optimization, And Hepatoprotective Evaluation of a Novel Herbal Capsule Using Factorial Design

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KEYWORDS

Polyherbal formulation, Hepatoprotective activity, Factorial design, Antioxidants, Acute toxicity

ABSTRACT:

The present study explores the formulation and evaluation of a polyherbal capsule comprising equal ratios of Ludwigia adscendens, Launaea pinnatifida, and Carica papaya extracts. Granules were prepared using a wet granulation method and encapsulated in size "00" hard gelatin capsules. A 3^2 full factorial design was employed to optimize the concentrations of ethyl cellulose and microcrystalline cellulose. The optimized formulation demonstrated acceptable pre-compression parameters (bulk density: 0.38 ± 0.05 g/cm³, angle of repose: 39.36 ± 2.67°) and capsule characteristics (disintegration time: 11 ± 0.34 min, moisture content: 3.6 ± 0.22%). Stability studies confirmed formulation integrity over three months. In vivo evaluations revealed no acute toxicity at doses up to 2000 mg/kg. The polyherbal formulation showed significant hepatoprotective effects against paracetamol and CCl_4-induced hepatotoxicity in Wistar rats, evidenced by normalized serum markers (AST, ALT, ALP, bilirubin), improved antioxidant status (SOD, GSH), reduced lipid peroxidation (MDA), and histological restoration of liver architecture. These findings suggest the formulation as a promising hepatoprotective agent with good stability and safety profiles.

1. INTRODUCTION

Liver diseases remain a global health concern, contributing significantly to morbidity and mortality due to factors such as drug toxicity, alcohol consumption, viral infections, and metabolic disorders. The liver, being the primary site of detoxification, is vulnerable to a wide range of xenobiotics, leading to hepatocellular injury and compromised physiological functions **(Kim *et al.,* 2018)**. Conventional pharmacotherapy often lacks efficacy or carries the risk of adverse effects, prompting a growing interest in alternative approaches, especially phototherapeutics, for liver protection and restoration. Herbal medicine, particularly polyherbal formulations, has gained increasing attention due to its synergistic therapeutic benefits and minimal side effects. The concept of polyherbalism, rooted in Ayurvedic and traditional medicine systems, involves the combination of multiple plant extracts to enhance efficacy and reduce toxicity **(Patel *et al.,* 2022)**. Recent studies emphasize the potential of such formulations in mitigating hepatotoxicity through antioxidant, anti-inflammatory, and cytoprotective mechanisms **(Zhao *et al.,* 2021)**. Despite the therapeutic potential of individual plant extracts, challenges remain in ensuring consistency, bioavailability, and targeted delivery, underscoring the importance of optimized formulation strategies. The use of factorial design and response surface methodology (RSM) in pharmaceutical formulation has revolutionized the optimization process. These statistical tools allow for the systematic evaluation of multiple formulation variables and their interactive effects, thereby enabling the development of robust and reproducible products **(Suresh & Vasudha, 2019)**. Ethyl cellulose and microcrystalline cellulose (MCC), for instance, are widely used excipients that influence disintegration, drug release, and mechanical properties of solid oral dosage forms. Their optimization through

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factorial design can significantly impact the performance of herbal formulations. In this context, the present study was undertaken to develop and evaluate a novel polyherbal capsule containing *Ludwigia adscendens, Launaea pinnatifida*, and *Carica papaya* extracts—plants traditionally acclaimed for their hepatoprotective properties. *Ludwigia adscendens* has been reported to exhibit significant antioxidant activity by scavenging free radicals and stabilizing cellular membranes **(Ahmed *et al.,* 2020)**. *Launaea pinnatifida* has demonstrated hepatocellular regeneration potential and protective effects against chemically induced liver damage **(Jayasinghe *et al.,* 2021)**. *Carica papaya* is known for its rich phytochemical profile, including flavonoids and vitamins that contribute to its liver-protective role **(Basnet *et al.,* 2023).** The formulation process utilized a wet granulation technique followed by encapsulation in size "00" hard gelatin capsules. A 3^2 full factorial design was employed to optimize the concentrations of ethyl cellulose and MCC, with disintegration time and weight variation as key response variables. In vivo assessments, including acute toxicity testing and hepatoprotective evaluation against paracetamol- and carbon tetrachloride (CCl_4)-induced hepatotoxicity, were conducted in Wistar rats. Biochemical markers, oxidative stress parameters, and histological evaluations were carried out to substantiate the protective effects of the formulation. This study not only highlights the therapeutic promise of a rationally designed polyherbal capsule but also underscores the relevance of factorial optimization in enhancing formulation efficiency and ensuring reproducibility in herbal drug development.

2. MATERIALS AND METHODS

2.1 Preparation materials

The polyherbal formulation was developed using equal proportions (1:1:1) of *Ludwigia adscendens*, *Launaea pinnatifida*, and *Carica papaya* extracts, which were dried and powdered. These plant materials were blended with pharmaceutical excipients including ethyl cellulose, microcrystalline cellulose (MCC), sodium benzoate, dibasic calcium phosphate, starch, colloidal silicon dioxide, talc, and magnesium stearate to ensure proper formulation characteristics. The granules were prepared using the wet granulation method with povidone as a binder, sieved, dried, lubricated, and finally encapsulated into size "00" hard gelatin capsules using a manual MF-30 capsule filling machine. Pre-compression and post-compression parameters were evaluated to ensure formulation quality.

2.2 Characterisation and Evaluation of blended powder for granules (Srinath *et al.,* 2011)

The blended powder of all trial batches were analysed for its flow characteristics like bulk density, tap density, compressibility index, Hausner's ratio and angle of reponse**.**

2.2.1 Bulk Density

A certain weight of granules was taken in a 100 ml measuring cylinder without compacting and maintain the proper level of granules and measure the volume, Vo (bulk volume) and calculated according to the formula given below.

Bulk density = Weight of powder taken/Bulk volume

2.2.2 Tapped density

A certain weight of granules was taken in a 100 ml measuring cylinder and tapped for 100 times. The volume of the granules was measured after complete tapped and got tapped volume. Calculate the tapped density according to the following formula:

Tapped density= Weight of granules/Tapped volume

2.2.3 Carr's index ratio

This was determined using bulk density and tapped density using the formula mention below. **Carr's index ratio = [(Tapped density–Bulk density)/Tapped density] × 100**

2.2.4 Hausner's ratio

Hausner ratio is used to determine the flow property of powder. Lower the Hausner ratio betters the flow property or vice versa. This was calculated from bulk density and tapped ensity using the formula given below.

Hausner's Ratio = Tapped density/Bulk density

2.2.5 Angle of repose

The prepared granules were allowed to pass through a funnel and the height of the pile (h) and radius of the pile (r) are measured. From this, the angle of repose, i.e., the angle between the height of the pile and radius

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of the pile is calculated with the help of the following formula.

Angle of repose Θ = tan-1(h/r)

2.3 Preparation of Granules and Capsules (Kawano *et al.*, 2010)

Wet granulation method was used. Extracts and excipients were mixed and granulated using povidone binder, sieved, dried, and lubricated. The granules were filled into size "00" gelatin capsules using MF-30 manual capsule filling machine.

Table 1: Composition of optimized capsule

Formulation Code	Exatract of *Ludwigia adscenden, Launaea pinnatifida* and *Carica papaya(1:1:1)*	Ethyl cellulose	Microcrystalline cellulose	Magnesium Stearate	Dibasic calcium phosphate	Starch	Sodium Benzoate	Total wt
PFGr	225 mg	25	30	5	3	5	2	295

2.4 Optimization of the capsule using Design of Experiments

The capsule formulation was optimized using a 3^2 full factorial design through Design Expert® software to evaluate the combined effects of two independent formulation variables: ethyl cellulose (X_1, 15–45 mg) and microcrystalline cellulose (MCC, X_2, 10–45 mg). These excipients were selected due to their influence on drug release and capsule integrity. The primary dependent variables assessed were disintegration time (Y_1) and weight variation (Y_2), as per Indian Pharmacopoeia standards. The design allowed for systematic evaluation of the interactions between variables and their individual contributions to the formulation quality, providing an efficient and statistically robust method for capsule optimization. This approach is widely recognized in pharmaceutical research for improving formulation performance and ensuring product consistency across batches **(Montgomery, 2017; Bolton and Bon, 2009).**

2.5 Standardization of capsules

The formulated capsules were standardized by evaluating key quality control parameters as per Indian Pharmacopoeia guidelines. Physical appearance was assessed visually for uniformity, color, and integrity. Weight uniformity was determined by individually weighing 20 randomly selected capsules and comparing the individual weights to the average. The pH of a 1% aqueous solution of capsule content was measured using a calibrated digital pH meter. Moisture content was evaluated using the Loss on Drying (LOD) method, where capsule contents were dried at 105 °C until a constant weight was achieved. Disintegration time was tested using a standard disintegration apparatus in distilled water at 37 ± 2 °C, and the time taken for complete disintegration of the capsule was recorded **(Indian Pharmacopoeia, 2018; Aulton and Taylor, 2018).**

2.6 Stability Study

The stability of the formulated capsules was assessed through an accelerated stability study conducted as per ICH guidelines. The capsules were stored at a controlled temperature of 40 ± 2 °C and relative humidity of 75% ± 5% for a period of three months. At monthly intervals, the samples were evaluated for any changes in physical appearance (color, odor, texture) and disintegration time to monitor formulation integrity and performance over time **(ICH, 2003).**

2.7 In Vivo Studies

2.7.1 Animals and treatment

Wistar rats of weighing 185±15 g were selected and procured from PBRI animal house. The animals were maintained under standard conditions of humidity, temperature (25±2 ◦C) and light (12 h light/dark). They

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were fed with standard rat pellet diet and water ad libitum.

2.7.2 Acute toxicity studies

Acute oral toxicity study of E1, E2, E3 extracts and formulations were studied according to OECD-423 guidelines in mice. Four dose levels were selected for acute oral toxicity. 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg were used as dose range **(Bedi and Krishan, 2020)**.

2.7.3 Paracetamol induced hepatotoxicity study

Animal were divided into seven groups of six animals each group. The first group (Group1) received only normal saline 5 ml/kg for 7 days. Groups II animals were administered PCM (500 mg/kg) single dose on 7th day, Group III received silymarin 25 mg/kg (p.o) orally for 7 days once daily and PCM on 7th day and Group IV received *Ludwigia adscendens* 200mg/kg (E1) and PCM on 7th day, Group V received *Launaea pinnatifida* 200 mg/kg (E2) orally for 7 days and PCM on 7th day, Group VI received *Carica papaya* 200mg/kg (E3) orally daily for seven days and PCM on 7th day Group VII received *Formulation (granules)* orally daily for seven days and PCM on 7th day respectively. On the eight day after 24 h of respective treatments the blood samples were collected from retro orbital plexus for the estimation of biochemical marker enzymes.

Serum was separated by centrifugation at 2000 rpm for 10 min at 4°C in cooling microfuge. Liver function tests were performed by measuring the levels of serum enzymes: aspartate aminotransferase (AST), alanine aminotrasferase (ALT) and bilirubin. All determinations were carried out on day's 8–10 post administration **(Edo *et al.,* 2025; Koshak *et al.,* 2023)**.

All animals were sacrificed by cervical decapitation. Immediately after the sacrifice, the livers were isolated and washed with ice-cold saline. A small piece of the central lobe of liver of each rat was fixed in 10% [v/v], formalin solution for the histopathological examination.

2.7.4 CCl4 induced hepatotoxicity study

Animals were divided into seven groups of six rats each. Rats of the 1st group will receive vehicle and served as normal control group. Group II inducer, treated with CCl4 1.5 ml/kg body weight. Group III received standard silymarin at an oral dose of 50 mg/kg along with CCl4 Group IV received sample *Ludwigia adscendens* 200mg/kg bw (E1) orally along with CCl4 administration. Group V received sample *Launaea pinnatifida* 200 mg/kg bw (E2) orally along with CCl4 administration. Group VI received sample *Carica papaya* 200mg/kg bw (E3) orally along with CCl4 administration. Group VII received *Formulation (granules)* along with CCl4 administration. The treatment was continued for 21 days, once daily. On 22nd day from groups III-VII, 30 min post-dose of test sample administration animals received CCl4 at the dose of 1.5 ml/kg body weight (1:1 of CCl4 in olive oil) orally. The animals were sacrificed after 24 h after administration of acute dose (1.5 ml/kg bw) of CCl4. The blood was collected by retro-orbital puncture for biochemical parameters. The serum was separated out and used for estimation of AST, ALT, ALP, total bilirubin using commercial kit diagnostics respectively. Analysis was done using Rapid Bio-autoanalyzer (Star 21) **(Ben *et al.,* 2022; El Rabey *et al.,* 2021)**.

2.8 Oxidative markers

The antioxidant parameters were evaluated using standard protocols. Superoxide dismutase (SOD) activity was assessed using a tetrazolium-based assay, Lipid peroxidation was measured as malondialdehyde (MDA) levels through the thiobarbituric acid reactive substances (TBARS) method, reduced glutathione (GSH) levels were estimated using Ellman's reagent following protein precipitation with TCA and EDTA.

2.9 Histopathology

The liver tissues were immersed in 10% formalin solution for histopathological examination. These tissues were processed, dehydrated in different grades of alcohol, cleared in toluene, and impregnated in molten paraffin wax for specified periods. Processed tissues were embedded in fresh molten paraffin wax and allowed to set. Sections were at 3 µ and dried on a hot plate for 15 min and stained with hematoxylin and 1% aqueous eosin to demonstrate general tissue structure. Stained slides were dehydrated in various ascending grades of alcohol, cleared in xylene, and mounted in Canada balsam. Sections were viewed microscopically using ×10 objective lenses **(Chan *et al.,* 2023)**.

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2.10 Statistical Analysis

Results are provided as Mean ± SD (n=6). Results were analyzed statistically using one-way analysis of variance (ANOVA) followed by Bonferroni t-test. $P < 0.05$ was considered as level of significance while comparison between groups.

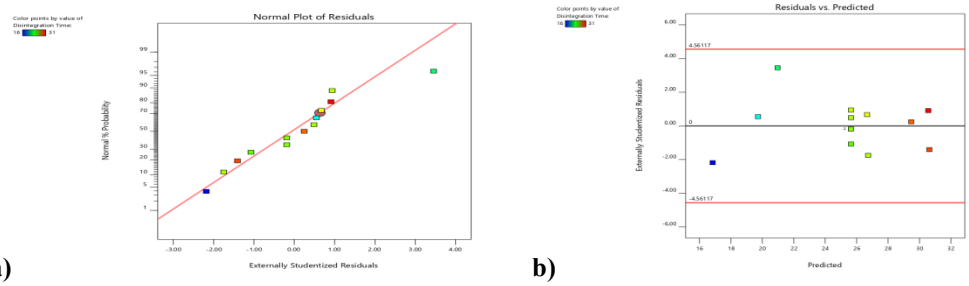
3. RESULTS

3.1 Granule Evaluation

Parameter	Value (PFGr Optimized Batch)
Bulk Density	0.38 ± 0.05 g/cm³
Tapped Density	0.47 ± 0.01 g/cm³
Compressibility Index	23.26 ± 2.54%
Hausner Ratio	1.22 ± 0.02
Angle of Repose	39.36 ± 2.67°

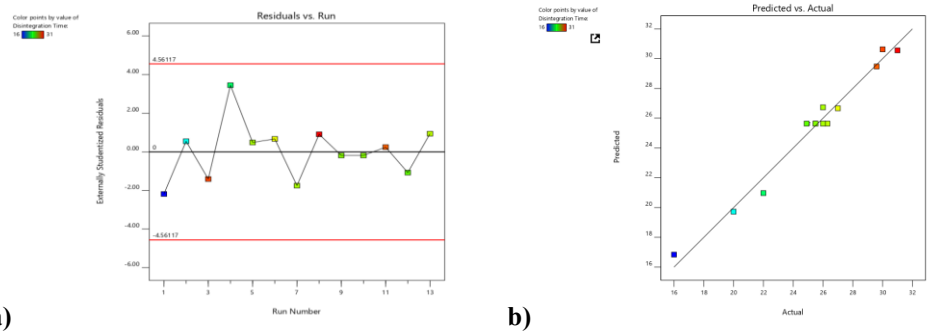
As per the standards, the flow properties of the blend granules to be filled in the capsules were in good range and was confirmed by the above parameters. PFGr optimized granules showed excellent flow characters and that batch was taken for capsule filling. The flow properties were Excellent and all parameter were within the Specified limits. So, third trial was chosen for further studies.

3.2 Optimization of the capsule via stat ease software (Design expert DoE)



a) b)

Graph 1: a) scatter plot of residuals b) residual vs predicted value of dependent variable (Disintegration time)



a) b)

Graph 2: a) residual vs run b) predicted vs actual value of dependent variable (Disintegration time)

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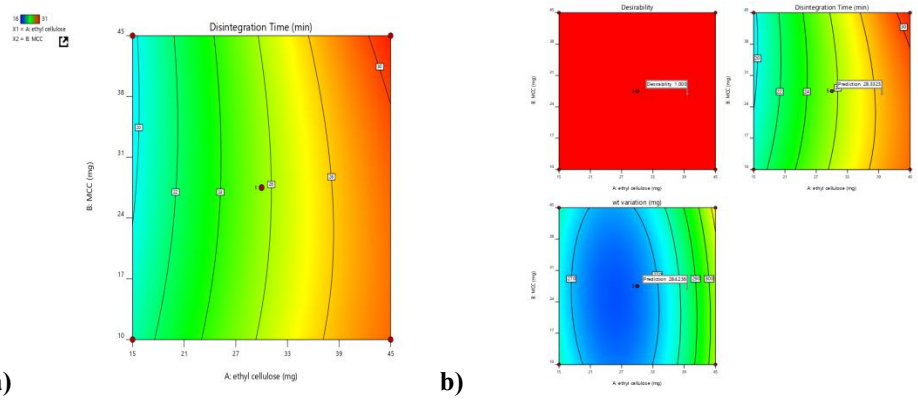
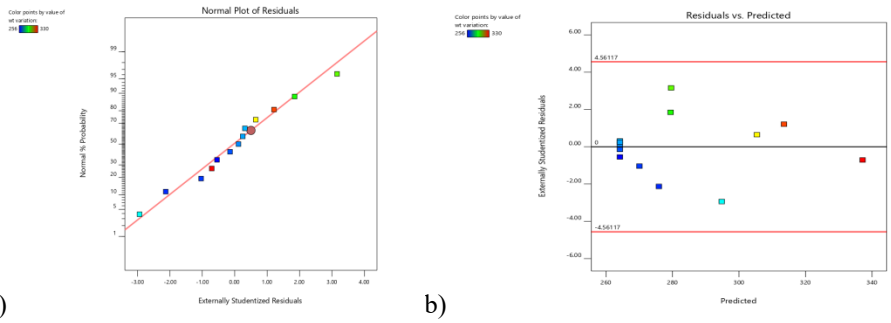


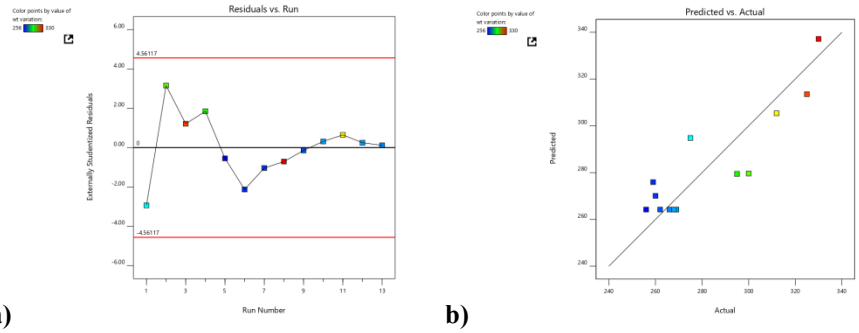
a) b)

Graph 3: a) Contour plot of dependent variable distintegartion time b) desirability of independent and dependent variables



a) b)

Graph 4: a) scatter plot of residuals b) residual vs predicted value of dependent variable (Disintegration time)



a) b)

Graph 5: a) residual vs run b) predicted vs actual value of dependent variable (Disintegration time)

Table 2: actual and predicted value of wt variation

Run Order	Actual Value	Predicted Value	Residual	Internally Studentized Residuals	Externally Studentized Residuals	Influence on Fitted Value DFFITS	Standard Order
1	275.00	294.83	-19.83	-2.031	-2.935	-3.790(1)	5
2	300.00	279.60	20.40	2.090	3.157	4.075(1)	3
3	325.00	313.55	11.45	1.174	1.212	1.565	4
4	295.00	279.45	15.55	1.593	1.847	2.384(1)	1

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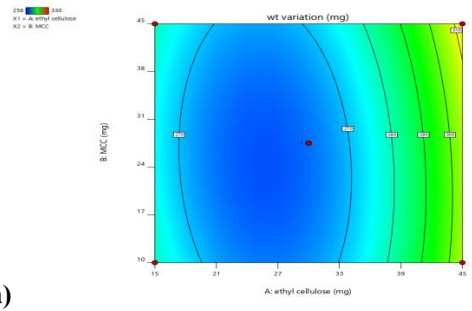
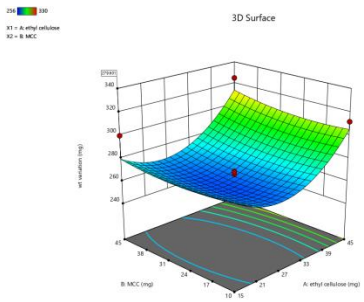
5	256.00	264.20	-8.20	-0.575	-0.546	-0.273	10
6	259.00	275.93	-16.93	-1.735	-2.127	-2.747(1)	8
7	260.00	270.07	-10.07	-1.032	-1.037	-1.339	7
8	330.00	337.17	-7.17	-0.735	-0.709	-0.915	6
9	262.00	264.20	-2.20	-0.154	-0.143	-0.072	12
10	269.00	264.20	4.80	0.337	0.314	0.157	11
11	312.00	305.40	6.60	0.676	0.648	0.836	2
12	268.00	264.20	3.80	0.267	0.248	0.124	13
13	266.00	264.20	1.80	0.126	0.117	0.059	9

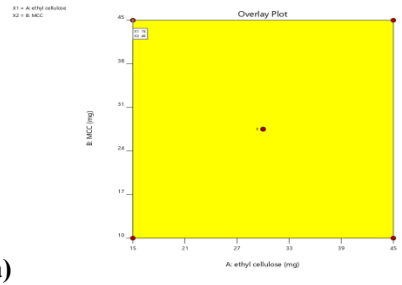


a)



b)

Graph 6: a) Contour plot of dependent variable wt variation b) response surface plot for the wt variation against independent variable



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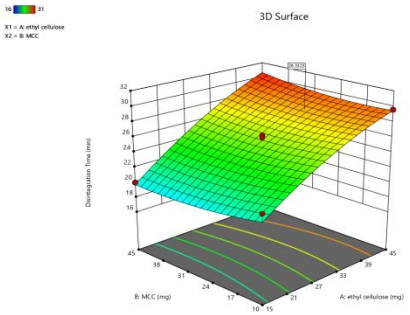

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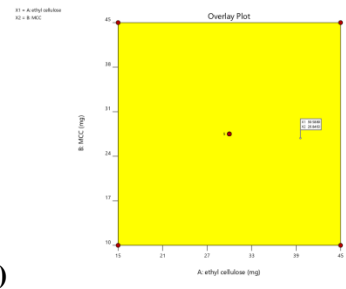

c)

Graph 7: a) overlay plot for the wt variation b) response surface plot c) overlay plot for the disintegration time for the disintegration time against independent variable

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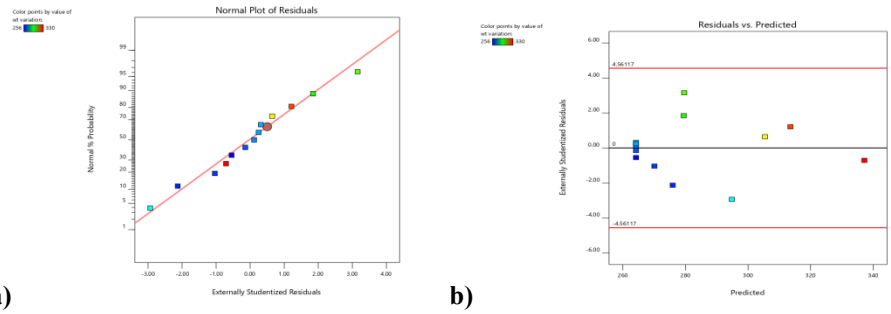
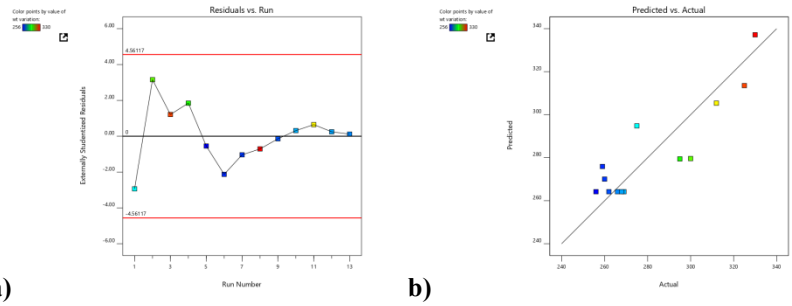


a) b)

Graph 8: a) scatter plot of residuals b) residual vs predicted value of dependent variable (Disintegration time)



a) b)

Graph 9: a) residual vs run b) predicted vs actual value of dependent variable (Disintegration time)

Table 3: actual and predicted value of wt variation

Run Order	Actual Value	Predicted Value	Residual	Internally Studentized Residuals	Externally Studentized Residuals	Influence on Fitted Value DFFITS	Standard Order
1	275.00	294.83	-19.83	-2.031	-2.935	-3.790[(1)]	5
2	300.00	279.60	20.40	2.090	3.157	4.075[(1)]	3
3	325.00	313.55	11.45	1.174	1.212	1.565	4
4	295.00	279.45	15.55	1.593	1.847	2.384[(1)]	1
5	256.00	264.20	-8.20	-0.575	-0.546	-0.273	10
6	259.00	275.93	-16.93	-1.735	-2.127	-2.747[(1)]	8
7	260.00	270.07	-10.07	-1.032	-1.037	-1.339	7
8	330.00	337.17	-7.17	-0.735	-0.709	-0.915	6
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10	269.00	264.20	4.80	0.337	0.314	0.157	11
11	312.00	305.40	6.60	0.676	0.648	0.836	2
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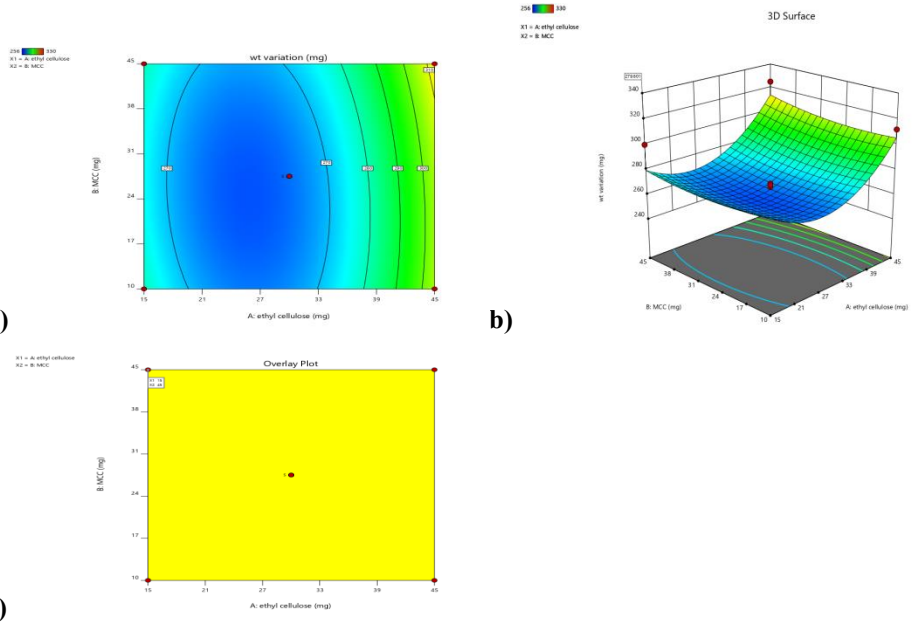

a) b) c)

Graph 10: a) Contour plot of dependent variable wt variation b) response surface plot for the wt variation c) overlay plot against independent variable

3.3 Standarization of herbal Capsules

The general appearance of a capsule, its visual identity and overall "elegance" is essential for consumer acceptance. The color, shape, odor and surface texture are all noted for the capsules prepared.

Table 4: Organoleptic characters

Name of test	Observations
Description	Pale brown powder contained in green cap/ body "00" size capsule
Color	Greenish brown powder
Odour	Characteristic odour
Taste	Bitter

3.4 Capsule Evaluation

Parameter	Observation
Moisture Content	3.6 ± 0.22%
Weight Uniformity	295.6 ± 4.5 mg
Disintegration Time	11 ± 0.34 min
pH (1% solution)	6.8 ± 0.21

3.5 Stability Testing

Table 5: Stability study

S. No.	Sample No.	Month	Condition	Color/ Odor	Disintegration Time
1		1	40°C/75 % RH	No change	11 min
2			40°C/75 % RH	No change	12 min
3			40°C/75 % RH	No change	12.5 min

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4	**PFGR1**	2	40°C/75 % RH	No change	11.5 min
5			40°C/75 % RH	No change	11.9 min
6			40°C/75 % RH	No change	12 min
7		3	40°C/75 % RH	No change	11min
8			40°C/75 % RH	No change	12 min
9			40°C/75 % RH	No change	13min

3.6 Acute Oral Toxicity

The acute oral toxicity study of extracts E1, E2, E3, and their formulation was conducted at graded doses of 5, 50, 300, and 2000 mg/kg in rats. No mortality or signs of toxicity were observed in any group during the 14-day observation period. All animals survived until the end of the study, indicating that the LD_{50} of each test sample is greater than 2000 mg/kg. Additionally, the body weight of all animals increased gradually across all dose groups, suggesting normal growth and no adverse effects on metabolic functions. This absence of lethality and the steady increase in body weight across treated groups confirm the safety of E1, E2, E3, and the formulation at the tested dose range.

3.7 Paracetamol-Induced Hepatotoxicity

3.7.1 Body weight changes during the study

Table 6: Body weight changes in normal control, inducer and Test samples E1, E2, E3 (200 mg/kg bw), formulation and standard

Group No.	Treatment	Body weight (gm)	
		Initial	Final
I.	Normal Control (Vehicle treated)	188.83±6.43	210.83±4.36
II.	Vehicle + Paracetamol	205.33±3.01	186.67±7.37
III.	Standard Silymarin 25 mg/kg + Paracetamol	187.83±7.52	203.33±7.20 ^{ns}
IV.	E1 (200 mg/kg)+ Paracetamol	191.00±2.68 ^{ns}	202.78±4.00 ^{ns}
V.	E2(200 mg/kg)+ Paracetamol	196.67±6.02 ^{ns}	209.33±1.37 ^{ns}
VI.	E3(200 mg/kg)+ Paracetamol	199.67±3.27 ^{ns}	213.50±4.85 ^{ns}
VII.	Formulation granules + Paracetamol	192.83±2.86 ^{ns}	208.33±2.50*

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ^{ns} non-significant compared to the Group II (Control).

3.7.2 Liver enzyme parameters

Table 7: Liver function test normal control, inducer and Test samples E1, E2, E3 (200 mg/kg bw) and standard

Group No.	Group	Parameter			
		AST (IU/dL)	ALT (IU/dL)	ALP (IU/dL)	Bilirubin (mg/dL)

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I	**Normal Control (Vehicle treated)**	53.03±1.273	33.27±3.798	125.83±4.875	0.40±0.025
II	**Vehicle + Paracetamol**	114.63±7.667	97.88±5.584	213.31±7.592	1.31±0.081
III	**Standard Silymarin 25 mg/kg + Paracetamol**	62.70±3.222**	44.83±5.115**	116.13±6.911**	0.57±0.043**
IV	**E1 (200 mg/kg)+ Paracetamol**	94.33±6.022 ns	82.67±5.854 ns	159.67±7.941**	0.82±0.057**
V	**E2(200 mg/kg)+ Paracetamol**	89.12±7.787 ns	81.33±6.110 ns	145.20±4.441**	0.76±0.020**
VI	**E3(200 mg/kg)+ Paracetamol**	72.67±8.819**	64.71±8.573**	128.21±10.948**	0.65±0.076**
VII	**Formulation granules + Paracetamol**	65.66±5.574**	55.33±5.007**	122.16±2.858**	0.61±0.058**

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ns non-significant compared to the Group II (Control).

3.7.3Oxidative stress parameters

Table 8: Oxidative parameters SOD in normal control, inducer and Test samples E1, E2, E3 (200 mg/kg bw) and standard

Group No.	Treatment	SOD (Unit/mg tissue)
I	**Normal Control (Vehicle treated)**	169.39±16.591
II	**Vehicle + Paracetamol**	85.38±3.839
III	**Standard Silymarin 25 mg/kg + Paracetamol**	162.80±5.476*
IV	**E1 (200 mg/kg)+ Paracetamol**	117.71±24.691 ns
V	**E2(200 mg/kg)+ Paracetamol**	130.46±10.835 ns
VI	**E3(200 mg/kg)+ Paracetamol**	146.17±5.666 ns
VII	**Formulation granules + Paracetamol**	163.25±15.576*

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ns non-significant compared to the Group II (Control).

Table 9: Oxidative parameters LPO in normal control, inducer and Test samples E1, E2, E3 (200 mg/kg bw) and standard

Group No.	Treatment	LPO (nmol MDA/mg tissue)
I	**Normal Control (Vehicle treated)**	22.33±2.944
II	**Vehicle + Paracetamol**	79.43±8.060
III	**Standard Silymarin 25 mg/kg + Paracetamol**	30.33±5.428*
IV	**E1 (200 mg/kg)+ Paracetamol**	57.67±8.430 ns
V	**E2(200 mg/kg)+ Paracetamol**	51.00±7.014 ns

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VI	**E3(200 mg/kg)+ Paracetamol**	45.33±11.075 ^{ns}
VII	**Formulation granules + Paracetamol**	34.67±6.772**

Values are expressed as MEAN±SD at n=6,One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ^{ns} non-significant compared to the Group II (Control).

Table 10: Oxidative parameters GSH in normal control, inducer and Test samples E1, E2, E3 (200 mg/kg bw) and standard

Group No.	Treatment	GSH (nmol/mg tissue
I	**Normal Control (Vehicle treated)**	0.91±0.132
II	**Vehicle + Paracetamol**	0.29±0.011
III	**Standard Silymarin 25 mg/kg + Paracetamol**	0.79±0.090**
IV	**E1 (200 mg/kg)+ Paracetamol**	0.57±0.092 ns
V	**E2(200 mg/kg)+ Paracetamol**	0.61±0.026 ns
VI	**E3(200 mg/kg)+ Paracetamol**	0.64±0.065*
VII	**Formulation granules + Paracetamol**	0.69±0.041*

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ^{ns} non-significant compared to the Group II (Control).

3.7.4 Histology

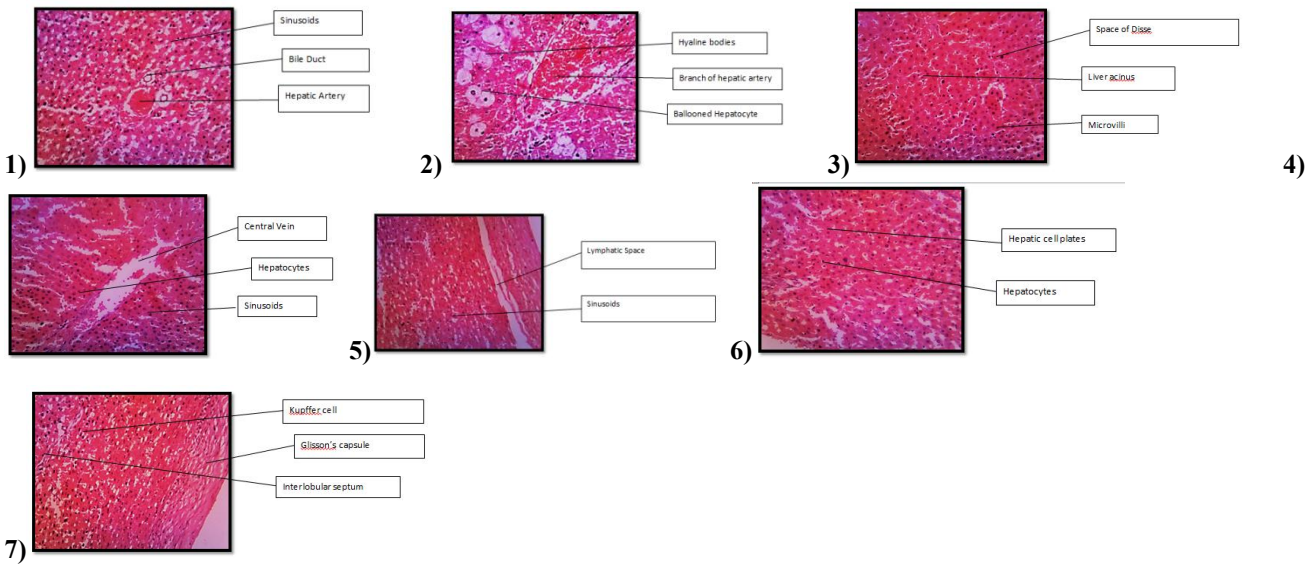

Figure 1 Histology of Paracetamol induced hepatotoxic liver and its subsequent groups

Histological evaluation revealed that the normal control group showed intact liver architecture, while the paracetamol-induced group exhibited severe hepatic damage with inflammatory cell infiltration, hyaline bodies, and ballooned hepatocytes. The standard-treated group restored normal hepatic structure. E1-treated rats showed mild central vein inflammation and fatty changes, whereas E2 treatment resulted in minimal inflammation with preserved sinusoids. E3-treated rats displayed cytoplasmic vacuolization and hepatic disarrangement. Notably, the formulation-treated group showed well-preserved liver architecture with visible

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Glisson's capsule, interlobular septa, Kupffer cells, and normal hepatocytes.

3.8 CCl_4-Induced Hepatotoxicity

3.8.1 Body weight changes during the study

Table 11: Body weight changes in normal control, inducer, Test samples E1, E2, E3 (200 mg/kg bw), standard and formulation granules

Group No.	Group	Parameter Body weight (gm)		
		0 day	7 day	21 day
I	Normal Control (Vehicle treated)	198.33±5.007	210.00±3.795	212.50±2.074
II	Vehicle + CCl4	196.83±5.419	208.17±3.312	222.33±4.412
III	Standard Silymarin 25 mg/kg + CCl4	203.77±3.125 ns	213.50±4.231 ns	222.50±6.156 ns
IV	E1 (200 mg/kg)+ CCl4	198.50±7.868 ns	210.50±4.680 ns	223.33±7.528 ns
V	E2(200 mg/kg)+ CCl4	202.12±3.219 ns	213.71±4.476 ns	226.00±2.000 ns
VI	E3(200 mg/kg) + CCl4 14	203.52±3.500 ns	211.00±3.606 ns	223.67±3.615 ns
VII	Formulation granules + CCl4	205.17±3.189 ns	214.17±4.167 ns	224.50±3.674 ns

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ns non-significant compared to the Group II (Control).

3.8.2 Liver enzyme parameters

Table 12: Liver function test normal control, inducer and Test samples E1, E2, E3 (200 mg/kg bw) and standard

Group No.	Group	Parameter			
		AST (IU/dL)	ALT (IU/dL)	ALP (IU/dL)	Bilirubin (mg/dL)
I	Normal Control (Vehicle treated)	40.29±6.507	31.09±4.122	79.66±7.763	0.22±0.013
II	Vehicle + CCl4	111.03±6.278	92.14±2.467	231.66±5.391	2.217±0.343
III	Standard Silymarin 25 mg/kg + CCl4	41.50±3.619**	34.16±4.750**	116.50±9.566**	0.41±0.014**
IV	E1 (200 mg/kg)+ CCl4	61.74±2.350**	46.58±4.261**	132.87±5.102**	0.526±0.016**
V	E2(200 mg/kg)+ CCl4	50.00±4.561**	41.00±2.191**	130.16±1.169**	0.50±0.010**
VI	E3(200 mg/kg) + CCl4	49.75±4.699**	39.30±3.044**	128.69±8.499**	0.497±0.059**
VII	Formulation granules + CCl4	48.85±2.969**	27.89±3.089**	121.33±4.320**	0.433±0.016**

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Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ns non-significant compared to the Group II (Control).

3.8.3 Oxidative stress parameters

Table 13: Oxidative parameters SOD in normal control, inducer and Test samples E1, E2, E3 (200 mg/kg bw) and standard

Group No.	Treatment	SOD (Unit/mg tissue)
I	**Normal Control (Vehicle treated)**	151.90±23.923
II	**Vehicle + CCl4**	86.63±6.614
III	**Standard Silymarin 25 mg/kg + CCl4**	165.07±11.597**
IV	**E1 (200 mg/kg)+ CCl4**	86.63±6.614 ns
V	**E2(200 mg/kg)+ CCl4**	96.65±9.599 ns
VI	**E3(200 mg/kg) + CCl4**	97.22±14.76 ns
VII	**Formulation granules + CCl4**	149.5±7.065**

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ns non-significant compared to the Group II (Control).

Table 14: Oxidative parameters LPO in normal control, inducer and Test samples E1, E2, E3 (200 mg/kg bw) and standard

Group No.	Treatment	LPO (nmol MDA/mg tissue)
I	**Normal Control (Vehicle treated)**	24.67±6.532
II	**Vehicle + CCl4**	70.33±3.881
III	**Standard Silymarin 25 mg/kg + CCl4**	29.67±4.967*
IV	**E1 (200 mg/kg)+ CCl4**	49.33±9.266 ns
V	**E2(200 mg/kg)+ CCl4**	46.33±8.892 ns
VI	**E3(200 mg/kg) + CCl4**	38.00±6.325*
VII	**Formulation granules + CCl4**	27.50±5.050*

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ns non-significant compared to the Group II (Control).

Table 15: Oxidative parameters GSH in normal control, inducer and Test samples E1, E2, E3 (200 mg/kg bw) and standard

Group No.	Treatment	GSH (nmol/mg tissue
I	Normal Control (Vehicle treated)	0.90±0.093
II	Vehicle + CCl4	0.32±0.045
III	Standard Silymarin 25 mg/kg + CCl4	0.83±0.132*
IV	E1 (200 mg/kg)+ CCl4	0.62±0.058 ns

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V	E2(200 mg/kg)+ CCl4	0.69±0.099 ^{ns}
VI	E3(200 mg/kg) + CCl4	0.74±0.059 ^{ns}
VII	Formulation granules + CCl4	0.76±0.119*

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050, **P<0.001 and ^{ns} non-significant compared to the Group II (Control).

3.8.4 Histology

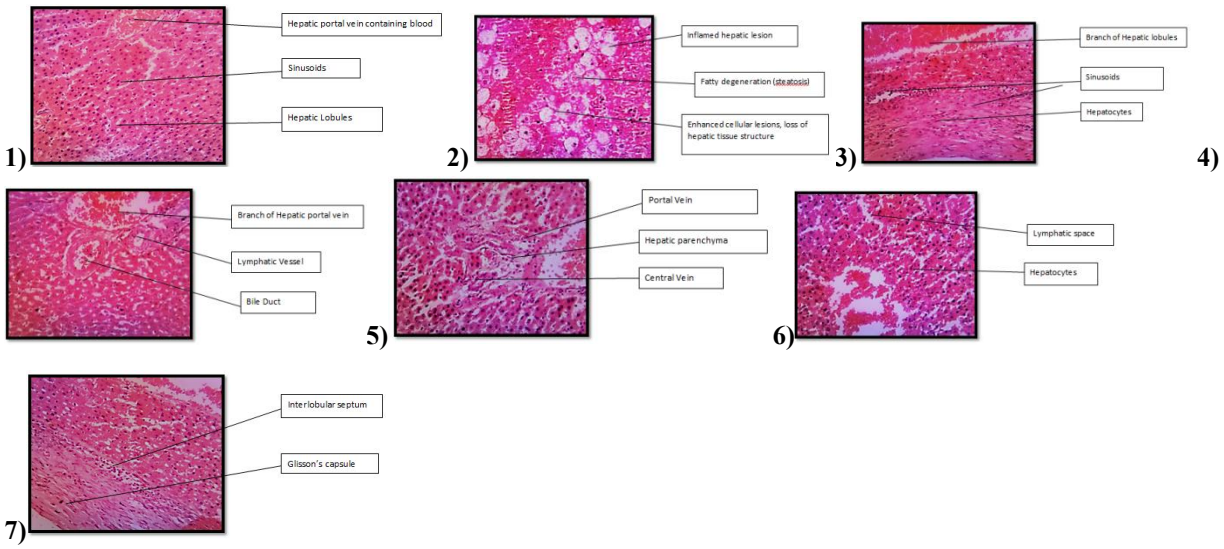

Figure 2 Histology of CCl4 induced hepatotoxic liver and its subsequent groups

Histopathological analysis revealed that the normal control group showed intact liver architecture, while the CCl_4-induced group exhibited severe hepatic damage, including inflammation, hepatocyte loss, and fatty changes. Silymarin-treated rats showed partial restoration with visible hepatic lobules. E1 and E2 treated groups displayed varying degrees of inflammation in lymphatic vessels, bile ducts, and hepatic structures. E3 treatment showed mild inflammation with largely normal hepatocytes. The formulation-treated group demonstrated near-normal liver histology, with preserved Glisson's capsule and hepatocytes, indicating a protective effect.

4. DISCUSSION

The polyherbal formulation comprising *Ludwigia adscendens, Launaea pinnatifida*, and *Carica papaya* extracts demonstrated promising hepatoprotective potential, supported by comprehensive physicochemical and in vivo evaluations. Granule flow characteristics such as angle of repose (39.36°), Hausner's ratio (1.22), and Carr's index (23.26%) confirmed excellent flow ability for capsule filling. Optimization using a 3^2 full factorial design effectively fine-tuned the concentrations of ethyl cellulose and microcrystalline cellulose to achieve ideal capsule disintegration time and weight uniformity. The capsules showed acceptable quality control parameters including a disintegration time of 11 minutes, moisture content below 4%, and consistent weight uniformity. Acute toxicity testing revealed no signs of toxicity or mortality at doses up to 2000 mg/kg, indicating a wide safety margin for the formulation. In both paracetamol and CCl_4-induced hepatotoxicity models, treatment with the polyherbal formulation significantly reduced serum levels of AST, ALT, ALP, and bilirubin compared to toxic controls, aligning with previous studies demonstrating the hepatoprotective effects of polyherbal interventions **(Pramyothin *et al.*, 2010; Devaraj *et al.*, 2011)**. The formulation also effectively restored antioxidant enzyme levels—SOD and GSH—while reducing lipid peroxidation (MDA), supporting its role in oxidative stress attenuation **(Zhao *et al.*, 2014)**. Histopathological findings confirmed biochemical observations: while the

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toxic control groups displayed severe hepatic damage, the formulation-treated groups showed restored architecture, with well-preserved hepatocytes, central veins, and Kupffer cells. Notably, the combined extract formulation outperformed individual plant extracts in efficacy and tissue recovery, suggesting synergistic hepatoprotective effects. This supports the current trend in phytomedicine favoring standardized polyherbal formulations due to their multifactorial bioactivity **(Patwardhan *et al.*, 2005; Singh *et al.*, 2020)**. In conclusion, the developed polyherbal capsule displayed excellent pharmaceutical characteristics, safety, and potent hepatoprotective efficacy, making it a viable candidate for future clinical translation.

5. CONCLUSION

The present study successfully developed and optimized a novel polyherbal capsule containing *Ludwigia adscendens, Launaea pinnatifida*, and *Carica papaya* extracts using a factorial design approach. The formulation exhibited favorable physicochemical properties, stability, and disintegration characteristics. In vivo evaluations confirmed its safety with no observed toxicity up to 2000 mg/kg and demonstrated significant hepatoprotective effects in both paracetamol and CCl_4-induced liver injury models. The formulation effectively normalized biochemical markers, enhanced antioxidant defense, and preserved liver histoarchitecture, indicating its potential as a safe and effective herbal hepatoprotective agent.

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