Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43B, No.2S,
July-December 2024: P. 1518-1530

Print version ISSN 0970-0765
Online version ISSN 2320-3188

Original Research Article

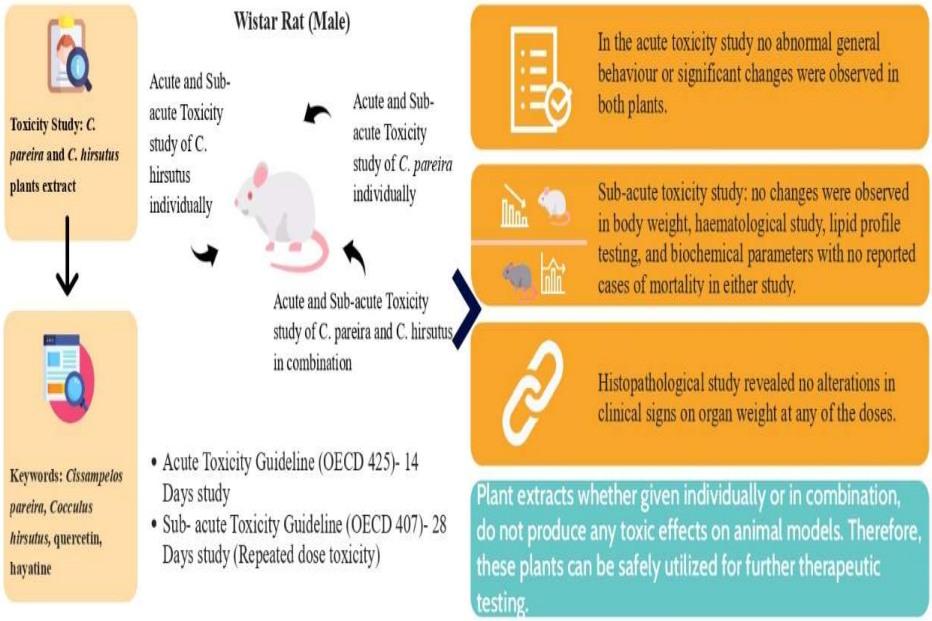
Content Available online at: www.bpasjournals.com

ASSESSING THE INDIVIDUAL AND COMBINED TOXICITY PROFILE OF CISSAMPELOS PAREIRA AND COCCULUS HIRSUTUS IN ANIMAL MODELS

Ashmun Nisha[1], [2], Aleza Rizvi[2]*, Arshiya Shamim[1], Bhagyashree Goswami[2], Wasim Akhtar[1],[2], Rizwan Ul Hasan[3]

[1]Faculty of Pharmacy, Integral University, Lucknow, Uttar Pradesh 226026, India
[2]Hygia Institute of Pharmacy, Lucknow, Uttar Pradesh 226020, India
[3]Era College of Pharmacy, Era University, Lucknow, 226003, UttarPradesh, India.

Graphical Abstract



Abstract

The toxicity profiles of *C. pareira*and *C. hirsutus*, commonly used in traditional medicine, were investigated using animal models in this study to assess the safety of their medicinal use. Acute toxicity testing involved a single oral dose of the plant extracts individually and in combination in rats over a 14-day period, while sub-acute toxicity testing included oral administration of the extracts for 28 days. Body weight, behavioral performance, adverse effects, and mortality were monitored during both studies. No abnormal behavior, changes in body weight, or significant alterations in hematological, lipid, or biochemical parameters were observed. Additionally, no mortality was reported, and histopathological examination showed no organ abnormalities at any dose. These findings suggest that the plant extracts do not pose toxic effects and can be safely used for further therapeutic experiments.

Keywords: Combination toxicity, quercetin, hayatine, LD_{50} (Lethal Dose), ED_{50} (Effective Dose).

Introduction

Throughout history, herbal drug extracts have been used in traditional medicine systems to

treat various health conditions. In contemporary times, there is a growing interest in using herbal drug extracts as alternative or complementary medicine (Dilshad *et al.* 2022, Tilburt *et al.* 2008). However, before these extracts can be considered safe and effective for consumption, a thorough toxicity investigation is necessary. The aim of this research is to evaluate the toxicity of different herbal drug extracts and determine their potential risks to human health (Ramesh *et al.* 2018). Traditional herbal medications are plant-derived materials used in healing practices without significant industrial processing. The goals of the toxicity study on herbal drug extracts include assessing acute and chronic toxicity, determining safe dosages, identifying toxic components, providing usage recommendations, contributing to knowledge, supporting decision-making in healthcare, and establishing guidelines for safe use in healthcare settings (Balunas *et al.* 2005, Majaz *et al.* 2016).

Herbal medicines have been widely used in both developed and developing countries due to their natural origin and minimal side effects. Approximately 75-80% of the global population, especially in developing nations, relies on herbal remedies as a primary form of healthcare (Parasuraman *et al.* 2011, Ghosh *et al.* 2019, Kabir *et al.* 2018). Toxicological assessments are crucial in developing new drugs, and regulatory authorities emphasize evaluating the toxicity and effects of compounds. Many developing countries incorporate herbs into healthcare practices, and a significant portion of allopathic medicines are derived from natural sources like plants. Approaches to evaluating the toxicity of herbal drug extracts include in vitro studies, animal studies, and clinical trials (Kumar *et al.* 2022, Arome *et al.* 2013).

The outcomes of toxicity studies on herbal drug extracts provide essential information on adverse effects, safe dosages, toxic components, and overall safety evaluation for healthcare use. These studies contribute to understanding the risks and safety of using herbal drug extracts in healthcare settings and can help guide decision-making for healthcare providers and regulatory agencies (Teke *et al.* 2014, Dubey *et al.* 2004, Middleton *et al.* 2000). The toxicity study is a non-clinical safety assessment to gather data on the toxicological characteristics of test compounds to ensure their safety before clinical trials. Herbal drug combinations have shown improved efficacy and decreased side effects compared to individual herbal drugs. Flavonoids, a group of plant-derived compounds, have gained attention for their potential health benefits, including antiviral, antibacterial, medicinal, and anti-carcinogenic activities (Williams *et al.* 2004). Examples of toxicity studies on specific herbal plants such as *C. pareira*, *C. hirsutus*, Sinococuline, and *Aegle marmelos* have demonstrated their safety for consumption through various animal models and tests. These studies highlight the importance of assessing the toxicity of different plant extracts to determine their suitability for human use (Panda *et al.* 2022).

1. Materials and methods

1.1.Chemicals and test materials

The desiccated foliage of the *C. pareira*and *C. hirsutus*plants were procured from a local market in Lucknow, India. All additional substances utilized were of analytical quality and obtained from CDH suppliers and Bharti Scientific Store, also located in Lucknow, India.

1.2.Methods

The study uses a randomized controlled trial with mice to test the toxicity of *C. pareira*extract. Male and female rats of the same strain, weighing 100-150 grams, will be used in the study. Sick or injured rats will not be included. The survival rate of the mice after receiving the extract will be measured. Data will be analyzed using a one-way ANOVA test to determine toxicity significance. Approval for the study was obtained from the Institutional Animal Ethical Committee (IAEC).

1.3.Experimental animal

Juvenile albino Wistar rats were obtained from Hygia Institute of Pharmaceutical Education and Research in Lucknow. They were acclimated for a week in standard cages with access to food and water. Environmental conditions were controlled with room temperature set at 23±2˚C, relative humidity at 50±5%, and a 12-hour light/dark cycle. The rats were healthy, weighing 100-150 grams and aged 10-11 weeks, before the study began.

1.4.Experimental design

Dose calculation: Various doses of a single plant extract and a combination of extracts were selected for toxicity studies following OECD guidelines and previous research findings. The doses were determined based on the ED50 value and toxicological profiles of each drug. The study followed the OECD guideline protocol, as there was limited information on the combination dosage in existing literature. Doses ranged from 250 mg/kg/day to 2000 mg/kg/day, with adjustments made based on body weights. These doses were used to evaluate acute and sub-acute toxicity of the individual plants and their combination in the study groupsin table **1** (Rasmussen *et al.* 2005, Bansod *et al.* 2010, Noorani *et al.* 2022).

1.5. Drug administration

The researchers chose to administer the herbal extracts of both plants individually and in combination orally, directly to the stomach using a gavage needle. Prior to dosing for acute toxicity, the animals underwent a 16-hour fast overnight. They were provided with ad libitum access to drinking water and feed, with feeding occurring approximately 4 hours after dosing (Ududua *et al.* 2019). In the sub-acute toxicity study, the plant extract was orally administered once daily for 28 days without the requirement of fasting.

Table 1. Protocol for administering *C. pareira*and *C. hirsutus*plant extracts individually and in combination to albino wistar rats for the purpose of conducting acute and sub-acute toxicity studies.

S. No.	Group name	Group detail	Dosing schedule
Acute toxicity study			
1	NC	Normal control of acute toxicity	Normal Saline
2	TG1	Acute toxicity group 1	250 mg/kg orally
3	TG2	Acute toxicity group 2	500 mg/kg orally
4	TG3	Acute toxicity group 3	1000 mg/mg orally
5	TG4	Acute toxicity group 4	2000 mg/kg orally
Sub-acute toxicity study			
1	NC	Normal control of acute toxicity	Normal Saline
2	TG1	Sub-Acute toxicity group 1	250 mg/kg orally
3	TG2	Sub-Acute toxicity group 2	500 mg/kg orally
4	TG3	Sub-Acute toxicity group 3	1000 mg/mg orally
5	TG4	Sub-Acute toxicity group 4	2000 mg/kg orally

1.6.Acute toxicity study

An acute toxicity study was conducted following OECD guidelines on rats divided into groups receiving different doses of plant extracts orally. The study aimed to evaluate any potential toxic effects of the extracts. The rats were observed for 14 days post-dosing for any signs of toxicity, changes in behavior, and various physiological parameters. The doses ranged from 250 mg/kg to 2000 mg/kg, with the highest dose given if the animals survived the previous one. Monitoring included body weight, urination, food and water intake, respiration, convulsions, tremors, constipation, and changes in eye and skin color. Measurements were

taken at different time intervals over the 14-day period. The study provided valuable information on the safety profile of the plant extracts at different doses (Ganguly *et al.* 2007).

1.7.Sub-acute toxicity study

A sub-acute toxicity study was conducted following OECD guidelines on five groups of rats. One group served as the control and received saline, while the rest were given the herbal extract at varying doses for 28 days. The rats' weights were monitored regularly, and on the 29th day, blood samples were collected for analysis. After euthanization, their organs were removed, rinsed, weighed, and preserved for histopathological examination. The study aimed to assess the effects of the herbal extract on the rats over a prolonged period. The mortality rate in each group was also recorded throughout the 28-day study period. This study provides valuable information about the potential toxic effects of the herbal extract at different doses, aiding in determining safe dosage levels for future use (Tiwari *et al.* 2021).

1.8. Body weight and relative organ weight

During the sub-acute toxicity study, animals' body weight was monitored daily with a digital balance. After 28 days, the animals were euthanized and their organs were harvested and weighed. Blood samples were collected from each organ and their relative weight was calculated. The formula used was: (Weight of organ / weight of animal on the day of sacrifice) × 100 / weight of animal on the day of sacrifice.

2.9. Parameters

Hematological analysis included parameters such as red blood cell count, hemoglobin concentration, and platelet count, white blood cell count, packed cell volume, mean corpuscular volume, and mean corpuscular hemoglobin concentration (Balogun *et al.* 2016, Afolabi *et al.* 2012). The biochemical parameters tested included Serum Glutamic Pyruvate transaminase, Serum Glutamic Oxaloacetic Transaminase, and Aspartate aminotransferase (Wilkinson *et al.* 1969, Doumas*et al.* 1981). Protein content was estimated through total protein, albumin, globulin, and serum globulin levels (Fossati *et al.* 1986, Buege *et al.* 1978, Shamim *et al.* 2018). Kidney function tests assessed various biomarkers and electrolyte levels (Choe *et al.* 2023, Ansari *et al.* 2021). Lipid profile estimation included high density lipoprotein, low density lipoprotein, very low density lipoprotein, total cholesterol, and triglycerides (Misra *et al.* 1972, Amresh *et al.* 2008). Antioxidant enzymes were measured, including glutathione, catalase, superoxide dismutase, and glutathione peroxidase (Amresh *et al.* 2007, Aydın *et al.* 2016). Histopathological parameters involved examining organs for abnormalities and conducting tissue analysis using Hematoxylin and Eosin staining (Sushruta *et al.* 2007, Alsarhan *et al.* 2021). Statistical analysis was done using Graph Pad Prism software, with significance levels set at $p<0.05$.These tests were conducted to evaluate the overall health and functioning of the animals after being exposed to a particular treatment or condition. The results were analyzed to determine any significant differences between the treatment group and the control group. The findings were presented as mean values with standard deviations to show the variability in the data. Various statistical tests were used to compare the results, and any differences above the set threshold were considered significant (Hayes *et al.* 2014; Ukwuani *et al.* 2012). Overall, the study aimed to assess the impact of the treatment on the animals' physiological parameters and organ function, providing valuable insights into the effects of the treatment on the body. The results obtained from these tests are crucial in understanding the treatment's safety and efficacy and guiding further research and development in the field.

2. Results

2.1. Effect of plant extract on acute toxicity study

Animals were given extracts individually or in combination with a comparison group of vehicle, and were monitored at various time intervals for up to 14 days. Body weights of the

animals were recorded, showing no significant differences between groups. Organ weights were also compared, indicating no toxicity. No clinical or behavioral changes were noted at any dose compared to the control group. The LD_{50} was determined to be greater than 2000mg/kg/day body weight. Overall, the study found no evidence of toxicity in the animals treated with the extracts in Table 2.

2.2.Effect of plant extract on sub-acute toxicity

In a study on repeated dose toxicity, doses of 250-2000 mg/kg/day of a drug showed no signs of toxicity or mortality in animal subjects. Microscopic examination of organs at necropsy did not show any abnormalities.

2.3. Effect of plant extract on average and relative organ weight

The study found that animals in various groups had normal body weights with minor alterations. There were no significant changes in organ weights among the groups. The tables show the effects of plant extracts on average weight and relative organ weight. No significant changes were seen in individual organ weights, indicating no toxic effects on essential organs. Statistical analysis comparing organ weights of different extracts to the control group showed non-significant results. Therefore, it was concluded that the extracts did not have toxic effects on organs during treatment in figure 1.

Table 2. The impact of plant extracts on the physical and behavioural parameters of rats was investigated during a 14-day acute toxicity study.

Parameter	Normal control (NC)			250 mg/kg (TG1)			500 mg/kg (TG2)			1000 mg/kg (TG3)			2000 mg/kg (TG4)		
Extracts- A: *C. pareira*, B: *C. hirsutus*, C: Combined extract of *C. pareira* and *C. hirsutus*															
Groups	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Body weight	100 gm.	100 gm.	100 gm.	120 gm.	110 gm.	110 gm.	120 gm.	110 gm.	110 gm.	120 gm.	120 gm.	110 gm.	110 gm.	120 gm.	110 gm.
Food consumption (24 h)	40 gm.	50 gm.	50 gm.	40 gm.	48 gm.	58 gm.	38 gm.	48 gm.	48 gm.	47 gm.	50 gm.	50 gm.	48 gm.	48 gm.	50 gm.
Water consumption (24 h)	90 ml	90 ml	90 ml	80 ml	100 ml	100 ml	90 ml	90 ml	98 ml	90 ml	90 ml	90 ml	100 ml	95 ml	95 ml
Body temperature	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Visible abnormalities	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Rate of respiration	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Drowsiness	Not observed	Not observed	Not observed	Not observed	Not observed	Observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed
Lethargy	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed
Stool color	Dark black	Dark black	Dark black	Dark black	Dark black	Dark black	Dark black	Dark black	Dark black	Dark black	Dark black	Dark black	Dark black	Dark black	Dark black
Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Diarrhea	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mucoid stool	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Eye color/ pigmentation	Normal (Pink)	Normal (Pink)	Normal (Pink)	Normal (Pink)	Normal (Pink)	Normal (Pink)	Normal (Pink)	Normal (Pink)	Normal (Pink)	Normal (Pink)	Normal (Pink)	Normal (Pink)	Normal (Pink)	Normal (Pink)	Normal (Pink)
Skin color	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Rashes	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen
Mobility	30 min	35 min	35 min	35 min	30 min	35 min	35 min	35 min	35 min	30 min	35 min	30 min	35 min	30 min	35 min
Paw jumping	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Paw licking	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Seen
Paw biting	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed
Mortality	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive

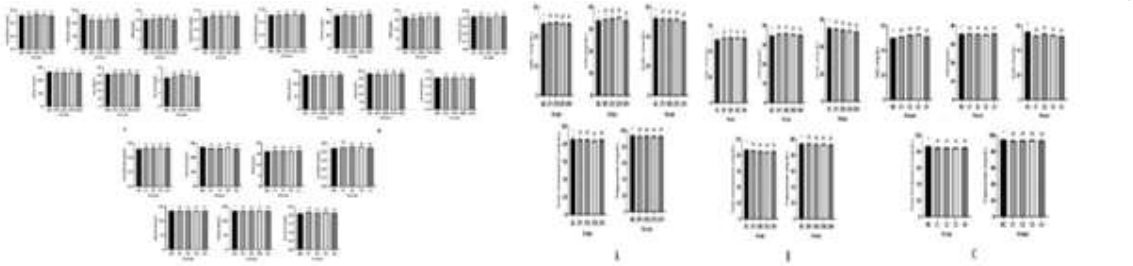


Figure 1. The impact of extract on the mean organ weight of rats (A: *C. pareira,* B:*C. hirsutus,* C: Combined extract of *C. pareira*and *C. hirsutus*). **Effect of plant extract on haematological parameters**

No significant toxicological differences were observed between the treated groups and the control group throughout the entire duration of the experiment, with a significance level of $p<0.05$. The results for all tested parameters are in Table. 3.4.1.

2.4. Effect of plant extract on biochemical estimation and protein estimation

When various oral extracts were given at doses of 250, 500, 1000, and 2000 mg/kg/day, no statistically significant alterations were observed in the levels of AST, ALT, GGT, and ALP in the respective groups compared to the normal control group ($p>0.05$) as indicated. There are

no notable alterations observed in the concentrations of total protein, globulin, albumin, and A/G ratio among the various treatment groups in comparison to the control groups in figure. 2.

2.5.Effect of plant extract on kidney function test and lipid profile testing

No notable alterations in the concentrations of BUN, total bilirubin, creatinine, glucose, sodium, urea, and uric acid were observed when comparing the treatment groups to the normal control groups, as indicated. When comparing all the treated groups to the control groups, the data indicates non-significant differences and demonstrates a lack of toxic effects, as evidenced by p-values greater than 0.05 in figure 3.

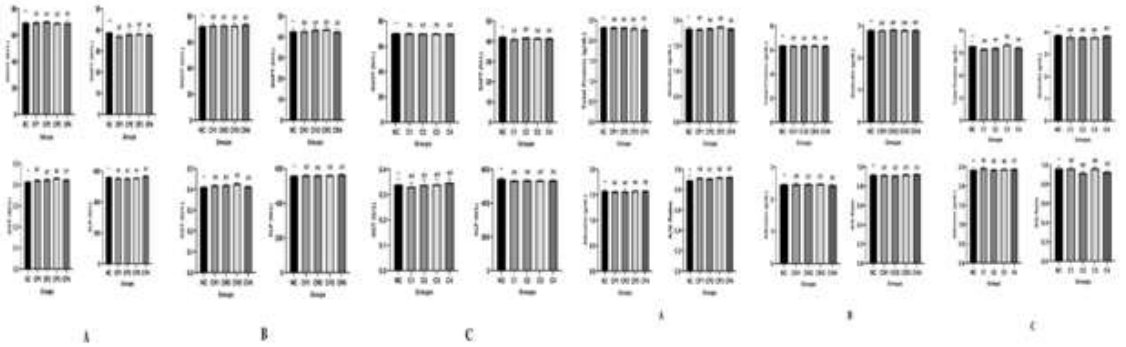


Figure 2. The impact of extract on biochemical parameters and protein content(A: *C. pareira,* B:*C. hirsutus,* C: Combined extract of *C. pareira*and *C. hirsutus*).

Organ	Hematological parameter (**Extracts-**A: *C. pareira,* B: *C. hirsutus,* C: Combined extract of *C. pareira* and *C. hirsutus*)														
	Normal control (NC)			**250 mg/kg (TG1)**			**500 mg/kg (TG2)**			**1000 mg/kg (TG3)**			**2000 mg/kg (TG4)**		
Groups	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Neutrophils (%)	26±1.581	27.2±0.836	31.6±1.816	30.2±0.836	31.6±1.816	31.6±1.140	26.2±0.836	31.2±1.303	32.8±2.774	30.4±0.547	32.4±1.140	32.4±1.516	27.8±1.303	27.6±1.673	32.2±1.483
Lymphocyte (%)	54.4±2.073	52.6±2.607	51.8±1.483	52.8±1.923	52±1.581	52.4±1.816	52.8±2.280	52.6±2.073	51.6±1.140	53.6±3.049	53.2±1.923	52±1.870	52.2±1.923	53.6±1.140	51.6±1.140
Monocyte (%)	0.3±0.158	0.32±0.083	0.32±0.164	0.36±0.230	0.32±0.083	0.34±0.151	0.34±0.167	0.26±0.134	0.44±0.114	0.36±0.151	0.34±0.089	0.44±0.114	0.3±0.070	0.28±0.130	0.34±0.194
RBC (106/µl)	5.728±0.052	5.738±0.033	4.942±0.034	5.42±0.238	4.95±0.031	4.938±0.025	5.38±0.286	4.4±0.316	4.936±0.027	5.44±0.270	5.48±0.319	4.936±0.019	5.34±0.194	5.38±0.334	4.95±0.038
WBC (103/µl)	7110±14.142	7140±54.772	6623.4±18.105	6460±114.017	6652±45.497	6638.8±14.618	6628±23.874	7098±56.745	6639±27.901	6458±53.103	6720±15.811	6647±32.756	7120±130.384	6914±11.401	6640±24.494
Platelet (106/µl)	213400±1140.17	213840±820.36	251000±707.10	193000±1581.13	203620±1527.08	251440.2±1125.73	193200±836.66	214160±114.01	251800±836.66	195403±366.46	211200±836.66	253800±2167.94	212106.2±77.988	213660±559.46	251900±894.42
PCV (L/L)	44.8±1.643	43±1.581	43±3.082	42.4±2.073	42.4±2.073	43±2.236	43.4±1.140	42.2±1.788	42.8±2.280	43±2	41.6±1.140	44±1.581	44±2	43±3.162	43.4±2.966
MCV (L/L)	64±1.581	64.4±1.140	62±64.121	62.4±2.073	62.4±1.673	64±67.1622	62.6±1.673	63±1.581	62.6±63.494	63.2±2.280	61.6±1.816	63.2±65.480	61.6±2.607	64.8±1.923	64.2±66.587
MCHC (g/ µl)	43.2±2.280	42.2±1.788	35±2.236	34±2.236	34±3.162	34.4±2.701	41.8±1.483	33±1.581	34.8±2.387	35.2±2.280	34.4±1.516	33±2.387	42±1.581	43.6±1.516	34±2.549
Eosinophil's (%)	0.4±0.122	0.3±0.1	0.42±0.286	0.44±0.114	0.44±0.114	0.42±0.164	0.34±0.151	0.28±0.130	0.38±0.148	0.38±0.148	0.3±0.158	0.44±0.260	0.52±0.130	0.34±0.194	0.42±0.148
Basophils (%)	0.8±0.836	2±1.581	1.2±0.836	1.2±0.836	1.4±0.894	2±0.707	0.6±0.547	1±0.707	2.2±1.095	0.6±0.547	1.2±0.836	1.2±0.836	1±1	1.4±0.894	1.6±1.140
Hemoglobin (%)	14.36±0.313	14.58±0.228	13.46±0.194	14.3±0.122	13.48±0.286	13.5±0.1	14.18±0.192	14.34±0.207	13.34±0.089	14.34±0.270	14.46±0.194	13.48±0.083	14.48±0.303	13.6±0.291	13.56±0.114

Table 3. The impact of plantextracts on haematological parameters.

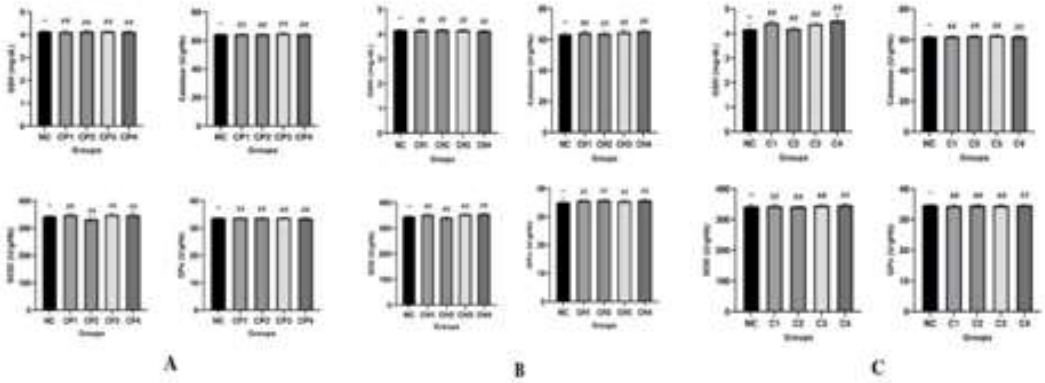


Figure 3. The impact of extract on renal function testing and lipid profile(A: *C. pareira,* B:*C. hirsutus,* C: Combined extract of *C. pareira*and *C. hirsutus*).

2.6. Effect of plant extract on Antioxidant enzymes

When the groups treated with the extract are compared to the normal control groups, all of

them exhibited non-significant results, indicating that the drug is non-toxic, as evidenced by a p-value greater than 0.05 in figure 4.

2.7.Histopathological Examination

Based on the findings, no adverse histological effects were observed in the liver, heart, brain, kidney, lungs, and spleen of the groups treated with the extract, as compared to the normal control groups in figure 5.

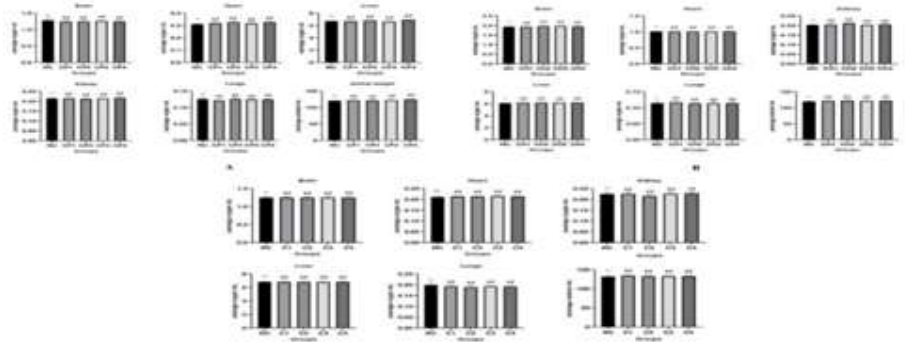


Figure 4. The impact of extract on antioxidant enzymes(A: *C. pareira,* B:*C. hirsutus,* C: Combined extract of *C. pareira*and *C. hirsutus*).

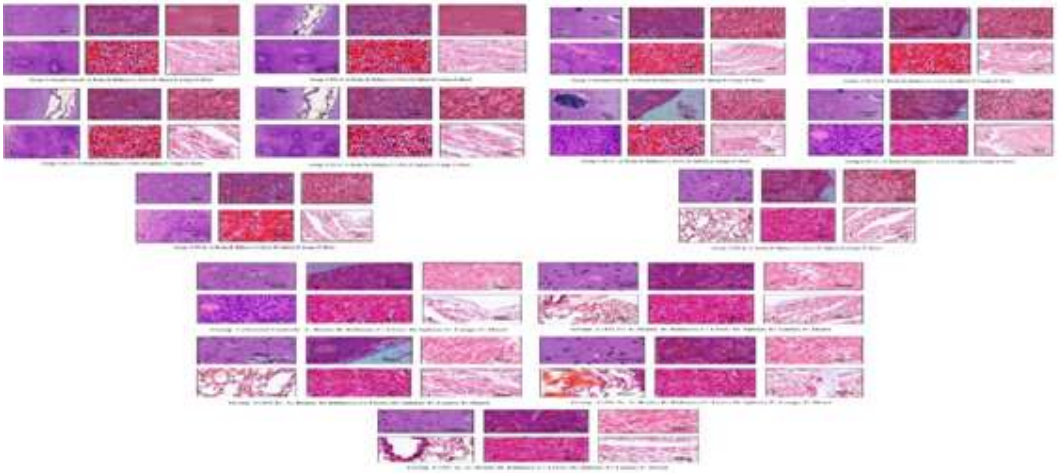


Figure 5. The impact of (A: *C. pareira,* B:*C. hirsutus,* C: Combined extract of *C. pareira*and *C. hirsutus*) on different organs.**4. Discussion**

The research involved male and female rats of varying ages, distributed randomly into groups of five animals each to explore the toxicity of *C. pareira*and *C. hirsutus*. The findings indicated no toxicity in the animal models, with no significant weight loss, organ damage, or mortality observed (Wang *et al.* 2017, Adeneye *et al.* 2009, Kunimatsu *et al.* 2004). Further investigations are needed to understand the mechanisms of toxicity for both plants and identify potential therapeutic targets, especially in human cells and tissues.

Research also highlighted the potential anti-inflammatory and antioxidant properties of *C. pareira*, suggesting its promise for therapeutic applications (Wasim *et al.* 2023, Abbott *et al.* 2013). Acute and sub-acute toxicity studies showed no adverse effects in mice and rats, indicating the safety of *C. pareira*(Piao *et al.* 2013, Arsad *et al.* 2013, Mukinda *et al.* 2007). The study emphasized the importance of conducting comprehensive toxicity studies on herbal drug extracts to ensure their safety and efficacy in medical applications. Additionally, the study found that a combination of herbal plant extracts could have potential health risks and necessitated further research (Nfozon *et al.* 2019). Acute toxicity testing of individual and

combined plant substances in rats showed no significant adverse effects, indicating their safety at certain doses (Beitollahi *et al.* 2022). Sub-acute toxicity assessments revealed no toxicity during repeated exposures to the plant extracts, suggesting their safety for clinical trials. Overall, the study provides valuable insights into the toxicity profiles of *C. pareira*and *C. hirsutus* and their potential applications in herbal medicine (Karincaoglu *et al.* 2005, Shariq *et al.* 2022).

5. Conclusion

The toxicological screening results showed normal parameters and no significant deviations for the substances studied. Histopathological studies confirmed no major changes in organ structure. No deaths occurred in single or multiple dose toxicity studies, indicating the LD_{50} of the plant extracts is over 2000 mg/kg body weight, making them safe for further research. However, more testing is needed to determine their effectiveness and safety in humans.

Acknowledgements
The authors are highly thankful to Honorable Founder and Chancellor, Prof. Syed Waseem Akhtar, Integral University and Vice-Chancellor, Prof. Javed Musarrat, Integral University, for providing an excellent research environment and facilities. The authors also express their gratitude towards Prof. Syed Misbahul Hassan, Dean, Faculty of Pharmacy, Integral University for his motivation and support.I am also thankful to Honourable Chairman Mr. Ishrat Hussain Jafri and Prof. (Dr.) Udayvir Singh Sara, Director, Hygia Institute of Pharmaceutical Education and Research. The authors offer their thanks to R and D department of Integral University for providing technical support and assigning Manuscript Number: IU/R&D/2024-MCN0002604.
Research Funding
No funding.
Author's contribution
All authors have accepted the responsibility and approved the manuscripts submission.
Competing interest
The author shows no conflict of interest and data available by the request on the first author AN.
Informed consent
Informed consent was obtained for all research participants.
Ethics approval
Approval for the study was obtained from the Institutional Animal Ethical Committee (IAEC) under IAEC no. HIPER/IAEC/131/03/2023.
Consent for Publication
Authors/institutions consented to manuscript publication.

5. References

1. Abbott, L.C. (2013). Selecting optimal animal models to investigate environmental toxicology. *Poultry Fisheries & Wildlife Sciences*, 1(1).
2. Adeneye, A.A. (2009). Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of Carica papaya Linn in Wistar rats. *Biol Med*, 1(1), 1-1.
3. Afolabi, S.O.(2012). A 90 day chronic toxicity study of Nigerian herbal preparation DAS-77 in rats. *BMC Complementary Alternative Med*icine, 12(1), 79.
4. Alsarhan, A. (2021). Review on some Malaysian traditional medicinal plants with therapeutic properties. *Journal of Basic & Applied Science*, 10, 149-159.

5. Amresh, G. (2007). Antinociceptive and antiarthritic activity of Cissampelos pareira roots. *Journal of Ethnopharmacol*gy, 111(3), 531-536.
6. Amresh, G. (2008). Toxicological screening of traditional medicine Laghupatha (Cissampelos pareira) in experimental animals. *Journal of Ethnopharmacol*ogy, 116(3), 454-460.
7. Ansari, A. (2021). Areca catechu: A phytopharmacological legwork. *Food Frontiers*, 2, 163-183.
8. Arome, D. (2013). The importance of toxicity testing. *J Pharm BioSci*., 4, 146-148.
9. Arsad, S.S.(2013). Evaluation of acute, subacute and subchronic oral toxicity of Rhaphidophora decursiva (Roxb.) Schott extract in male Sprague Dawley rats. *J Med Plant Res*, 7(41), 3030-3040.
10. Aydın, A. (2016). A guidance manual for the toxicity assessment of traditional herbal medicines. *Natural Product Communications*, 11(11), 1763-1773.
11. Balogun, F.O. (2016). Acute and subchronic oral toxicity evaluation of aqueous root extract of Dicoma anomala sond. In wistar rats. *Evidence-Based Complementary and Alternat Med*icine, (1), 3509323.
12. Balunas, M.J. (2005). Drug discovery from medicinal plants. *Life Sci*ences, 78(5), 431-441.
13. Bansod, M.S. (2010). Evaluation of analgesics and anti-inflammatory activity of a poly-herbal formulation. *Int J PharmTech Res*., 2(2), 1520-1527.
14. Beitollahi, M. (2022). *Assessment of Salivary and Serum Antioxidant Status in Patients with Recurrent Aphthous Stomatitis*.
15. Buege, J.A. (1978). Microsomal lipid peroxidation. *Methods in enzymology*, 52, 302-310.
16. Choe, H. (2023). Accuracy of albumin, globulin, and albumin-globulin ratio for diagnosing periprosthetic joint infection: A systematic review and meta-analysis. *Journal of Clinical Med*ecine, 12(24).
17. Dilshad, R. (2022). Chemical composition and biological evaluation of Typha domingensis pers. To ameliorate health pathologies: In vitro and in silico approaches. *Biomed Research International*, 2022, 8010395.
18. Doumas, B.T. (1981). A candidate reference method for determination of total protein in serum. I. Development and validation. *Clinical Chemistry,* 27(10), 1642-1650.
19. Dubey, N.K. (2004). Kumar R, Tripathi P. Global promotion of herbal medicine: India's opportunity. *Current science*., 86, 37-41.
20. Fossati, P. (1986). A kinetic colorimetric assay of gamma-glutamyltransferase. *Clinical Chem*istry, 32(8), 1581-1584.
21. Ganguly, M. (2007). Antifertility activity of the methanolic leaf extract of Cissampelos pareira in female albino mice. *Journal of Ethnopharmacology*, 111(3), 688-691.
22. Ghosh, D. (2019). Mondal S, Ramakrishna K. Acute and sub-acute (30-day) toxicity studies of Aegialitis rotundifolia Roxb., leaves extract in Wistar rats: safety assessment of a rare mangrove traditionally utilized as pain antidote. *Clinical Phytoscience*, 5(1).
23. Hayes, A.W. (2014). Principles and Methods of Toxicology*, CRC Press*. CRC Press.
24. Kabir, I.(2018). Ansari I. A review on in vivo and in vitro experimental models to investigate the anti-inflammatory activity of herbal extracts. *Asian J Pharm Clin Res*, 11(11), 29.
25. Karincaoglu, Y. (2005). The levels of plasma and salivary antioxidants in the patient with recurrent aphthous stomatitis. *Journal of Oral Pathology & Medicine,* 34(1), 7-12.
26. Kumar, V. (2022). Biogenic Phytochemicals Modulating Obesity: From Molecular Mechanism to Preventive and Therapeutic Approaches. *Evidence-Based Complementary and Alternative Medicine*, 6852276.

27. Kunimatsu, T.(2004). Evaluation for reliability and feasibility of the draft protocol for the enhanced rat 28-day subacute study (OECD Guideline 407) using androgen antagonist flutamide. *Toxicology*, 200(1), 77-89.
28. Majaz, Q., (2016). Herbal medicine: A comprehensive review. *International journal of pharmaceutical research*, 8, 1-5.
29. Middleton, E. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological Review*, 52(4), 673-751.
30. Misra, H.P. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chem*istry, 247(10), 3170-3175.
31. Mukinda, J.T. (2007). Acute and chronic toxicity of the aqueous extract of Artemisia afra in rodents. *Journal of Ethnopharmacology*, 112(1), 138-144.
32. Nfozon, J.N. (2019). Acute and sub-chronic toxicity evaluation of triplotaxis stellulifera (Benth.) hutch and crasssocephalum bougheyanum CD Adams methanol extract on mice. *Biochemistry and Analytical Biochemistry*, 8(3), 1-1.
33. Noorani, A.A. (2022). Hepatoprotective Effect of Cocculus Hirsutus Linn. against Ethanol-Induced Liver Damage in Albino Wistar Rats.
34. Panda, A.K. (2022). Ethnomedicinal and ethnobotanical investigations and documentation of plants used by traditional healers of Eastern India. *Current Traditional Medicine*, 8(6).
35. Parasuraman, S., (2011). Journal of pharmacology & pharmacotherapeutics.
36. Piao, Y. (2013). Change trends of organ weight background data in sprague dawley rats at different ages. *Journal of Toxicologic Pathology*, 26(1), 29-34.
37. Ramesh, P. (2018). Effect of antimicrobial activity of Eupatorium odoratum against clinical microbes. *Int J Sci Res Biol Sci*; 5(5), 30-35.
38. Rasmussen, S.E. (2005). Dietary proanthocyanidins: occurrence, dietary intake, bioavailability, and protection against cardiovascular disease. *Molecular Nutrition & Food Research*, 49(2), 159-174.
39. Shamim, A. (2018). Lipids: An insight into the neurodegenerative disorders. *Clinical Nutrition Exp*erimental, 20, 1-19.
40. Shariq, M.(2022). Preparation, characterization and safety assessment of combinatorial nanoparticles of carvedilol and sericin. *International Journal of Applied Pharmceutics*, 80-85.
41. Sushruta, K. (2007). Evaluation of the blood-glucose reducing effects of aqueous extracts of the selected umbelliferous fruits used in culinary practices. *Tropical Journal of Pharmaceutical Research*, 5(2).
42. Teke, G.N. (2014). Acute and Subacute Toxicities of African Medicinal Plants. *Toxicological Survey of African Medicinal Plants*. Elsevier.
43. Tilburt, J.C. (2008). Herbal medicine research and global health: an ethical analysis. *Bulletin of the World Health Organization,* 86(8), 594-599.
44. Tiwari, R. (2021). Correction to: An exploratory analysis on the toxicity & safety profile of Polyherbal combination of curcumin, quercetin and rutin. *Cliinical Phytoscience*., 7(1).
45. Ududua, U.O. (2019). Evaluation of acute toxicity of the ethanolic leaf extract of Brachystegia eurycoma in albino wistar rats. *Journal of Complementary and Alternative Medical Research,* 7(1), 1-7.
46. Ukwuani, A.N. (2012). Toxicological studies of hydromethanolic leaves extract of Grewia crenata. *Int J Pharm Sci Drug Res*, 4(4), 245-249.
47. Wang, J. (2017). Safety assessment of vitacoxib: Acute and 90-day sub-chronic oral toxicity studies. *Regulatory Toxicology and Pharmacology,* 86, 49-58.
48. Wasim, R. (2023). Entanglement of AGE-RAGE axis in cardiac pathosis. *bioRxiv*.

49. Wilkinson, J.H. (1969). Evaluation of a new system for the kinetic measurement of serum alkaline phosphatase. *Clinical Chem*istry, 15(6), 487-495.
50. Williams, R.J. (2004). Flavonoids: antioxidants or signalling molecules? *Free Radical Biology Med*icine, 36(7), 838-849.